CONCEPTS IN MARINE POLLUTION MEASUREMENTS

Edited by Harris H. White

A Maryland Sea Grant Publication
University of Maryland
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Contents

Chapter 4. Bioaccumulation Tests

Chapter 5. Chemical Measurements and Effects Criteria

Chapter 6. Anomalies in Field Specimens

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Preface

Problems of the environment have received considerable attention over the past decade. Concern over pollution of the marine environment has been particularly strong and has been the focus of innumerable conferences and publications. Unfortunately, the strategies and techniques used to measure marine pollution and its effects have not always been based on solid scientific methods, and many studies have thereby cast some doubt on the results of pollution effects measurements in general.

A workshop on "Meaningful Measures of Marine Pollution Effects" was convened in Pensacola, Florida, on April 26-29, 1982. The purpose of the workshop was to discuss which attributes of pollution measurements contribute to their credibility and significance, and which detract. Contributed papers were solicited from several dozen scientists with longstanding experience both in the techniques of marine pollution and the strategies those techniques fit into. Authors were asked not to dwell on the details of methodology, but rather to comment on the strong as well as the debilitating features of whole categories of techniques. This was a challenging experience for most of the contributors, but the reader will be gratified to find that many authors successfully transcended the narrow confines of their own work.

The papers were subjected to a rigorous peer review, which was deemed essential despite the resulting delay in publication. The papers are organized into chapters which loosely move from controlled, laboratory assessment strategies to field strategies. Between these two lie chapters on several categories of pollution measurements that typically form the backbone of laboratory and field work. Most common measurement techniques are discussed;

unfortunately this attempt at completeness has rendered the volume's organization almost as undisciplined as the field of environmental science itself.

Authors were encouraged to be penetrating and critical, rather than merely to "review" some body of literature. Conclusions drawn by several authors are shocking, if not downright heretical. Yet most of the papers include hopeful suggestions for remedying present weaknesses. The authors of the final chapter not only summarize the many critical views presented in this volume, but they also present their own roadmap for righting the dimly conceived strategies that have ruled the field.

It is hoped that this effort will spawn others just like it, not only with respect to marine pollution, but also for the whole of environmental science. Present frustrations, confusion and backtracking simply cry for a renewed atmosphere of critical thought and scientific rigor. Serious environmental protection demands as much, and honest science allows nothing less.

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Editor

Chapter 1. Toxicity Tests

Introduction

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Toxicity tests have provided, and will continue to provide, valuable information on the impact and potential impact of pollutants on the marine environment. The manner in which these tests are conducted and interpreted continues to evolve. Many of the tests described in this chapter did not exist five years ago and have been changed drastically within the last ten years. Toxicity tests can be used to compare toxicity of compounds and sensitivity of species and to determine mechanisms of effect, thus providing the cornerstones of a hazard assessment process.

A hazard assessment may be defined as "a process whose objective is to provide information regarding the safety or risk of a pollutant in the environment" (Cairns et al. 1978). In this context, safety is a value judgment of the acceptability of risk, risk being defined as the scientific judgment regarding probability of harm to the aquatic environment resulting from known or predicted environmental concentrations. According to this approach, a hazard assessment is made to determine risk to the environment, and is an objective but probabilistic measure requiring both empirical factors and scientific judgment. A value judgment concerning risk is then made.

It is important to realize that at least two types of hazard assessments exist. Although the processes are not mutually exclusive, they require different data inputs and are conducted with different objectives in mind. A "prospective" hazard assessment is exploratory, conducted before a substance is released in the environment, and is chiefly predictive in nature, whereas a "retrospective" hazard assessment is conducted after a substance is in the environment, and is often employed to describe present impact as well as to predict effects. Prospective assessments are used in pre-market testing activities, pesticide registration and National Pollution Discharge Elimination System permits and in establishing ecological baselines. Retrospective assessments are especially useful in assessing impact of pollutants on a living resource, such as the impact of a spill on specific fisheries. Both kinds of assessments are necessary, and both are only as good as the measurements of effects and environmental concentrations that compose them (J. Couch, personal communication). Each of the above conceptual approaches may combine data from laboratory tests and

field measurements for more complete assessments.

Toxicity and other tests that constitute a hazard assessment are often conducted under controlled laboratory conditions, but there is an overwhelming need to coordinate laboratory tests closely with field data. For example, over 600 contaminants were identified in a hazard assessment of Puget Sound (D. Malins, personal communication). Obviously, it is unlikely that laboratory single-species toxicity tests with individual contaminants could be integrated to predict the simultaneious effect of the 600 contaminants to organisms. A second and equally confounding factor is that laboratory tests are unlikely to simulate the complex environmental factors that affect toxicity and bioavailability in the field. Also, measurements of effects of simultaneous exposures to organisms in the polluted environment would not necessarily yield precise information on mechanisms of effects of individual chemicals. However, a combination of laboratory and field experiments could yield the necessary framework for the hazard assessment.

Unfortunately, predictive (and therefore prospective) hazard assessments must often be conducted with a paucity of laboratory-developed data and without any field data. Verification and predicted environmental effects and concentrations can occur only after the material is released into the environment. Nevertheless, the biological responses used to determine effects in the laboratory should be related to biological responses that occur and can be measured in the environment. Thus, there is a need for combining field and laboratory studies in both prospective and retrospective hazard assessments.

Laboratory and field comparisons can also be used to overcome a warranted criticism of toxicity tests conducted in the past--doubt that a toxicity value determined in the laboratory lies within a certain range of what occurs in the field. Such uncertain analyses lead one to consider the subject of field validation. It is through validation exercises, such as the Shaler Run Study (reported in this chapter), that the limits of applicability of laboratory data to field situations can be determined and quantified. Such studies are already under way as part of a few marine and freshwater hazard assessment activities.

Also at issue is the manner in which organisms are exposed to pollutants in the laboratory, compared with their exposure in the natural environment. Most flowing-water toxicity tests involve continual additions of the pollutant in order to maintain a desired concentration level in the water. However, pollutants are often added in discontinuous "pulses" in the environment. The exposure regime of the laboratory test should be as similar as possible to that in the field, unless the experiment is conducted strictly to determine relative toxicity of a compound to several species of test organisms. More importantly, an estimate should be made of the potential error resulting from discrepancy between laboratory- and

field-exposure regimes.

The necessity for more effort in relating laboratory and field tests has already been discussed. The correct balance of studies in the laboratory and field is more difficult to establish, but this balance must be evaluated on a case-by-case basis. Toxicity tests are designed only to characterize the wastes, and ecologists should provide the toxicologists with criteria for effects in the field before more meaningful laboratory and field studies can be accomplished. Obviously, in some instances, field tests are a waste of resources; in others, understanding mandates their use. Concern about proper balance of laboratory and field tests may be somewhat alleviated if the uncertain analyses discussed previously are applied to the laboratory tests. Balance would then be defined by users.

The diversity of types of toxicity tests available for hazard assessments and other purposes is represented by the variety of papers in this chapter. State-of-the-art toxicity tests evaluate organism responses from the cellular to multispecies and system levels of biological and ecological organization. Although the various tests discussed have different levels of precision and significance, it is often necessary to have such diversity to assess impact or potential impact of a pollutant on the environment.

The first paper (Hinton and Couch) presents pathobiological approaches to measuring the effects of marine pollution. The discipline of pathobiology includes such techniques as structure/function analyses of organ, tissues, cells and subcellular components (organelles, cytosol, enzymes and other macromolecules). These components are subjected to morphologic examination, marker-enzyme tests, and cellular and organ dysfunction tests. By using morphometry, the volume, surface area, and a number of structural features can be objectively and reproducibly determined. Enzyme activity can be measured quantitatively and accurately in a given fraction of biological sample, but often spatial relationships between organelles and cells are lost. Organ dysfunction tests include measurements of substances circulating in the blood which depend upon a specific organ for production or removal, assay of serum constituents and plasma disappearance rates. The overall objective of pathobiologic studies is to correlate pollutant exposure with altered structure and function in the various levels of biological organization. It provides the tools most likely to link cause and effect through understanding mechanisms of effects and injury. Although most pathobiologic studies have been conducted under controlled conditions in the laboratory, it is frequently possible to apply existing laboratory methodologies to the field. In addition, since cells and their organelles are fundamental units of all living species, data at this level of organization are important because the similar level of organization exists in organisms from unicellular to humans.

The next paper (Hansen) discusses the utility of toxicity tests in measuring the effects or potential effects of marine pollution. Acute and chronic tests with single species, multispecies tests and biological accumulation of pollutants are presented. The design, uses, species most commonly tested and results indicating the advantages and disadvantages of each type of test also are illustrated. Acute tests are standardized through the American Society of Testing and Materials, Standard Methods and other publications. These tests are used as measures of the comparative toxicity of chemicals, in prioritizing tests and as a scale for comparison of longer duration tests. Chronic test uses include estimates of Maximum Acceptable Toxicant Concentration, development of most sensitive "end-points" in a hazard evaluation and estimates of population effects. Disadvantages to this approach include the time and cost of the research. Multispecies tests can yield valuable information regarding the impact of pollutants on structure and function of test communities and provide a valuable link for laboratory-to-field extrapolations. The paper concludes by suggesting the need for prioritization of pollutant testing so that the cost of obtaining information through effects assessment is proportional to the level of environmental hazard.

A closely related paper that follows (Gentile and Schimmel) illustrates the manner in which toxicological data are applied to regulatory decisions. A brief historical perspective is given to relate state-of-the-art toxicity testing and legislative mandates of the Clean Water Act. Development of water quality criteria and subsequent use of the criteria in site-specific regulatory actions are presented. The regulatory strategy for site-specific criteria not only involves biological-effects data but also includes data on environmental concentrations of pollutants for protection of fish and wildlife that consume contaminated organisms. The strategy is designed to prevent commercially and recreationally important organisms from exceeding Food and Drug Administration action levels. Among the assumptions underlying such strategies are these: laboratory exposure conditions closely reflect those of environment in terms of bioavailability of toxicant or toxic components; biological responses of pollutant action are similar in laboratory and field; and sensitivity of laboratory test populations is representative of similar populations in the environment and, therefore, can be used to protect untested species. The validity of such assumptions is discussed. Several case histories are presented in which criteria effects and other assessments components are utilized to give a hazard assessment. The author reiterates the need for current and future strategies to integrate realistic exposure conditions in laboratory studies and to verify, in the field, relevant hypotheses.

The final paper (Cairns and Buikema) deals with system-level

toxicity tests and suggests that regulation based on toxicity tests must be scientifically justified and subject to validation. The difference between statistical significance and biological significance of toxicity tests and the necessity for both in a hazard assessment are discussed. Biological assessments should include a determination of the relationship between sublethal effects in organisms and effects of the toxicant on survival, growth and reproduction of the organisms in their natural habitat. The need for single-species tests is put in perspective with the need for validating or verifying predictions of harm and safety for the "real" world. Case studies of toxicity tests with single species, multispecies and natural communities are presented. In community tests, functional change can be predicted from structure and vice versa (e.g., ATP used to estimate biomass). Research is presented in which perturbation of community structure and function are measured by utilizing the ratio of ATP-estimated biomass/chlorophyll or estimated biomass (heterotrophic index) and colonization rates. Both indices of perturbation were applied to laboratory and field communities and indicated a similar response in both settings. This is cited as evidence that some laboratory tests may be good predictors of field responses when the tests and predictions are made at the same level of biological organization. Furthermore, it supports the concept that field validity of laboratory tests can, indeed, be determined and that common failure to do so is not always due to technical difficulty.

In summary, discussions and presentations in the toxicity chapter indicate that measurements of toxicity at the single, multispecies, community and system levels are useful in ranking chemicals by their toxicity and in hazard evaluations. However, relating the results of those tests directly to the environment is especially risky under these circumstances:

1. The manner in which laboratory organisms are exposed to pollutants differs from exposure in the environment.
2. Laboratory tests deal with single chemicals, and organisms are exposed to complex mixtures in the environment.
3. Criteria for effects in the laboratory are not important functional end points in population and system dynamics.

The dominant theme of this chapter is the necessity for an appropriate balance between laboratory and field studies. The need for this balance is easily articulated, but will require concerted effort to achieve.

REFERENCES

Cairns, J., Jr., K. L. Dickson and A.W. Maki (eds.). 1978. Estimating the Hazard of Chemical Substances to Aquatic Life. ASTM STP 657. American Society for Testing and Materials. Philadelphia, Pa.

Pathobiological Measures of Marine Pollution Effects

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INTRODUCTION

The class of techniques embodied in the term "pathobiology" includes structure/function analyses of organs, tissues, cells and subcellular components (organelles, cytosol, enzymes and other macromolecules) derived--in experimental toxicology--from control and diseased* organisms. Since the cell is the functional unit of all plant and animal organization, pathobiologic mechanisms apply to all phyletic systems. Rationale and methodology of pathobiology are found in extensive reviews (Forbus 1952; Majno et al. 1960; Hill 1975; Trump and Arstila 1975; Scarpelli and Trump 1971). A few basic principles are particularly relevant in this workshop. Disease is not associated with new, different structure and function; rather, quantitative alterations (increase or decrease) of existing structure and function occur (Forbus 1952). To understand disease, we must understand alterations in structure/function occurring in response to various injuries. Although the number of etiologic agents

*In this paper, disease includes all reactions to injury associated with pollutants in the aquatic environment and may incorporate infectious disease.

may include many pollutants and pathogenic organisms, we can be encouraged by the fact that a long list of etiologic agents of disease does not reflect an equally long list of pathogenetic mechanisms (Hill 1975). An organism's genetic composition is of importance relative to initial susceptibility to injury; however, the manifestation of disease reflects disturbances in enzymatic activity. Since enzymes are associated with specific organelles of cells, altered enzyme activity is often reflected as structural alteration (Hill 1975; Trump and Arstila 1975; Scarpelli and Trump 1971) underscoring the need for correlated structure/function studies. Since experimental procedures in pathobiology include morphological, biochemical and physiological tests, it is impossible in one review to cite all work encompassed by this term. Rather, the intent is to review major categories of pathobiological research and to present strengths and weaknesses of each. Where appropriate, examples from the aquatic toxicology literature are cited. Finally, the application of the pathobiologic approach to field studies and the importance of findings in aquatic species to other species including man are discussed.

GENERAL METHODOLOGY OF PATHOBIOLOGICAL ASSESSMENT

Table 1 presents some of the laboratory pathobiological tests used in determining pollutant effect. Hypotheses, assumptions, controls and the type data are given for each test with some information on statistical evaluation.

Morphologic Examination

This includes gross inspection of fresh or fixed individuals to yield information on weight, size, shape, number, coloration and surface texture. Microscopic inspection uses magnification to display structures in greater detail. The underlying assumption of morphological approaches in toxicity studies is that the investigator can distinguish between "normal" and altered structure and, among the latter, premortem (i.e., toxicant induced) from postmortem (autolytic) change. Although all fixed tissues have been removed from their blood supply and can be considered irreversibly injured, the morphologic analysis of adequately fixed material may reveal the following general populations of cells: those not altered by toxicant prior to somatic death and maintained in a normal, nonaltered state; those altered but not killed prior to somatic death, in which certain features associated with sublethal injury (swelling, fat vacuolation, or accumulation of stainable cytoplasmic inclusions) would be seen (Trump and Arstila 1975); and those altered lethally (before somatic death), in which autolysis (necrosis) would have occurred (Scarpelli and Trump 1971) prior to fixa-

Table 1. Considerations for validity of experimental design in laboratory pathobiological tests.

Test	Hypothesis[a]	Assumptions	Controls	Data/Evaluation
Routine morphologic	Exposure results in structural alteration at cell, tissue, organ or organism level.	Normal structure known at all levels of organization.	Paired for sex, age, size; all conditions same except no toxicant; region and orientation of tissue identical in exposed and control animals.	Target organ determination; degree injury established; determine number affected at each toxicant concentration (chi-square); determine % incidence of lesion in each experimental group.
Morphometric	Exposure results in quantifiable response at cell, tissue, or organ level.	Structural entities recognizable at each level of organization; 3-dimensional information can be obtained from 2-dimensional sections.	See controls for routine morphologic test; randomization must be ensured.	Volume compartment; surface density; numerical density and length of tissue components in reference volume of cell or tissue. Data are numerical and are amenable to statistical evaluation.

[a] As used here is overall (not statistical) hypothesis.

Table 1. Continued

Test	Hypothesis[a]	Assumptions	Controls	Data/Evaluation
Marker enzyme activity in cells of target tissues of organism exposed to toxicant	Exposure results in altered enzyme activity of specific cellular organelles.	Recovery of specific subcellular fractions identical in control and treated animals.	Control conditions of the routine morphologic test must be observed. Estimates of recovery of marker enzyme activity in treated and control population essential.	Data (numerical) are amenable to statistical evaluation.
Serum enzyme activity in organisms exposed to toxicant	Exposure results in change in serum enzyme (or isozyme) levels.	Damaged cell populations release tissue-specific recoverable in serum.	See controls of routine morphologic test. Same conditions for enzyme assays in sera from both.	Data are amenable to statistical evaluation.
Other fluids in organism	Elevation or depression of specific molecular entities (e.g., ions, sugars, fats, proteins) follows exposure.	Toxicant-induced dysfunction results in imbalance.	See controls of routine morphologic and serum enzyme tests.	Data are amenable to statistical evaluation.

Staining (histochemistry)	Exposure results in appearance or loss of reaction product of enzyme or cellular component (glycogen, fat, mucus) in affected cell populations.	Quantitative difference will be detectable.	See controls of routine morphologic test plus biologic and "no substrate".	Presence or absence of change scored per individual; strength of reaction graded semiquantitatively (+1, +2, etc.) using well-defined criteria. If reaction stoichiometric, can quantify using scanning microdensitometer. If areal analysis of affected foci conducted, statistics can be applied (Volume affected area/Volume organ).

[a] As used here is overall (not statistical) hypothesis.

tion. In addition, certain cells which responded to chemotactic factors released by dead and dying cells (Ward 1975) in the living organism may be found as accumulations in tissue sites not normally the loci for these cells.

The scope of this conference does not permit detailed description of techniques used in the morphologic phase of pathobiologic studies. Therefore, only brief mention will be made of procedures essential for validity of morphologic examination. Suitable procedures of necropsy, fixation, dissection/trimming, processing and light microscopic analysis of aquatic animals including zooplankton, sponges, hydroids, anemones, corals, worms, barnacles, bivalves, gastropods, cephalopods, crustaceans, echinoderms and fish have been compiled (Galigher and Kozloff 1971; Couch et al. 1974; Yevich and Barszcz 1982; Hinton et al. 1973). Fixatives suitable for both routine light and electron microscopy are available (Ito and Karnovsky 1968; McDowell and Trump 1976) and use of these is recommended unless decalcification is considered essential, in which case use of alternative fixatives may be necessary (Yevich and Barszcz 1982). In addition to fixation, orientation of organs prior to embedment in paraffin or plastic is important to ensure comparison between identical regions of organs and tissues in control and exposed animals. Distinguishing premortem from postmortem change is also important. Studies of controlled in vitro autolysis (Majno et al. 1960; Trump and Arstila 1975; Trump et al. 1971) have shown that lethally injured cells undergo a recognizable series of morphologic changes during the necrotic phase (degradative reactions occurring after cell death—collectively termed autolysis (Trump and Arstila 1975)). Direct chemical fixation of cells causes death without necrosis (Trump and Arstila 1975). However, in the case of improperly fixed tissue, distinguishing lethally injured cells produced by toxicant from those which underwent autolysis between necropsy (at which time tissue was removed from blood supply) and actual time of chemical fixation, becomes difficult. Data on controlled in vitro autolysis include invertebrates (Sparks 1972) and fishes (unpublished data of Gardner discussed in Couch et al. 1974). An understanding of these processes in tissues of test organisms helps achieve meaningful morphologic data.

By means of a routine light microscopic morphologic approach, the target organ of a given pollutant can be determined. Specific regions of tissue response within the target organ can also be established. When marine shrimp Penaeus duorarum were exposed to cadmium dissolved in seawater, gross and light microscopic examination revealed gill alteration which appeared as blackened regions (Couch 1977). More detailed analysis revealed foci of alteration primarily in terminal filaments. When analysis by electron microscopy was performed, organelle alteration including nuclear lysis, mitochondrial swelling and myelin-line membrane accumulation

was consistent with cellular necrosis. Hawkes (1980) reviewed literature on effects of xenobiotics on marine fish tissues after exposure to chlorobiphenyls or crude oil. Morphologic analysis after laboratory exposure shows olfactory epithelium as a target tissue in Menidia menidia exposed to crude oil. Other sites showing structural evidence of toxicity after exposure to crude oil included lens and intestine. When juvenile Chinook salmon, Oncorhynchus tshawytscha, were fed a diet containing a model mixture of petroleum hydrocarbons, chlorinated biphenyls or both (Hawkes et al. 1980), cellular change, primarily in columnar cells of intestinal mucosa, indicated toxicity in this organ. When the estuarine fish, the sheepshead minnow, Cyprinodon variegatus, was exposed to Kepone, an organochlorine, scoliosis resulted (Couch et al. 1977). In addition, light microscopic and x-ray analysis revealed breaking of centra of vertebrae at the epicenter of the spinal flexure.

All the above relate to laboratory exposures of organisms to specific pollutants. Sindermann (1979) reviewed lesions which have been found in feral marine organisms after gross and microscopic examination. These included fin erosion, ulcers, shell disease of crustacea, lymphocystis, stress provoked latent infections, neoplasms, skeletal anomalies and genetic abnormalities.

Data derived from routine morphologic analysis are valuable in determining which experimental probes should follow (i.e., ultrastructural study, analysis of organ homogenates or analysis of cellular and/or subcellular fractions from specific cell types after their separation). If the number of affected individuals can be determined and expressed for each toxicant concentration, data are amenable to statistical evaluation by tests such as the chi-square. Another application of a routine morphologic approach is in chemical carcinogenesis research using teleost species, where the end point, a clearly defined structural entity with specific characteristics, is usually detected by routine light microscopic techniques and the incidence data for tumors in each experimental group are listed (Ishikawa et al. 1975; Aoki and Matsudairo 1977; Pliss and Khudoley 1975; Hendricks et al. 1980 a,b). The routine morphologic approach is of additional importance when one considers that it provides a relatively quick initial screen to gain information from a variety of organ systems simultaneously.

Morphometry

Since the overall objective of the pathobiologic approach to toxicity studies is correlation of altered structure and function, it becomes necessary to employ tests which may provide for integration of morphological, biochemical and physiological studies. The non-quantitative appearance of morphologic data (i.e., micrographs) has seriously hampered full integration of studies incorporating cellular fractionation on one hand and electron microscopy on the other (Reide and Reith 1980). However, methods to extract

numerical data from microscopic images have evolved (Reide and Reith 1980; Bolender 1980, 1981; Elias et al. 1971; DeHoff and Rhines 1968; Underwood 1970; Weibel and Bolander 1973; Weibel 1979, 1980). Based upon fundamental principles defined in mathematical terms (Elias et al. 1971; Weibel 1979, 1980), morphometry permits the quantitative study of tissue and cell structures. Coupled with computer assisted technology, quantitative work can be performed in reasonable time (Rohr et al. 1976). Thus, morphometry is regarded as a necessary element in interdisciplinary studies such as toxicology which involve morphologists, biochemists, physiologists or pharmacologists (Bolender 1980). By using morphometry, the volume, surface area and number of structural features can be objectively and reproducibly determined.

For the determination of volume, a point network (lattice) with total points = P_t is superimposed over the micrograph or the projected image of the glass slide and point counts within a given profile (P_i) are determined. This requires a judgment call by the observer at each point and is recorded for each. The volume density of the structure i (V_{vi}) is proportional to P_i/P_t which is proportional to P_{Pi}. The total number of test points that must be applied to one representative sample unit depends on V_v and on the relative error considered acceptable. Formulae (Weibel 1979) exist for determining the number of test points needed per representative sample. In practice, one roughly estimates the order of magnitude of V_v on a few micrographs and then decides upon the total points (P_t). Table 2 illustrates the multistage morphometric analysis. At stage 1, the weight and volume of the organ or possibly organism (some invertebrates) is determined. The second stage of investigation, using light microscopic observation of fixed material, is conducted at the organ/tissue level of organization. At this level, for example, points lying over parenchyma, stroma, blood vessels and ducts would be determined. The subsequent stage of examination, at the level of individual cells, requries high resolution light microscopy (Epon or methacrylate sections) or, more commonly, low magnification electron microscopy. Analysis of these sections determines the total number of points lying over nuclei and cytoplasm of all individual cell types. In addition, the total number of points over extracellular space is determined. Linear intercepts with structures such as nuclear and plasma membranes are quantified to determine surface density of each. The fourth level of organization involves subcellular structures (organelles). Here the total number of points lying over specific organelles and the linear intercepts with membranous structures are determined to derive the volume density and surface area of structures. This level of investigation is done under medium to high magnification using the electron microscope. Procedures for morphometry require that randomization be carried out at the animal level, at the level of tissue blocks, and within areas of sectioned and stained material. Collecting tissues for morphometric examination can follow gener-

Table 2. The multistage morphometric analysis[a].

Stage	Level of Structural Organization	Data
1	Organ or organism	Weight, volume
2	Organ/Tissue	P_i parenchyma, stroma, blood vessels, ducts, etc.
3	Cell	P_i nuclei and cytoplasm of all individual cell types P_i extracellular space P_i mitochondria I_1 nuclei
4	Organelle	P_i mitochondria I_i endoplasmic reticulum

[a] For complete details see Rohr et al. (1976)

P_i = total points over tissue component

I_1 = linear intercepts with structure

al procedures used in routine morphologic examination. In addition, the investigator, using the multistage morphometric analysis (Table 2) controls the final level of organization from which information is sought. It may not be necessary in all instances to proceed to the electron microscope. Once the material has been collected for such analysis, the option is there, should such investigation prove necessary.

Hughes (1972) applied morphometric examination in studies of gas exchange regions of gill in tench, trout, dogfish, icefish and catfish. Expressing gill area per body weight, he found no good correlation among species. When distribution of gill area was analyzed, filament lengths varied as a function of their number. Shape

of secondary lamellae differed at various sites along filaments. On the basis of the above findings, Hughes recommended analysis of the following parameters in gill toxicity studies: relative proportion of specific cell types and extracellular material in secondary lamellae; surface area of blood channels in secondary lamellae; and effect of pollutants on different parts of the water/blood barrier. In a subsequent study, Hughes et al. (1979) used morphometric techniques to determine O_2 diffusion capacities in gills of control, nickel-, chromium- and cadmium-treated trout. Based on measurements of blood/water barrier, nickel exposure produced a marked harmful effect in diffusion capacity. Recovery to normal values was seen in trout 21 days after exposure. These workers concluded that morphometric methods can be combined with physiological data to have a quantitative, and possibly a predictive value, for assessing the effects of pollution on gills and on other organ systems.

Zuchelkowski et al. (1980) studied the effects of acid water (H_2SO_4 addition, pH 5.7-6.15) on alcian blue-periodic acid Schiff's stainable mucosubstances of epidermis in brown bullhead catfish, Ictalurus nebulosus. Standard morphometric techniques were used to determine mucous cell volume density and number density. Mucous cell volume density was significantly increased in fish exposed to acid stress for 5 days. Since the number of mucous cells was nearly twice that of controls while the volume of individual cells was the same, these workers concluded that acid stress results in increased number (hyperplasia) but not volume of individual mucous cells.

Subsequently, Zuchelkowski and Lantz (1981) reported sexual dimorphism in brown bullhead skin with respect to mucous cells. Control female bullheads had more mucous cells per volume of epidermis than did males. After acid stress, the sex differences were not seen. If male fishes predominate in assay populations, greater responses of mucous cells to acid stress will be encountered (Zuchelkowski and Lantz 1981).

Sex associated differences in epidermis may be due to hormonal levels in individual fish. Schwerdtfeger (1979a,b) used morphometric methodology to quantitatively examine epidermis of the guppy, Poecilia reticulata, following treatment with prolactin, thyroxine, and testosterone. When freshwater adapted guppies were treated with prolactin, morphologic parameters in epidermis which were increased included number of mucous cells, number of cell layers and the number of subepidermal capillaries. Upon adaptation to seawater (Schwerdtfeger 1979a), epidermis of guppies showed increased surface extent of superficial cells, an increase in the number of small, electron-dense vesicles and increased occurrence of chloride cells. Thyroxine in low doses caused an increase in number of mucous cells and small, electron-dense vesicles as well as increased height of glycocalyx (Schwerdtfeger 1979b).

In addition to the above, morphometry has been applied to analysis of changes during carcinogenesis in liver of Japanese medaka, Oryzias latipes (Hinton et al. 1982a).

Marker Enzyme Tests

This third category of laboratory pathobiological tests (Table 1) measures "marker" enzyme activity in cells of target tissues. Before this type of biochemical data can be collected, an organ must be homogenized and fractionated. Unfortunately, spatial relationships of organelles in a single cell and/or organelles from several different types of cells are lost (Bolender 1980). The strength of the biochemical approach resides in the accuracy with which an enzyme activity can be measured in a given fraction. Within the framework of analytical fractionation, the amount of activity in the various fractions can be compared with that in the original homogenate by calculating recoveries (DeDuve and Berthet 1954). Subcellular fractionation of rainbow trout liver homogenates has been studied to assess homogeneity of fractions (Statham et al. 1977), and a refined fractionation protocol has been developed. While such methods can be used to identify membrane marker heterogeneities, the initial homogenization procedure resulted in a mixture of membranes from a variety of cell types. Therefore, the cellular origin of the heterogeneities remains obscured (Bolender 1980). If we consider teleost liver in general, abundant morphologic heterogeneity is present, and the following cell types are seen: hepatocytes, endothelial cells, Kupffer cells, fat-storing cells, bile ductular epithelial cells, fibroblasts, macrophages, circulating blood cells and exocrine pancreatic cells (Hinton and Pool 1976). From this the need for "coupled" morphologic and biochemical assays (Bolender 1980) is readily apparent.

Organ Dysfunction Tests

These may be grouped into four broad categories. The first includes measurement of substances circulating in the blood, which depend upon a specific organ for their production or removal. The second group of tests assays substances in the serum which indicate abnormal function. In the third, plasma disappearance rates are determined using dyes and other substances which, following administration, are removed by a specific organ. Last, tests can be administered to determine the capacity of an organ to synthesize a given product when an essential precursor is furnished. Organ dysfunction tests are reviewed in standard texts on clinical laboratory diagnosis (Davidsohn and Henry 1974) or in monographs on specific organs such as the liver (Levy 1965). Some substances released from damaged cells are useful in the diagnosis of cell damage. Detectable in very low concentrations, enzymes reach a peak in the plasma following release from damaged cells. All cells have similar intracellular enzymes with similar organelle localization. How-

ever, in the human, the use of organ-specific isoenzymes (Zimmerman and Henry 1974) has greatly enhanced identification of the injured organ. Although isoenzymes of phosphoglucomutase, isocitrate dehydrogenase, and glucose-6-phosphate dehydrogenase specific for teleost lever and muscle are known to exist (Allendorf et al. 1977), little use in toxicologic assays has been forthcoming.

In an effort to determine the response of rainbow trout (Salmo gairdneri) liver to a proven hepatotoxic compound, carbon tetrachloride (CCl_4), studies were performed using serum enzymes (Statham et al. 1978; Pfeifer et al. 1980) and the dye, sulfobromophthalein (BSP) (Gingerich et al. 1978), which is selectively cleared from plasma by the liver and excreted in the bile. Exposure caused elevation of serum enzymes (Statham et al. 1978; Pfeifer et al. 1980) and induced retention of BSP (Gingerich et al. 1978). Chemical assay of liver revealed ^{14}C residue of CCl_4 in hepatic homogenate and showed increased diene conjugation indicating membrane toxicity (Statham et al. 1978). Recently, Gingerich (1982) reviewed literature on hepatic toxicology and covered the plasma enzymes and clearance studies in detail.

Urine, another body fluid available for assay (Table 1), has been studied in trout before and after CCl_4 exposure (Pfeifer and Weber 1980). Decreased urine flow, increased urine osmolality and proteinuria characterized trout exposed to CCl_4.

In a study of the effects of chlorine produced oxidants (CPO) on juveniles of the marine fish, Leiostomus xanthurus, Middaugh et al. (1980) used analysis of blood pH, oxygen uptake and gill morphology to establish toxicity. At sublethal concentrations of CPO, blood pH was decreased, oxygen uptake was depressed and gill morphology was altered. The latter included lesions of oxygen exchange portions of the gill and included sloughing of respiratory epithelium from underlying vasculature. These workers felt that the gill alterations could easily account for the change in blood pH and oxygen uptake.

Although relatively little application of serum and other body fluid assays is evident in aquatic toxocologic investigations, the above studies clearly indicate their merit. It should be noted that careful consideration of microchemical assays will have to be given when specimens of species with small body size are used.

Staining (Histochemistry)

This technique (see Table 1) represents a powerful tool for localization of enzymes and other cellular components (secretion products, hormones, lipids, proteins and carbohydrates) within tissues and, when applied at the cytochemical level, within cells. Although not directly quantifiable, except in instances where stoichiometric reaction can be demonstrated, the techniques provide a method for in situ characterization of sites of enzyme alteration. Studies of mucosubstances in skin and intestinal tract of teleosts

commonly employ these techniques (Reifel and Travill 1979; Zuchelkowski et al. 1980; Hinton et al. 1982b). Although as yet rarely employed in aquatic carcinogenesis studies (Scarpelli et al. 1963), enzyme histochemistry has been a valuable tool in characterization of small foci and other putative precursor lesions for epithelial tumors in mammalian carcinogen bioassay (Kalengayi and Desmet 1975; Lipsky et al. 1981).

Structure/Function Correlation

As was stated in the introduction, this is the goal of pathobiologic investigation. This objective, pursued in vitro using teleost kidney preparations (Forster 1948; Trump and Bulger 1971; Trump et al. 1975; Trump and Jones 1977), involved a variety of inhibitors and conditions to definitively modify functional parameters (transport of dye). Identical preparations, functionally defined, were then processed for ultrastructural analysis. In these correlated studies membranes of various organelles, especially plasma and mitochondrial membranes, revealed alterations concomitant with reduced transport function. A pattern of morphologic reaction is emerging in transporting epithelium which can be linked to functional impairment (Pritchard and Miller 1980).

The studies by Hughes et al. (1979) are particularly encouraging. Gill structure/function alteration was produced by laboratory exposure, and, under conditions of recovery, values were obtained which indicated a return to the control state.

One response, common across phyla including both aquatic and terrestrial organisms, has been the morphologic alteration of hepatic and/or hepatopancreatic endoplasmic reticulum after exposure to certain xenobiotics, specifically petroleum hydrocarbons and chlorobiphenyls (Couch and Nimmo 1974; Sabo and Stegeman 1977; Hinton et al. 1978; Lipsky et al. 1978; Klaunig et al. 1978; Schoor and Couch 1979; Hawkes 1980). Structure/function correlation has been shown in biochemical and coupled morphologic (quantitative) studies of rat liver endoplasmic reticulum after phenobarbital exposure (Bolender 1980). In fish exposed to polychlorinated biphenyls or aflatoxin (Klaunig et al. 1978; Scarpelli 1976) biochemical induction correlated with an apparent increase in endoplasmic reticulum. Clearly morphometric analysis of this alteration is needed to correlate with functional data.

Bolender (1980) emphasizes the necessity for a common unit of reference if correlation between biochemical and morphologic data are to be obtained. Where some workers failed to demonstrate correlation of morphologic and chemical (serum enzyme) data, a lack of common reference unit is apparent. For example, light microscopic subjective morphologic evaluation of liver (Pfeifer et al. 1980) and of kidney (Pfeifer and Weber 1980) toxicity following CCl_4 failed to show correlation with elevated serum enzyme levels and urine parameters respectively. Both serum enzyme elevations

and proteinuria imply cellular alteration, yet routine histologic techniques, which yield little if any information at the cellular level were used in the companion morphologic studies. For a common unit of reference to exist, morphologic probes at the cellular and/or subcellular level would be necessary and morphometric studies would be needed to provide objective numerical data for comparison.

Application of Pathobiologic Tests to Field Studies

Provided a power source and equipment are available for fractionation, freezing, and fixation of tissues, no apparently insurmountable difficulty exists in on-site collection of field samples for subsequent laboratory pathobiologic examination. It is clear that the tests can be performed in a reproducible manner, are scientifically valid and, when administered properly, yield data amenable to statistical evaluation. The central problem in the application of pathobiologic as well as other forms of tests to field studies is illustrated in Figure 1 which was modified from a figure in the elegant paper by Snieszko (1974) which describes many of these interactions in detail. Conditions affecting the field test can change with respect to the animal (host), the environment and the pathogenic agent(s). A partial listing of host factors (circle at top left) includes age, sex, genetic strain, nutritional status and the history of prior exposure to xenobiotics which might influence the rate of metabolism of potentially pathogenic chemicals (Lech and Bend 1980). Environment is represented in the bottom circle of Figure 1. Environmental factors and the physiologic adaptations of fishes have been the subject of an in depth and recent review (Love 1980). In that review, specific factors in addition to those given in Figure 1 include stress other than crowding; geographical influence; and depth, including pressure and illumination. The overlapping of the circles (host and environment) in Figure 1 signifies interaction. Since even minor variations in the external environment require adjustment by the cell or organism, any major variations of course require major adjustments. The inherent dynamic nature of the environment and the host adds additional complexity to experimental design since factors may change acutely or remain altered for some time (chronic interaction). The circle at the top right in Figure 1 has been labeled "pathogenic agent" to signify xenobiotics as well as infectious disease organisms. Excreta were included in this circle since work by Smith and Piper (1975) has clearly demonstrated metabolic ammonia as one cause of pathologic alteration in teleost gill. The concentration of pathogenic agents present in a given site is clearly subject to change relative to the source and extent of pollution or to factors governing density of infectious organisms. If certain factors are present in the host (e.g., genetic susceptibility) and if pathogenic agent(s) are present in sufficient concentration in an altered environment (viz., elevated in tempera-

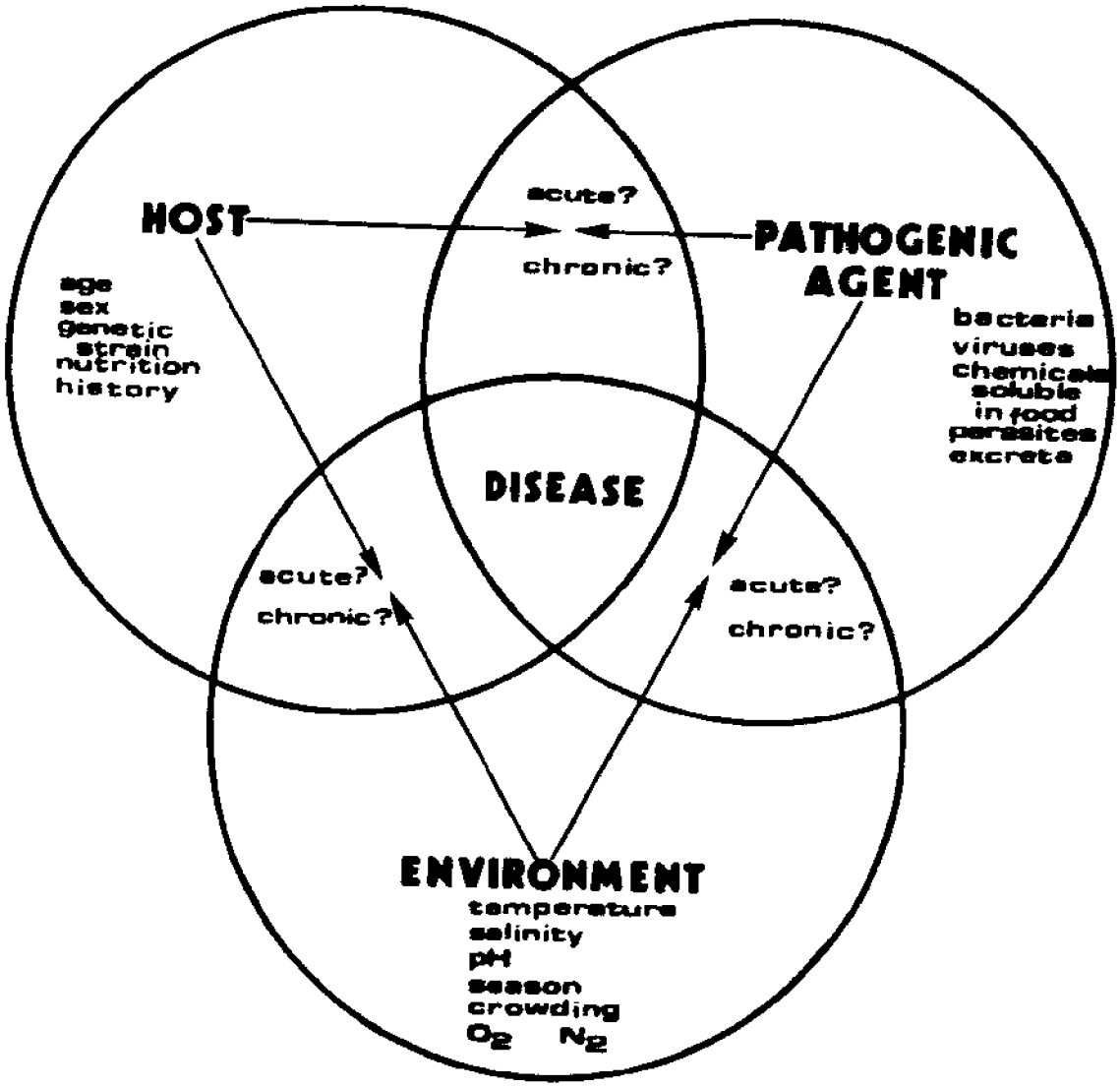

Figure 1. Interactions of environment, host and pathogenic agent(s). (Modified from Snieszko 1974.)

ture (Roberts 1975)) disease may occur (Snieszko 1974). Thus it is important to possess data on host factors, environmental quality and concentration of pathogenic agents.

Examples of important host factors, in addition to those reviewed by Love (1980), are seen in recent studies with the microsomal mixed-function oxidase system of teleost liver. This multienzyme system, induced by specific chemical compounds, plays a central role in xenobiotic biotransformation including activation and detoxification (Lech and Bend 1980; Stegeman 1981; Lech et al. 1982). Stegeman and Chevion (1980) demonstrated sex differences in mixed function oxidase activity in gonadally mature rainbow trout. Stegeman (1981) cites other studies indicating variation in response with age and sex of exposed fish. Pedersen et al. (1976) studied mixed function oxidase activity in six strains of rainbow trout and found differences in constitutive enzyme activity between strains. Following exposure to an inducing agent, differences in response of various genetic strains were evident. Gingerich (1982) reviewed literature on hepatic toxicology of fishes and cited studies indicating that the nutritional status was one of the most important factors in determining the ultimate response of liver tissue to toxicants. In these studies with proven hepatotoxic agents, diet formulations given fish prior to and during exposure to the toxicant greatly affected the extent of toxicity. Following laboratory exposure to certain polycyclic hydrocarbons, polychlorinated biphenyls, polybrominated biphenyls or dioxins, several species of freshwater and marine fish exhibited induction of the hepatic microsomal mixed function oxidase system (see reviews by Lech and Bend 1980; Stegeman 1981). Although environmental pollution has not been conclusively linked to this enzyme response, higher levels of mixed function oxidase components were seen in killifish (Fundulus heteroclitus), from the site of an oil spill 8 years earlier, than were seen in killifish from "nonpolluted" reference marshes (Stegeman 1978). The high level of mixed-function oxidase activity seen in scup (Stenotomous versicolor; see discussion in Stegeman (1981)) and occasionally in sheepshead (Archosargus probatocephalus) (James and Bend 1980) collected from the field, may indicate prior exposure to inducing agents. Since enzyme induction can exert dramatic effects upon the rate of formation of toxic and nontoxic metabolites, carcinogenesis and other endpoints may be affected in populations with prior history of induction (Lech and Bend 1980).

Although the major thrust of laboratory aquatic toxicology studies has centered on host and pathogenic agent(s), recent studies have begun to assess environmental factors mediating response(s) of host to toxicants. Of those factors, temperature has received the most attention. The examples cited here relate to the metabolism of xenobiotics by the microsomal and mixed-function oxidase system. Other examples have been reviewed recently (Love

1980). Under controlled laboratory conditions, temperature has been shown to modify interaction between a xenobiotic compound and the host. James and Bend (1980) collected sheepshead for studies in summer when the water temperature was 26°C and in winter when the water temperature was 14°C. Under summer temperature conditions, exposure to the inducing agent 3-methylcholanthrene caused maximum activity in one microsomal enzyme at 3 days with a return to control values by 14 days after a single dose. Under winter conditions, maximum activity was not seen until 8 days after treatment but elevated activities were still observed at 28 days. The effect of environmental temperature on naphthalene metabolism was studied in the starry flounder (Platichthys stellatus) under controlled laboratory conditions (Varanasi et al. 1981). Flounder, maintained at either 4° or 12°C, were force fed isotopically labeled naphthalene. Analysis of both groups at 24 h post exposure indicated a higher level of metabolites and parent compound in tissues of fish maintained at the lower water temperature. Stegeman (1981) discusses seasonal variations in polycyclic hydrocarbon (benzo-a-pyrene) content in mussels; the highest levels are seen in winter. Since laboratory experimentation has been carried out with oysters (Couch et al. 1979) and shrimp (Couch 1977) the possibility exists for determination of effects of temperature and other environmental factors on toxicity in other marine organisms. Review of the literature on aquatic toxicology serves to underscore further the need for continued effort to define environmental factors and to determine, under controlled laboratory conditions, the extent to which these factors modify the host/pathogenic agent interaction. It is obvious that the work has only begun and that more complex designs for laboratory experiments are necessary to evaluate simultaneously interactions among host, pathogenic agent(s) and environment.

Figure 2 illustrates the role that pathobiology plays in environmental risk assessment. Pathobiologic techniques analyze damage at the various levels of structural organization seen within an organism. Once the extent of the damage has been determined and the number of affected individuals established versus the dose of the toxicant delivered, attempts can be made to predict effects upon populations and species. In addition feral populations can be sampled and alterations described using pathobiologic approaches. The review by Sindermann (1979) cites numerous examples of such studies. When an effect (end point) is seen, retrospective analysis attempts by means of mechanistic connection to link cause and effect (Figure 2). The requirements for construction of a firm association between a disease end point and environmental pollution have been defined in detail (Sindermann 1979). General agreement exists concerning the approach necessary to reach such an important objective. Carefully controlled experimental conditions are necessary to characterize thoroughly the etiology of the process

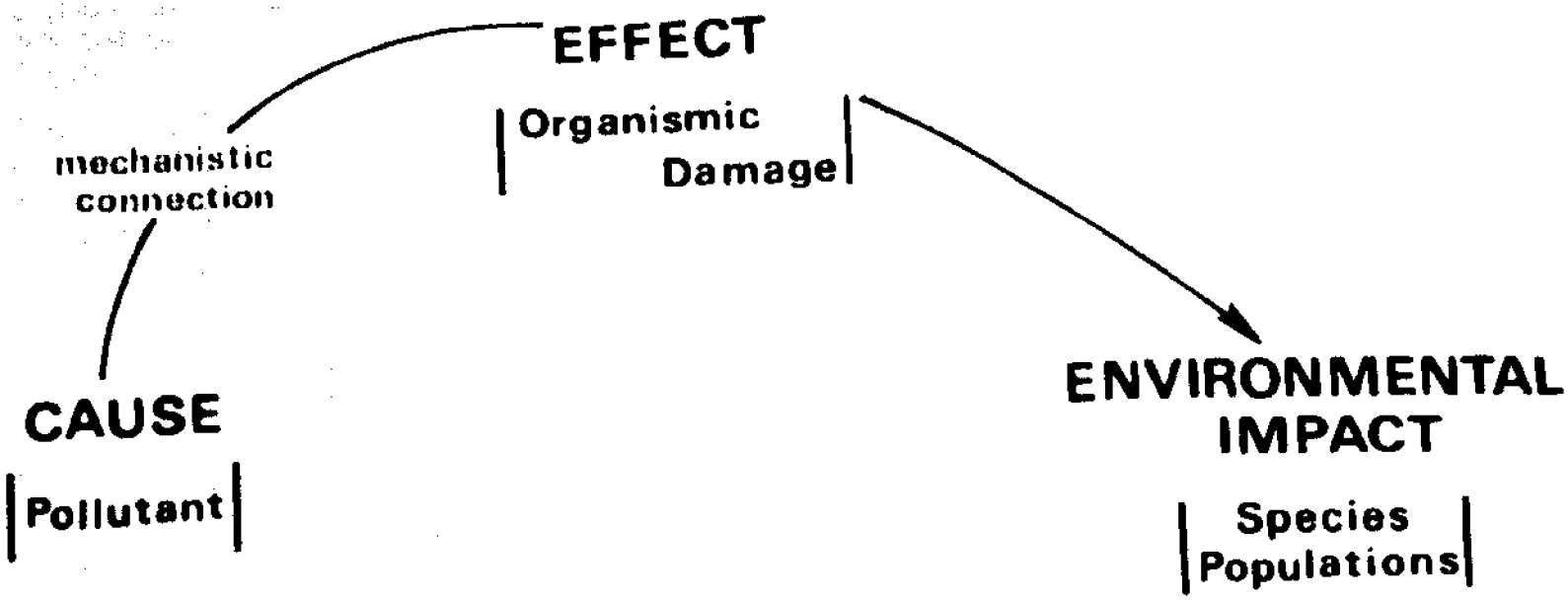

Figure 2. Role of pathobiology in environmental risk assessment. Initial investigation determines effect on organism. Prospective studies based on numbers of individuals affected can be used to predict possible environmental impact. If effect is known, retrospective analysis may lead to cause-effect relationship by means of mechanistic connection. Controlled laboratory tests employing specific chemical toxicants form basis for postulated cause-effect relationship which can then be tested (field validation). (From unpublished work by one of us (J.C.)).

and to determine the chemicals which can cause the toxic response (Lech and Bend 1980; and Bend and Weber 1980).

Application of Data from Pathobiology to Other Species Including Humans

Since cells and their organelles are the fundamental units in all living species, data at this level of organization, whether obtained in an aquatic or in a terrestrial species, are important since the identical level of organization is shown in humans. Transphyletic similarity exists in enzyme systems which convert pollutants to biologically active (toxic) or inactive metabolites (Bend and Weber 1980). Pathobiology brings together the disciplines of anatomy, biochemistry, pharmacology, physiology and pathology. When coupled with a comparative approach, building upon simpler organization of structure/function and proceeding to more complex systems these tools can lead to an understanding of basic biological mechanisms important in understanding marine pollution effects.

REFERENCES

Allendorf, F.W., N. Mitchell, N. Ryman and G. Stahl. 1977. Isozyme loci in brown trout (Salmo trutta, L.): Detection and interpretation from population data. Hereditas 86: 179-190.

Aoki, K. and H. Matsudairo. 1977. Induction of hepatic tissue tumors in a teleost (Oryzias latipes) after treatment with methylazoxymethanol acetate: Brief communication. J. U.S. Natl. Cancer Inst. 59: 1747-1749.

Bend, J.R. and L.J. Weber. 1980. Aquatic animals as models in biomedical research/comparative pharmacology in aquatic species. Fed. Proc. 14: 3183.

Bolender, R.P. 1980. Morphometric analysis in the assessment of the response of the liver to drugs. In: D.W. Fawcett and J.S. Newberne (eds.), American Society for Pharmacology and Experimental Therapeutics, Workshop on Cellular and Molecular Toxicology, pp. 429-443. Williams and Wilkins, Baltimore, Md.

Bolender, R.P. 1981. Stereology: Applications to pharmacology. Ann. Rev. Pharmacol. Toxicol. 21: 549-573.

Couch, J.A. 1977. Ultrastructural study of lesions in gills of a marine shrimp exposed to cadmium. J. Invertebr. Pathol. 29: 267-288.

Couch, J.A. and D.R. Nimmo. 1974. Ultrastructural studies of shrimp exposed to the pollutant chemical polychlorinated biphenyl (aroclor 1254). Bull. Soc. Pharmacol. Environ. Pathol. 2: 17-20.

Couch, J.A., G. Gardner, J.C. Harshbarger, M.R. Tripp and P.P. Yevich. 1974. Histological and physiological evaluations in some marine fauna. In: G. LaRoche (ed.), Marine Bioassays, pp. 156-172. American Petroleum Inst., U.S. Environmental Protection Agency and Marine Technology Society, Washington, D.C

Couch, J.A., J.T. Winstead and L.R. Goodman. 1977. Kepone-induced scoliosis and its histological consequences in fish. Science 197: 585-587.

Couch, J.A., L.A. Courtney, J.T. Winstead and S.S. Foss. 1979. The American oyster (Crassostrea virginica) as an indicator of carcinogens in the aquatic environment. In: E. Scarpelli, S. Nielsen and G. Migaki (eds.), Animals as Monitors of Environmental Pollutants, pp. 65-84. National Academy of Sciences, Washington, D.C.

Davidsohn, I. and J.B. Henry. 1974. Clinical Diagnosis by Laboratory Methods. W.B. Saunders Co., Philadelphia, Pa.

DeDuve, C. and J. Berthet. 1954. The use of differential centrifugation in the study of tissue enzymes. Int. Rev. Cytol. 3: 225-275.

Dehoff, R.T. and F.N. Rhines. 1968. Quantitative Microscopy. McGraw-Hill, New York.

Elias, H., A. Henning and D.E. Schwartz. 1971. Stereology: Application to biomedical research. Physiol. Rev. 51: 150-200.

Forbus, W.D. 1952. Reaction to Injury: Pathology for Students of Disease. Williams and Wilkins, Baltimore, Md.

Forster, B.F. 1948. Use of thin kidney slices and isolated renal tubules for direct study of cellular transport kinetics. Science 108: 65-67.

Galigher, A. and E. Kozloff. 1971. Essentials of Practical Microtechnique, 2nd ed. Lea and Febiger, Philadelphia, Pa.

Gingerich, W.H. 1982. Hepatic toxicology of fishes. In: L.J. Weber (ed.), Aquatic Toxicology, Vol. 1, p. 55-105. Raven Press, New York.

Gingerich, W.H., L.J. Weber and R.E. Larson. 1978. Carbon tetrachloride-induced retention of sulfobromophthalein in the plasma of rainbow trout. Toxicol. Appl. Pharmacol. 52: 347-350.

Hawkes, J.W. 1980. The effects of xenobiotics on fish tissues: Morphological studies. Fed. Proc. 39: 3230-3236.

Hawkes, J.W., E.H. Gruger, Jr. and O.P. Olson. 1980. Effects of petroleum hydrocarbons and chlorinated biphenyls on the morphology of the intestine of chinook salmon (Oncorhynchus tshawytscha). Environ. Res. 23: 149-161.

Hendricks, J.D., R.A. Scanlan, J.L. Williams, R.O. Sinnhuber and M.P. Grieco. 1980a. Carcinogenicity of N-methyl-N'-nitro-N'-nitrosoguanidine to the livers and kidneys of rainbow trout (Salmo gairdneri) exposed as embryos. J. U.S. Natl. Cancer Inst. 64: 1511-1519.

Hendricks, J.D., R.O. Sinnhuber, J.E. Nixon, J.H. Wales, M.S. Masri and D.P.H. Hsieh. 1980b. Carcinogenic response of rainbow trout (Salmo gairdneri) to aflatoxin Q and synergistic effect of cyclopropenoid fatty acids. J. U.S. Natl. Cancer Inst. 64: 523-527.

Hill, R.B. 1975. Pathobiology and the medical sciences. In: M.F. LaVia and R.B. Hill (eds.), Principles of Pathobiology, 2nd ed., pp. 3-8. Oxford Univ. Press, New York.

Hinton, D.E. and C.R. Pool. 1976. Ultrastructure of the liver in channel catfish (Ictalurus punctatus) Rafinesque. J. Fish. Biol. 8: 209-219.

Hinton, D.E., M.W. Kendall and B.B. Silver. 1973. Use of histologic and histochemical assessments in the prognosis of the effects of aquatic pollutants. In: J. Cairns, Jr. and K.L. Dickson (eds.), Biological Methods for the Assessment of Water Quality, pp. 194-208. ASTM STP 528, American Society for Testing and Materials, Philadelphia, Pa.

Hinton, D.E., J.E. Klaunig and M.M. Lipsky. 1978. PCB-induced alterations in teleost liver: A model for environmental disease in fish. Mar. Fish. Rev. 40: 47-50.

Hinton, D.E., R.C. Lantz and J.A. Hampton. 1982a. Effect of age and exposure to a carcinogen on the structure of the medaka liver: A morphometric study. Proceedings of EPA/NCI. Symposium on the Use of Small Fish Species in Carcinogenicity Testing. J. U.S. Natl. Cancer Inst. Monogr. Ser.

Hinton, D.E., E.R. Walker, C.A. Pinkstaff and E.H. Zuchelkowski. 1982b. A morphological survey of teleost organs important in carcinogenesis with a brief note on fixation. Proceedings of EPA/NCI. Symposium on the Use of Small Fish Species in Carcinogenicity Testing. J. U.S. Natl. Cancer Inst. Monogr. Ser.

Hughes, G.M., 1972. Morphometrics of fish gills. Respir. Physiol. 14: 1-25.

Hughes, G.M., S.F. Perry and V.M. Brown. 1979. A morphometric study of effect of nickel, chromium and cadmium on the secondary lamellae of rainbow trout gills. Water Res. 13: 665-679.

Ishikawa, T., T. Shimamine and S. Takayama. 1975. Histologic and electron microscopy observation on diethylnitrosamine-induced hepatomas in small aquarium fish (Oryzias latipes). J. U.S. Natl. Cancer Inst. 55: 909-916.

Ito, S. and M.J. Karnovsky. 1968. Formaldehyde-glutaraldehyde fixatives containing trinitro compounds. J. Cell Biol. 39: 1689.

James, M.O. and J.R. Bend. 1980. Polycyclic aromatic hydrocarbon induction of cytochrome P-450-dependent mixed-function oxidases in marine fish. Toxicol. Appl. Pharmacol. 54: 117-133.

Kalengayi, M.M. and V.J. Desmet. 1975. Sequential histological and histochemical study of the rat liver during aflatoxin B_1-induced carcinogenesis. Cancer Res. 35: 2854-2858.

Klaunig, J.E., M.M. Lipsky, B.F. Trump and D.E. Hinton. 1978. Biochemical and ultrastructural changes in teleost liver following subacute exposure to PCB. J. Environ. Pathol. Toxicol. 2: 953-963.

Lech, J.J. and J.R. Bend. 1980. Relationship between biotransformation and the toxicity and fate of xenobiotic chemicals in fish. Environ. Health Perspect. 34: 115-131.

Lech, J.J., M.J. Vodicnik and C.R. Elcombe. 1982. Induction of monooxygenase activity in fish. In: L.J. Weber (ed.), Aquatic Toxicology, Vol. 1, pp. 107-148. Raven Press, New York.

Leevy, C.M. 1965. Evaluation of Liver Function in Clinical Practice. H. Popper and C.S. Davidson (eds.) Lilly Research Labs., Indianapolis, Ind.

Lipsky, M.M., J.E. Klaunig and D.E. Hinton. 1978. Comparison of acute response to polychlorinated biphenyl in liver of rat and channel catfish: A biochemical and morphological study. J. Toxicol. Environ. Health. 4: 107-121.

Lipsky, M.M., D.E. Hinton, J.E. Klaunig, P.J. Goldblatt and B.F. Trump. 1981. Biology of hepatocellular neoplasia in the mouse. II. Sequential enzyme histochemical analysis of BALB/C mouse liver during safrole-induced carcinogenesis, J. U.S. Natl. Cancer Inst. 67: 377-392.

Love, R.M. 1980. Reactions and adaptations to the environment. In: R.M. Love (ed.), The Chemical Biology of Fishes, Vol. 2, Advances 1968-1977, pp. 230-349. Academic Press, London.

Majno, G., M. Lagattuta and T.E. Thompson. 1960. Cellular death and necrosis: Chemical, physical and morphologic changes in rat liver. Virchows Arch. A. Pathol. Anat. Histol. 333: 421-465.

Middaugh, D.P., L.E. Burnett and J.A. Couch. 1980. Toxicological and physiological responses of the fish, Leiostomus xanthurus exposed to chlorine produced oxidants. Estuaries 3: 132-141.

McDowell, E.M. and B.F. Trump. 1976. Histologic fixatives suitable for light and electron microscopy. Arch. Pathol. Lab. Med. 100: 405-414.

Pedersen, M.G., W.K. Hershberger and M.R. Juchau. 1976. Hepatic biotransformation of environmental xenobiotics in six strains of rainbow trout. J. Fish. Res. Bd. Canada 33: 666-675.

Pfeifer, K.F., L.J. Weber. 1980. The effect of carbon tetrachloride treatment on urine flow rate of the rainbow trout (Salmo gairdneri). Toxicol. Appl. Pharmacol. 52: 347-350.

Pfeifer, K.F., L.J. Weber and R.E. Larson. 1980. Carbon tetrachloride-induced hepatotoxic response in rainbow trout (Salmo gairdneri), as influenced by two commercial fish diets. Comp. Biochem. Physiol. 67: 91-96.

Pliss, G.B. and V.V. Khudoley. 1975. Tumor induction by carcinogenic agents in aquarium fish. J. U.S. Natl. Cancer Inst. 55: 129-136.

Pritchard, J.B. and D.S. Miller. 1980. Teleost kidney in evaluation of xenobiotic toxicity and elimination. Fed. Proc. 39: 3207-3212.

Reifel, C.W. and A.A. Travill. 1979. Structure and carbohydrate histochemistry of the intestine in ten teleostean species. J. Morphol. 162: 343-360.

Riede, U.N. and A. Reith. 1980. Morphometry in Pathology. Gustav Fisher Verlag, New York.

Roberts, R.J. 1975. The effects of temperature on diseases and their histopathological manifestations in fish. In: W.E. Ribelin and G. Migaki (eds.), The Pathology of Fishes, pp. 477-496. Univ. of Wisconsin Press, Madison, Wisc.

Rohr, H.P., M. Oberholzer, G. Bartsch, G. and M. Keller. 1976. Morphometry in experimental pathology: Methods, baseline data and application. Int. Rev. Exper. Pathol. 15: 233-325.

Sabo, D.J. and J.J. Stegeman. 1977. Some metabolic effects of petroleum hydrocarbons in marine fish. In: F.J. Vernberg, A. Calabrese, F.P. Thurberg and W.B. Vernberg (eds.), Physiological Responses of Marine Biota to Pollutants, pp. 279-287. Academic Press, New York.

Scarpelli, D.G. 1976. Drug metabolism and aflatoxin-induced hepatoma in rainbow trout (Salmo gairdneri). Prog. Exp. Tumor Res. 20: 339-350.

Scarpelli, D.G. and B.F. Trump. 1971. Cell Injury. (Prepared by Universities Associated for Research and Education in Pathology, Inc.) Upjohn Co., Kalamazoo, Mich.

Scarpelli, D.G., M.H. Greider and W.J. Frajola. 1963. Observations on hepatic cell hyperplasia, adenoma, and hepatoma of rainbow trout (Salmo gairdneri). Cancer Res. 23: 848-857.

Schoor, W.P. and J.A. Couch. 1979. Correlation of mixed-function oxidase activity with ultrastructural changes in the liver of a marine fish. Cancer Biochem. Biophys. 4: 95-103.

Schwerdtfeger, W.K. 1979a. Morphometrical studies of the ultrastructure of the epidermis of the guppy, Poecilia reticulata Peters, following adaptation to seawater and treatment with prolactin. Gen. Comp. Endocrinol. 38: 476-483.

Schwerdtfeger, W.K. 1979b. Qualitative and quantitative data on the fine structure of the guppy (Poecilia reticulata Peters) epidermis following treatment with thyroxine and testosterone. Gen. Comp. Endocrinol. 38: 484-490.

Sindermann, C.J. 1979. Pollution--associated diseases and abnormalities of fish and shellfish: A review. Fishery Bull. 76: 717-749.

Smith, C.E. and R.G. Piper. 1975. Lesions associated with chronic exposure to ammonia. In: W.E. Ribelin and G. Migaki (eds.), The Pathology of Fishes, pp. 497-514. Univ. of Wisconsin Press, Madison, Wisc.

Snieszko, S.F. 1974. The effects of environmental stress on outbreaks of infectious diseases of fishes. J. Fish Biol. 6: 197-208.

Sparks, A.K. 1972. Invertebrate Pathology Non-communicable Diseases. Academic Press, New York.

Statham, C.N., S.P. Szyjka, L.A. Menaham and J.J. Lech. 1977. Fractionation and subcellular localization of marker enzymes in rainbow trout liver. Biochem. Pharmacol. 26: 1395-1400.

Statham, C.N., W.A. Croft and J.J. Lech. 1978. Uptake, distribution, and effects of carbon tetrachloride in rainbow trout (Salmo gairdneri). Toxicol. Appl. Pharmacol. 45: 131-140.

Stegeman, J.J. 1978. Influence of environmental contamination on cytochrome P-450 mixed-function oxygenases in fish: Implications for recovery in the Wild Harbor Marsh. J. Fish. Res. Bd. Canada 35: 668-674.

Stegeman, J.J. 1981. Polynuclear aromatic hydrocarbons and their metabolism in the marine environment. In: H. Gelboin and P.O. Ts'O (eds.), Polycyclic Hydrocarbons and Cancer, Vol. 3, pp. 1-60. Academic Press, New York.

Stegeman, J.J. and M. Chevion. 1980. Sex differences in cytochrome P-450 and mixed function oxygenase activity in gonadally mature trout. Biochem. Pharmacol. 39: 553-558.

Trump, B.F. and A.U. Arstila. 1975. Cellular reaction to injury. In: M.F. LaVia and R.B. Hill (eds.), Principles of Pathobiology, 2nd ed. pp. 97-140. Oxford Univ. Press, New York.

Trump, B.F. and R.E. Bulger. 1971. Experimental modification of lateral and basilar plasma membranes and extracellular compartments in the flounder nephron. Fed. Proc. 30: 22-41.

Trump, B.F. and R.T. Jones. 1977. Correlation of structure and active transport in the teleost nephron. J. Exp. Zool. 199: 365-382.

Trump, B.F., B.P. Croker and W.J. Mergner. 1971. The role of energy metabolism, ion and water shifts in the pathogenesis of cell injury. In: G.W. Richter and D.G. Scarpelli (eds.), Cell Membranes: Biological and Pathological Aspects, pp. 82-128. Williams and Wilkins, Baltimore, Md.

Trump, B.F., R.T. Jones and S. Sahaphong. 1975. Cellular effects of mercury on fish kidney tubules. In: W.E. Ribelin and G. Migaki (eds.), The Pathology of Fishes, pp. 585-612. Univ. of Wisconsin Press, Madison, Wisc.

Underwood, E.E. 1970. Quantitative Sterology. Addison-Wesley, Reading, Mass.

Varanasi, U., D.J. Gmur and W.L. Reichert. 1981. Effect of environmental temperature on naphthalene metabolism by juvenile starry flounder (Platichthys stellatus). Arch. Environ. Contam. Toxicol. 10: 203-214.

Ward, P.A. 1975. Inflammation. In: M.F. LaVia and R.B. Hill (eds.), Principles of Pathobiology, 2nd ed., pp. 97-140. Oxford Univ. Press, New York.

Weibel, E.R. 1979. Stereological Methods, Vol. 1. Academic Press, London.

Weibel, E.R. 1980. Stereological Methods, Vol. 2. Academic Press, London.

Weibel, E.R. and R.P. Bolender. 1973. Stereological techniques for electron microscopic morphometry. In: M.A. Hayat (ed.), Principles and Techniques of Electron Microscopy, Van Nostrand Reinhold Co., New York.

Yevich, P.P. and C.A. Barszcz. 1982. Preparation of aquatic animals for histopathological examination. In: C. Weber (ed.), Biological Field and Latorabory Methods for Measuring the Quality of Surface Waters and Wastes. U.S. Environmental Protection Agency, Cincinnati, Ohio.

Zimmerman, H.J. and J.B. Henry. 1974. Serum enzyme determinations as an aid to diagnosis. In: I. Davidsohn and J.B. Henry, (eds.), Clinical Diagnosis by Laboratory Methods, pp. 837-869. W.B. Saunders Co., Philadelphia, Pa.

Zuchelkowski, E.M. and R.C. Lantz. 1981. Recruitment of mucous cells in epidermis of acid-stressed brown bullhead catfish (Ictalurus nebulosus). Anat. Rec. 199: 288a.

Utility of Toxicity Tests to Measure Effects of Substances on Marine Organisms

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INTRODUCTION

Toxicity tests using single species, microcosms and communities are the foundation for laboratory evaluations of marine (estuarine and oceanic) pollution effects. Hazard evaluation techniques use toxicity tests to predict the need for environmental concern on new and old chemicals. Interpretation of the results of field investigations can be aided through the use of laboratory tests, where variables can be controlled and effects quantified. Although not a panacea, laboratory toxicity tests are generally accepted by industry, university and government scientists as a useful technique for measuring the effects of marine pollution.

Discussions in this paper will emphasize acute, early life stage, life cycle and community toxicity tests with marine (estuarine or oceanic) species as conducted at the U.S. EPA Environmental Research Laboratory at Gulf Breeze, Florida. These tests are emphasized because of their importance in the hazard evaluation process, as discussed in workshop proceedings by Cairns et al. (1978) and Dickson et al. (1979). Individual papers in these two publications detail hazard evaluation techniques used by the American Institute of Biological Science, American Society for Testing and Materials, Monsanto Company, U.S. Environmental Protection Agency and Japanese and French scientists. In addition, testing requirements for effects assessments have been recently identified for development of water quality criteria by the U.S. EPA (1980a) and the U.S. Army (Pearson and Glennon 1979). Although the applications of these hazard evaluation procedures differ considerably, the princi-

pal effects testing requirements include those discussed herein. Laboratory effects tests have been pivotal in most evaluations of hazard because the evaluation processes occur before chemical impact on the environment, and they are used only rarely to measure the impact in an already contaminated environment.

Acute, early life stage, life cycle and community toxicity tests will be discussed separately. As a part of each discussion, the design of the test, uses of the data, sources of variability, species amenable to testing, availability of standardized methods and relationships to data from other test types will be discussed where appropriate. Much of the summary information was assembled as a result of developing water quality criteria for the consent decree chemicals or from the data used in the criteria documents (U.S. EPA 1980a,b).

ACUTE TOXICITY TESTS

Results from acute toxicity tests are extremely useful, but have probably been more maligned than results from any other type of toxicity test. Because acute toxicity tests are completed in a short time, they are relatively inexpensive and usually provide the first data on the toxicity of individual chemicals or complex mixtures. Standardized methods for conducting acute toxicity tests are available (ASTM 1980; APHA-AWWA-WPCF 1980), and information useful in design or interpretation of results is also available (Sprague 1969, 1970, 1971; Buikema et al. 1982). Unfortunately there are no standards for using or evaluating the results in environmental situations where concentrations and bioavailability change over time; herein lies the problem. Acute toxicity test data can be particularly useful in the first stages of product development. Data from acute toxicity tests are used to select exposure concentrations for chronic toxicity, bioconcentration and other tests used in the evaluation process. The comparative acute sensitivities of species and life stages of marine organisms to a particular chemical can aid in identifying populations at risk.

Acute toxicity tests can be used to measure the bioavailability/toxicity of complex wastes. Chemical fractionation of wastes followed by toxicity tests can aid in identification of the toxic fraction (Walsh and Garnas, 1983). Conversely, data from tests of individual chemicals rarely can be used to predict the overall effect of complex wastes or contaminated sediments that contain mixtures of a great many chemicals.

The most appropriate use of acute toxicity tests is to measure the toxicity of substances or sensitivity of species to substances, not to predict environmental hazard directly. In some instances, however, results from acute toxicity tests may be directly applicable to situations in which environmental and acutely lethal concentrations and availability are similar.

Relatively few marine species have been used in acute toxicity tests, and most species tested have been fishes or arthropods. For example, the 64 water quality criteria documents concerning the consent decree chemicals, which include many of the intensively studied metals and pesticides, report acute toxicity test data on only 76 species of marine animals (Table 1). Arthropods and fishes account for 75% of the animal species tested. Only 10% of the species used were tested on more than 10 of the chemicals. Eleven species of mollusks were tested; four were gastropods, accounting for only 11% of the data on mollusks. Thirty percent of the 76 animal species were tested with only one chemical. The lack of data on comparative sensitivities of species of phytoplankton and macroalgae is even greater for consent decree chemicals than that for tests on animals. Because most of the acute toxicity test data are concentrated on a small number of species in a few phyla, our knowledge of the relative sensitivities of untested or little tested taxa is minimal.

Table 1. Number of species from various phyla of animals, phytoplankton and macroalgae tested acutely against greater than 10, from 6 to 10 or from 1 to 5 consent decree chemicals. Of the 64 water quality criteria documents developed for the consent decree chemicals, 51 contained acute toxicity data on one or more animal species and 38 contained toxicity data on phytoplankton and macroalgae.

	Number of Species			
Phylum	1-5 Chemicals	6-10 Chemicals	> 10 Chemicals	Total Species
Arthropoda	22	7	2	31
Chordata	17	5	4	26
Mollusca	8	2	1	11
Annelida	6	-	1	7
Echinodermata	1	-	-	1
Total Species	54	14	8	76
Phytoplankton	31	1	1	33
Macroalgae	7	1	-	8

Variation in the relative acute sensitivity of animal species to chemicals is considerable. For those pesticides or metals for which marine water quality criteria are available, the ratios of LC50 values for the species least sensitive to the species most sensitive to a given chemical ranged from 20 to 18,000, averaging 600 for metals and 3800 for pesticides (Table 2). The variation in sensitivity among species is far greater than the variation that occurs within a species as a function of the acute toxicity test method. The degree of variation in acute sensitivity of species perhaps is a toxicological property of individual chemicals that is important in determining the adequacy of data for decision-making.

A second factor important to the adequacy of data is the need for information on the sensitivity of a variety of phylogenetic groups to identify the most sensitive taxa and to optimize testing. Certain types of organisms such as arthropods, including penaeid shrimp, mysids and copepods, have been generally recognized as extremely sensitive. Water quality criteria documents indicate that these taxa frequently are extremely sensitive, but can also be relatively insensitive (Table 2). Therefore, assessments of acute hazard or specific substances may require a reasonable spread in the kinds of species tested to identify phylogenetic groups that are more sensitive.

Measurement of the variability of data from standardized acute toxicity tests is important if results are to be used in decision-making. Schimmel (1981) reports on an interlaboratory comparison of the results of acute toxicity tests that used the method described in ASTM (1977). The six participating laboratories conducted static 96-h toxicity tests on sheepshead minnows (Cyprinodon variegatus), the bay mysid (Mysidopsis bahia) and the copepod (Acartia tonsa), using the pesticide endosulfan and silver ($AgNO_3$). Flow-through tests were also conucted on the first two organisms and these chemicals. An analysis of the results reported by Schimmel (1981) indicated that, except for the copepod tests where biological problems existed, the variability of the results was acceptable (Table 3). The coefficient of variation for the fish and mysid data ranged from 0.27 to 0.62 (average 0.43), depending on the test type or chemical. Results are similar, even though laboratories had varied experience with the test and the test species, laboratories failed to follow completely all requirements of the test, some fish were feral and some lab-raised, and the source of dilution water and organisms varied, being from estuaries along the Gulf of Mexico to New England.

Table 2. Variation in acute sensitivities of marine species to chlordane, DDT, dieldrin, endosulfan, endrin, heptachlor, cadmium, chromium, copper, mercury, nickel, selenium, silver and zinc. Values are grand averages across substances; ranges for each substance are in parentheses.

Item	Pesticides	Metals
Number of species tested	16 (8-21)	20 (10-26)
LC50 least sensitive/LC50 most sensitive	3800 (70-18,000)	600 (20-2,300)
Sensitive phyla[a]	Arthropoda Chordata	Arthropoda Mollusca Chordata Annelida
Percentile Rank Of		
Acartia spp.	33	28 (8-50)
Mysids	46 (29-67)	27 (3-70)
Penaeids	7 (5-12)	46 (38-61)

[a] Listed when a species from the phylum was one of the two most sensitive to the substances.

Table 3. Interlaboratory comparison of the results from static or flow-through acute toxicity tests. Results are mean 96-hour LC50s in µg/L for six laboratories; coefficient of variation in parentheses (s/x) was calculated from LC50s reported by Schimmel (1981). LC50s were calculated from nominal concentrations in exposure water in static tests and measured concentrations in flow-through tests.

Species	Chemical	Static	Flow-through
C. variegatus	Endosulfan	2.40(0.37)	0.82(0.38)
	Silver	1,122(0.34)	1,216(0.46)
M. bahia	Endosulfan	0.80(0.62)	0.90(0.40)
	Silver	210(0.27)	192(0.58)
A. tonsa	Endosulfan	0.22(0.82)	-
	Silver	38.5(0.42)	-

CHRONIC TOXICITY TESTS

Life Cycle and Early Life Stage Toxicity Tests

Entire life cycle toxicity tests begin with fish embryos (Figure 1) or newly released invertebrate larvae, and partial life cycle toxicity tests begin with juveniles; both continue through development to mature individuals and production of progeny. Test duration ranges from a few weeks for invertebrates to 5 to 8 months for fish. Toxicologists who wish to conduct life cycle tests with marine organisms must use procedures in published literature because standard methods are not available. Use of procedures in APHA-AWWA-WPCF (1980) should not generally be attempted without consulting original references.

The primary end points include determination of effects on survival, growth and reproduction. Secondarily, pathological, behavioral and physiological effects and bioconcentration of the chemical may be measured. The lowest concentration that causes statistically significant effects on the primary end points and the highest concentration at which statistical effects were not detected are

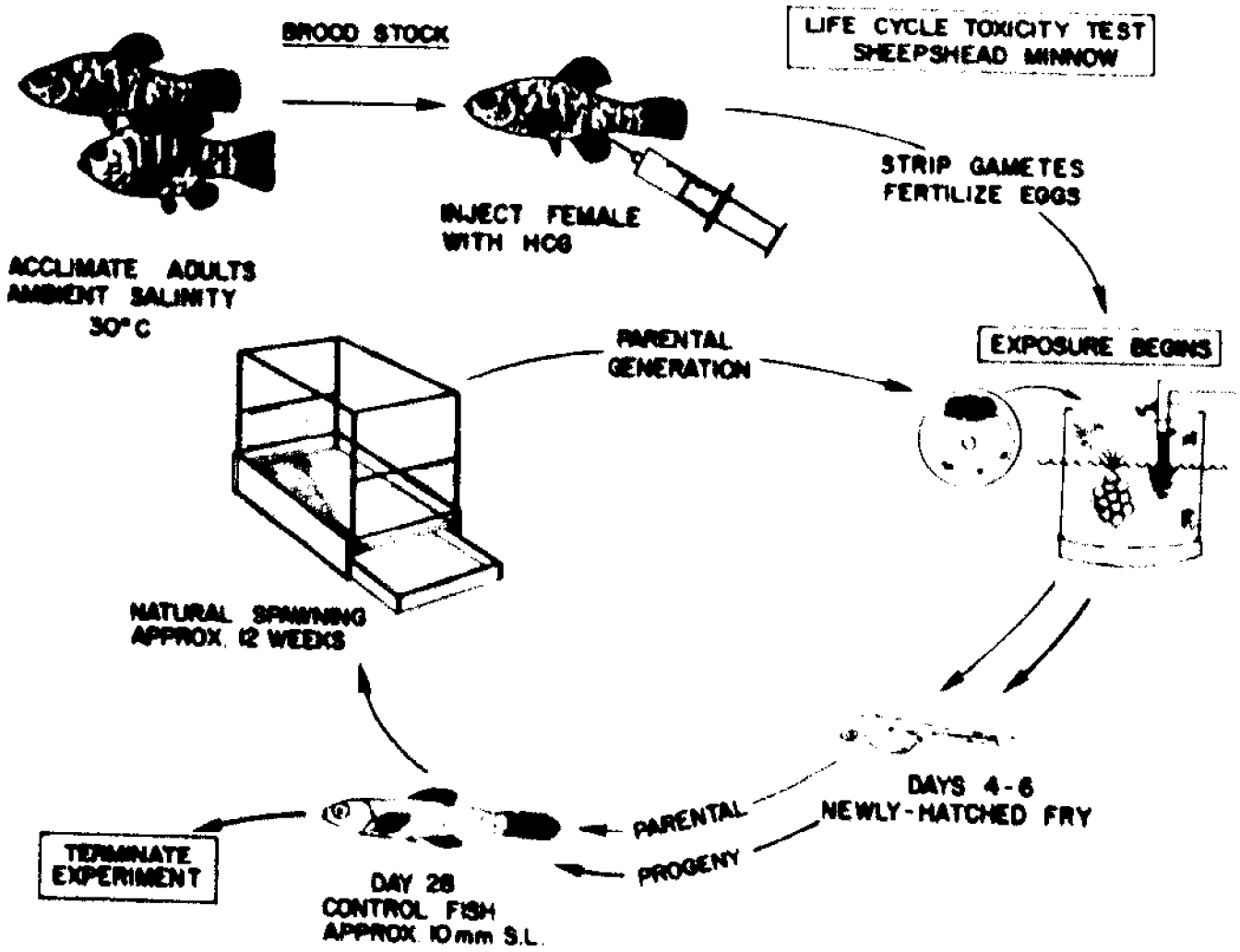

Figure 1. Diagram of the sheepshead minnow (Cyprinodon variegatus) entire life cycle toxicity test.

used to set limits termed the maximum acceptable toxicant concentration (MATC). The primary end points can also be used with population dynamics models to measure the effect of substances on the intrinsic rate of population increase (Allen and Daniels 1980; Daniels and Allen 1981; J. Gentile et al. 1982). The MATC or intrinsic rate of increase is used to estimate the "safe" concentration of a chemical for a given species.

Only a few marine species have been successfully used in life-cycle toxicity tests, and only two have been tested with enough chemicals to provide comparative toxicological information (Table 4). Life cycle tests with the bay mysid (Nimmo et al. 1978) and the sheepshead minnow (Hansen et al. 1978) probably provide more than 75% of all available data on the life cycle effects of substances on marine organisms. More than 30 tests have been conducted on these two species. In contrast, only one or two life cycle tests have been completed with species such as the mollusk Crepidula fornicata (Nelson et al. in press), the copepods Acartia tonsa (Ward et al. 1979) and Eurytemora affinis (Daniels and Allen 1981), the grass shrimp Palaemonetes pugio (Tyler-Schroeder 1979) and the mysid Mysidopsis bigelowi (J. Gentile et al. 1982; S. Gentile et al. 1982).

Early life stage toxicity tests with marine fish begin with embryos and continue through embryonic development, hatching and growth to the juvenile state. Duration is typically 4 to 8 weeks, depending upon species or the need to extend the exposure because effects continue beyond the usual test duration. Consensus early life stage toxicity test methods for marine and freshwater fishes are being developed (ASTM 1982). Few marine species have been used in early life stage toxicity tests; these include the sheepshead minnow Cyprinodon variegatus (Goodman et al. 1978), the silversides Menidia peninsulae (Goodman et al. 1983) and M. menidia, the California grunion Leuresthes tenius and the Gulf toadfish Opsanus beta (Table 4). Sheepshead minnow is the only species that has been tested with several chemicals. The primary end points of early life stage tests include determination of effects on embryonic development, hatching success, survival of hatched fish and growth. Secondarily, pathological, behavioral and physiological effects and bioconcentration of the chemical can be measured.

Results from early life stage toxicity tests with freshwater fishes have demonstrated that MATCs from life cycle toxicity tests can usually be estimated within a factor of 2, using estimated MATCs derived from survival or growth data from early life stage tests (McKim 1977; Macek and Sleight 1977). The only data base for marine fishes is that from life cycle and early life stage tests with sheepshead minnows. Slightly more than 70% of the life cycle MATCs were estimated within a factor of 2 from estimated MATCs derived from early life stage tests within life cycle tests on this species (Table 5). This relationship between early life stage and life cycle MATCs is not as clear if data are used from early

Table 4. Species of marine fishes and invertebrates that have been tested in life cycle or early life stage toxicity tests, and approximate number of tests completed.

Test	Species	Number of Tests
Partial or entire life cycle	Cyprinodon variegatus	>10
	Mysidopsis bahia	>25
	M. bigelowi	2
	Palaemonetes pugio	2
	Acartia tonsa	1
	Crepidula fornicata	1
Early life stage	Cyprinodon variegatus	>25
	Menidia menidia	2
	M. peninsulae	1
	Leuresthes tenius	1
	Opsanus beta	1
	Fundulus similis	1

Table 5. Summary of the chemicals where the MATCs from life cycle toxicity tests were predicted within a factor of 2 by estimated MATCs from early life stage portions of life cycles tests. The estimated MATCs used were from the progeny portions of partial life cycle tests or from the progeny or parental portions of entire life cycle tests.

MATCs Predicted	MATCs Not Predicted
Carbofuran, Chlordane, Endrin, Heptachlor, Kepone, Malathion, Methoxychlor, Pentachlorophenol	Diazinon, EPN, Trifluralin

life stage tests that began with embryos from unexposed parents. Only seven chemicals have been so tested; four (57%) predicted life cycle MATCs poorly. Overall, however, the early life stages of both marine and freshwater fishes are as sensitive, or more sensitive, than later life stages including reproduction. Therefore, early life stage tests acceptably predict life cycle MATCs.

Examination of the frequency of occurrence of survival, growth or reproduction as the most sensitive end point in chronic toxicity tests with marine fish and invertebrates (Table 6) is especially interesting relative to the conclusions reached in the comparisons made above. For early life stage toxicity tests, survival and growth were equally sensitive; in some instances, both were reduced at the lowest effect concentration. In life cycle toxicity tests with sheepshead minnows, grass shrimp and bay mysids, reproduction (the number of embryos or young released per female, or the percentage fertility) was usually the most sensitive end point. Reproductive impairment was the most sensitive end point in sheepshead minnow life cycle tests, and this may seem to contradict the conclusion that early life stage tests can be useful in estimating life cycle test results. However, there is no contradiction, because reproductive effects frequently occur at the same concentration, or at a concentration within a factor of 2 of the concentration that affected survival or growth of early life stages. In those instances where early life stage tests are poor predictors of life cycle MATCs because the reproductive process was particularly sensitive, no clues may be evident in the early life stage test. However, signs of poisoning, including physiological or behavioral effects, at concentrations that do not affect survival or growth, may indicate that reproductive effects could occur and that life cycle tests may be desirable. This is particularly true when environmental concentrations are close to those that affect the early life stages of fishes.

Table 6. Frequency of occurrence of survival, growth or reproduction (eggs/female or fertility) as end points in early life stage or life cycle toxicity tests with the fish (Cyprinodon variegatus), grass shrimp (Palaemonetes pugio) and bay mysid (Mysidopsis bahia).

Test	Number of Tests	Most Sensitive End Point		
		Survival	Growth	Reproduction
Early life stage (fish)	23	52%	52%	---
Life cycle (fish)	11	45%	27%	77%
Life cycle (grass shrimp)	2	0%	0%	100%
Life cycle (bay mysid)	21	57%	19%	57%

Chronic toxicity tests provide data useful in predicting "safe" concentrations. Mount and Stephan (1967), Eaton (1973) and Buikema et al. (1982) discuss the use and limitations of empirically derived application factors, the ratio of the MATC to the LC50, in predicting "safe" concentrations when only acute toxicity data are available for other more sensitive species. Mount (1977) summarized data on freshwater fishes to determine whether the application factor for any one chemical was similar for different fishes tested in life cycle tests. For the ten chemicals with tests on two to six freshwater fishes, the spread of application factors across species was reasonably narrow, from 2.4 to 5.8, but for the remaining five chemicals the spread was large, from 13 to 206. Mount concluded that the spread in numerical values was unacceptable and that a better method than the application factor concept was needed to predict concentrations that will not affect survival, growth or reproduction.

An examination of the application factor concept using data from life cycle tests with sheepshead minnows and data on freshwater fishes and the same chemicals may be useful. Of the eleven pesticides tested in life cycle exposures using sheepshead minnows, six have also been tested with one to four species of freshwater fish (Table 7). These data support the application factor concept because, within a factor of 4, the application factors are similar between species for the same chemical; the effect of diazinon on flagfish is the lone exception. Therefore, extrapolation of application factors that have been experimentally determined for a particular chemical to other fishes and that chemical may be justified, particularly in instances where data on more than one species support their applicability. However, because application factors for freshwater fishes are quite variable or may be completely inappropriate, and data on the chronic sensitivity of additional marine fishes are unavailable, additional research is needed to identify new techniques for predicting safe concentrations in aquatic environments.

The interlaboratory variability of chronic toxicity tests, including the life cycle toxicity test using Mysidopsis bahia (Nimmo et al. 1978) and the early life stage toxicity test using Cyprinodon variegatus (ASTM 1982), is being determined. Six laboratories conducted the mysid test with both silver ($AgNO_3$) and endosulfan (McKenney 1982). Only three laboratories completed acceptable tests with silver and four laboratories with endosulfan. Tests were judged unacceptable if critical requirements in the method were violated. The average of the geometric means of the MATC limits of the three tests using silver was 42 µg/L; coefficient of variation was 0.57. The average of the geometric means of the MATC limits of the four tests with endosulfan was 0.34 µg/L; coefficient of variation was 0.38. The average of the two coefficients of variation was 0.48, a value similar to the overall coefficient of variation

Table 7. Application factors (no-effect concentration in a partial or entire life cycle toxicity test divided by the 96-hour LC50) for the sheepshead minnow (an estuarine fish) and freshwater fishes.

Chemical	Application Factor	
	Sheepshead Minnow	Freshwater Fishes
Diazinon	<0.0003	0.03[a], <0.0004[b], <0.0007[c]
Endrin	0.35	0.26[a]
Heptachlor	0.09	0.12[b]
Kepone	0.001	0.004[b]
Malathion	0.08	0.02[b], 0.03[d], 0.06[a]
Trifluralin	0.007	0.017[b]

[a] Jordanella floridae
[b] Pimephales promelas
[c] Salvelinus fontinalis
[d] Lepomis macrochirus

(0.43) of acute toxicity tests. Seven laboratories have completed duplicate early life stage toxicity tests with C. variegatus, using endosulfan and pentachlorobenzene. Although final analyses of data and test acceptability have not been completed, variability appears similar to that seen in the acute toxicity tests and the mysid life cycle toxicity tests.

Community Toxicity Test

A common criticism of toxicity tests in which species are exposed separately is that extrapolation of results to protect communities which consist of a large number of interacting species is not justified. In an attempt to examine species interactions, a toxicity test has been developed at the Gulf Breeze Laboratory.

The effects of substances on development and structure of estuarine communities in sand-filled aquaria can be assessed by comparing numbers, species and phyla of benthic animals that grow from planktonic larvae entering replicated control aquaria and aquaria contaminated with different concentrations of a substance (Hansen and Tagatz 1980). This approach has advantages over single species toxicity tests, microcosms and field studies in obtaining insight into community impacts. In single species tests and microcosms, an adequate number of species may not have been tested, methods used may be inappropriate, species interactions may be absent, and species composition may be artificial; field studies usually lack adequate controls and replication.

The apparatuses used in these experiments were variations of two primary designs. In the first type, raw seawater with its natural component of plankton was pumped into head boxes and then flowed by gravity into four apparatuses; one served as a control and three received different concentrations of the substance to be tested. Each apparatus consisted of 6, 8 or 10 replicate aquaria (Hansen 1974; Tagatz and Ivey 1981; Tagatz et al. 1982a). The surface area of aquaria averaged approximately 400 cm^2; each contained 5 cm of sand as a substrate for colonization, covered by 3 cm of water. Flow to each aquarium was maintained at 200 mL/min. In these tests, the larvae, settling stages, juveniles and adults were exposed in the laboratory to the test substance for 2 to 4 months.

The second type, used in field studies, consisted of individual aquaria 32 cm^2 and 6 cm deep which were filled with sand and placed in the estuary. After about 8 weeks of colonization, the 32 aquaria were removed and placed in the laboratory for 1 to 2 weeks. Eight received seawater only and served as controls, and three groups of eight received three different concentrations of the test substance. Communities colonized in the field and placed in the laboratory for the exposure generally contain the more resistant juvenile and adult life stages. Following both types of exposure, the animals were collected (by siphoning the contents of each aquarium into a 1 mm mesh sieve), preserved and identified. Data were then analyzed, and effects on community structure, including numbers of individuals, number of species, species diversity indices, and occasionally effects on community function, were determined.

Results from 18 community tests conducted over the last decade (Table 8) demonstrate that a variety of species of marine organisms will settle and grow in this system (Hansen 1974; Hansen and Tagatz 1980; Tagatz and Tobia 1978; Tagatz and Ivey 1981; Tagatz et al. 1977; 1978 a,b; 1979 a,b; 1981; 1982a, b, unpublished data on carbophenothion and pentachlorophenol and di-n-butyl phthalate). A total of 54,000 individuals representing 223 species from 11 phyla have been identified from these 18 experiments.

Table 8. Numbers of individuals, species and phyla in eighteen laboratory- and field-colonized estuarine benthic community toxicity tests (range in parentheses).

	Individuals	Species	Phyla
Number per test (n=18)	3,000(180-12,000)	44(24-81)	7(5-9)
Average per laboratory-colonized test (n=14)	3,600(270-12,000)	42(24-81)	7(5-9)
Average per field-colonized test (n=4)	1,000(180-2,300)	50(32-65)	7(6-9)
Total all tests	54,000	223	11

The numbers of individuals, species and phyla differed considerably among tests, probably as a function of the seasonal variations in reproductive patterns of species and water quality. The test-to-test differences were greatest in the number of individual organisms, 180 to 12,000 (average 3,000); differences were less for the number of species per test, 24 to 81 (average 44); and phyla per test, 5 to 9 (average 7). Of the 223 species in the 18 tests, 45% were annelids, 23% mollusks, and 18% arthropods (Table 9); species from the remaining eight phyla constituted only 12% of the total. Thus, the test was generally most effective in detecting effects on species in the three dominant phyla.

A comparison of communities that developed in laboratory and field experiments (Table 8) shows that test-to-test variations are greatest in the number of individuals and smallest in the number of species and phyla. Tagatz and Deans (1983) discuss the structural similarity of benthic communities in the field and those that develop in experimental aquaria in the field and laboratory, and the relative merits of toxicity tests using laboratory- and field-developed communities. They conclude that, although each test has merit, the laboratory test is operationally simpler and can be more sensitive because early life stages of settling organisms are exposed.

Table 9. Numbers of species, by phylum, from eighteen laboratory- and field-colonized estuarine benthic community toxicity tests.

Phylum	Number of Species
Annelida	100
Mollusca	55
Arthropoda	40
Echinodermata	8
Chordata	5
Nemertinea	5
Coelenterata	4
Platyhelminthes	2
Sipunculoidea	2
Hemichordata	1
Phoronida	1
Total - 11 Phyla	223 Species

The data from the 18 community tests with pesticides, formulated products, organic chemicals, and muds used in drilling oil wells provide insight into the sensitivity of phylogenetic groups not possible from toxicity tests in which species are exposed individually (Table 10). As would be expected, arthropods were particularly sensitive to the five pesticides tested. Arthropods were not particularly sensitive to the other substances, except Dowicide G-ST which contains 79% sodium pentachlorophenate. Mollusca was the most frequently sensitive phylum. Its sensitivity to pesticides rivaled that of the arthropods and probably would not have been detected from acute mollusk larval toxicity tests or oyster shell deposition tests. Annelids, chordates (tunicates), echinoderms and coelenterates, phyla rarely tested as individual species in acute and chronic toxicity tests (Tables 1 and 4), were also identified as par-

Table 10. Phyla whose numbers of individuals or species were reduced in estuarine benthic community toxicity tests with individual pesticides (chlorpyrifos, fenvalerate, pentachlorophenol, sevin or toxaphene); with formulated products (Dowicide-GST or Surflo-883); with non-pesticide organics (Aroclor 1254 or di-n-butyl phthalate) or insoluble energy-related products (barite, drilling muds incorporated in sand or muds delivered in suspension). Two tests were conducted with chlorpyrifos, fenvalerate and di-n-butyl phthalate and four tests with pentachlorophenol.

Phylum	Number of tests in which phylum was reduced				
	Pesticides	Formulations	Organics	Muds	Total
Arthropoda	6	0	1	0	7
Mollusca	5(1[a])	2	2	0	9(1[a])
Annelida	1	0	1	2	4
Chordata	1	0	1(1[a])	1	3(1[a])
Echinodermata	0	0	1	0	1
Coelenterata	0	0	0	1	1
Total tests	10	2	3	3	18

[a] Numbers were increased in the presence of specific chemicals.

ticularly sensitive to certain organic chemicals and drilling muds. Surprisingly, decreases in numbers of individuals or species were compensated for by increases in other taxa only twice, with toxaphene and Aroclor 1254.

A comparison with results from single species tests demonstrates that community tests (1) provide information on the sensitivity of species that cannot be determined from tests of species separately; (2) can be as sensitive as, or more sensitive than, chronic exposures of individual species because the often most sensitive early life stages of a wide variety of species are exposed; (3) provide insight into the limits of applicability of single species tests to predict impacts on communities consisting of many interacting species, some of which are phylogenetically related to species that are sensitive in single species tests. The test in which communities develop in the laboratory as they are exposed is the

most sensitive. However, caution must be used in extrapolation of the results from both of these community tests to environmental effects. Test communities are not exact replicates of field communities, because larger benthic and water column species are absent and exposure conditions vary between the laboratory and the field.

UTILITY OF TOXICITY TESTS

The ultimate purpose of toxicity tests is to determine the toxicity of substances and the sensitivity of species, to use these data, along with data on environmental concentration, to predict with known accuracy the potential for environmental hazard, and to do so at minimum cost (Figure 2). For the tests to be cost effective, those substances with minimum or no likelihood for environmental harm must be rapidly identified with minimal expenditure of resources. It is essential that the majority of resources be available to study those substances that possess the greatest potential for hazard. Single species toxicity tests, or structure-activity relationships derived from these tests and from physical-chemical properties of classes of substances, are likely to be the most cost-effective technique of identifying substances that have a low degree of hazard.

Accurate predictions when hazard is likely to be high (i.e., where effect and environmental concentrations are similar) require a maximum of resources and testing beyond that of single species toxicity tests in which species and water differ from that of the specific site of concern (Figure 3). Even in such instances, testing will generally begin with standardized single species toxicity tests that have the high degree of control and replicability needed to determine the toxicity of the substance, the species most sensitive, the stages of the life cycle susceptible to sublethal exposure, the effect of site water quality on toxicity and the bioavailability of the substance. These results are then used to select exposure concentrations and appropriate test design in the latter stages of the evaluation, where toxicity tests are conducted that measure effects on systems of interacting species, such as those found in microcosms, and in tests to measure impacts on community structure and function. Finally, in those instances where the substance is already in the marine environment, field studies may be conducted. Few field studies have attempted to provide data linking all aspects of this evaluative process. This is probably because of the expense and the fact that, except in engineered systems, the controls and replication so important in experimental design are lacking. Effective assessment of substances when the hazard is high

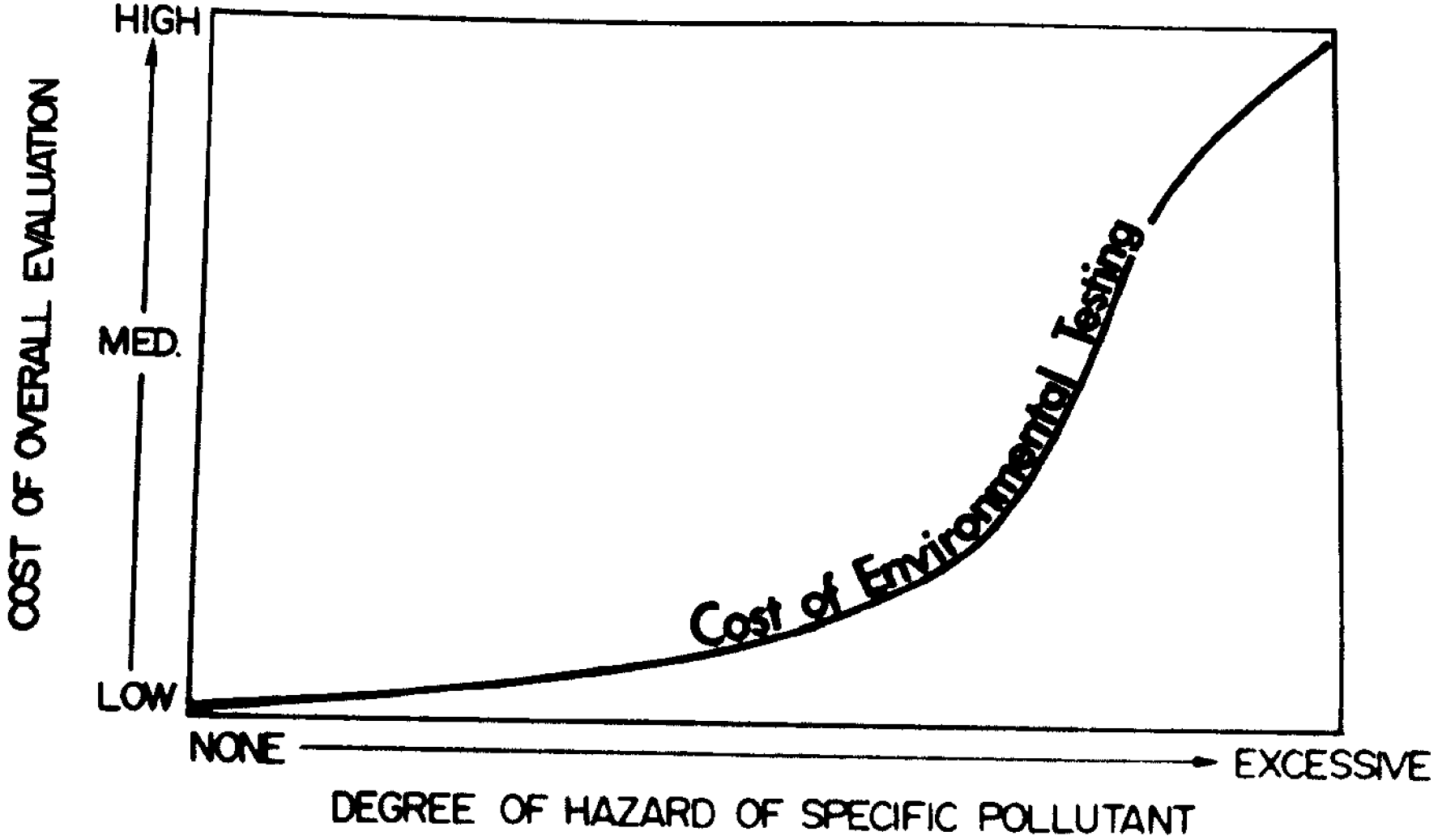

Figure 2. Optimization of the hazard evaluation process to minimize testing effort for substances with a low potential for hazard and to maximize effort for substances with the greatest potential hazard.

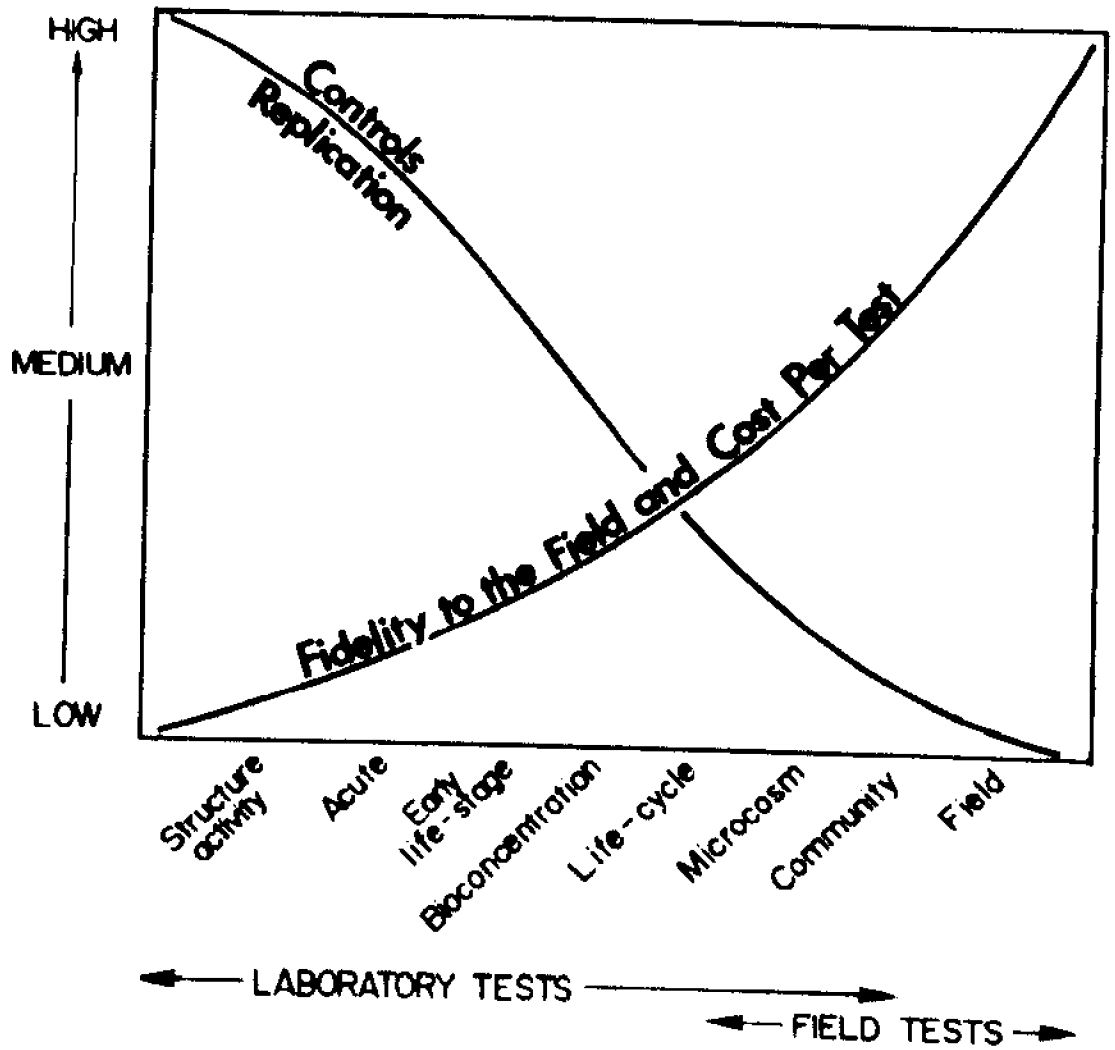

Figure 3. Relationship between the degree of control, replication, cost and fidelity to the field of laboratory and field effects assessment methodologies.

requires integration of critical laboratory and field studies. Environmental scientists should emphasize the overall process of hazard evaluation and how it can be more efficacious, rather than debating over who has the best test this week.

REFERENCES

Allen, J.D. and R.E. Daniels. 1980. Sublethal effects of pollutants: Life table evaluation of chronic exposure of Eurytemora affinis (Copepoda) to Kepone. Tech. Rep. No. 61, Water Resources Research Center, Univ. of Maryland, College Park, Md.

APHA-AWWA-WPCF (American Public Health Association-American Water Works Association-Water Pollution Control Federation). 1980. Standard methods for the examination of water and wastewater. Fifteenth Edition, Am. Publ. Health Assoc., Am. Water Works Assoc., and Water Pollut. Control Fed.

ASTM (American Society for Testing and Materials). 1977. Standard practice for conducting basic acute toxicity tests with fishes, macroinvertebrates, and amphibians. Draft No. 6, E-35.21, ASTM, Philadelphia, Pa.

ASTM (American Society for Testing and Materials). 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. In: Annual Book of ASTM Standards, E729-780. ASTM, Philadelphia, Pa.

ASTM (American Society for Testing and Materials). 1982. Proposed standard practice for conducting toxicity tests with the early life stages of fishes. S.C. Schimmel, subcommittee chairman, E-47.01, ASTM, Philadelphia, Pa.

Buikema, A.H., Jr., B.R. Niederlehner and J. Cairns, Jr. 1982. Biological monitoring. Part IV. Toxicity Testing. Water Res. 16: 239-262.

Cairns, J., Jr., K.L. Dickson and A.W. Maki (eds.). 1978. Estimating the Hazard of Chemical Substances to Aquatic Life. ASTM STP 657, American Society for Testing and Materials. Philadelphia, Pa.

Daniels, R.E. and J.D. Allen. 1981. Life table evaluation of chronic exposure to a pesticide. Can. J. Fish. Aquat. Sci. 30(5): 485-494.

Dickson, K.L., A.W. Maki and J. Cairns, Jr. 1979. Analyzing the Hazard Evaluation Process. American Fisheries Society, Water Quality Section, Bethesda, Md.

Eaton, J.G. 1973. Recent developments in the use of laboratory bioassays to determine "safe" levels of toxicants for fish. In: G.E. Glass (ed.), Bioassay Techniques and Environmental Chemistry, pp. 107-115. Ann Arbor Science Publ., Ann Arbor, Mich.

Gentile, J.H., S.M. Gentile, N.G. Hairston, Jr. and B.K. Sullivan. 1982. The use of life-tables for evaluating the chronic toxicity of pollutants to Mysidopsis bahia. Hydrobiologia 93: 179-187.

Gentile, S.M., J.H. Gentile, J. Walker and J.F. Heltshe. 1982. Chronic effects of cadmium on two species of mysid shrimp: Mysidopsis bahia and Mysidopsis bigelowi. Hydrobiologia 93: 195-204.

Goodman, L.R., D.J. Hansen, J.A. Couch and J. Forester. 1978. Effects of heptachlor and toxaphene on laboratory-reared embryos and fry of the sheepshead minnow. Proceedings, 30th Annual Conference Southeastern Association Game and Fish Commission, pp. 192-202.

Goodman, L.R., D.P. Middaugh, D.J. Hansen, P.K. Higdon and G.M. Cripe. 1983. Early life stage toxicity test using tidewater silversides (Menidia peninsulae) and results obtained with chlorine produced oxidants. Environmental Toxicol. Chem.

Hansen, D.J. 1974. Aroclor 1254: Effect on composition of developing estuarine animal communities in the laboratory. Contrib. Mar. Sci. 18: 19-33.

Hansen, D.J. and M.E. Tagatz. 1980. A laboratory test for assessing impacts of substances on developing communities of benthic estuarine organisms. In: J.G. Eaton, P.R. Parrish and A.C. Hendricks (eds.) Aquatic Toxicology, pp. 40-57. ASTM STP 707, American Society for Testing and Materials, Philadelphia, Pa.

Hansen, D.J., P.R. Parrish, S.C. Schimmel and L.R. Goodman. 1978. Life cycle toxicity test using sheepshead minnows (Cyprinodon variegatus). In: Bioassay procedures for the ocean disposal permit program, pp. 109-115. EPA-600/9-78-010, U.S. Environmental Protection Agency.

Macek, K.J. and B.H. Sleight, III. 1977. Utility of tests with embryos and fry of fish in evaluating hazards associated with the chronic toxicity of chemicals to fishes. In: F.L. Mayer and J.L. Hamelink (eds.), Aquatic Toxicology and Hazard Evaluation, pp. 137-146. American Society for Testing and Materials, Philadelphia, Pa.

McKenney, C.L., Jr. 1982. Interlaboratory comparison of chronic toxicity test using the estuarine mysid (Mysidopsis bahia). U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Fl. (unpublished report).

McKim, J.M. 1977. Evaluation of tests with early life stages of fish for predicting long term toxicity. J. Fish. Res. Board Can. 34: 1148-1154.

Mount, D.I. 1977. An assessment of application factors in aquatic toxicology. In: R.A. Tubb (ed.), Recent Advances in Fish Toxicology, pp. 183-190. EPA-600/3-77-085, U.S. Environmental Protection Agency, Office of Research and Development.

Mount, D.I. and C.E. Stephan. 1967. A method for establishing acceptable toxicant limits for fish-malathion and the butoxyethanol ester of 2,4-D. Trans. Am. Fish. Soc. 96(2): 185-193.

Nelson, D.A., A. Calabrese, R.A. Greig, P.P. Yevich and S. Chang. Long-term silver effects on the marine gastropod (Crepidula fornicata). Marine Ecology Progress Series (In press).

Nimmo, D.R., T.L. Hammaker and C.A. Sommers. 1978. Entire life cycle toxicity test using mysids (Mysidopsis bahia) in flowing water. In: Bioassay procedures for the ocean disposal permit program, pp. 64-78. EPA-600/9-78-010, U.S. Environmental Protection Agency.

Pearson, J.G. and J.P. Glennon. 1979. A sequential comprehensive hazard assessment strategy for estimating water quality criteria. In: K.L. Dickson, A.W. Maki and J. Cairns, Jr. (eds.), Analyzing the Hazard Evaluation Process, pp. 30-49. American Fisheries Society, Water Quality Section, Bethesda, Md.

Schimmel, S.C. 1981. Results: Interlaboratory comparison-acute toxicity tests using estuarine animals. EPA-600/4-81-003, U.S. Environmental Protection Agency.

Sprague, J.B. 1969. Review Paper: Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3(11): 793-821.

Sprague, J.B. 1970. Review Paper: Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results. Water Res. 4: 3-32.

Sprague, J.B. 1971. Review Paper: Measurement of pollutant toxicity to fish. III. Sublethal effects and "safe" concentration. Water Res. 5: 245-266.

Tagatz, M.E. and C.H. Deans. 1983. Comparison of field- and laboratory-developed estuarine benthic communities for toxicant-exposure studies. Water Air Soil Pollut.

Tagatz, M.E. and J.M. Ivey. 1981. Effects of fenvalerate on field- and laboratory-developed estuarine benthic communities. Bull. Environ. Contam. Toxicol. 27: 256-267.

Tagatz, M.E. and M. Tobia. 1978. Effect of barite ($BaSO_4$) on development of estuarine communities. Estuar. Coast. Mar. Sci. 7: 401-407.

Tagatz, M.E., J.M. Ivey, J.C. Moore and M. Tobia. 1977. Effects of pentachlorophenol on the development of estuarine communities. J. Toxicol. Environ. Health 3: 501-506.

Tagatz, M.E., J.M. Ivey and M. Tobia. 1978a. Effects of Dowicide G-ST on development of experimental estuarine macrobenthic communities. In: K. Ranga Rao (ed.), Pentachlorophenol, pp. 157-161. Plenum Press, New York.

Tagatz, M.E., J.M. Ivey, H.K. Lehman and J.L. Oglesby. 1978b. Effects of a lignosulfonate-type drilling mud on development of macrobenthic communities. Northeast Gulf Sci. 2(1): 35-42.

Tagatz, M.E., J.M. Ivey and J.L. Oglesby. 1979a. Toxicity of drilling-mud biocides to developing estuarine macrobenthic communities. Northeast Gulf Sci. 3(2): 88-92.

Tagatz, M.E., J.M. Ivey, H.K. Lehman and J.L. Oglesby. 1979b. Effects of sevin on development of experimental estuarine communities. 1979. J. Toxicol. Environ. Health 5: 643-651.

Tagatz, M.E., J.M. Ivey, N.R. Gregory and J.L. Oglesby. 1981. Effects of pentachlorophenol on field- and laboratory-developed estuarine benthic communities. Bull. Environ. Contam. Toxicol. 25: 137-143.

Tagatz, MdE., J.M. Ivey, C.E. DalBo and J.L. Oglesby. 1982a. Responses of developing estuarine macrobenthic communities to drilling muds. Estuaries 5(2): 131-137.

Tagatz, M.E., N.R. Gregory and G.R. Plaia. 1982b. Effects of chlorpyrifos on field- and laboratory-developed estuarine benthic communities. J. Toxicol Environ. Health. 10: 411-421.

Tyler-Schroeder, D.B. 1979. Use of grass shrimp (Palaemonetes pugio) in a life cycle toxicity test. In: L.L. Marking and R.A. Kimerle (eds.), Aquatic Toxicology, pp. 159-170. ASTM STP 667, American Society for Testing and Materials, Philadelphia, Pa.

U.S. EPA (Environmental Protection Agency). 1980a. Guidelines for deriving water quality criteria for the protection of aquatic life and its uses. U.S. Fed. Regist. 45(231): 79341-79347.

U.S. EPA (Environmental Protection Agency). 1980b. Ambient water quality criteria for acenaphthene through zinc. EPA-440/5-80-015 to EPA-440/5-80-079, U.S. EPA.

Walsh, G.E. and R.L. Garnas. 1983. Determination of bioactivity of chemical fractions of liquid wastes using freshwater and saltwater algae and crustaceans. Environmental Sci. Technol. 17(3): 180-182.

Ward, T.J., E.D. Rider and D.A. Drozdowski. 1979. A chronic toxicity test with the marine copepod Acartia tonsa. In: L.L. Marking and R.A. Kimerle (eds.), Aquatic Toxicology, pp. 148-158. ASTM STP 667, American Society for Testing and Materials, Philadelphia, Pa.

Strategies for Utilizing Laboratory Toxicological Information in Regulatory Decisions

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INTRODUCTION

Five major pieces of federal legislation define the role of the Environmental Protection Agency (EPA) with regard to marine and estuarine pollution: the Federal Water Pollution Control Act (1965); the Marine Protection, Research and Sanctuaries Act (1972); the Resource Conservation and Recovery Act (1976); the Toxic Substances Control Act (1976); and the Federal Insecticide, Fungicide and Rodenticide Act (1975). Each of these acts charges the administrator of EPA with a variety of responsibilities that are generally summarized as follows: develop comprehensive programs to conserve waters for the propagation and protection of fish, aquatic life and wildlife; regulate the dumping of all types of materials into ocean waters, and prevent or strictly limit the dumping into ocean waters of any material that would adversely affect human health, the marine environment, ecological systems or economic potentialities; and develop adequate data with respect to the effect of chemical substances and mixtures on health and the environment.

Implicit in the implementation of the goals of these regulatory programs is the use of laboratory test data to determine the toxicological properties of the chemical substances being regulated. This information is then used to predict the potential impact of a material on the environment. Since the implementation of the acts, considerable research effort has been devoted to developing a wide variety of laboratory toxicity tests designed to define the ef-

fects of chemicals on a variety of biological processes. A detailed analysis of these tests, their expected variances, and the degree of confidence in the types of data generated from these tests has been reviewed in this volume by Hansen.

The purpose of this paper is to discuss how various types of toxicological data are organized, interpreted and utilized in making regulatory decisions. To illustrate this, two approaches will be examined in detail: first, the strategy used for developing water quality criteria for the protection of aquatic life; second, the concept of hazard assessment is examined and compared with the criteria strategy in terms of both similarities and differences. Finally, a retrospective case study is presented for each strategy to illustrate the relationship between the predictions derived from laboratory toxicity tests and field observations.

WATER QUALITY CRITERIA

A water quality criterion is a numerical value that identifies the highest water concentration of a compound that will produce a water quality generally suited to the maintenance of aquatic life and its uses. Publication of water quality criteria has been an evolution and ongoing process involving EPA and its predecessor agency, the Federal Water Pollution Control Administration, since the publication of the first compilation of water quality criteria, the "Green Book" in 1968 (U.S. Department of the Interior 1968). Since then, water quality criteria have been revised and expanded with the publication of the "Blue Book" (U.S. EPA 1972) and the "Red Book" (U.S. EPA 1976). Criteria published within this time period were characteristically derived by a panel of experts from very limited and non-quality-assured data bases. In addition to combining criteria for fresh- and saltwaters, the derivations often reflected an inconsistent use of data and a large measure of subjectivity. Furthermore, there was no consistent rationale for defining appropriate data and their use in deriving a criterion.

The general approach that characterizes the period (prior to 1976) is summarized in Figure 1. For pollutants whose bioavailability was known to be influenced by water quality (e.g., hardness), the aquatic life criterion was derived by multiplying the LC50 (concentration lethal to 50% of the exposed population) of the sensitive important species tested in site water by a compound-specific, lab-derived application factor. The application factor is defined as the Maximum Acceptable Toxicant Concentration (MATC) from a chronic test, divided by the 96-h LC50. In the case of non-water quality related chemicals (e.g., most pesticides) two procedures were available. First, the lowest LC50 from the most sensitive species was multiplied by a general application factor of 0.01 or the lowest concentration not affecting survival, growth or re-

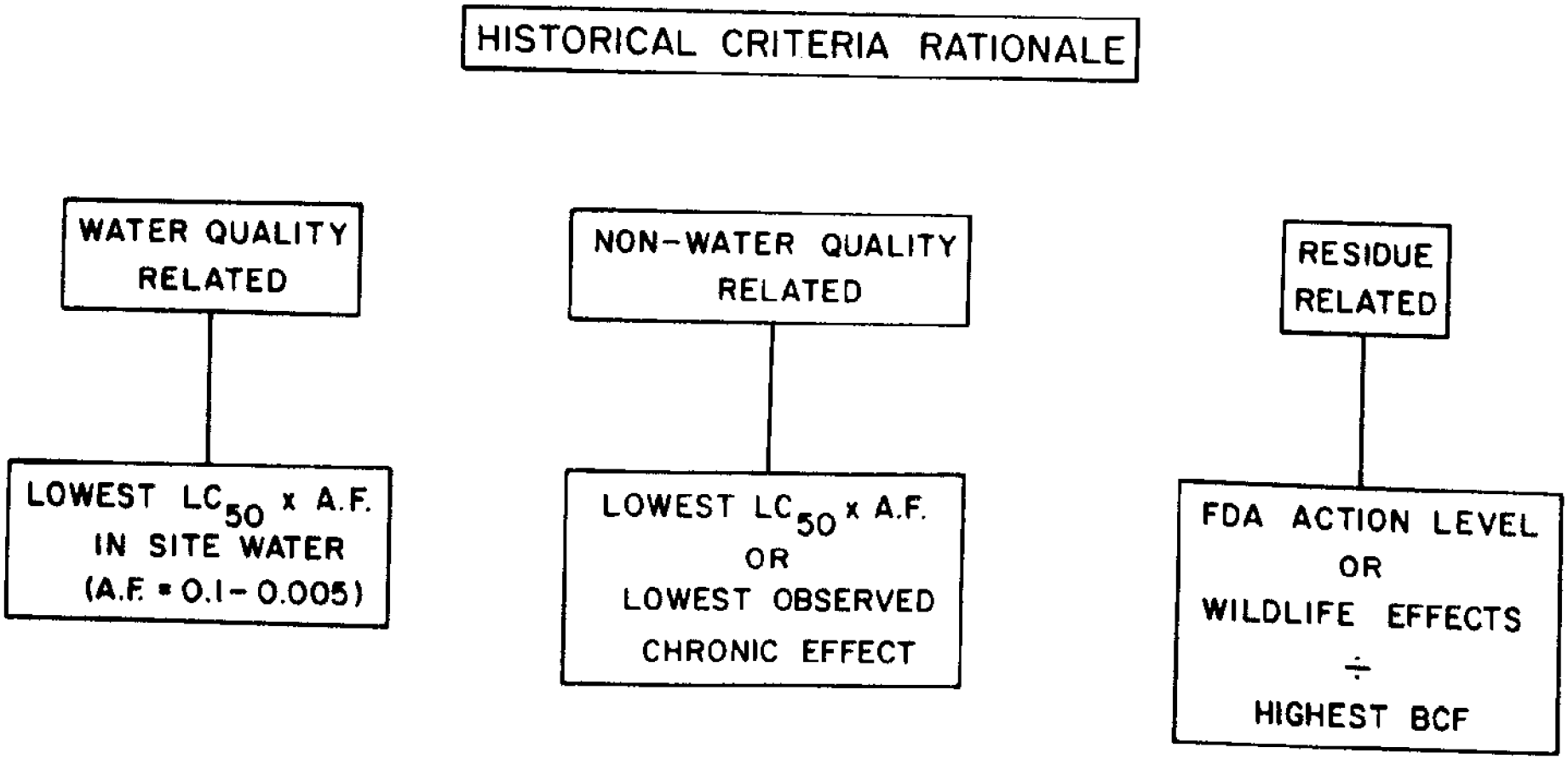

Figure 1. Three approaches used in the development of Water Quality Criteria from 1968 through 1976.

production in a chronic test was utilized. The second procedure for developing criteria was applied to those chemicals known to bioaccumulate extensively and for which either FDA action levels for humans or wildlife effects studies were available. In this approach, the maximum permissible tissue concentration (FDA action level) is divided by the highest bioconcentration factor (BCF = edible tissue concentration $(mg\ kg^{-1})$/water concentration $(mg\ L^{-1})$) to give an ambient water concentration that cannot be exceeded. These procedures were used to calculate the maximum pollutant concentration that was deemed protective of aquatic life, and in the case of tissue residues, assured the marketability of fish and shellfish.

As mentioned previously these procedures suffered from the lack of an internally consistent rationale for selecting and interpreting data to eliminate subjectivity. Recognition of the need for scientific guidelines was reinforced when EPA was directed in 1977, by court order (NRDC et al., v. Train 1976) to publish criterion documents for 65 chemicals or groups of chemicals that Congress designated as toxic. To satisfy this legal mandate a systematic toxicological testing program was instituted to develop comprehensive data bases on freshwater and saltwater species, and a task force of EPA scientists was constituted to develop the scientific rationale that forms the basis for developing water quality criteria. The Guidelines for the Development of Water Quality Criteria (U.S. EPA 1980) represent a rationale that describes the collection, review, selection and categorizing of toxicological information. The categories include information on acute and chronic toxicity to animals and plants, bioaccumulation data, and other data such as those derived from studies on physiology, biochemistry, community structure and function, and bioaccumulation. The guidelines also define minimum data requirements in the various categories that are necessary to formulate criteria and prescribe methods for using the data in the formulation of criteria. Consequently, the guidelines provide an internally consistent rationale and well-defined procedures for deriving criteria that eliminate the problems of subjectivity inherent in the earlier process. This, coupled with a systematic and quality-assured data base, represents a major advancement in the application of toxicological information to the regulatory process. The intent of this rationale and the resulting criteria is the protection of most of the species most of the time but not all the species all of the time. Further, it assumes that if most species are protected, maintenance of community function will be assured.

Numerical criteria derived using the current guidelines are expressed as two numbers: a maximum concentration, and a 30-day average concentration (Figure 2). The two-number criterion is intended to identify the highest average concentration consistent with the maintenance of aquatic life while restricting the extent and duration of excursions over the average so that the total expo- will not cause unacceptable adverse effects.

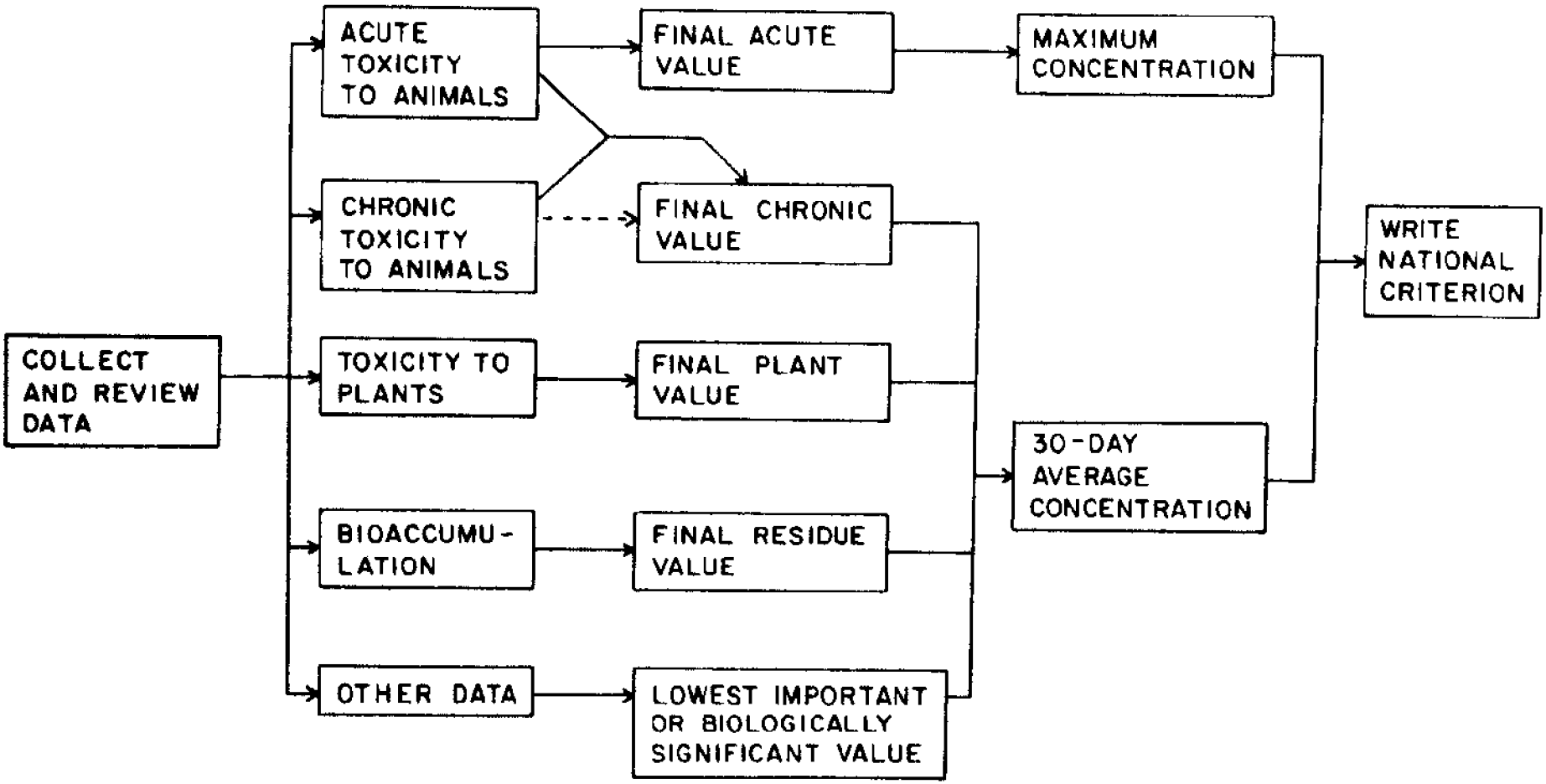

Figure 2. Schematic of the rationale used currently to develop Water Quality Criteria for the protection of aquatic life and wildlife.

The maximum component of the criterion is designed to limit acute lethality and is based upon the compilation and analysis of acute toxicity data from a minimum of eight taxonomic saltwater families. Specifically, for a saltwater criterion, acute toxicity data from the following taxa are required: two different families in the phylum Chordata, a family in a phylum other than Chordata and Arthropoda, either the Mysidae or Penaeidae family, three other families not in the phylum Chordata, and any other family. The species acute toxicity data are arranged in families which are then ranked from most to least sensitive. Regression analysis is employed to determine the Final Acute Value (Erickson and Stephan 1982).

The Final Acute Value, which is an estimated LC50, is multiplied by 0.5 to give the MATC which is an estimated LC0 or nonlethal concentration. This value is intended to protect 95% of a group of families with the same sensitivity as those tested, when the cumulative deviation of excursions above the specified 30-day average concentration is limited to 96 hours in any 30 consecutive days. Inherent in the rationale for determining the MATC are the following assumptions: (1) the range of species sensitivity from laboratory tests is an unbiased estimator of the range of sensitivity of species in natural communities; (2) the variability of species within families is less than that between species across higher taxonomic groups; (3) the ranking of family sensitivities of laboratory populations is not different from that of field populations; (4) laboratory test data are not intrinsically biased, though never originally chosen to provide a random reflection of the species in the environment; (5) the maximum concentration is designed to protect most of the species most of the time; (6) by protecting species composition, community function is protected.

Derivation of the 30-day average component of a criterion involves the interpretation of data bases on chronic toxicity to animals, toxicity to plants, bioaccumulation and other biologically significant effects on ecologically or economically important resource populations (Figure 2).

There are two methods for determining chronic toxicity to animals. The first is to perform a regression analysis on eight families of chronic tests. Usually, this option is not practical because of the limited number of chronically testable saltwater species and the prohibitive cost of such testing. The second method uses the acute-chronic ratio concept. To determine an acute-chronic ratio for a given species, a measure of acute toxicity, defined as a 96-h LC50, is divided by a measure of chronic toxicity, defined as the geometric mean of the highest no effect and lowest effect concentration. Effect and no effect are defined as being statistically different or indistinguishable from appropriate controls. The guidelines recommend that acute-chronic ratios represent a minimum of three families--one must be saltwater and must have a fish and an

invertebrate--to be a satisfactory estimator of the relationship between acute and chronic effects. The geometric mean of these acute-chronic ratios is divided into the Final Acute Value in order to provide a chronic exposure concentration protective of untested species. The assumptions inherent in this approach are that acute-chronic ratios from laboratory tests are unbiased estimators of those expected from natural populations. Further, for certain compounds the acute-chronic ratio is similar across species and consequently can be used to extrapolate to untested species. In contrast, the acute-chronic ratio for some compounds decreases with increasing species sensitivity, and this relationship has been used to predict chronic sensitivities for untested species. Since the range of acute toxicity is often two or more orders of magnitude it is more important to have a good estimate of the range of species acute sensitivities, to which the acute-chronic ratio can be applied (Hansen, this volume) than to have a large number of acute-chronic ratios.

The Final Residue Value (Figure 2) is utilized in developing criteria to protect marketability by preventing commercially or recreationally important aquatic organisms from containing amounts of chemicals exceeding relevant FDA action levels (maximum permissible tissue concentrations) and to protect wildlife, including fishes and birds that eat aquatic organisms. This concept is not unique to the current guidelines and, in fact, has been utilized in limited cases in previous criterion documents. The quality assurance and minimum data requirements are what is different. In addition, for lipophilic material the bioconcentration factor (BCF) (the quotient of the concentration of a substance in all or part of an aquatic organism divided by the concentration in water to which the organism has been exposed) is normalized to 1% lipid. This adjustment seeks to make all the measured BCFs comparable regardless of the species or tissue for which the BCF was measured. The final residue value is derived by dividing the maximum permissible tissue concentration by an appropriate BCF and multiplying this by the greatest percentage of lipid in a marketable species. The final residue, adjusted for highest lipid, defines the pollutant concentration in the water that cannot be exceeded to protect marketability or wildlife consumers of aquatic life.

The remaining categories of data that can contribute to estimating the chronic 30-day average component of the criteria are plant data and other data, including physiological, biochemical, and behavioral data on ecologically or commercially important species and data on impacts on community structure and function. Information in these two categories is generally supportive of criteria derived from chronic animal tests and residue values but has not been the basis for criteria derivation.

The derivation of the 30-day average criterion is based upon systematic comparison of data from all four categories of chronic

tests and selection of the lowest value. This value is deemed protective of aquatic life from chronic effects, and when coupled with the MATC to protect for acute effects, provides the scientific information necessary for this specific management decision. Thus, recommendations of chemically specific water quality criteria derived by using the current guidelines are based upon (1) a defined and consistent rationale for utilizing all available pertinent data, (2) quality-assured data bases; (3) minimum data requirements for all information categories; (4) analysis of data using statistical procedures.

The guidelines procedure is utilized to predict the water concentration of a chemical that is suited to the maintenance of aquatic life. Another way of viewing the guidelines then is as a predictive method based on the synthesis and interpretation of toxicological data. Prediction is the key to the regulatory process since EPA is mandated to make decisions on the registration and disposal of chemicals prior to their use (TSCA Section 4, Premanufacture Notice/Pesticide Registration). After development of a predictive rationale such as the guidelines, the next step is to evaluate the accuracy of its predictions. This involves a field applicability or verification study. Unfortunately, we do not have a direct field verification study in marine waters for the current guidelines. However, we do have a freshwater field case study, Shayler Run, that tested the principles of the current guidelines and provides insights into the limits of applicability of laboratory studies for predicting responses in the field.

Shayler Run

The purpose of the Shayler Run study, 1967-1972 (Geckler et al. 1976), was to determine the usefulness of laboratory toxicity tests in predicting water quality criteria for protection of freshwater aquatic life. The study was designed so that the results from standard laboratory and streamside acute and chronic toxicity tests could be compared with the results of long-term stream exposure. The field verification portion of this study lasted 4 years and 9 months. The exposure concentration in the stream was selected by using acute toxicity data for 10 fish species and chronic toxicity data for fathead minnows so that the most resistant fish species would be protected from chronic effects and the most sensitive would not be protected. Since this study predates the current guidelines it is not intended as a direct verification of the latter. It does, however, provide insight into the appropriateness of many of the concepts and assumptions in the current guidelines and is useful therefore in assessing their validity.

The regulatory climate prevailing at the time of the study reflected the 1965 Federal Water Pollution Control Act, which required water quality standards to protect designated uses. Uses could be defined, for example, as maintenance of a salmonid, a

warmwater sport or a roughfish fishery, maintenance for recreational uses or merely for navigation. Within this context, broader ecological concepts such as community structure and function and species diversity were not specifically studied. The objectives and hypotheses implicit within the Shayler Run study are these: (1) the conditions acceptable for the designated use also provide satisfactory conditions for the support of ecosystem components (e.g., food species); (2) the exposure-response relationships determined for individual species using laboratory effect measurements are not different from responses determined in the field for the same species; (3) the relative sensitivity of the same species is not different in lab tests and in the field; (4) indirect effects due to the toxicant on other components of the field system are not significant in altering the responses of the test species; (5) the laboratory-derived acute-chronic ratio can be used to protect fishes in the field.

The experimental design for the Shayler Run studies consisted of three components: (1) acute and chronic toxicity tests in standard laboratory water; (2) streamside acute and chronic tests with stream species; and (3) field studies. Acute toxicity tests were conducted to determine the effect of changes in the water quality of Shayler Run on toxicity and the relative sensitivity of 10 fish species common in the stream. Chronic toxicity studies were used to define the copper concentrations affecting survival, growth and reproduction in fathead minnows and to measure the ratio of acute to chronic toxicity. This ratio was then applied to the acute tests for the other 10 fish species in Shayler Run water, to predict safe concentrations for untested species. These values were used to select the copper field exposure concentration of 120 $\mu g\ L^{-1}$. Field studies included eight biannual (spring and fall) samplings of fish populations using electric seining techniques, daily collections of vertebrates and invertebrates from weir screens, and fish feeding patterns from analysis of stomach contents. Reproduction and growth rates were estimated from fry collection traps and weirs and from direct spawning observations. Nonfish studies included the examination of benthic macroinvertebrates and periphyton communities.

The results of the field study indicated that, as predicted, the most sensitive fish species in Shayler Run were adversely affected by copper. However, with one exception, copper adversely affected almost every common species of fish that spawned in the exposure and recovery areas by restricting spawning locations. Generally, spawning occurred within reaches of the exposure portion of the stream where copper concentrations were 60-90 $\mu g\ L^{-1}$ (a gradient of copper concentrations commonly existed in the 900-meter exposure area). Four of the five most abundant macroinvertebrate populations in the exposure area were eliminated by copper. Although dietary shifts were observed in two of the fish species, indirect effects on growth of the fish populations through effects on the aquatic food chain could not be demonstrated.

We have discussed this study because it is an indirect field verification of several of the concepts and hypotheses implicit in the current guidelines for developing water quality criteria. The conclusions that can be drawn from this study are totally consistent with our original hypothesis. First, the dose-response relationships and sensitivity ranking of resident fish species in laboratory tests were within a factor of 2 of those determined in the field for the same species. The greater sensitivity in the field was due to field-observed avoidance of concentrations of copper between 90 and 120 $\mu g\ L^{-1}$, a behavior not accounted for during standard toxicity tests. Second, indirect ecological effects (e.g., dietary shifts) did not alter the field responses. The fact that results of laboratory toxicity tests, within the context of the experimental design, were within a factor of 2 of the field observations is both remarkable and yet totally consistent with the expected variability in toxicological tests conducted under the most rigid laboratory-controlled conditions (Hansen, this volume). Regarding the second hypothesis, concentrations of copper determined to be protective of the organisms of interest (e.g., bluegill and other sunfish) were not protective of many species of macroinvertebrate or other ecosystem characteristics. Because the experimental design did not include toxicity information on other ecosystem components (e.g., invertebrates, plants) their sensitivities were not accounted for and thus the degree of protection afforded to these groups was not determined. This study also provides a first-order estimate of the confidence of our laboratory predictions and identifies a series of issues that would further enhance the credibility of laboratory data. Recently, Hansen and Garton (1982) assessed the ability of a standard set of freshwater, single species toxicity tests to predict accurately the effects of an insecticide on complex laboratory stream communities. In this study, the single species tests adequately predicted the concentrations of the insecticide diflubenzuron that affected the stream communities, but they were less successful in predicting the exact nature of the effects at the community level. Those effects resulting from direct lethality to component species were clearly predicted; indirect effects due to altered species interactions could be predicted only with an a priori knowledge of the system's trophic dynamics. What is important is that the strategy of defining a wide range of species sensitivities from acute tests and coupling them with acute-chronic ratios can be a highly accurate predictive tool when properly applied. Within the context of the needs of the regulatory process, we would offer that the guidelines constitute a strategy that is in effect a very meaningful measure of marine pollution. This is a particularly important point to understand. We are not talking of a single test method any longer but a rationale for organizing and interpreting a variety of test data from which we can derive predictions with a specified degree of confidence.

HAZARD ASSESSMENT

The Toxic Substances Control Act (TSCA), enacted in 1976, provides that no person may manufacture a new chemical substance or manufacture or process a chemical substance for a new use without obtaining clearance from the EPA. TSCA represents an attempt to establish a mechanism whereby the hazard to human health and the environment of a chemical substance is assessed before it is introduced into the environment (Cairns et al. 1978). The enactment of TSCA provided the stimulus for both government and industry to develop testing and decision-making procedures that represent a systematic and comprehensive approach to the problem of predicting chemical hazard to the environment. The initial premise of aquatic hazard evaluation procedures is that toxicological data are interpreted in relation to the expected or measured exposure concentration of the material in the aquatic environment (Kimerle et al. 1978). This leads to a description of the relationship between the generic types of information that form the basis for a hazard assessment. It is important to point out at this time that the Water Quality Criteria and the Hazard Assessment Strategies both utilize laboratory data on aquatic toxicity. Hazard assessment, however, requires additional laboratory information to predict chemical concentrations in the environment. This requirement is one of the principal differences between the two approaches and determines how they are applied in the regulatory process. The other difference relates to the amount of information required to make a decision. The criteria rationale requires very comprehensive minimum data requirements to derive a prediction because these chemicals have been identified as hazardous. Hazard assessment does not require as structured a data set; instead the testing is designed to be sequential. Moving between each level or hierarchy of testing is controlled by specific criteria. Decisions can be made at each level on the basis of varying amounts and complexities of information. The details of hazard assessment are available in an excellent review by Cairns et al. (1978). For the purposes of this paper we will focus on the types of biological test data used in hazard assessment and illustrate how this is coupled with predicted or measured exposure to determine potential hazard. Two case studies representing major environmental problems will be retrospectively analyzed to illustrate the applicability of this approach as a meaningful measure and predictor of marine pollution.

CASE HISTORIES

Few case histories exist that describe (1) the release of a chemical contaminant from a point source into an estuarine environment; (2) the subsequent monitoring of the pollutant; and (3) the

correlation of the field data with a substantial toxicity data base. Two such case histories concern the presence of polychlorinated biphenyl (PCB) Aroclor 1254 in Pensacola Bay, Florida, and the insecticide Kepone in the James River, Virginia, estuary.

Aroclor 1254

In the case of Aroclor 1254, an industrial plant released a quantity of the chemical into the Escambia River (Duke et al. 1970). Residues of Aroclor 1254 contaminated the biota and sediment of the bay system. Because they are toxic, persistent and bioaccumulative, and because they are present in some aquatic habitats worldwide, Aroclors were added to the U.S. Environmental Protection Agency's (EPA) Consent Decree Chemical list (U.S. EPA 1980a) and provided the data for generating a water quality criterion for PCBs (U.S. EPA 1980b).

Figure 3 illustrates much of the available marine toxicity data base on PCBs. Data for the acute toxicity screening tests encompass the Aroclors 1016, 1248, 1254 and 1260. The acute toxicity data base should be interpreted with caution, however, because the solubility of PCBs is frequently exceeded in these tests. Of the six data points, only one (96-h EC50 as shell deposition in the eastern oyster, Crassostrea virginica, 14 $\mu g\ L^{-1}$) was for Aroclor 1254 (personal communication, J.I. Lowe, U.S. EPA, Environmental Research Laboratory, Gulf Breeze, Florida). Oyster shell deposition studies with other PCBs provided EC50 values that were within a factor of 6 of the Aroclor 1254 value (10.2 $\mu g\ L^{-1}$, Hansen et al. 1974, to 60 $\mu g\ L^{-1}$, personal communication, J.I. Lowe). The 96-h LC50 values for brown shrimp, Penaeus aztecus, and grass shrimp, Palaemonetes pugio, exposed to Aroclor 1016 were also within the range observed with the oysters (10.5 and 12.5 $\mu g\ L^{-1}$, respectively, Hansen et al. 1974).

Several long-term exposures of juvenile or adult marine animals to PCBs were conducted. Hansen et al. (1971) exposed juvenile spot, Leiostomus xanthurus, and pinfish, Lagodon rhomboides, to 5 $\mu g\ L^{-1}$ of the PCB for 14 to 45 days. They reported significant incidence of hemorrhagic lesions and death in both species. Nimmo et al. (1971) exposed pink shrimp, Penaeus duorarum, to Aroclor 1254 for 15 days; they reported 51% mortality in animals exposed to 0.94 $\mu g\ L^{-1}$ of the chemical. A second study by Nimmo and Bahner (1976) reported a comparable value (1.0 $\mu g\ L^{-1}$) as the 15-day LC50 of pink shrimp.

Only two early life stage toxicity tests were conducted on marine animals with Aroclor 1254; both involved the sheepshead minnow, Cyprinodon variegatus. Schimmel et al. (1974) reported a 21-day LC50 of 0.93 $\mu g\ L^{-1}$ for embryos and larvae of the species; MATC was 0.098 $\mu g\ L^{-1}$. Hansen et al. (1973) exposed adult sheepshead minnows to Aroclor 1254 for 4 weeks, injected the females for egg production, and held the subsequent embryos and lar-

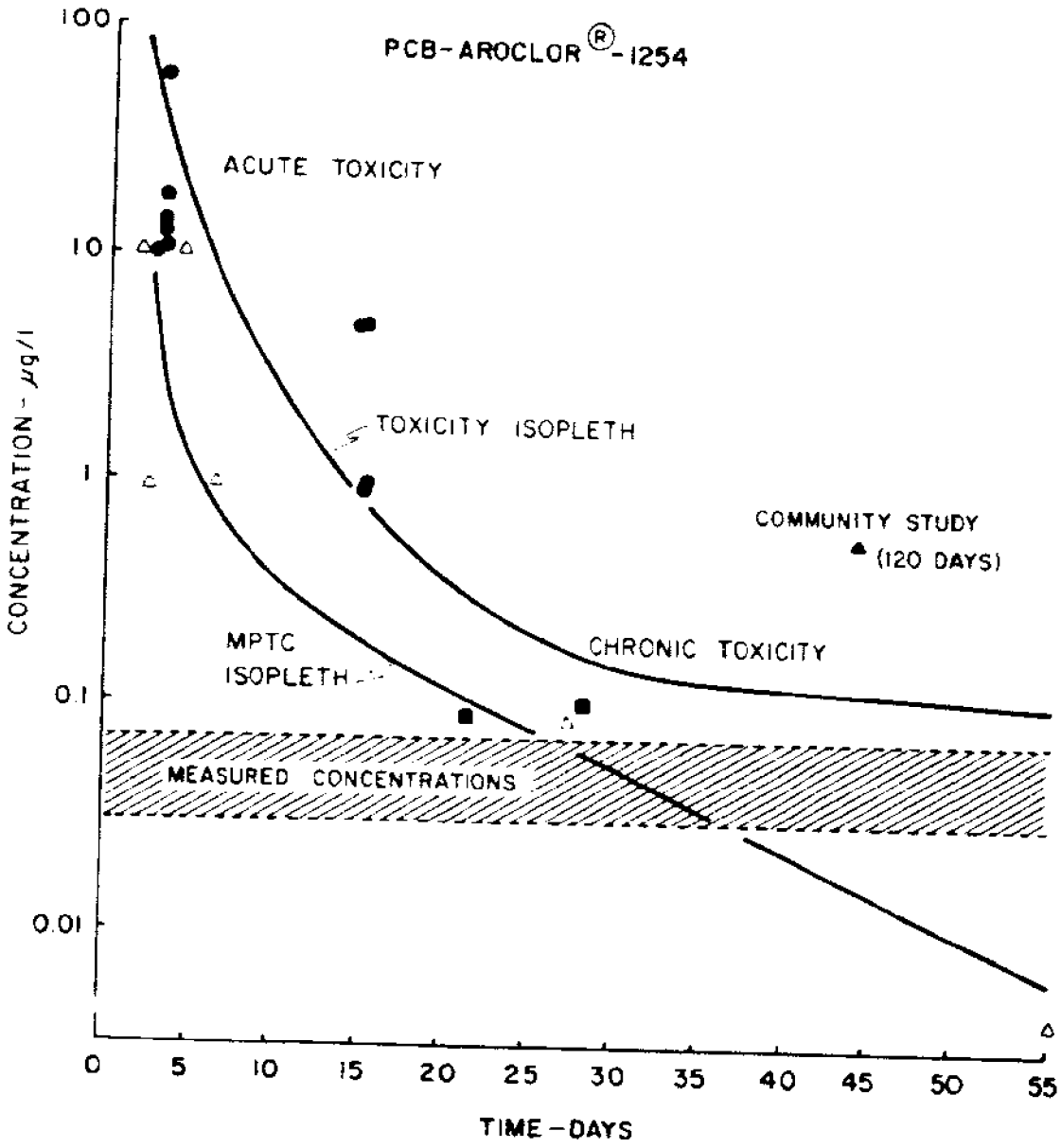

Figure 3. Laboratory-derived toxicity (closed data points) and bioconcentration (open triangles) of the polychlorinated biphenyl Aroclor 1254, compared with measured water concentrations of the chemical in Escambia Bay, Florida. MPTC is the maximum permissible tissue concentration (5 mg kg^{-1}) allowed in fish and shellfish.

vae in PCB-free water for an additional 4 weeks. They reported that survival of the early life stage was significantly reduced when adult fish were exposed in a measured PCB concentration of 0.14 $\mu g\ L^{-1}$; no significant effects were observed in progeny from adults exposed to 0.09 $\mu g\ L^{-1}$. In this instance mortalities were caused by PCB within the eggs in amounts greater than 7 $mg\ kg^{-1}$. This body burden-related effect is seldom observed and would have been overlooked in this test had not the animals been placed in clean seawater.

The last data point on Figure 3 refers to a settling community study by Hansen (1974). In that study, unfiltered seawater received three concentrations of Aroclor 1254 and flowed over clean sand in several aquaria. Planktonic larval stages of estuarine animals in the seawater settled and grew in the aquaria for a 4-month period. Significant differences in community composition were observed in aquaria receiving PCB concentrations $\geq$ 0.6 $\mu g\ L^{-1}$.

As illustrated in Figure 3, the increasing complexity of testing, the longer periods of exposure, and the testing of the more sensitive portions of the life cycle provide lower effect concentrations. It is evident that the acute toxicity tests would not predict the adverse chronic effects such as those observed in fish early life stage toxicity tests. Indeed, the acute toxicity of Aroclor 1254 is more than two orders of magnitude greater than its chronic toxicity (Figure 3). The similarity of the concentrations of PCBs measured in Escambia Bay, Florida (<0.05 to 0.07 $\mu g\ L^{-1}$; Schimmel et al. 1974 and Nimmo et al. 1971) to those producing adverse chronic effects in the laboratory (0.098 $\mu g\ L^{-1}$; Schimmel et al. 1974) was close enough to cause concern.

For those chemicals that have been assigned an FDA action limit, one can also establish an isopleth based on the maximum permissible tissue concentration (MPTC) (U.S. EPA 1980a). An MPTC isopleth for Aroclor 1254 (5.0 $mg\ kg^{-1}$) is illustrated in Figure 3. The amount of any chemical taken up from water into tissues of aquatic animals is dependent upon factors such as the chemical's concentration in water, its octanol/water partition coefficient, the duration of exposure, gill ventilation rates of the exposed animal, etc. Bioconcentration studies with Aroclor 1254 using various estuarine species, exposure concentrations, and durations of exposure indicate that the FDA action level can be exceeded in spot exposed to 1.0 $\mu g\ L^{-1}$ of the PCB in 7 days (Hansen et al. 1971). The longer the exposure, the more PCBs are accumulated until steady state is reached. Female sheepshead minnows exposed to 0.09 $\mu g\ L^{-1}$ for 28 days accumulated PCBs to one-half the action level (Hansen et al. 1973) while oysters, C. virginica, exposed for 56 days to 0.005 $\mu g\ L^{-1}$ (measured concentrations) accumulated to 1.65 $mg\ kg^{-1}$ or 33% of the action level (Parrish et al. 1974). In another study (Lowe et al. 1972), oysters exposed to 10 $\mu g\ L^{-1}$ Aroclor 1254 for 24 weeks concentrated the chemical in their tissues

101,000 X the exposure concentration (20 X the action level). It is obvious from the MPTC isopleth that exposure concentrations measured in Escambia Bay, Florida, could result in residues approaching or exceeding the action level, and in fact they did (Nimmo et al. 1971).

Kepone

Release of the chlorinated insecticide Kepone (chlordecone) into the James River, Virginia, in the 1960s and 70s, and the subsequent contamination of estuarine water, sediment and biota are well documented (Onishi and Ecker 1978; Bender et al. 1977; Huggett et al. 1980). Kepone concentrations in James River oysters varied by season, averaging 0.16 mg kg^{-1} at eight locations; amounts in finfish averaged from 2.7 mg kg^{-1} for white perch, a long-term resident, to 0.81 mg kg^{-1} for spot, a short-term resident (Bender et al. 1977). Kepone concentrations in sediment varied considerably but were related to the proximity from the source of the release (Hopewell, Virginia), and correlated with sediment organic content (Nichols and Trotman 1979). Dissolved Kepone in water samples collected from the estuarine portion of the James River averaged 0.01 $\mu g\ L^{-1}$ (Onishi and Ecker 1978).

A substantial laboratory data base exists on the acute toxicity of Kepone to estuarine animals (Figure 4). The 96-h LC50 for *Mysidopsis bahia* was 10.1 $\mu g\ L^{-1}$ (Nimmo et al. 1977), and that for the blue crab *Callinectes sapidus* was >210 $\mu g\ L^{-1}$ (Schimmel and Wilson 1977). Estuarine finfish 96-h LC50 values for six species ranged from 6.6 $\mu g\ L^{-1}$ for the spot to 69.5 $\mu g\ L^{-1}$ for sheepshead minnows (Butler 1963; Schimmel and Wilson 1977; Roberts and Bendl 1982).

Three studies were conducted involving the sensitive life stages of estuarine animals (Nimmo et al. 1977; Hansen et al. 1977; Goodman et al. 1982). Nimmo et al. (1977) conducted a full life cycle toxicity test with *M. bahia* and reported that the MATC (based on reproductive impairment) was less than 0.39 $\mu g\ L^{-1}$, the lowest concentration tested. In a separate test, juvenile *M. bahia* were exposed to five concentrations of Kepone for 14 days. They reported that growth was affected at 0.075 $\mu g\ L^{-1}$ but not at 0.026 $\mu g\ L^{-1}$, giving an MATC of 0.043 (Figure 4). Hansen et al. (1977) conducted toxicity tests using various life stages of the sheepshead minnow. Results of their studies indicated that the 28-day LC50 for adult fish was 1.3 $\mu g\ L^{-1}$. However, growth of juvenile progeny in that study was significantly reduced by exposure to 0.08 $\mu g\ L^{-1}$ Kepone, the lowest concentration tested. Therefore, the calculated MATC would be less than 0.08 $\mu g\ L^{-1}$ (Figure 4). Goodman et al. (1982) conducted an entire life cycle toxicity test with sheepshead minnow. The most sensitive end point in the study was growth of the progeny at 0.12 $\mu g\ L^{-1}$ Kepone. The calculated MATC was 0.094 (geometric mean of 0.074 and 0.12 $\mu g\ L^{-1}$).

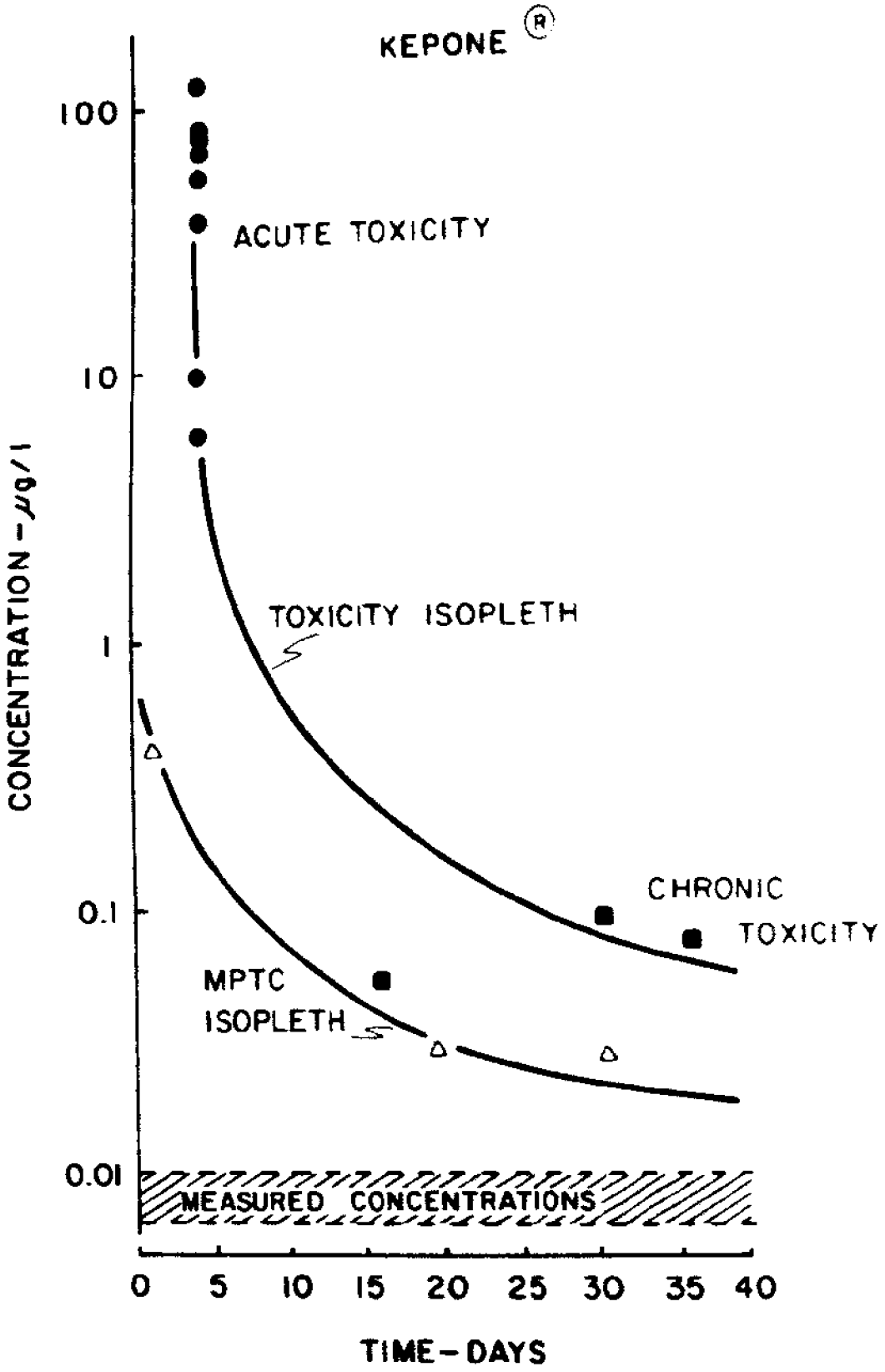

Figure 4. Laboratory-derived toxicity (closed data points) and bioconcentration (open triangles) of the insecticide Kepone, compared with measured water concentrations of the chemical in the James River Estuary, Virginia. MPTC is the maximum permissible tissue concentration (0.3 mg kg^{-1}) allowed in fish and shellfish.

As with the data shown with PCBs, it is evident that by increasing the duration of exposure to Kepone, by using more sensitive life stages and more discriminatory end points, the chronic toxicity is revealed. In doing so, we can better define the ultimate risks to estuarine species. In the case of Kepone, we see that growth for both mysid shrimp and sheepshead minnows was affected at Kepone concentrations between 0.04 and 0.08 $\mu g\ L^{-1}$; the best estimate of Kepone concentrations in the James River is 0.01 $\mu g\ L^{-1}$. The small difference between the two values suggests a relatively high probability of adverse biological effects associated with Kepone in the James River.

As for PCBs, the U.S. Food and Drug Administration (FDA) has assigned an action level (MPTC) for Kepone in fish and shellfish (0.3 $mg\ kg^{-1}$). Laboratory studies and chemical analysis of aquatic life in the James River prove the significant bioaccumulative properties of Kepone. In laboratory studies, sheepshead minnows surviving 96-h exposures bioconcentrated Kepone as much as 1548 times the concentration measured in the exposure water (Schimmel et al. 1977). Given an MPTC of 0.3 $mg\ kg^{-1}$, the maximum acute exposure of Kepone allowed to this fish would be 0.000194 $mg\ L^{-1}$ or 0.194 $\mu g\ L^{-1}$ (0.3 ÷ 1548). Results of longer term bioconcentration studies at or near steady state indicate higher bioconcentration factors (BCF). Bahner et al. (1977) exposed C. virginica to Kepone and reported BCF values of approximately 9300 X in 19 days, while the crustaceans M. bahia and P. pugio, bioconcentrated 13,473 and 11,425 in 21 and 28 days, respectively. The BCF of Kepone in fish at steady state was lower than that shown by invertebrate species (7100 X for C. variegatus after a 30-day exposure; Bahner et al. 1977). Using the highest BCF generated, (13,473 X for mysids) and the 0.3 $mg\ kg^{-1}$ action level, we calculate that a maximum, steady state exposure of 0.02 $\mu g\ L^{-1}$ could produce tissue concentrations exceeding the MPTC. This water concentration is only a factor of 2 greater than that measured in the James River (Onishi and Ecker 1978), and certain species contained Kepone concentrations in excess of the MPTC.

DISCUSSION

Implicit in hazard assessment are several concepts: (1) there is a coupling of the biological effects observed and the environmental exposure expected; (2) the procedure is predictive in its intent, not post facto monitoring; and (3) the monitoring aspect is a feedback procedure to verify or compare the results with the prediction.

The two estuarine case histories are obviously post facto observations of environmental contamination. The purpose of their description here is to illustrate that within the context of a thorough hazard assessment and strict adherence to the recommendations

derived from that assessment, neither episode would probably have occurred. In the case of PCBs, several properties of the chemical should have provided clues to its ultimate hazard. First, the higher chlorinated PCBs, including Aroclor 1254, have very high octanol/water partition coefficients and as such would be expected to concentrate in biological tissues (e.g., lipids). This single test (octanol/water partition determination) is a valuable first level test in identifying chemical hazard through bioaccumulation potential. Second, simple hydrolysis and photolysis studies would indicate negligible degradation through those pathways. Results of Aroclor 1254 laboratory bioconcentration studies indicated a steady state BCF of more than 100,000 X (Lowe et al. 1972). When combined with the FDA action limit of 5.0 mg kg^{-1}, the maximum allowable PCBs in seawater should not exceed 0.05 $\mu g\ L^{-1}$. Results of field-derived BCFs indicate that the field BCF is greater than 670,000 X for spotted seatrout, Cynoscion nebulosus (Nimmo et al. 1971). This difference between the laboratory and field-derived values may be due to inaccurate estimates of PCBs in Escambia Bay waters or possibly an underestimation of the importance of aquatic food chains in the accumulation of PCBs. The inability to differentiate those chemicals that bioconcentrate from those that bioconcentrate and accumulate substantially within food chains (or the lack of appreciation of some chemical to do so) may be the weakest aspect of hazard assessment, and deserve more thorough evaluations.

On the effects side of hazard assessment, PCBs have moderately high acute toxicity ranging between 10 and 100 $\mu g\ L^{-1}$. More important are the effects on biota in tests of longer duration, specifically, the effects on energetics, growth and reproduction. We can see from Figure 3 that the lowest value producing adverse biological effects (0.098 $\mu g\ L^{-1}$ in a C. variegatus early life stage test; Schimmel et al. 1979) was close to the range of environmental water concentrations in Escambia Bay, and that there probably existed no margin of safety. Indeed, on the basis of their known insensitivity to other pollutants it would be naive to assume that the sheepshead minnow would be the most sensitive of all the species resident in the bay. Had PCBs been subjected to the rigors of a hazard assessment process, responsible decision-makers would have concluded that PCBs should not be allowed to approach environmental concentrations that would be expected to produce tissue concentrations exceeding the MATC (0.05 $\mu g\ L^{-1}$) or adversely affecting fishes (0.098 $\mu g\ L^{-1}$). The amount of safety margin (risk) they would apply to PCBs before manufacture would depend upon these data and the myriad of social and economic issues relating to the specific chemical under evaluation, and the resources at risk.

The Kepone case history contains many of the same clues that should allow the hazard assessment process to identify the potential threats of this insecticide to estuarine systems, and in fact

that is why this chemical was not registered for use in this country. Careful exposure assessment through laboratory studies would identify several characteristics of Kepone including (1) Kepone's extreme refractory nature (Bourquin et al. 1978; Garnas et al. 1977); (2) its strong affinity for sediments, particularly the organic constituents (Nichols and Trotman 1978; Huggett and Bender 1980; Garnas et al. 1978); (3) low volatility (Dawson et al. 1978); and (4) high bioaccumulative capacity as determined by the log of the octanol/water partition coefficient, 5.7 (personal communication, R.L. Garnas, U.S. EPA National Enforcement Investigations Center, Denver, Colorado) and laboratory BCF studies (Bahner et al. 1977; Schimmel and Wilson 1977; Hansen et al. 1977). Results of these data predict that Kepone's release into an estuarine system would contaminate water, sediment and biota of all trophic levels. Degradation of the material would be negligible as would loss due to volatilization. Therefore, loss of the chemical from the aquatic system could be extremely slow, possibly taking decades.

Like the exposure assessment, the effects assessment scheme should predict the hazard of Kepone in estuarine environments. Kepone was toxic to a wide variety of organisms including plants, crustaceans, molluscs, and finfish (Walsh 1977; Schimmel and Wilson 1977; Butler 1963; Hansen et al. 1977; Nimmo et al. 1977; Roberts and Bendl 1980). The ratio of the acute effect concentrations to the chronic effect concentrations ranged from 235 X (Nimmo et al. 1977) to greater than 862 X (Hansen et al. 1977). The necessity for conducting chronic tests in hazard assessment using environmental fate triggers is obvious.

One unexpected result of the exposure assessment procedure was the accumulation of Kepone in James River blue crabs. Schimmel and Wilson (1977) exposed blue crabs to Kepone in acute tests and reported that the BCF in that period was approximately 8 compared with a high of 1,548 for sheepshead minnows. However, male blue crabs collected from the James River contained an average of 0.81 mg kg^{-1}; females contained an average of 0.19 mg kg^{-1} (Bender et al. 1977). One hypothesis for explaining the difference between low laboratory residues and significant Kepone residues in field-collected blue crabs was that the crab's food may be a major source of Kepone. Schimmel et al. (1979) tested this hypothesis and reported that indeed food (oysters) was the major contributing factor for Kepone residues. In addition, crabs that accumulated approximately 0.07 mg kg^{-1} Kepone suffered significant mortality and molted less frequently than those fed Kepone-free oysters. Others attempted to reproduce these effects, but were unsuccessful, and their studies were not reported in the peer-reviewed literature. The adverse effects due to body burden and the contribution of food to that body burden were not expected and most likely would be overlooked in the hazard assessment protocol.

It is clear in the case histories illustrated here that the hazard assessment scheme identifies many of the properties that are potentially deleterious to the aquatic environment. One hazard that was not identified in either case history is that related to probable importance of food chain transfer and resulting body burdens. It is likely that only a few chemicals (like PCBs and Kepone) would produce such a hazard. However, we must learn from post facto exposures such as those illustrated here and should develop definitive predictive tests, or adapt existing tests to identify hazards that may not be predicted.

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REFERENCES

Bahner, L.H., A.J. Wilson, Jr., J.M. Sheppard, L.R. Goodman, G.E. Walsh and J.J. Patrick, Jr. 1977. Kepone bioconcentration, accumulation loss, and transfer through estuarine food chains. Chesapeake Sci. 18: 299-308.

Bender, J.E., R.J. Huggett and W.J. Hargis. 1977. Kepone in Chesapeake Bay biota. Proceedings of the Kepone II Seminar, Easton, Md., Sept. 19-21, pp. 14-37. U.S. EPA, Region III, Philadelphia, Pa.

Bourquin, A.W., P.H. Pritchard and W.R. Mahaffey. 1978 Effects of Kepone on estuarine microorganisms. Dev. Indus. Micro. 19: 489-497.

Butler, P.A. 1963. Pesticide-wildlife studies--A review of fish and wildlife service investigations during 1961 and 1962. U.S. Fish Wildl. Circ. 167: 11-25.

Cairns, J., Jr., K.L. Dickson and A.W. Maki. 1978. Estimating the Hazard of Chemical Substances to Aquatic Life. ASTM STP 657, American Society for Testing and Materials, Philadelphia, Pa.

Dawson, G.W., S.J. Shupe and W.C. Weimer. 1978. Properties of Kepone. In: The Feasibility of Mitigating Kepone Contamination in the James River Basin, Contract EY-76-C-06-1830. U.S. Dept. of Energy.

Duke, T.W., J.I. Lowe and A.J. Wilson, Jr. 1970. A polychlorinated biphenyl (Aroclor 1254) in the water, sediment and biota of Escambia Bay, Florida. Bull. Environ. Contam. Toxicol. 5: 171-180.

Erickson, R.J. and C.E. Stephan. 1982. Calculation of the final acute value for water quality criteria for aquatic life. U.S. EPA, Environmental research Laboratory, Duluth, Minn. (Unpublished manuscript).

FWPCA (Federal Water Pollution Control Administration). 1968. Water quality criteria: Report of the National Technical Advisory Committee to the Secretary of the Interior. U.S. Dept. of Interior, FWPCA.

Garnas, R.L., A.W. Bourquin and P.H. Pritchard. 1977. The fate and degradation of 14C-Kepone in estuarine microcosms. Contrib. No. 351, U.S. EPA, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Fla.

Garnas, R.L., A.W. Bourquin and P.H. Pritchard. 1978. The fate of 14C-Kepone in estuarine microcosms. Abstract, American Chemical Society National Meeting, March 1978, Anaheim, Calif.

Geckler, J.R., W.B. Horning, T.M. Neiheisel, Q.H. Pickering, E.R. Robinson and C.E. Stephan. 1976. Ecological Research Series, EPA 600/3-76-116, U.S. Environmental Protection Agency.

Goodman, L.R., D.J. Hansen, C.S. Manning and L.F. Fass. 1982. Effects of Kepone on the sheepshead minnow in an entire life-cycle toxicity test. Arch. Environ. Contam. Toxicol. 11: 335-342.

Hansen, D.J. 1974. Aroclor 1254: Effect on composition of developing estuarine animal communities in the laboratory. Contrib. Mar. Sci. 18: 19-33.

Hansen, D.J. 1983. Utility of toxicity tests to measure effects of substances on marine organisms. (This volume.)

Hansen, D.J., P.R. Parrish, J.I. Lowe, A.J. Wilson Jr. and P.D. Wilson. 1971. Chronic toxicity, uptake, and retention of aroclor 1254 in two estuarine fishes. Bull Environ. Contam. Toxicol. 6: 113-119.

Hansen, D.J., S.C Schimmel and J. Forester. 1973. Aroclor 1254 in eggs of sheepshead minnows: Effect on fertilization success and survival of embryos and fry. Proc., 27th Annual Conference of South East Association of Game and Fish Commission, pp. 420-426.

Hansen, D.J., P.R. Parrish and J. Forester. 1974. Aroclor 1016: Toxicity to and uptake by estuarine animals. Environ. Res. 7: 363-373.

Hansen, D.J., L.R. Goodman and A.J. Wilson, Jr. 1977. Kepone: Chronic effects on embryo, fry, juvenile, and adult sheepshead minnows (Cyprinodon variegatus). Chesapeake Sci. 18: 227-232.

Hansen, S.R. and R.R. Garton. 1982. Ability of standard toxicity tests to predict the effects of the insecticide diflubenzuron on laboratory stream communities. Can. J. Fish. Aquat. Sci. 39: 1273-1288.

Huggett, R.J. and M.E. Bender. 1980. Kepone in the James River. Environ. Sci. Tech. 14: 918-923.

Huggett, R.J., M.M. Nichols and M. Bender. 1980. Kepone contamination of the James River estuary. In: R.A. Baker (ed.), Contaminants and Sediments, Vol. 1, pp. 33-52. Ann Arbor Science Publishers, Inc., Ann Arbor, Mich.

Kimerle, R.A., W.E. Gledhill and G.J. Levinskas. 1978. Environmental safety assessment of new materials. In: J. Cairns, Jr., K.L. Dickson and A.W. Maki (eds.), Estimating the hazard of chemical substances to aquatic life, ASTM STP 657, American Society for Testing and Meterials, Philadelphia, Pa.

Lowe, J.I., P.R. Parrish, J.M. Patrick, Jr. and J. Forester. 1972. Effects of the polychlorinated biphenyl Aroclor 1254 on the American oyster Crassostrea virginica. Mar Biol. 17: 209-214.

NRDC (Natural Resources Defense Council) et al., v. Train, 8E. R.C. 2120. 1976.

Nichols, M. and R. Trotman. 1978. The role of sediments in the storage, movement and biological uptake of Kepone in estuarine environments: Bed sediments and suspended sediments. In: Proc. Kepone Seminar II. EPA - 903/9-78-011. U.S. Environmental Protection Agency, Region III, Philadelphia, Pa. pp. 363-456.

Nimmo, D.R. and L.H. Bahner. 1976. Metals, pesticides and PCB: Toxicities to shrimp singly and in combination. In: Estuarine Processes. I. Uses, Stresses and Adaptation to the Estuary, pp. 523-531. Academic Press, New York.

Nimmo, D.R., R.R. Blackman, A.J. Wilson, Jr. and J. Forester. 1971. Toxicity and distribution of Aroclor 1254 in the pink shrimp Penaeus duorarum. Mar. Biol. 3: 191-197.

Nimmo, D.R., L.H. Bahner, RA. Rigby, J.M. Sheppard and A.J. Wilson, Jr. 1977. Mysidopsis bahia: An estuarine species suitable for life-cycle toxicity tests to determine the effects of a pollutant. In: F.L. Mayer and J.L. Hamelink (eds.), Aquatic Toxicology and Hazard Evaluation, pp. 109-116. ASTM STP 634, American Society for Testing and Materials, Philadelphia, Pa.

Onishi, Y. and R.M. Ecker. 1978. The movement of Kepone in the James River. In: The Feasibility of Mitigating Kepone Contamination in the James River Basin, Chapter VII. Contract EY-76-C-06-1830, U.S. Dept. of Energy.

Parrish, P.R. 1974. Aroclor 1254, DDT and DDD, and Dieldrin: Accumulation and loss by American oysters (Crassostrea virginica). Proc. Nat. Shellfish Assoc. pp. 64:7.

Roberts, M.H. and R. Bendl. 1982. Acute toxicity of Kepone to selected freshwater fishes. Estuaries.

Schimmel, S.C. and A.J. Wilson, Jr. 1977. Acute toxicity of Kepone to four estuarine animals. Chesapeake Sci. 18: 224-227.

Schimmel, S.C., D.J. Hansen and J. Forester. 1974. Effects of Aroclor 1254 on laboratory-reared embryos and fry of sheepshead minnows (Cyprinodon variegatus). Trans. Am. Fish. Soc. 103: 582-586.

Schimmel, S.C., J.M. Patrick, Jr., L.F. Faas, J.L. Oglesby and A.J. Wilson, Jr. 1979. Kepone: Toxicity and bioaccumulation in blue crabs. Estuaries. 2: 9-15.

U.S. EPA (Environmental Protection Agency). 1972. Water quality criteria 1972. A Report of the Committee on Water Quality, Environmental Studies Board, National Academy of Sciences, National Academy of Engineering, Washington, D.C.

U.S. EPA (Environmental Protection Agency). 1976. Water quality criteria for aquatic life. U.S. Environmental Protection Agency.

U.S. EPA (Environmental Protection Agency). 1980a. Water quality criteria documents: Availability. Fed. Regist. 45: 231 (Friday, Nov. 28, 1980).

U.S. EPA (Environmental Protection Agency). 1980b. Polychlorinated biphenyl criteria document. EPA-440/5-80-068, U.S. EPA.

Walsh, G.E. 1977. Toxicity and uptake of Kepone in marine unicellular algae. Ches. Sci. 18: 222-223.

Verifying Predictions of Environmental Safety and Harm

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INTRODUCTION

Regulations based on toxicity tests and other estimates of hazard should be scientifically justifiable. Determining scientific validity should include validation or verification of these predictions either in "the real world" or in a demonstrably suitable surrogate of the real world. Such predictions rarely have been verified, presumably because discharges or uses of chemicals based on these predictions were not known to have adverse environmental consequences. However, in absence of an organized study of the environment receiving the chemicals, the so-called verification is really based on an absence of evidence (that is, no fish kills were seen, etc.) rather than on a body of evidence gathered to test the hypothesis that a particular concentration of a chemical would not prove harmful. Several important questions that should be answered follow.

1. Are single species laboratory tests accurate predictors of the response of the particular species in natural systems?
2. Can single species tests be used to predict responses at other levels of biological organization, such as populations, communities, and ecosystems?
3. To what degree is environmental realism, including variability and complexity, important in establishing accurate estimates of environmental hazard?
4. Can complex systems be used for toxicity tests in such a way that replication is possible?

5. To what degree can one extrapolate results from one community or complex ecosystem to another?
6. How much information redundancy exists at levels of biological organization above single species?

Few substantive data exist that bear on these extremely important questions, and this paper could not possibly address all of them in detail. This paper describes a series of field and laboratory studies designed to validate predictions of environmental response beginning with the single species level and progressing to higher levels of biological organization.

Renewed interest has surfaced recently in the well-recognized, but regularly ignored, distinction between statistical significance and biological significance of toxicity testing or hazard evaluation results (e.g., Stephan 1982). Of course, both are essential to the process of hazard evaluation. Statistical significance must be determined if the soundness of the science is to be explicitly stated, and biological significance must be determined in order to evaluate suitability of information generated for a specific purpose. Information content and quality are dependent not only on method of data acquisition and analysis, but also on how effectively information is used in decision making and, of course, how suitable it is for the intended purpose.

Biological assessment (including toxicity testing) of potentially harmful chemicals is for determining conditions of exposure that are biologically harmful. However, determination of harm to the biota includes alteration of biological processes, including, but not limited to, mortality, reproductive success, growth, behavior, competitive interactions, predator-prey relationships, energy cycling, nutrient transfer, alteration in autotrophic/heterotrophic ratios, diversity and species richness, rate of colonization and succession and a variety of other characteristics for different levels of biological organization from subcell to ecosystem. Biological assessments are rarely explicitly stated in these terms, but the general public almost certainly has the impression that they are designed to protect living organisms at all levels of biological organization such that both normal structure and function are maintained. This paper examines that general impression with site-specific evidence. Ours is by no means a new viewpoint (e.g., see Wilson et al. 1974; Doudoroff 1976; Cowell 1978; Heath 1980). Doudoroff (1976) stated the problem:

> ...a pickle jar by any other name, or having any other shape or purpose intended by its manufacturer, and with or without constant-flow renewal of its contents, is still essentially a pickle jar. When narrowly confined within its walls, the fish is unlikely to entertain the illusion that this new, in vitro or plastic-lined environment of his is the real world, and to behave accordingly....

> Few...investigators have demonstrated much awareness of, or interest in, the essential differences between the experimental conditions created by them in the laboratory and the natural conditions of existence of their test subjects....They may never have even wondered whether or not there is any correlation between the intensity of a measured response of their test organisms to sublethal levels of different toxicants and the ability of these different toxicants to interfere with the survival, growth, and reproduction of the organisms in their natural environments.*

Reflection on present capabilities for evaluating hazard of chemicals to aquatic life brings to mind the story about an inebriated gentleman frantically searching around the base of a street light. "What are you doing?" asked a policeman. "Searching for a key I lost up the street in the dark," was the reply. "Why are you searching here?" asked the policeman. "Because the light is better," responded the inebriated gentleman. Is the situation in hazard evaluation much different? Pickle jar ecology is almost certainly used even with a full awareness that Doudoroff was right! Pickle jar tests with single species do shed some light on the problem, but the key to scientifically sound hazard evaluations probably lies, at least partly, up the street in the dark area of complex system response to stress. No guilt should be associated with use of single species toxicity tests, not because of their extensive use, but because single species testing was a rational way to begin when biological tests were poorly accepted in pollution assessment. Concern should be apparent, however, because few attempts have been made to validate or verify in any scientifically justifiable way the predictions of harm and safety made, presumably for the real world, from single species test data. Perhaps going beyond single species testing is not needed, but the decision should be based on scientifically sound evidence.

The single species toxicity test has also provided a degree of protection far beyond that existing before these tests were frequently used. However, even scientists most knowledgeable about single species toxicity tests (e.g., Mount 1979) acknowledge that more complex multispecies systems may respond differently than single species. The present situation in estimating the hazard of chemical and other anthropogenic stresses upon aquatic ecosystems is clearly in a state of marked transition because of two factors.

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*The Doudoroff quotations appear in Biological Monitoring of Water and Effluent Quality and are reproduced through the kind permission of Robert Meltzer, American Society for Testing and Materials, Philadelphia, Pa.

First, technology based standards (Best Available Technology (BAT) and Best Practical Technology (BPT)) still appear to be scientifically unjustifiable (Cairns 1982; Loehr 1981). Second, no compelling evidence exists that natural systems are or are not protected by these standards. On a case-by-case basis, protection depends on size of the receiving system, type of material being treated, and a number of other factors. Unquestionably, both BAT and BPT afford some measure of protection to the environment, but, in the absence of biological information regarding their impact, precision in estimating hazard is not possible. Mount (1979) identified the difficulties of going beyond present methods quite clearly: (1) failure of the public to grasp complex issues of ecosystem stress and response, and (2) difficulty in separating normal community variability from stress responses. Odum et al. (1979) discuss the problem of ecological enhancement caused by waste discharges that may be ecological subsidies, which simultaneously might impair other desirable ecological qualities. Elsewhere, Odum (1981) calls attention to the reemergence of nonlaboratory science just described.

Predictions of hazard required by Toxic Substances Control Act (TSCA) cannot be made with any degree of precision. Many industrial managers are conducting single species tests required by law, but the question is whether tests conducted at lower levels of biological organization are sufficient for a scientifically defensible hazard evaluation. Many managers are not convinced that the risks involved by not regularly generating information at levels of organization above single species are sufficient to justify the additional expense of providing this information. Wrenn and Forsythe (1978) demonstrated that laboratory data overestimated thermal effects in experimental ecosystems that presumably are more environmentally realistic than laboratory tests. Geckler et al. (1976) showed that copper produced an effect in a natural stream at a concentration approximately 50% of that predicted to have no effect, on the basis of laboratory data. However, the effect detected in the field was behavioral avoidance that unfortunately had not been measured in laboratory tests.

A large number of professionals who depend almost entirely on single species toxicity tests for hazard evaluation agree that a more ecological approach is preferred but feel it is limited by our primitive understanding of ecosystem function and the processes that control community structure. We agree that our general understanding of these factors is not adequate for immediate large scale implementation of multispecies and system-level toxicity testing. However, the parallels to single species toxicity testing in the mid-1940s are striking. When Hart et al. (1945) proposed one of the first standard methods for acute toxicity testing of fish, our understanding of such tests was equally primitive. Progress in the last 35 years is partly a consequence of calling attention to the

need for such tests, which was then not widely recognized or accepted. Even today our ability to predict the response of one species from the response of another is severely limited, and our understanding of the mechanisms that produce the toxic response is nearly as primitive as our understanding of ecological processes. This paper does not resolve the problem but merely calls attention to it and offers some suggestions for exploratory approaches that show some promise. It is important to emphasize that we do not believe that present understanding of ecological systems permits routine use of complex tests. There is no question that this approach is still in the developmental stage, but the rate of development will be minuscule if more emphasis is not placed on the need for such tests.

The cost of multispecies, community and ecosystem level testing should not inhibit acquisition of the type of data needed to determine the hazard of chemicals to ecosystems. When the kind of evidence needed to make a scientifically sound decision is known, then a cost/benefit analysis can be carried out. It is worth remembering, however, that not all tests at levels of biological organization higher than the single species are expensive. Some studies included in this paper demonstrate that they are not expensive. Professional ecologists, unfortunately, have not firmly established a single standard method based on multispecies, community or ecosystem analyses. Until ecologists agree on parameters to be measured and methods for measuring, the amount of testing needed to determine the relationship between single species testing and testing at higher levels of biological organization will not be adequate. Since a single paper cannot address all problems and possible solutions in detail, this paper describes some field and laboratory studies designed to validate predictions of environmental response starting with the single species level and progressing to higher levels of biological organization (i.e., community and ecosystems).

CASE STUDIES

Many types of toxicological studies have been conducted and can be divided basically into single species tests, multiple species tests, and natural community tests. Multiple species tests can be subdivided further into (a) tests conducted independently on an array of single species (e.g., hazard evaluation protocol), (b) an artificial collection of species presumed to interact with each other in a meaningful way or (c) a community acquired from a natural system. The remainder of this paper addresses selected research within these areas.

Single Species Studies

Single species studies are of limited value in predicting ecosystem effects of toxicants (Baker 1976; Buikema et al. 1982; Cairns 1980, 1981; Wilson et al. 1974). Properly conducted single species tests may be useful in understanding short-term responses of populations exposed to toxic stress (e.g., mortality) but rarely are they useful in predicting effects due to long-term exposure (e.g., impairment of growth or reproduction). Acute toxicity tests may be most useful in ecological investigations after an episodic spill. Before toxicity tests can be used to assess ecological effects, four critical properties of toxicity tests must be addressed (Baker and Crapp 1974): (1) each test must be designed for making predictions about a particular kind of community or habitat, and should be based upon field studies in that community or habitat; (2) the test must be capable of predicting mortalities among the key species of the community; (3) the effects upon mortalities of such factors as seasonal and behavioral variations must be appreciated; (4) the ecological consequences of these mortalities must be understood. In this way, single species tests can be used post facto to understand in a general way the observed responses of field populations to perturbation (Baker and Crapp 1974; Wilson et al. 1974).

Behavioral studies in the laboratory can be used to predict field effects (e.g., Cairns et al. 1981; Cherry and Cairns 1982), and such studies have been useful in obtaining temperature variances from Section 316 of Public Law 92-500 and in the analysis of mixing zones and safe passage of aquatic organisms (e.g., Cherry et al. 1975, 1977, 1979; Cairns et al. 1981). The Glen Lyn power plant is a fossil-fueled plant located in southwestern Virginia and was selected as a study site for comparing laboratory and field data on preference-avoidance responses to temperature and chlorine (Cairns et al. 1981). Research included laboratory thermal preference studies using a temperature gradient, and temperature and chlorine avoidance studies using a two-chambered device, in which two streams of water at different temperatures enter at opposite ends of the chamber and exit at a center drain (Cherry et al. 1975). Field studies included sampling of fish within and outside a thermal plume and sampling of fish within a thermal plume in the presence and absence of chlorine.

Temperature preference and avoidance data for four species of fish (Campostoma anomalum (stoneroller), Ictaturus punctatus (channel catfish), Notropis rubellus (rosyface shiner), and Pimephales notatus (fathead minnow)) were studied. Differences between preferred temperatures in the field and laboratory were less than 5°C; for the channel catfish, the difference was less than 1°C. For fish acclimated to 30°C, the highest temperatures avoided in the laboratory ranged from 33°C to 35°C. In the field, the highest temperatures at which the fish were collected ranged from 34.4°C to 35°C. Close similarities observed in laboratory and field

studies indicate that fish can select optimal temperatures and avoid potentially lethal or damaging high temperatures. More important, these laboratory studies accurately predicted what would happen under natural conditions.

Data for avoidance to chlorine were obtained in the laboratory and field for two species of fish. Between 24°C and 30°C, field populations of the spotfin shiner (Notropis siplopterus) and whitetail shiner (N. galacturus) avoided a total residual chlorine concentration (TRC) of 0.18 mg L^{-1}. In the laboratory, they avoided a TRC of 0.11 to 0.22 mg L^{-1}. The total fish community overall avoided TRCs $\geq$0.15 mg L^{-1}. When these data were compared with individual data for 12 species studied in the laboratory, the TRC that was avoided by most laboratory populations was within the 95% confidence limits for summer populations of fish in the river system.

The correlation between single species laboratory and field data was quite good, indicating that if laboratory and field studies are properly carried out laboratory studies can be useful in predicting responses of single species in the field. Note that these data were measurements of behavioral responses, and no attempt was made to predict lethal or sublethal effects. The proximity of the laboratory to the field site that permitted use of natural river water directly from the river and reduced transport time for fish used in the laboratory was probably a major factor in producing a good correlation.

Microcosm Studies

Many types of microcosms have been proposed and used in attempts to measure and predict effects of contaminants to ecosystems. Basically, the two types of microcosms are model food chains and model ecosystems. Model food chains are simpler and easy to analyze in terms of population dynamics, competition, predator-prey interactions, trophic dynamics and so forth, but they are not ecologically realistic (Giddings 1980). Model ecosystems have been used to study such concepts as diversity, stability, nutrient cycling and energy flow under controlled conditions. Microcosm, especially model ecosystem, studies have used artificial and simplistic assemblages of presumably interactive populations (e.g., Metcalf et al. 1971), complex associates that are partially defined (e.g., Marshall and Mellinger 1980; Harte et al. 1980) and gnotobiotic microcosms (e.g., Taub and Crow 1978, 1980). In addition, microcosms have been allowed to reach their own equilibrium (e.g., Taub and Crow 1978), or species have been periodically introduced to simulate natural replacement of species (e.g., Taub and Crow 1978, 1980). Despite attempts to simulate natural systems so that effects of perturbation can be examined, microcosm studies have had many problems (e.g., Giesy 1980; Cooke 1977; Cairns 1981).

Marshall and Mellinger (1980) used small volume (8 and 25 L) in situ enclosures of natural freshwater plankton communities to determine effects of metal stress on community composition. The data were also compared with those for large in situ enclosures (1.5×10^5 L) in another lake of similar physical and chemical features. In the first experiments, small volume carboys were filled with ambient water containing natural populations of plankton inoculated with different concentrations of cadmium and incubated for varying lengths of time. The effect of cadmium addition was evaluated by comparing zooplankton abundance, community composition after three weeks as measured by two similarity indices, and changes in dissolved oxygen concentration. Zooplankton abundance was not significantly affected by cadmium concentrations below 1.6 $\mu g L^{-1}$, but reductions in dissolved oxygen and similarity indices among zooplankton communities occurred at concentrations ≥ 0.2 $\mu g L^{-1}$ cadmium. From these studies, the authors predicted the concentration of cadmium that would affect community composition in large volume enclosures. After three weeks of exposure, effects of cadmium on the percent similarity index in the large volume enclosures were within the 95% confidence limits predicted from the small volume enclosures. Whether this type of study can be used to predict an ecosystem effect remains to be seen because only water column effects were studied.

Microcosm studies as now conducted are most useful for screening contaminants for possible effects at levels of organization above the population, but their usefulness in predicting ecosystem effects is limited (King 1980; Heath 1979, 1980). The reason is that microcosms, as constructed, are not structurally and functionally similar to ecosystems; that is, they are not ecologically realistic (Blanck and Gustafsson 1978; Heath 1980). Further, community or ecosystem response cannot be characterized by measuring the response of a few component parts (Heath et al. 1969). Microcosms are not miniature ecosystems but fragments of an ecosystem designed to simulate selected characteristics. Microcosms are either dissimilar, analogous, or, at best, homomorphic, that is, presumed to be complex but lacking a one-to-one correspondence with reality (Heath 1980). Nature may be simulated by adding trophic levels (e.g., producers, consumers and decomposers) to an aquarium, but the behavioral interaction may not be similar to natural interactions. For a microcosm to have predictive capability, it must be isomorphic to natural ecosystems. That is, the two systems must exhibit identical dynamic behavioral responses to external stimuli or perturbation (Ashby 1956); how this behavior occurs is of secondary concern. Further, an identical response can occur even if the two systems are not identical in composition; that is, similar changes in community structure or function may occur even though community composition is not exactly the same. For example, perturbation may cause a decrease in photosynthetic activity in both pond (phytoplankton) and stream (periphyton) algal communities.

Perhaps the frustration at not being able to predict ecosystem effects has caused ecologists to ignore the concept of redundancy within biotic communities. In the transition from lower to higher levels of biological organization, some attributes become more complex and variable, while others become less complex and variable (Odum 1971). Functional measures (e.g., photosynthetic rate) of a community may be less variable than the rates of some of the component parts. Perhaps selection of key functional parameters, such as nutrient spiraling, might produce good evaluations of hazard in systems of modest complexity. If these parameters had high information redundancy with other critical parameters, the task would be further simplified.

Redundancy of components in natural, undisturbed communities has been well established (e.g., Kaesler et al. 1974) and mild perturbation may cause changes in component parts, among them deletions, additions and numerical changes, without a noticeable change in such functions as photosynthesis and fish production. Redundancy also exists in another dimension—the microbial "community" has many structural and functional similarities to the protozoan "community," benthic "community," planktonic "community," etc. All these "communities" have trophic structure, energy flow, etc. Each subset of the biotic community may respond behaviorally in a similar manner to external stimuli. If the idea of redundancy is accepted, then one "community" can be used to predict responses of other "communities" and, as a result, the response of one "community" can be useful in predicting responses of the entire biotic community.

Structure and Function Studies

Structure and function are both important in aquatic ecosystems, and biomonitoring studies should include a combined structure-function strategy (Cairns 1977; Matthews et al. 1982). The primary reason for this combined strategy is that structural or functional changes may occur without a concomitant change in the other, or, because of intimate links, one may change because the other changes. In the first instance, low levels of perturbation may cause changes in community composition or structure (i.e., loss of one or two species) without a change in function, assuming that the surviving members of the community can carry out similar functions (e.g., energy transfer). Alternatively, a rate process (function such as respiration or photosynthesis) may be reduced under sublethal stress but the community structure is left intact.

Prediction of functional changes from structural changes, or vice versa, is based on the assumption that a change in species numbers results in a concurrent change in functional complexity, for example, decrease in number of energy pathways (Cairns 1974). Some measurements of structure are intimately related to function (Matthews et al. 1982). For example, ATP can be used to estimate

viable biomass (e.g., Holm-Hansen 1970) and can also be correlated with changes in metabolism (e.g., Spencer and Ramsay 1978). Other examples of structure-function relationships include chlorophyll concentrations that can be compared with photosynthetic rates of communities, and nucleotide concentrations that can be related to microbial growth rates. The following compares the responses of different "communities" or subsets of the biotic community to perturbation, using structural, functional and structural-functional measures.

COMMUNITY RESPONSES TO STRESS

The research described here used measures of community structure and function in response to perturbation. Measures used were the ratio of ATP estimated biomass/chlorophyll a estimated biomass (=heterotrophic index = HI) (Matthews et al. 1980, 1982), macrobenthic invertebrate diversity and changes in proportion of functional groups (Matthews et al. 1980, 1982; Kontradieff et al., unpublished), and protozoan colonization rates (Cairns et al. 1980; Buikema et al. 1983).

The HI (previously called an autotrophic index) and protozoan colonization rates were measured from analyses of organisms that colonize a 6 x 5 x 5 cm polyurethane foam unit (PFU) substrate. Organisms that colonize these PFUs include representative diatoms, protozoans (both chlorophyllous and achlorophyllous forms), rotifers, bacteria and fungi. Analyses of the protozoan community inhabiting the sponge indicate that the PFU is colonized by the same interactive genera and species found in the interstices of algal and hyphal mats (Picken 1931; personal observation), and 53% of diatom species found in the sediments are found on the PFUs (Matthews 1981). For certain studies, the HI was measured simultaneously with protozoan colonization rates; in others, the HI was measured at the time macroinvertebrate communities were sampled. Macroinvertebrates were sampled using Surber and portable box samplers (Matthews et al. 1980). In other experiments, only invasion rates were compared under different conditions. In a separate set of experiments, simultaneous studies measuring colonization rates and HIs were conducted in the laboratory and field using natural waters containing dilutions of sewage treatment plant and electroplating plant effluent.

Field Experiments

Data were collected from Cedar Run, a small stream near Blacksburg, Va. that receives shopping center and apartment complex parking lot runoff, chlorinated sewage effluent and electroplating industrial effluent. Sampling stations (Figure 1) were selected near riffle zones. On Cedar Run, Station 1 was an upstream reference station, and Stations 2 through 10 were stressed stations

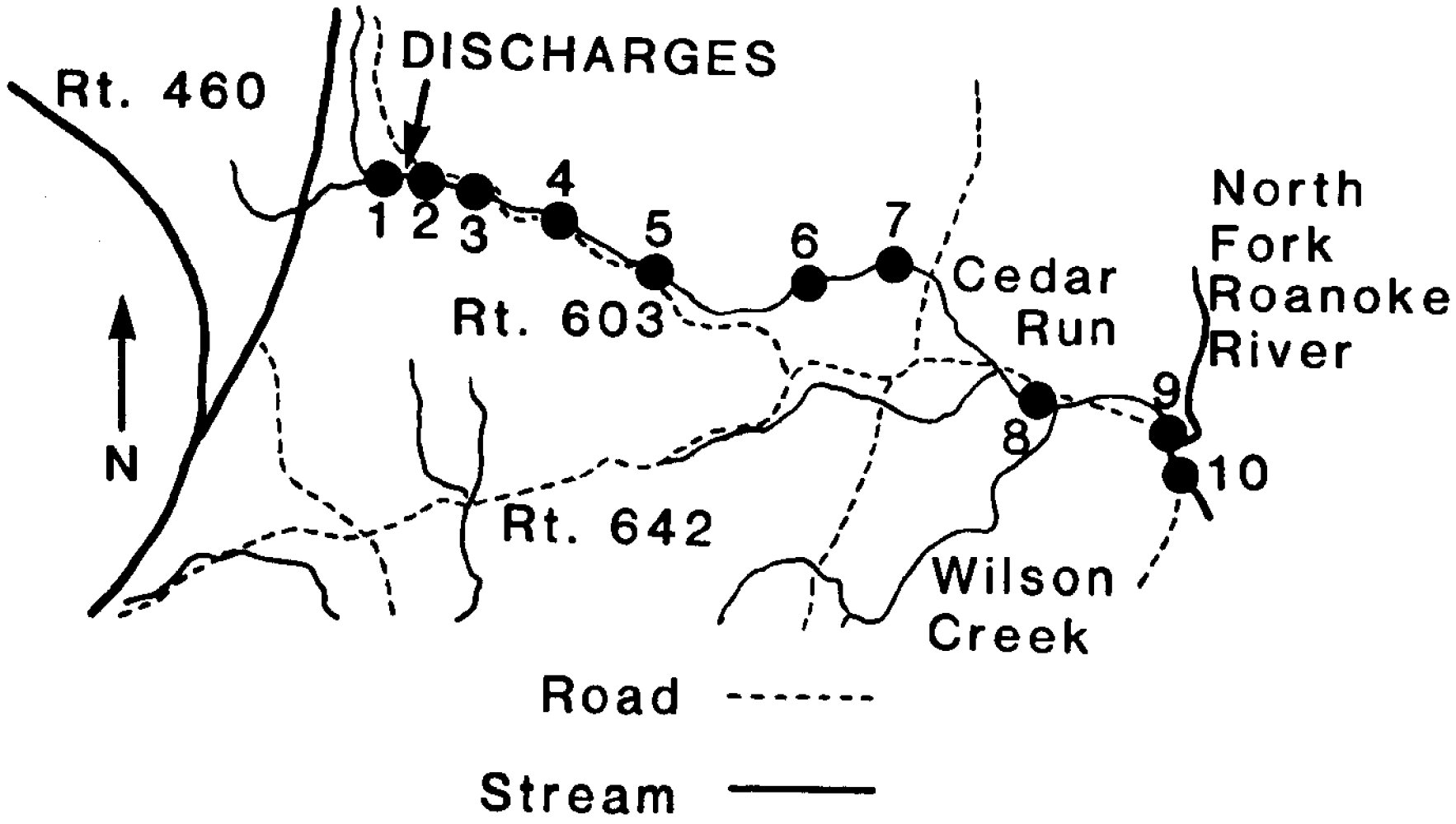


Figure 1. Sampling stations on Cedar Run, Montgomery County, Virginia.

at increasing distances downstream of the effluent outfalls and after confluence with two other major bodies of water.

Uniformly sized PFU substrates (6 x 5 x 5 cm) were used to sample the microbial community. Preliminary experiments in Cedar Run showed that these substrates were colonized rapidly in streams and contained many of the same numbers and kinds of organisms as did natural organic and sediment substrates (Matthews 1981). The PFU substrates were used rather than other natural or artificial substrates because they produced a large volume of extract. This volume allowed several samples to be taken from the same PFU community, permitted statistical analysis and improved replicability.

Substrates were allowed to colonize in the stream for one or more days. They were collected in self-sealing plastic bags and returned to the laboratory on ice within 3 h of collection. The microbial community was squeezed from the PFUs into clean beakers. This extract was sampled for protozoans and the HI. Protozoans were counted on two slides for each substrate. A time limit for examination was not set because densities never reached the level where time became a problem. Organisms were identified to species whenever possible, using standard protozoan keys, and the number of individuals of each species was also recorded. The HI was determined by the method described below.

Duplicate benthic macroinvertebrate collections were made on eight occasions in one of the experiments. A portable box sampler was used in most cases; when the water was too shallow, a Surber sampler was used. Substrate was scoured and agitated to a depth of 10 cm or until bedrock was reached. Invertebrates were floated in a saturated calcium chloride solution and skimmed from the surface (Hynes 1970), and the sediment was examined for specimens that did not float. The invertebrates were preserved in 70% ethanol, identified taxonomically and categorized into functional groups based on how food was acquired (Merritt and Cummins 1978).

Laboratory Experiments

Experiments were conducted using PFUs as artificial substrates. Many PFUs (6 x 5 x 5 cm) were tied firmly around the middle with nylon string, attached to a long nylon rope supported by floats, and anchored along the shore of Pandapas Pond in approximately 1 m of water or in flowing water of Cedar Run. Both habitats were located in Montgomery County, Va. The PFUs were squeezed when placed in the aquatic ecosystem to achieve saturation with ambient water. Pond PFUs were allowed to colonize for 21 days before they were used; stream PFUs were allowed to colonize 2 to 3 days before use.

An epicenter (colonized substrate) and uncolonized substrates were placed in plastic plant trays (54 x 26 x 5.5 cm). Two trays

were set up as controls, each containing 5 L of water from either Pandapas Pond or Cedar Run that had been pasteurized for 20 min at a temperature of 60°C. Other trays contained pasteurized pond or stream water with copper, sewage treatment effluent, or electroplating effluent or both effluents. One fluorescent bulb was positioned above each tray so that migration of phototrophic species would not be biased. Light was a 16L: 8D photoperiod at an intensity of about 1750 lux. A clear plastic hood covered the trays and reduced the chance of outside colonization by any airborne protozoans and reduced currents induced by wind. A sketch of one experimental system is provided in Figure 2. On days 1 and longer, one or two islands were examined for protozoans using the method outlined for field experiments. On day 15, sponges were also analyzed for the HI.

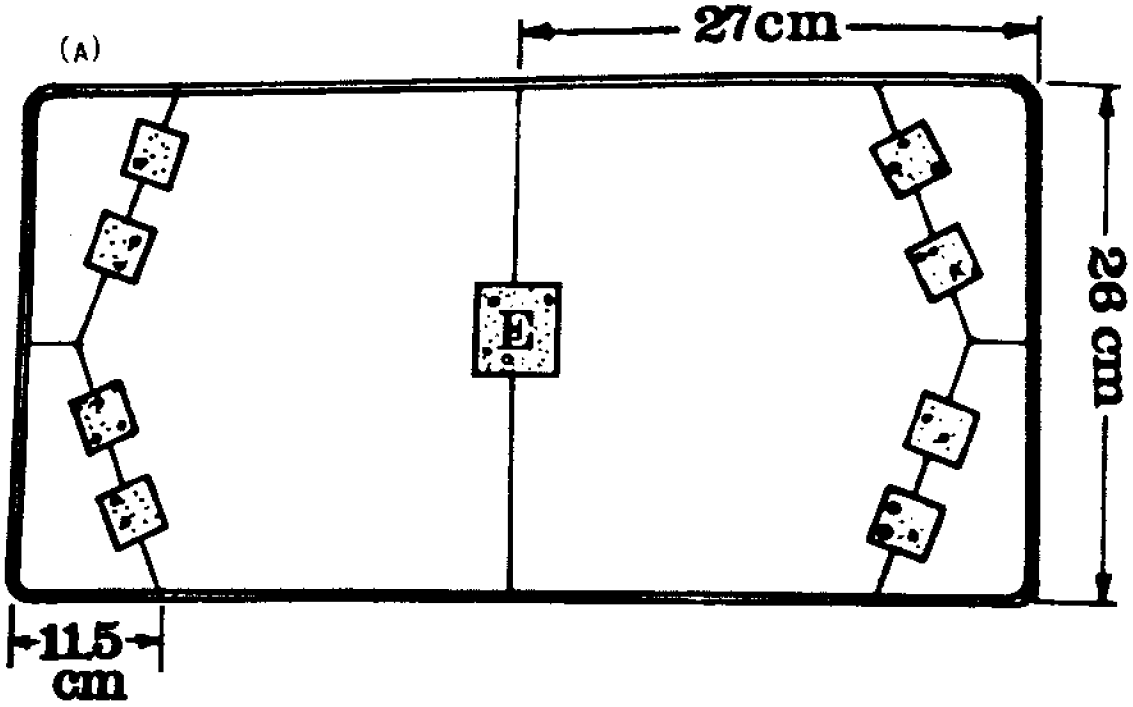
Heterotrophic Index

The ATP was extracted by injecting 1.0 L of the microbial community extract into 9 L of boiling tris buffer (Tris-(Hydroxymethyl) aminomethane, 0.02 M, pH 7.75), heating for 15 min, freezing at -20°C, and assaying within 7 days. Assays used standard bioluminescence techniques with firefly enzyme on a Labline #9140 ATP Photometer (APHA 1976).

Chlorophyll a levels were measured by injecting 1.0 mL of the microbial community into 9 mL of 100% spectrograde acetone (which produced a 90% acetone extraction medium) and extracting for 24 h at 4°C in the dark (Holm-Hansen and Riemann 1978). Chlorophyll a determinations were made using a calibrated Turner Designs fluorometer.

Replicate measurements of chlorophyll a and ATP using direct injection techniques for individual PFUs were low; coeeficients of variation were 1 and 12% (Matthews 1981). For duplicate PFUs at each site, chlorophyll a measurements varied more; coefficients of variation ranged from 14 to 23% and increased as pollution stress increased. Matthews (1981) determined that the coefficients of variation for ATP duplicates ranged from 30 to 72%; the highest coefficients occurred when the samples were contaminated with early instar macroinvertebrates or filaments of algae and were measured with a less sensitive JRB photometer. Subsequent measurements of ATP were made with a more sensitive photometer and purified luciferin-luciferase, and the coefficients of variation were much lower.

An HI similar to the autotrophic index proposed by APHA et al. (1976) was used to test whether the balance between autotrophy and heterotrophy was upset; however, the index was adapted to use ATP-based biomass estimates rather than ash-free dry weight (Equations 1 and 2):



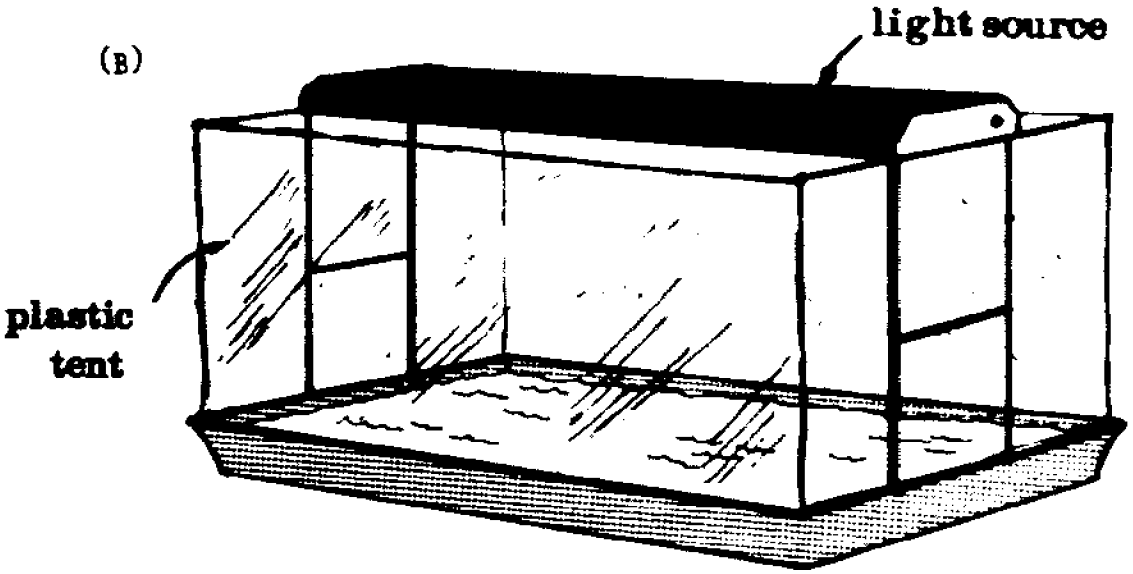


Figure 2. (a) Arrangement of epicenter (E) and PFU islands in tray. (b) Plant tray with a fluorescent light and clear plastic hood.

$$HI = \frac{\hat{B} \text{ total (mg/L)}}{\text{chlorophyll } \underline{a} \text{ (mg/L)}} \quad (1)$$

where

$$\hat{B} \text{ total} = \frac{\text{ATP (ng/L)}}{2{,}400} = \text{estimated total living biomass} \quad (2)$$

ATP has frequently been used to estimate total living biomass, although its use has been restricted predominantly to lentic habitats (e.g., Holm-Hansen and Booth 1966). Because a considerable portion of the organic matter in streams is nonliving and often allochthonous, ATP-based biomass estimates should provide a better approximation of the active microbial community than the traditional gravimetric measurements of ash-free dry weight.

RESULTS AND DISCUSSION

Comparison of components of field data revealed a good correlation between the mean HI and mean macroinvertebrate diversity and proportion of the macroinvertebrate community comprising those organisms that feed on aufwuchs (scrapers) (Figure 3). Before the effluents entered Cedar Run, the HI was low (indicating a large autotrophic component), and macroinvertebrate diversity was high. Further, the proportion of scrapers in the community was also high. Significant changes in both communities occurred downstream of the point of discharge of the effluents, and the stream in the area studied did not recover.

In laboratory experiments with copper, the mean number of species after 4 days exposure in previously uncolonized sponges was 9 (Figure 4), and after 15 days there were 12 species. After 4 days, there were 4 and 1 species, respectively, in sponges exposed to 0.1 and 0.5 mg L^{-1} copper. In the control, species equilibrium occurred in about 7 days, and for both concentrations of copper equilibrium was not reached after 15 days. After 15 days, the HI was lowest where copper concentration was highest, and vice versa.

In separate laboratory experiments with electroplating plant effluent, colonization occurred most rapidly in the controls, and inhibition of colonization rate was greater as the concentration of the effluent increased (e.g., Figure 5). The HI increased as proportion of electroplating effluent increased.

In the last experiment, sponges were placed at 10 stations in Cedar Run upstream and downstream of the sewage treatment and electroplating plant effluents. Several days later, water samples from each station were brought to the campus for laboratory colonization experiments. All water samples were analyzed for cadmi-

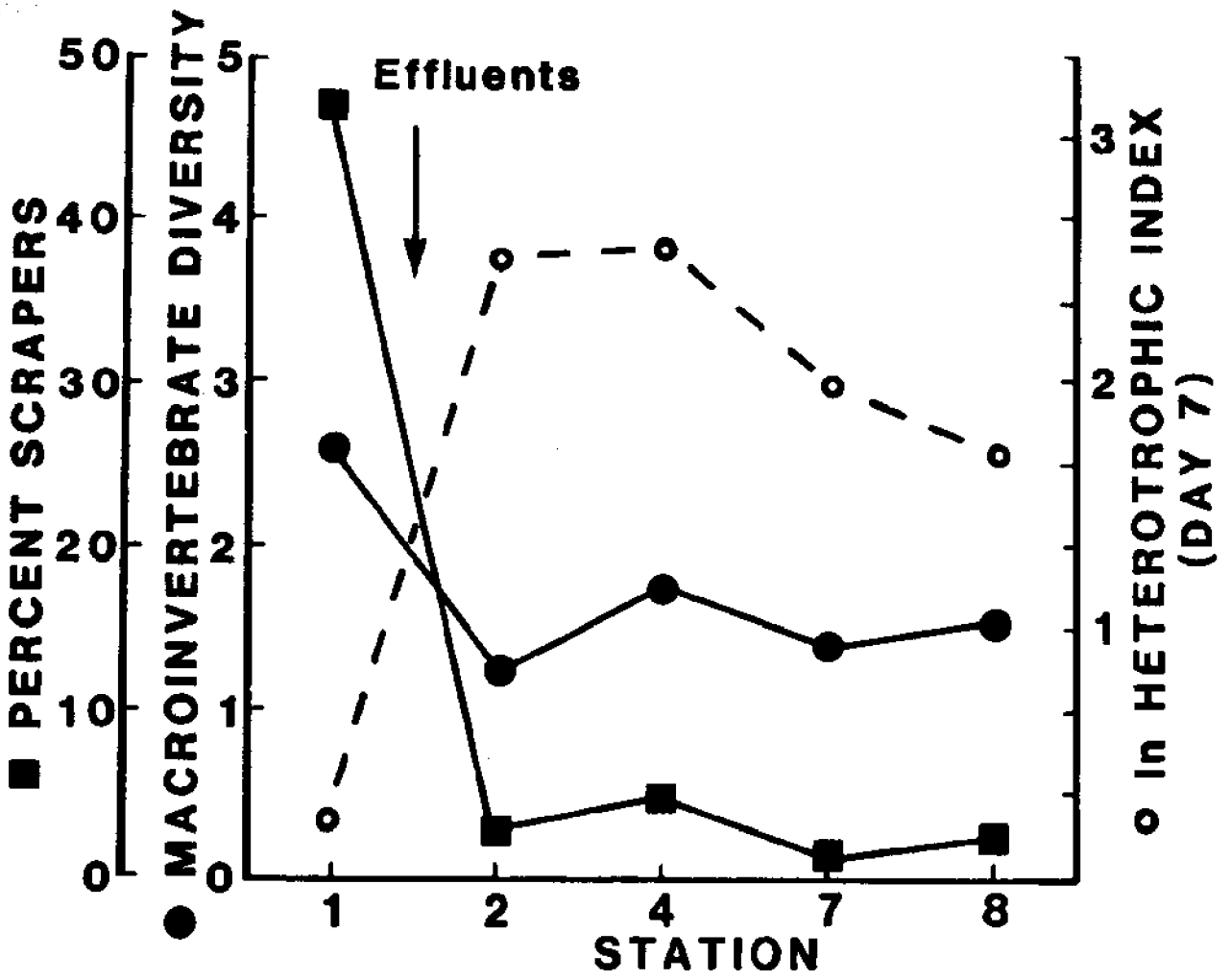


Figure 3. Yearly means of proportion of scrapers, macroinvertebrate diversity, and heterotrophic index (adapted from Matthews 1981 and Firth 1980).

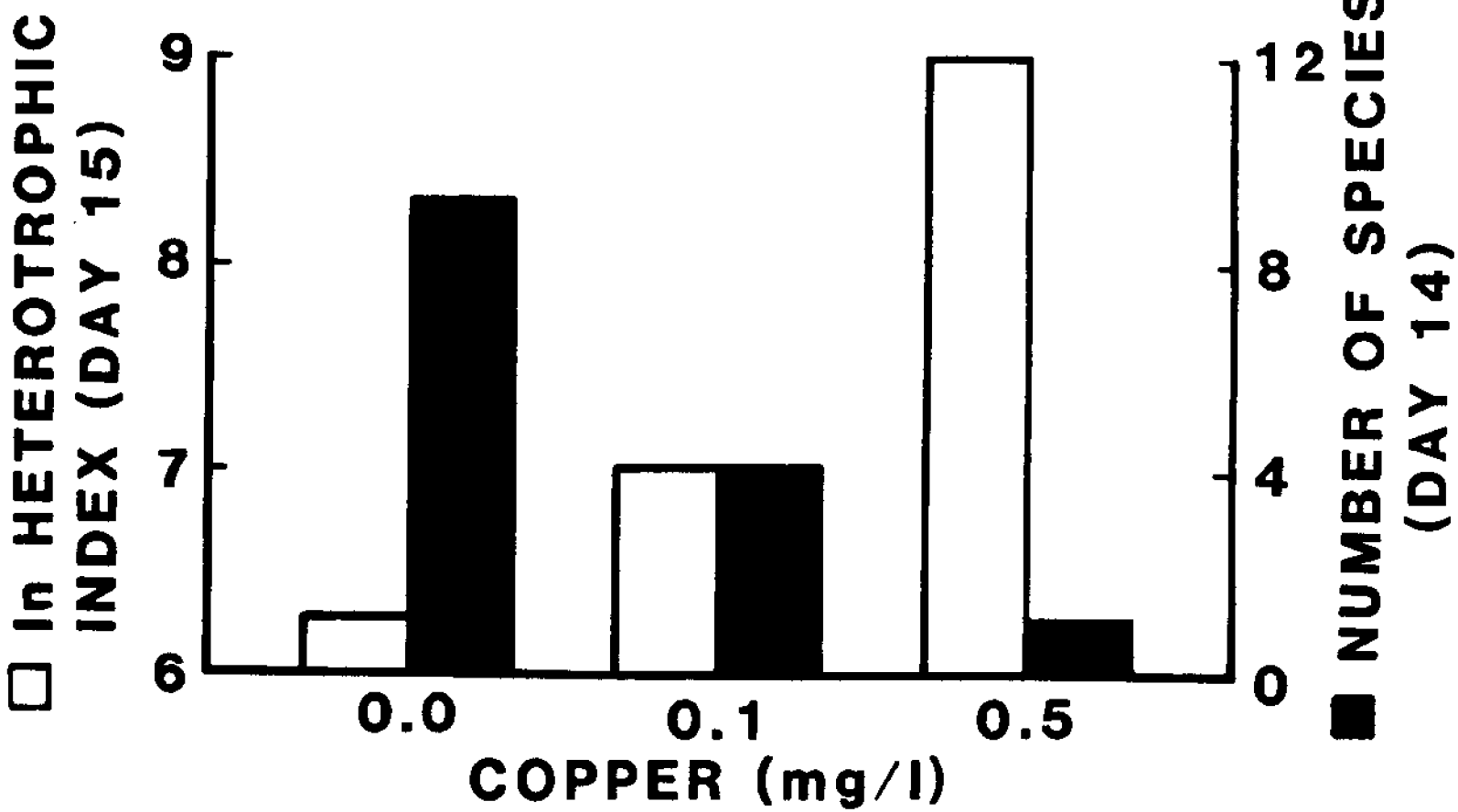


Figure 4. Relationships between heterotrophic index and number of protozoan species inhabiting a polyurethane foam sponge after exposure to copper sulfate.

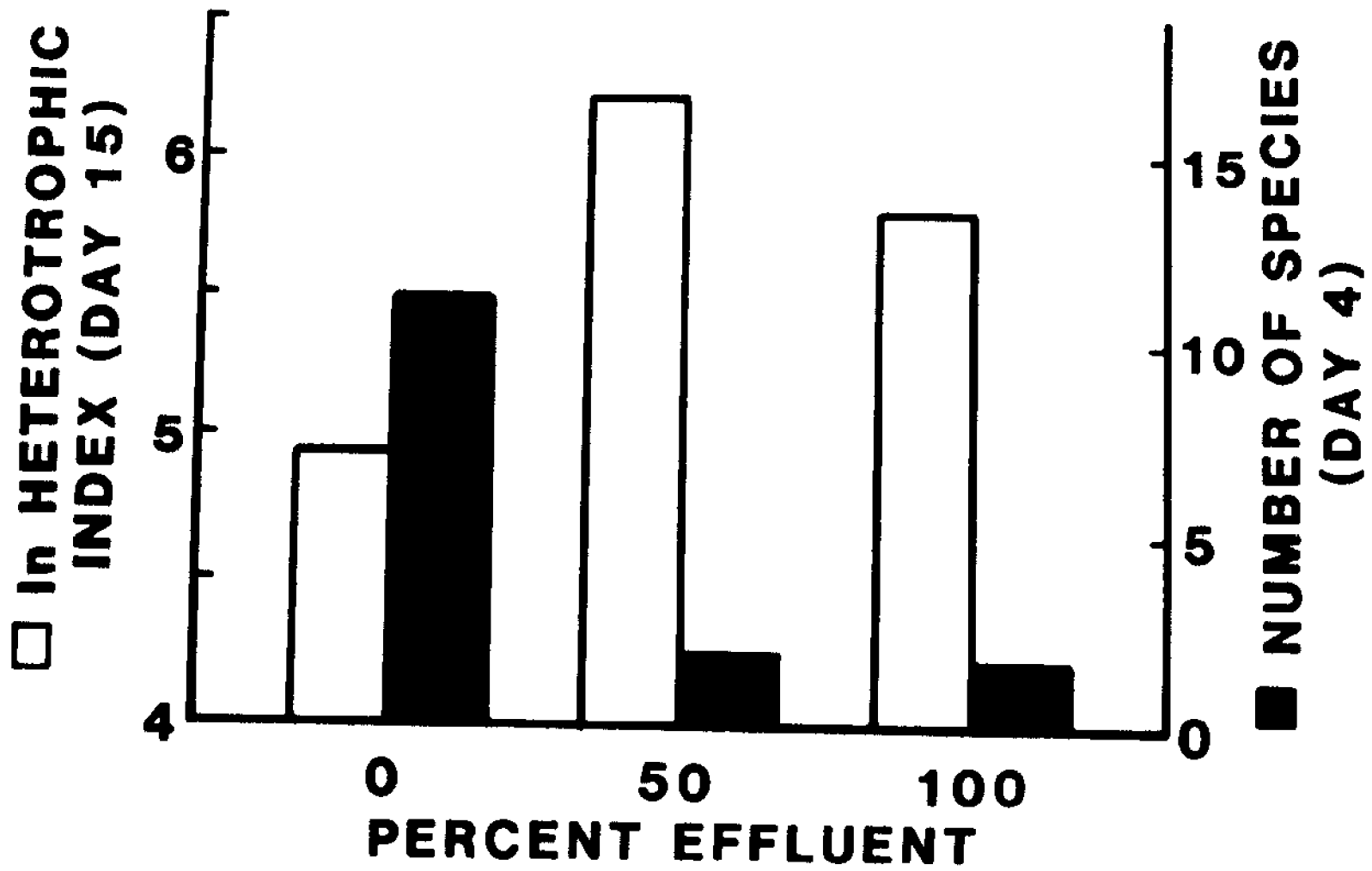


Figure 5. Relationships between heterotrophic index and number of protozoan species inhabiting a polyurethane foam sponge after exposure to dilutions of an electroplating plant effluent.

um, copper, lead, nickel and zinc, and these data were expressed as total heavy metal concentrations. When the total metal concentrations were high, the number of species colonizing the substrate was similar to that found at the reference station (Station 1). At the polluted stations, the HI increased. The low HI at Station 2 was attributed to a combination of a toxic effect and wash-in of upstream organisms.

The day the water was brought into the laboratory, the distribution and concentration of total metals varied, but in general concentrations (except for Station 9) decreased as the effluent moved downstream (Figure 6). The reference station (Station 1) also had metal concentrations higher than the downstream Station 10 and higher than that usually measured in the field (Figure 7). The cause of this difference is attributed to rain runoff from parking lots and the like. In spite of these variations, a pattern similar to that observed in the field was observed. As metal concentrations increased, the number of species colonizing substrates after 3 days decreased. The results of this research, which need additional confirmation in varied situations, indicate that the use of a microbial community, a subset of the biotic community, may be useful in assessing pollution effects and predicting ecosystem effects. By using substrates colonized by representative indigenous flora and fauna and using water from the receiving system in question, the isomorphic state proposed by Heath (1980) and others was approached. In the process, the potential for more accurate prediction of ecosystem effects increased. In both the laboratory and field, colonization rates and HIs responded in the same manner to perturbation and, therefore, not only corroborated each other but satisfied the need for a predictive system that exhibits the same behavioral responses that are observed in nature.

Identical behavior can occur even though the composition of the two systems is not identical (Heath 1980). This research and others support this statement. For example, when pond and stream communities are used to study the impact of sewage treatment effluent on colonization rates, the rates are highest in the control and progressively reduced as the proportion of effluent increases. Cairns et al. (1980) found that immature and mature communities, which differ substantially in species composition, respond in a similar manner to stress (Figure 8). However, the colonization rate from a mature community was not affected as dramatically as the rate from an immature community. The differences between rates were sufficient to suggest that community maturity can be an important factor in the sensitivity of ecosystems to chemical stress. The results for two tests were similar even though the species composition was different in each test. The test was not repeated in other ecosystems or with other concentrations of copper so it is not known if the response is general or limited. Last, as HIs increased, macroinvertebrate diversity and shifts in proportions of

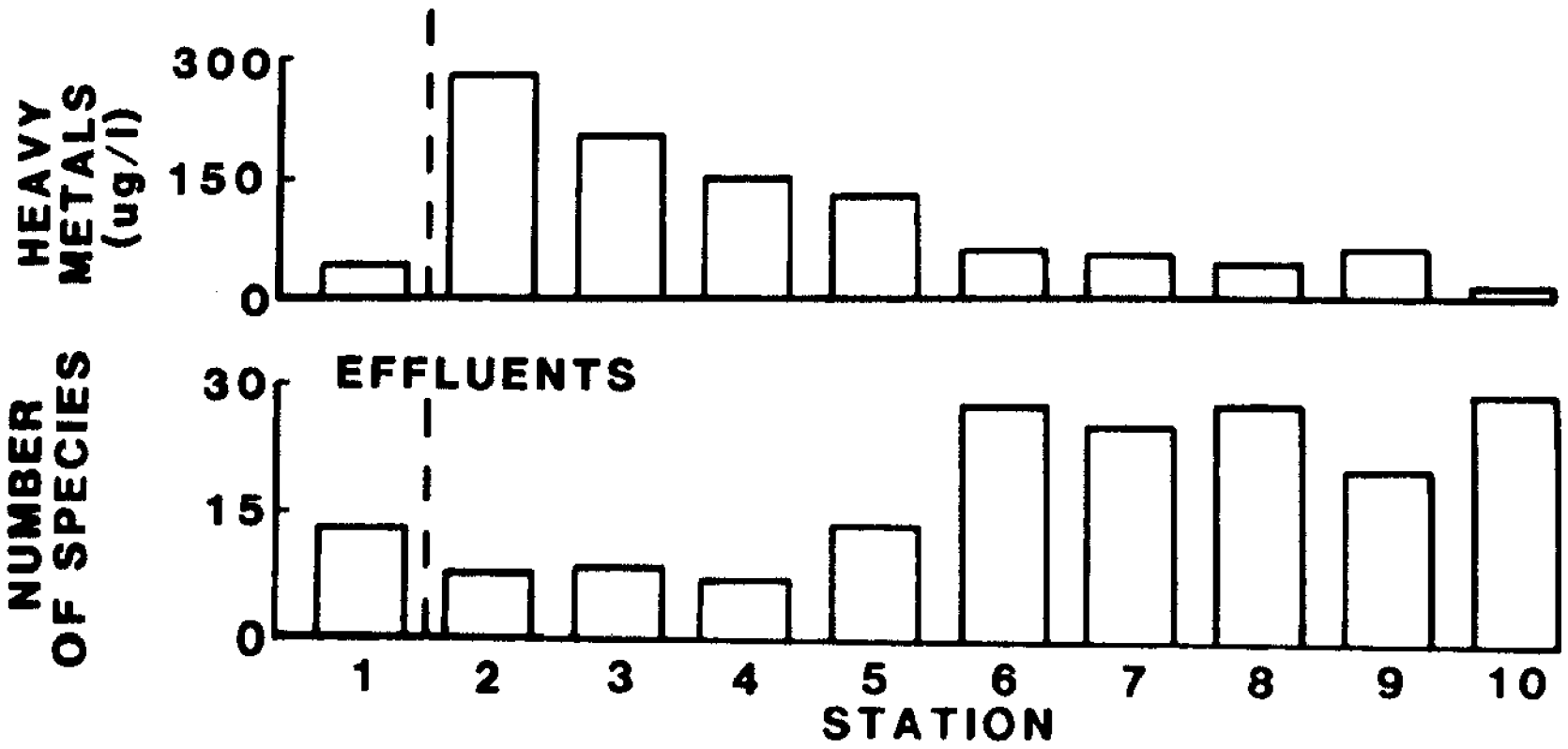


Figure 6. Effect of heavy metal concentrations on colonization rates of microbial communities observed in the laboratory.

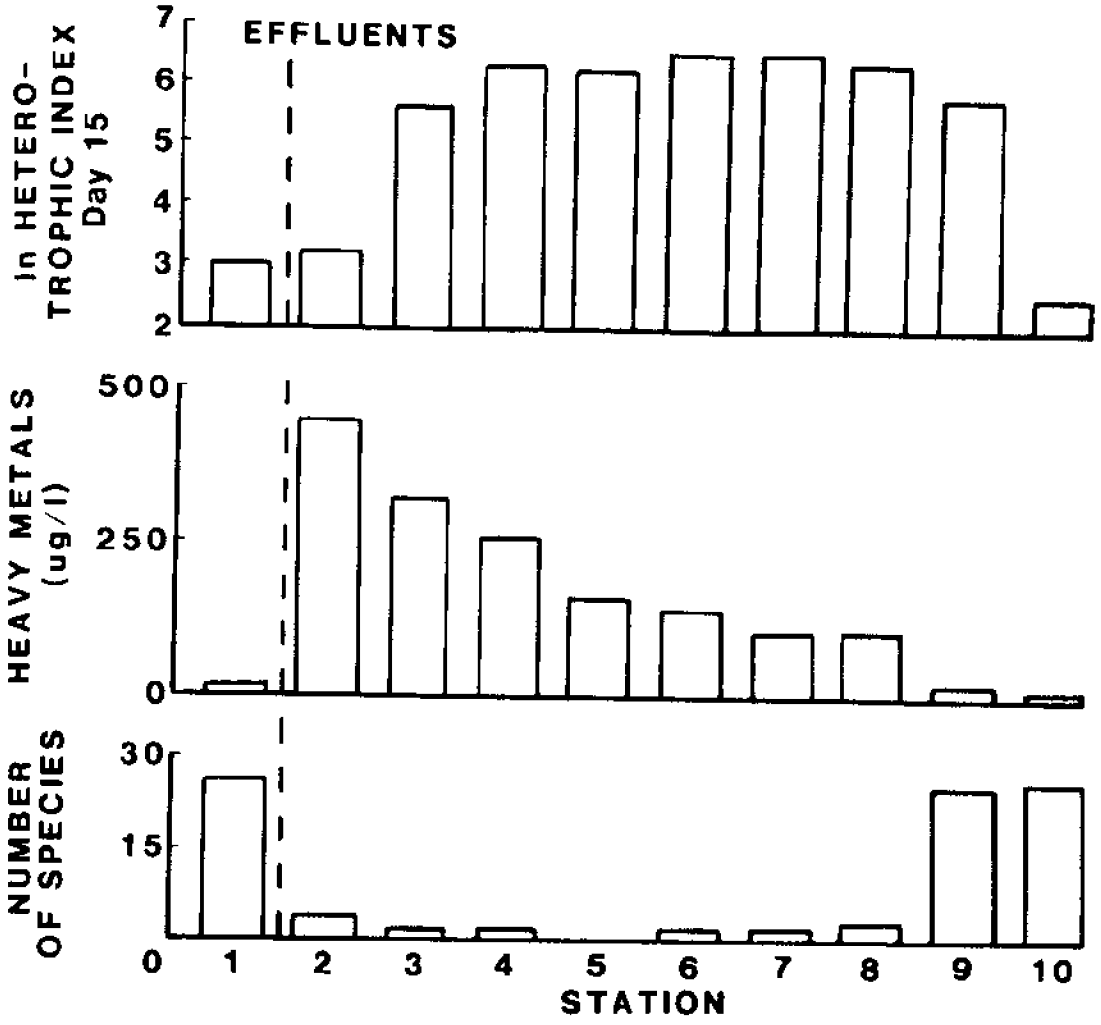


Figure 7. Effect of heavy metal concentrations on colonization rates of microbial communities observed in the field.

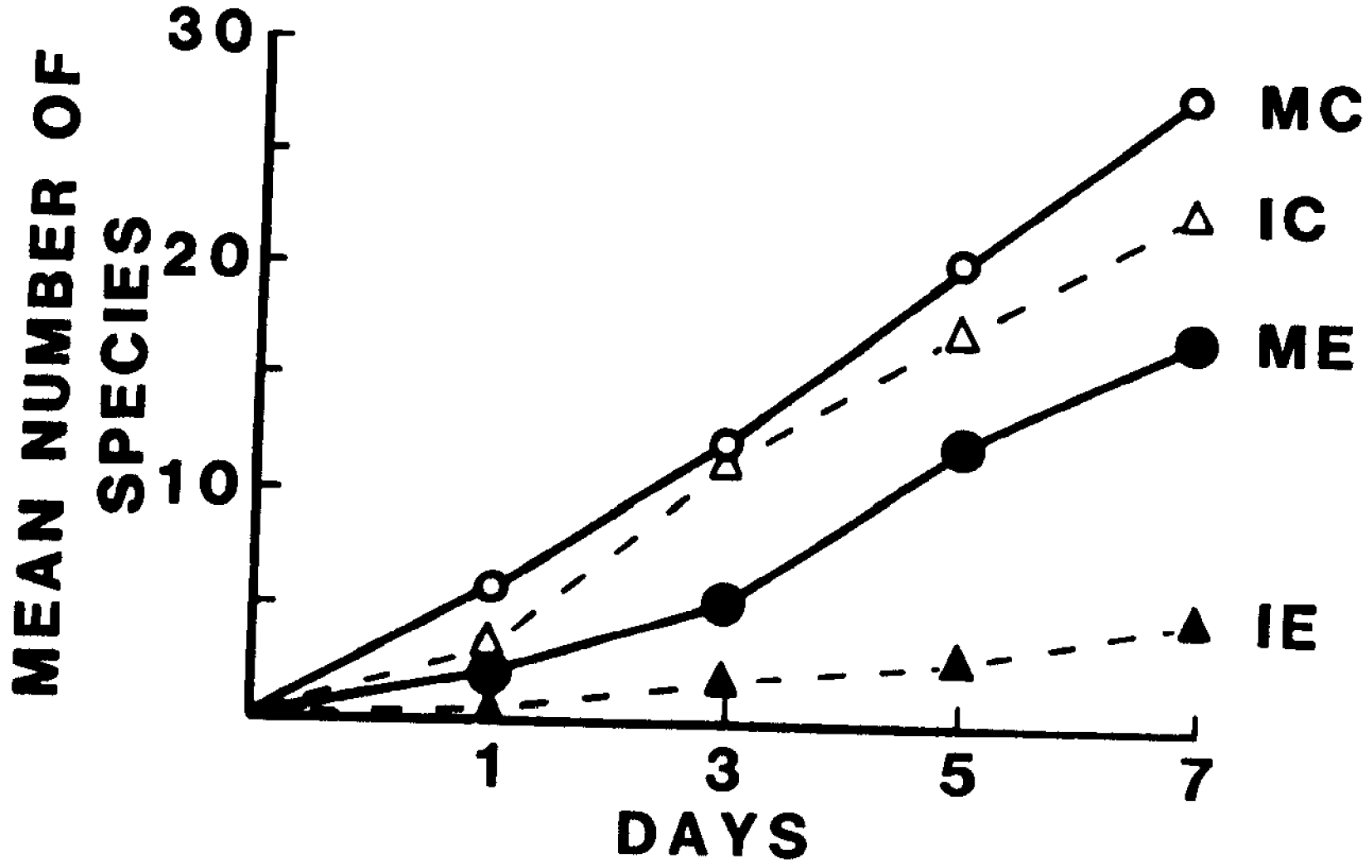


Figure 8. Colonization rates from mature (M) and immature (I) epicenter under control (C) conditions and exposure (E) to 6.42 mg L^{-1} copper sulfate (Adapted from Cairns et al. 1981).

functional groups of the macroinvertebrate community decreased (Figure 3; Matthews et al. 1980; Kondratieff et al., unpublished).

The advantages of using PFUs to monitor stress are simplicity and low cost/test. Taxonomic expertise is not important for counting species (although it is helpful) because only the number of different kinds of organisms is important for estimating invasion rate. Even though chlorophyll a and ATP measurements can vary among PFUs, the range between mean absolute values as a function of "healthy" versus "polluted" conditions was great, and the HIs at any one site rarely, if ever, indicated both a healthy and polluted effect. Carrying out valid toxicity tests at the community ecosystem level is beset with many pitfalls largely because of our inadequate knowledge of processes controlling ecological systems. Failure to begin to utilize tests because of an apprehension about the degree of understanding of the processes involved is reminiscent of the objections to using single species tests in the 1940s and early 50s. Despite the many conceptual obstacles, the tests proved to be useful, and we are convinced that beginning now even the most primitive multispecies tests will enhance our ability to estimate hazard to complex systems in a more scientifically justifiable way. Those who object to the use of multispecies tests because of our lack of understanding forget that the ultimate goal of all tests is to estimate hazard to the environment. Because we are now not doing this well, the real question is this: Will we be doing it any worse if we begin multispecies testing without a total and complete understanding of the complex processes involved?

CONCLUSIONS

The basic question is whether good science is being used to protect the environment. Technology based standards (BAT, BPT) are not science, but rather an attempt to substitute technology for science. Neither is the science sound when the main hypothesis is not verified or validated. In attempts to protect the environment against hazardous chemicals, the hypothesis (rarely explicitly stated) appears to have been that water quality criteria based on single species tests are adequate to protect higher levels of biological organization including communities and ecosystems. The limited evidence available to support this hypothesis is primarily circumstantial, and even the most charitable scientists do not feel that this hypothesis has been confirmed in a scientifically justifiable way. Mount (1979) is probably correct in his estimate that the probability of predicting community effects with laboratory data is quite high when the effects are gross and practically nonexistent when the effects are refined. The steepness of the curve in Figure 9 was probably deliberate to suggest that the ability to predict community response with single species tests declines markedly when one

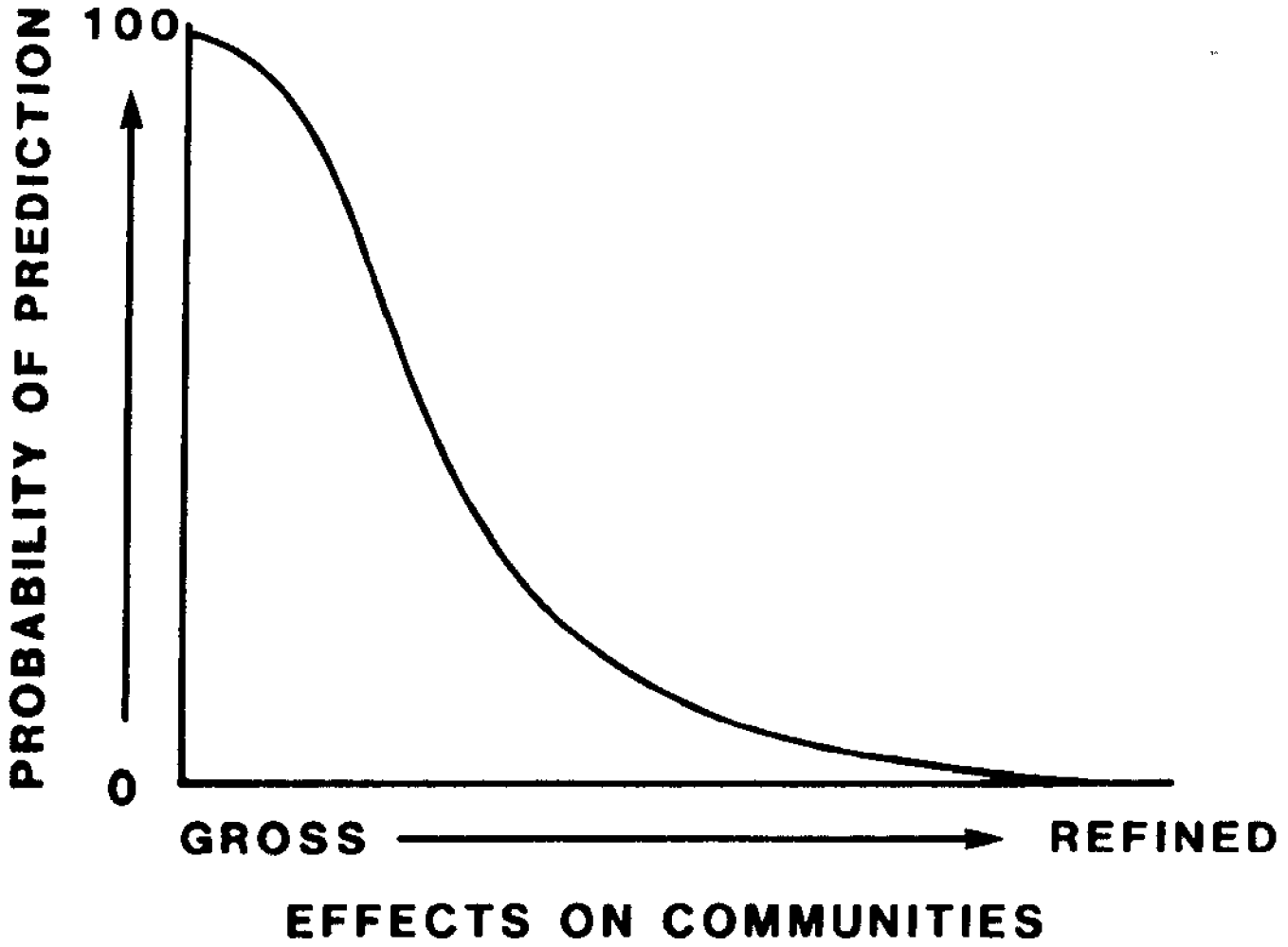


Figure 9. Probability of predicting community effects accurately from laboratory data (adapted from Mount 1979).

departs from gross effects. Even if the cost of measuring community response is high, investigators should, in the name of sound science, more carefully evaluate single species tests as predictors so that their strengths and limitations are better understood.

This paper has illustrated some important points:

1. Communities can be collected from natural systems on artificial substrates and brought into the laboratory. These communities, although not as easily replicated in terms of the kinds of species present as the assembled species frequently used in microcosms, do offer the opportunity to achieve a greater degree of environmental realism at low cost.
2. Although the communities collected in natural systems on artificial substrates rarely contain precisely the same species, there is evidence that the responses of two communities collected in the same way (artificial substrates) but composed of different species may be quite similar. Presumably this is because the range of sensitivity to the test chemical found in these communities is not as great as the differences in kinds of species present.
3. Good evidence exists that some types of laboratory tests may be good predictors of field responses when these are made at the same level of biological organization.
4. Considerable information redundancy in natural systems theoretically makes it possible to predict one quality of a system if another is known (in this instance, colonization rate and heterotrophic index correlated with each other as a function of stress).

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REFERENCES

APHA (American Public Health Association), American Water Works Association and Water Pollution Control Federation. 1976. Standard Methods for Examination of Water and Wastewater, 14th ed. Washington, D.C.

Ashby, W.R. 1956. An Introduction to Cybernetics. John Wiley and Sons, New York.

Baker, J.M. 1976. Experimental investigation of refinery effluents. In: J.M. Baker (ed.), Marine Ecology and Oil Pollution, pp. 247-254. Applied Science Publ., Essex, England.

Baker, J.M. and G.B. Crapp. 1974. Toxicity tests for predicting oil and emulsifier pollution. In: L.R. Beynon and E.B. Cowell (eds.), Ecological Aspects of Toxicity Testing of Oils and Dispersants, pp. 23-40. John Wiley and Sons, New York.

Blanck, H.G.D. and K. Gustafsson. 1978. An annotated literature survey of methods for determination of effects and fates of pollutants in aquatic environments. Report from the National Swedish Environmental Protection Board, Stockholm, Sweden.

Buikema, A.L., Jr., B.R. Niederlehner and J. Cairns, Jr. 1982. Biological monitoring. Part IV. Toxicity testing. Water Res. 16: 239-262.

Buikema, A.L., Jr., J. Cairns, Jr. and W.H. Yongue, Jr. 1983. Correlation between the autotrophic index and protozoan invasion rates as indicators of pollution stress. In: W.E. Bishop, R.D. Cardwell and B.B. Heidolph (eds.), Aquatic Toxicology and Hazard Assessment: Sixth Symposium ASTM STP 802, pp. 204-215. American Society for Testing and Materials, Philadelphia, Pa.

Cairns, J., Jr. 1974. Indicator species vs. the concept of community structure as an index of pollution. Water Resour. Bull. 10: 338-347.

Cairns, J., Jr. 1977. Quantification of biological integrity. In: R.K. Ballentine and L.J. Guarria (eds.), The Integrity of Water, pp. 171-187. U.S. Environmental Protection Agency, Washington, D.C.

Cairns, J., Jr. 1980. Beyond single species toxicity testing. Mar. Environ. Res. 3: 157-159.

Cairns, J., Jr. 1981. Biological monitoring. Part VI. Future needs. Water Res. 15: 941-952.

Cairns, J., Jr. 1982. Preface. In: J. Cairns, Jr., A.L. Buikema, D.S. Cherry, E.E. Herricks, R.A. Matthews, B.R. Niederlehner, J.H. Rogers, Jr. and W.H. van der Schalie (eds.), Biological Monitoring in Water Pollution, pp. vii-xi. Pergamon Press, Oxford.

Cairns, J., Jr., K.M. Hart and M.S. Henebry. 1980. The effects of a sublethal dose of copper sulfate on the colonization rate of freshwater protozoan communities. Am. Midl. Nat. 104: 93-101.

Cairns, J., Jr., D.S. Cherry and J.D. Giattina. 1981. Correspondence between behavioral responses of fish in laboratory and field heated chlorinated effluents. In: W.J. Mitsch, R.W. Bosserman and J.M. Klopatek (eds.), Energy and Ecological Modelling, pp. 207-215. International Society of Ecological Modelling, Louisville, Ky.

Cherry, D.S. and J. Cairns, Jr. 1982. Biological monitoring. Part V. Preference and avoidance studies. Water Res. 16: 263-301.

Cherry, D.S., K.L. Dickson and J. Cairns, Jr. 1975. Temperatures selected and avoided by fish at various acclimation temperatures. J. Fish. Res. Board Can. 32: 485-491.

Cherry, D.S., K.L. Dickson, J. Cairns, Jr. and J.R. Stauffer. 1977. Preferred, avoided and lethal temperatures of fish during rising temperature conditions. J. Fish. Res. Board Can. 34: 239-246.

Cherry, D.S., S.R. Larrick, J.D. Giattina and J. Cairns, Jr. 1979. Continuation of laboratory and field determined avoidance responses of Ohio River, New River and Lake Michigan fish to thermal and chlorinated discharges. American Electric Power Service Corp., Canton, Ohio.

Cooke, G.D. 1977. Experimental aquatic laboratory ecosystems and communities. In: J. Cairns, Jr. (ed.), Aquatic Microbial Communities, pp. 59-103. Garland Reference Library of Science and Technology, Vol. 15, Garland Publ., New York.

Cowell, E. 1978. Ecological monitoring as a management tool in industry. Ocean Manage. 4: 273-285.

Doudoroff, P. 1976. Reflections on pickle-jar ecology (Keynote Address). In: J. Cairns, Jr., K.L. Dickson and G. Westlake (eds.), Biological Monitoring of Water and Effluent Quality, pp. 3-19. American Society for Testing and Materials, Philadelphia, Pa.

Firth, P.L. 1980. Nutrient retention and macroinvertebrate community response to sewage stress in a stream ecosystem. M.S. Thesis, Virginia Polytechnic Inst. and State Univ., Blacksburg, Va.

Geckler, J.R., W.B. Horning, T.M. Neiheisel, Q.H. Pickering and E.L. Robinson. 1976. Validity of laboratory test for predicting copper toxicity in streams. EPA Ecology Research Ser. 600/3-76-116, U.S. Environmental Protection Agency, Cincinnatti, Ohio.

Giddings, J.M. 1980. Types of aquatic microcosms and their research applications. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp. 248-266. (CONF-781101, National Technical Information Service, Springfield, Va.).

Giesy, J.P., Jr. (ed.). 1980. Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978. (CONF-781101, National Technical Information Service, Springfield, Va.).

Hart, W.B., P. Doudoroff and J. Greenbank. 1945. The Evaluation of Toxicity of Industrial Wastes, Chemicals and Other Substances to Freshwater Fishes. Waste Control Lab., Atlantic Refinery Co., Philadelphia, Pa.

Harte, J., D. Levy, J. Rees and E. Saegebarth. 1980. Making microcosms an effective assessment tool. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp. 105-137. (CONF-781101, National Technical Information Service, Springfield, Va.).

Heath, R.T. 1979. Holistic study of an aquatic microcosm: The theoretical and practical implication. Int. J. Environ. Stud. 13: 87-93.

Heath, R.T. 1980. Are microcosms useful for ecosystem analysis? In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp. 333-347. (CONF-781101, National Technical Information Service, Springfield, Va.).

Heath, R.G., J.W. Spann and J.F. Kreitzer. 1969. Marked DDE impairment of mallard reproduction in controlled studies. Nature 224: 47-48.

Holm-Hansen, O. 1970. ATP levels in algal cells as influenced by environmental conditions. Plant Cell Physiol. 11: 689-700.

Holm-Hansen, O. and C.R. Booth. 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnol. Oceanogr. 11: 510-519.

Holm-Hansen, O. and B. Reimann. 1978. Chorophyll a determination: Improvements in methodology. Oikos 30: 438-447.

Hynes, H.B.N. 1970. The Ecology of Running Waters. Univ. of Toronto Press, Toronto, Canada.

Kaesler, R.L., J. Cairns, Jr. and J.S. Crossman. 1974. Redundancy in data from stream surveys. Water Res. 8(9): 634-642.

King, O.L. 1980. Some cautions in applying results from aquatic microcosms. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp. 164-191. (CONF-781101, National Technical Information Service, Springfield, Va.).

Loehr, R. C. 1981. Trichlorethylene standards. In: Proceedings, Seminar on Development and Assessment of Environmental Standards. American Academy of Environmental Engineers, George Washington Univ., Washington, D.C.

Marshall, J.S. and D.L. Mellinger. 1980. An in situ experimental method for toxicological studies on natural plankton communities. In: J.G. Eaton, P.R. Parrish and A.C. Hendricks (eds.), Aquatic Toxicology, pp. 27-29. ASTM STP 707, American Society for Testing and Materials, Philadelphia, Pa.

Matthews, R.A. 1981. Field testing of environmental stress effects in streams using microbial communities. Ph.D. Dissertation. Virginia Polytechnic Inst. and State Univ., Blacksburg, Va.

Matthews, R.A., P.F. Kondratieff and A.L. Buikema, Jr. 1980. A field verification of the use of the autotrophic index in monitoring stress effects. Bull. Environ. Contam. Toxicol. 25: 226-233.

Matthews, R.A., A.L. Buikema, Jr., J. Cairns, Jr. and J.H. Rodgers, Jr. 1982. Biological monitoring. Part II. Receiving system functional methods, relationships and indices. Water Res. 16: 129-139.

Merritt, R.W. and K.W. Cummins (eds.). 1978. An Introduction to Aquatic Insects of North America. Kendall-Hunt Publ., Dubuque, Iowa.

Metcalf, R.L., G.K. Saugha and I.P. Kapoor. 1971. Model ecosystem for the evaluation of pesticide biodegradability and ecological magnification. Environ. Sci. Technol. 5: 709-713.

Mount, D.I. 1979. Adequacy of laboratory data for protecting aquatic communities. In: K.L. Dickson, A. Maki and J. Cairns, Jr. (eds.), Analyzing the Hazard Evaluation Process, pp. 112-118. American Fisheries Society, Washington, D.C.

Odum, E.P. 1971. Fundamentals of Ecology, 3rd ed. W.B. Saunders Co., Philadelphia, Pa.

Odum, E.P. 1981. Revival of non-laboratory science. Assoc. Southeast. Biol. Bull. 28(3): 119-121.

Odum, E.P., J.T. Finn and E.H. Franz. 1979. Perturbation theory and the subsidy-stress gradient. BioScience 29: 349-352.

Picken, L.E.R. 1931. The structure of some protozoan communities. J. Ecol. 25: 368-384.

Spencer, M.J. and A.J. Ramsay. 1978. Bacterial populations, heterotrophic potentials, and water quality in three New Zealand rivers. New Zealand J. Mar. Freshwater Res. 12: 415-427.

Stephan, C.E. 1982. Chronic endpoints. In: Aquatic Toxicology Newsletter, March 12, pp. 6-7. American Society for Testing and Materials Subcommittee E47.01, Philadelphia, Pa.

Taub, F.B. and M.E. Crow. 1978. Loss of a critical species in a model (laboratory) ecosystem. Verh. Int. Verein. Limnol. 20: 1270-1276.

Taub, F.B. and M.E. Crow. 1980. Synthesizing aquatic microcosms. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp. 69-104. (CONF-781101, National Technical Information Service, Springfield, Va.).

Wilson, K.W., E.B. Cowell and L.R. Beynon. 1974. The toxicity testing of oils and dispersants: A European view. In: L.R. Beynon and E.B. Cowell (eds.), Ecological Aspects of Toxicity Testing of Oils and Dispersants, pp. 129-141. John Wiley and Sons, New York.

Wrenn, W.B. and T.D. Forsythe. 1978. Effects of temperature on production and yield of juvenile walleyes in experimental ecosystem. Am. Fish. Soc. Spec. Publ. 11: 66-73.

Chapter 2. Laboratory Microcosms

Introduction

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Microcosms, whether derived from natural communities or synthesized from laboratory cultures, are intermediate in complexity among the array of techniques for determining the effects of chemical pollutants in a marine environment. Microcosms lend themselves to valid experimental designs, but are open to question regarding claims that the responses are natural, or that the parameters measured are ecologically or economically important. The naturalness of the response is compromised whenever large scale processes are omitted, as they are in virtually all studies except full scale, open, natural communities (which by virtue of their scale and openness do not lend themselves to valid experimental design at reasonable costs). Most microcosm studies are limited to primary, secondary and detrital trophic levels, and do not include species of economic importance, such as tuna or salmon; rather, it is often argued that the higher trophic levels depend on lower trophic level success.

It must be noted that the term "microcosm" is defined in different ways in each of the three following papers. Each paper must be read in the context of the author's definition. The differences are important and the definitions represent important assumptions concerning the properties of ecosystems.

Pritchard and Bourquin define a microcosm as "a calibrated laboratory simulation of a portion of an ecosystem in which intact environmental components, in as undisturbed a condition as possible, are enclosed within definable physical and chemical boundaries, under a standard set of laboratory conditions (controlled lighting, humidity, aeration, mixing, temperature, etc)." They strive for a level of complexity that cannot be readily duplicated in the laboratory by assembling component parts or by making composites of individuals. By so defining a microcosm as an excised portion of a natural community, they exclude the other two papers from consideration as examples of microcosm research. Many researchers believe that natural ecosystems have unknown but important properties which

were acquired during coevolution of the component organisms over geological time periods. They may not be willing to accept that assemblages of organisms that have not evolved together can fully exhibit ecosystem properties. Studies of naturally excised communities are best used as subsystems to study the properties of the parent community. Pritchard and Bourquin's purpose is very similar to that of mesocosm studies: they attempt to scale for water flow, temperature and other local parameters, and they attempt to relate their measurements back to the parent environment. Excised natural communities are generally too complex taxonomically to provide much of a simplification from the parent system. If the concern is with the fate of a chemical, it may be argued that the taxonomy is unimportant as long as it is typical of the parent environment.

Leffler considers his microcosms to be "naturally derived, generic aquatic microcosms" because the organisms have accommodated to the demands of being a self-sustaining community in his laboratory for several months. He views his microcosms as distinct ecosystems in their own right rather than site-specific simulations of particular natural systems. He considers them to function as generalized simulations of a large class of ecosystems. Although his organisms are seeded from a large and variable number of natural sources, and the medium is a chemically defined solution, he considers the mixture to exhibit ecosystem level properties if the microcosms function as homeostatic, self-sustaining ecosystems capable of existing through time periods of at least a year. For a microcosm to be generic, he suggests, a minimum level of taxonomic or functional groups must be present. Leffler hypothesizes that the exact species composition is unimportant for detecting ecosystem level impacts as long as certain key functional groups are represented. His systems allow the measurement of species level variables, but most of his effort has been on variables that represent an integration of processes and reflect the state of the ecosystem such as pH, eH and community metabolism.

Taub's paper presents yet a different microcosm approach, that of synthesizing or assembling a model ecosystem from organisms that represent important ecological roles in aquatic microcosms. She hypothesizes that the community structure, including the organisms and their initial densities, will be important determinants of the responses to pollution or other stresses; a mathematical model and experimental data support the hypothesis, which is in direct conflict with Leffler's hypothesis. The ability to determine the species composition of the microcosm allows the taxonomic complexity to be an experimental variable, and, given its simplicity relative to natural ecosystems, allows the species level properties to be monitored more easily. The untested hypothesis in this work is that by controlling the species assemblage and chemical properties of the medium, different researchers could obtain similar re-

sponses if they tested the same concentration of test chemical. The ability to reproduce ecological results would increase confidence in the use of ecological properties to evaluate the safety of new chemicals in the environment.

In discussions with other researchers, several brought up issues that had not been covered in these papers, for example, what effect would the source of microcosm material have on the results in a site-specific microcosm. Would material excised from a heavily polluted ecosystem degrade a pollutant faster or to a different degree than material excised from a pristine community? Dr. Leffler was asked if microcosms initiated in the summer would give the same results as those initiated in the winter; he thought that season would have little effect because his stock community was adapted to laboratory conditions.

There was considerable interest in the relationship between mesocosms and microcosms. Specifically, several researchers questioned whether the same information could be obtained less expensively in microcosms. Pritchard and Bourquin, Leffler and Taub all agreed that mesocosm research and microcosm research served different purposes, and that researchers were usually asking different questions. If a researcher wanted to have maximum confidence in extrapolating back to a specific, large scale environment, the mesocosm served to provide greater realism in that it allowed more large scale processes to be included in the system. If simplification was the goal, either to semi-isolate certain components to determine their importance in degradation (Pritchard and Bourquin), or to provide more easily analyzed ecosystems for test purposes (Leffler and Taub), then microcosms were more appropriate. In many cases, to formulate and test hypotheses, researchers would be likely to search for information in the natural ecosystem, in mesocosms and in microcosms of varying complexity.

While there is no absolute separation between a mesocosm and a microcosm, in practice most mesocosms are outdoors where temperature and light intensity are ambient for the parent community, and most mesocosm researchers have tried to realistically scale for the factors they think are most important in controlling ecological relationships, for example, mixing and settling rates in MERL (see mesocosm papers). Most microcosms are incubated in the laboratory and are subject to greater environmental control. Microcosms are generally smaller, and more replicates are used. However, there is considerable overlap; for example, the microcosms of Pritchard and Bourquin were scaled for several natural parameters, and they do not mention replicates. Indeed the parent community of their microcosm is an artificial stream at Monticello, Minn., which might be considered by some to be a natural community and by others a mesocosm.

There was great interest in the ability of microcosm results to predict natural ecosystem responses. Dr. Pritchard mentioned that

the microcosms were not used to predict the natural environment as much as to explain and provide quantitative estimates of processes that had first been observed in the natural ecosystem. Dr. Leffler responded that he had developed his microcosms to rank the relative toxicity of test chemicals, and not to predict specific natural ecosystems. Dr. Taub stated that the responses of some natural communities corresponded to the microcosm results, but that different communities would respond differently to the same stress, as in the case of the CEPEX spring and summer communities (cited in her paper).

The authors were asked why microcosm researchers were so conservative in their claims for what their research could accomplish. Why did they set criteria for their efforts such as field validation or interlaboratory calibration that were not standard in other environmental techniques? What kinds of microcosms could be recommended for use in the near future for pollution studies? Answers varied. There was some discussion on potential disagreement as to the best type of microcosm to use. Dr. Leffler stated that this is becoming less of a problem since there seems to be a convergence in techniques. The very process of designing microcosms, it was pointed out, makes the researcher aware of the restrictions and limitations of the microcosms. The differences between a beaker in the laboratory and the bay outside were very obvious. Each of the microcosms presented had been designed to answer a different type of question, and each speaker believed that the specific question had been addressed rather well by the system used. However, none of the microcosms would have been suitable to answer all questions. As to the types of microcosms that are available for immediate use, suitability would depend on the questions being asked.

A Perspective on the Role of Microcosms in Environmental Fate and Effects Assessments

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INTRODUCTION

Comprehensive environmental risk analysis for chemical pollution in aquatic ecosystems must eventually attempt to characterize the waste assimilative capacity of any particular environment; that is, the potential of any environment, from a quantitative standpoint, to accept a certain level of pollution without adverse effects. (The definition of waste assimilative capacity differs slightly depending on the application: Cairns 1981; Campbell 1981; Goldberg 1979). A characterization of this type, of course, requires accurate measurements of the rates and dynamics of both fate and effects processes, including their complex interactions. It also requires determinations of how toxic materials will alter these processes quantitatively. This is a significantly separate approach from the current bank of qualitative tests which lead to risk assessments based primarily on relative comparisons of fate and effects test results. Relative fate and effects testing, of which the single-species bioassays and routine biodegradability testing are examples, uses "worst-case" and "ranking" criteria to provide consensus measurements of potential adverse environmental effects. Despite the evident success in applying relative fate and effects testing to pollution problems, more attention needs to be directed toward the measurement of waste assimilative capacity.

This emphasis is clearly evident in the risk assessment approach EPA has taken (i.e., determination of "unreasonable risk") in order to meet various responsibilities under TSCA (Federal Register 1979). Although current toxicological methodologies concentrate on the effects of chemicals on a few test species or isolated populations, the realistic environmental concern is the effect of these

chemicals on natural communities and ecosystems, particularly as they relate to disruptions of the delicate network of interactions that characterize the function, structure and homeostasis of natural ecosystems. Acknowledging this concern, EPA, in conjunction with the Ecosystems Research Center at Cornell University, has recently produced a strategy document for implementing a more quantitative research program in ecosystem-level toxicology and environmental fate (Levin 1982).

A similar concern in the area of environmental fate of chemicals has focused attention on biodegradation studies. Since standard biodegradability or persistence testing procedures do not produce the environmentally relevant information necessary for many current types of risk analysis, strategies concerned with the determination of biodegradation rate constants and examination of the site-specific environmental factors that control biodegradation rates have to be developed and implemented (Haque 1980; Bourquin and Pritchard 1979; Maki et al. 1980).

Given this established need for a more quantitative approach to risk assessment in aquatic environments, our paper attempts to illustrate how microcosm studies interface with both waste assimilative capacity determinations (regardless of the approach taken or endpoints selected) and other less quantitative types of assessments. Problems and inconsistencies observed in the interpretation and application of microcosm results are discussed.

A microcosm study is defined here as an attempt to bring an intact, minimally disturbed piece of an ecosystem into the laboratory for study in its natural state. The confinement of that piece of the ecosystem within definable physical and chemical boundaries under a standard set of experimental conditions (controlled lighting, humidity, aeration, mixing temperature, etc.) constitutes the microcosm system itself. The microcosm is theoretically meant to harbor a level of complexity that (1) is representative of that portion of the ecosystem from which it was sampled and (2) cannot be readily duplicated in the laboratory by assembling component parts or by making composites of individuals. It is considered an analog to the field, therefore, studies with a microcosm are surrogates for actual field studies. The simulation capacity of a microcosm is determined by calibration with the field, using criteria established through experimentation and the question to be addressed by research. Ideally this should be done by using measurements that reflect macroscopic, system-level properties that result from the integrative coupling among the biological, chemical and physical properties of an ecosystem. Some success has been apparent in this regard with the measurements of integrative biochemical properties, such as total CO_2 flux (Ausmus et al. 1978; Van Voris et al. 1980), nutrient cycling (Jackson et al. 1978; Jassby et al. 1977), pH-Eh or dissolved oxygen relationship (Waide et al. 1980; Giddings and Eddlemon 1978). A more extensive discussion of field calibration is given by Pritchard (1981).

THE ROLE OF MICROCOSMS IN ENVIRONMENTAL RISK ANALYSIS

The role of microcosm studies in environmental risk analysis is quite explicit, given the definition described above. A schematic representation of this role is shown in Figure 1. Risk analysis is divided into two categories: qualitative assessments and quantitative assessments. Quantitative assessments are synonymous with determinations of waste assimilative capacity. Qualitative assessments establish risk by comparing test information (fate or effects) for a chemical that is ranked within a data base against a large array of previously tested chemicals using the same, often highly standardized test method. The emphasis, in this latter case, is on screening large numbers of chemicals for information on their fate and effects. This approach requires consistency in test results and expediency in test method implementation.

The test methods are further divided into three levels of organization: (1) single species tests, such as those used in bioassays and certain biodegradation studies; (2) multispecies tests which use inocula (generally isolated populations) from the field in an experimentally convenient manner and generally ignore controlling factors associated with the physical integrity (intactness) of the field samples, natural population interactions, system homeostasis and trophic and biological structure; (3) microcosm studies (tests) which encompass all of the controlling factors not considered in the multispecies test.

The focus of Figure 1 is the flow of information (indicated by arrows). Single species tests and many multispecies tests support the qualitative or screening assessment, as would be expected. Many biodegradation studies are examples of multispecies tests in that they use inocula of natural water or sediment in a shake flask system. Since little regard is given to the natural physical integrity of these microbial communities, the results can be used only for qualitative assessments.

The community settling test of Hansen and Tagatz (1980) is a good example of a multispecies test used for effect studies. In this case, azoic sand exposed to a particular concentration of toxicant is colonized by invertebrate larvae in unfiltered sea water flowing over the sand. Adverse effects are indicated by statistically significant changes in population size and diversity relative to the unexposed controls. Only a qualitative assessment of toxic effects can be made; very little can be deduced about effects on population dynamics or on the permanent or long term damage to natural benthic communities.

Conceptually, multispecies tests are also the basis for quantitative assessments and predictions when used in conjuction with microcosm studies or actual field studies (depicted by the converging arrows in Figure 1). The microcosm serves as the interpreta-

tional tool for integrating information from multispecies test systems into predictions of environmental fate and effects. It, in fact, provides the basis for extrapolating multispecies test results to the field. Once this basis is established, the multispecies test for the particular application being considered becomes the principal source of information needed for all subsequent quantitative assessments. The microcosm results then are of little predictive value by themselves. Their usefulness in quantitative assessments is a direct function of the availability and experimental design of good multispecies tests.

THE ROLE OF MICROCOSMS IN QUALITATIVE ASSESSMENTS

To discuss the role of microcosms in qualitative assessments, it is necessary to ask how the data from a microcosm study can be used for screening purposes? The term "screening microcosms" originated from the fear that simplified fate tests, such as the BOD or river die-away methods and single species effects tests, were not comprehensively revealing all potential problems brought on by contamination of an ecosystem with a particular pollutant (Harris 1980). Undoubtedly, these tests lacked complexity. Microcosms seemed superficially capable of filling that void. Two assumptions are crucial to the screening microcosm approach. First, ecosystems as biogeochemical units have measurable properties (Schindler et al. 1980). These properties or ecosystem-level behaviors reflect the integration of many internal processes and components and their interactions. The ecosystem-level behaviors cannot be inferred or predicted from measurements on isolated components in the laboratory (Weiss 1971). Since microcosms are essentially analogs to certain pieces or parts of an ecosystem, they should also have measurable ecosystem-level properties.

Second, measurements of these integrative properties of natural ecosystems or microcosms possess a degree of consistency and reproducibility which allows toxic effects of a chemical to be distinguished from background noise and allows the effect of these properties on the fate of the chemical to be investigated.

Given these assumptions, a screening program can be set up in which the same type of microcosm is dosed with an array of chemicals and the resulting fate and effects information compared on a relative basis much like the assessment procedures used for toxicity and biodegradability information. Standardization of the microcosm study eventually will be required under this type of program. There is no need to extrapolate quantitatively the results to the field; in fact, it is not expedient in light of screening objectives because additional information will be required for extrapolation.

Undeniably, there would appear to be a role for screening microcosms because of the current interest in ecosystem-level fate

and effects studies (Levin 1982). However, the microcosms must be used correctly and with good foresight. Two criteria are proposed for the development and use of screening microcosms:

1. Care should be taken to assure that the conclusions or risk assessments obtained from a screening microcosm study cannot be obtained in some simpler way. For example, if a chemical is found to biodegrade more readily in a microcosm containing sediment, it may be because the system contains such a large amount of bacteria-laden sediment. The simpler shake flask studies with sediment-water slurries at different sediment concentrations, therefore, may provide the same information as the microcosm. Likewise, if in a microcosm effects study a toxic response by a community can be attributed to the response of a single species, then potentially a bioassay can be developed with that species and the same protective effect on the community realized through the single-species test. In both cases, the microcosm test becomes superfluous. The applicability of synthetic communities studies, pioneered by Metcalf et al. (1971), Gillette and Gile (1976) and Isensee et al. (1973), as potential screening microcosms has been criticized (Pritchard 1981; Branson 1978) because, in many cases, much of the same fate and effects information could be obtained in a clearer form with simpler tests.

   To be useful, the screening microcosm must provide insight into phenomena or events which are not readily examined or demonstrated in other previously established tests. It must be unique enough, for example, to be included in the "minimum ecological data set" of the water quality criteria program (Federal Register 1980). The soil core microcosms developed by Van Voris et al. (1980) and Jackson et al. (1977) represent a unique screening microcosm. These systems permit the screening of chemicals for their interference with the leaching of inorganic nutrient from the soil. It is not clear exactly which biotic components in soil control the leaching process, but their sensitivity to toxicants is readily demonstrable. No other existing tests provide information on leaching and there are few other good tests for examining soil processes. At some future time however, the sensitive factor(s) that controls the leaching process may be identified, thus providing the basis for a new bioassay which could then supplant the microcosm study.
2. Part of the concept of water quality criteria and its use of screening data is to establish MATC (maximum acceptable toxicant concentration) values based on the assumption that if the most sensitive species are protected, all other

species will be protected. If the results from a screening microcosm, therefore, are not unique relative to existing simpler tests (i.e., not routinely part of the minimum ecological data set) and if they are evaluated in the same qualitative manner as single-species test information, they then must indicate a greater sensitivity to a toxicant than results from other well established tests. For example, if a toxicant interferes with the metabolism of a special algal community in a microcosm at 10 times the concentration at which it reduces fish growth, then, from a regulatory point of view, an MATC to protect the fish (the assumed critical species in the environment) would also protect the algae. Thus, for relative ranking and worst-case analyses, a screening microcosm must demonstrate the existence of a more sensitive component of the ecosystem.

LIMIT TO THE DEGREE OF ENVIRONMENTAL SIMULATION IN MICROCOSMS

The means by which microcosms can aid in the extrapolation of laboratory data to the field or in determinations of waste assimilative capacity should be considered. Figure 1 indicates that microcosm results do not feed directly into waste assimilative capacity determinations; that is, extrapolation to field situations occurs in a more indirect manner. The reason for this approach lies in the fact that there are specific limits to the degree of environmental simulation possible in a microcosm and consequently, limitations on the extrapolation of microcosm data. Our focus is on simulation of the field in a microcosm, rather than duplication, and there are consequently certain limits to the exactness that can be expected. Thus, the deviation from the norm and the degree of the acceptable diversion will be a function of the experimental question posed and the intended applications. Because of these limits, it is very difficult to make quantitative or one-on-one extrapolations of a microcosm result to the field. It is entirely unreasonable to assume, for example, that one can multiply a microcosm result by some type of "expansion" factor and then equate it with a field situation. At best, the microcosm is a qualitative indicator of phenomena, rates and interactions that could occur in nature. The simulation capability of a microcosm merely enhances our confidence in the fact that the phenomena and reactions observed in the microcosm will more than likely occur in nature. Microcosm results alone disclose very little about how to predict quantitatively the dynamics of these phenomena and interactions in nature, particularly when considering the temporal and spatial variations in environmental parameters typical of many ecosystems. Predictions of dynamic events in nature will undoubtedly come from mathematical model-

ing efforts in which data from the analysis of processes and component interactions (multispecies test) are used. The key consideration is that a microcosm study provides the conceptual and experimental framework for developing a testable hypothesis and for recognizing and investigating the frequency distribution of the processes and component interactions operating within an ecosystem.

Several important factors can be cited in support of this approach. First, a microcosm, by design, isolates a community of some level of complexity that is representative of a portion of an ecosystem. Since the fundamental thermodynamic basis of ecosystems involves the generation of energy from the oxidation of degradable organic materials to immobilize inorganic nutrients produced from geochemical events external to the ecosystem, ecosystems are viewed as biogeochemical units that reflect the integration of a large variety of metabolic networks. A microcosm, therefore, will house a certain proportion of these networks but not all. This is shown diagrammatically in Figure 2. The microcosm design and the assumptions applied to its operation will determine the extent of the biogeochemistry of an ecosystem that is captured in a microcosm. Thus, a particular microcosm may or may not harbor a great deal of the biogeochemistry of an ecosystem (size of the microcosm circles), and it may or may not reflect the true dynamics and interactions of an ecosystem (overlap into the ecosystem circle). In most microcosm studies, the circles will be large, indicating the presence of many metabolic networks found in nature, but will have lesser degrees of overlap, indicating limitations or exaggeration of the dynamics of the networks in the microcosm.

Rarely, if ever, would a situation occur as shown in Figure 2B, in which a single community or microcosm would account qualitatively and quantitatively for all the biogeochemistry of the ecosystem. By establishing arbitrary boundary conditions of an environmental sample, as often effected in a microcosm, the biogeochemistry becomes uncoupled from the ecosystem. Although this uncoupling may not eliminate any critical components or change the number and types of interactions, it could greatly upset the rates at which these interactions occur. The usefulness of a microcosm is not impaired but if the controlling variables for the rates of these interactions are not understood, no attempt should be made to extrapolate the microcosm result quantitatively to the field.

A common example of the almost unavoidable uncoupling brought on by a microcosm study is the exclusion of higher trophic levels, such as herbivores and carnivores, from a microcosm. Several studies have shown that the effects of grazing on primary productivity may be substantial. Reductions in producer standing crops by a herbivore have caused increases in primary productivity (Cooper 1973), and deposit-feeding amphipods have been shown to affect the productivity of sediment microflora significantly (Hargrave 1970). Primary productivity of diatom communities is

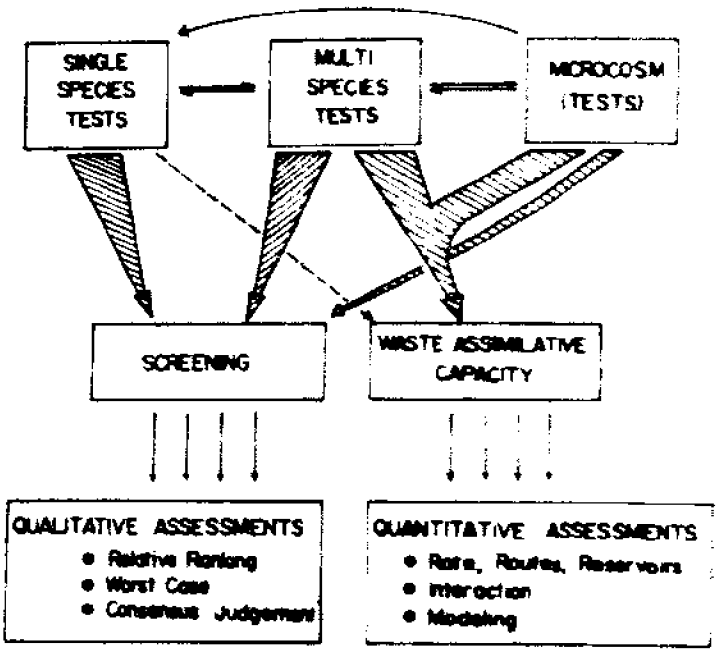

Figure 1. Schematic diagram of possible information flow from laboratory test systems to risk assessments. Differences between tests are explained in the text. Thickness of the arrows indicates amount of information flow. Cross hatched areas represent current flow of information.

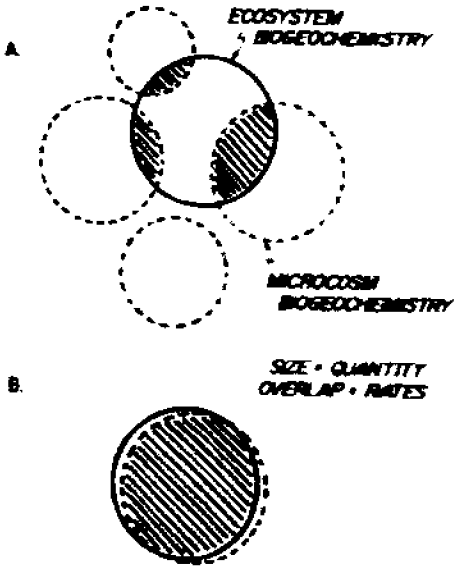

Figure 2. Schematic diagram portraying possible interrelationships between the biogeochemistry of an ecosystem and various microcosms. Size of the microcosm circles indicates extent of the ecosystem's biogeochemistry housed therein. Overlap of the circles indicates similarities in rates of biogeochemical processes in micrososms; the ecosystems A and B represent possible examples of these interrelationships.

known to be increased by crayfish grazing (Flint and Goldman 1975). With these types of potential uncouplings, it is precarious to extrapolate a microcosm result to the field.

Further evidence that a microcosm is unlikely to represent 100% of the ecosystem's biochemistry can be found in the spatial, and sometimes the temporal, assumption made in physically modeling a particular ecosystem (for example, the cross section of an estuarine ecosystem as shown in Figure 3). An assumption in modeling part of the ecosystem in a microcosm may be to establish a sediment/water system representing the average sediment surface area to water volume ratio of the bay (dotted line in figure). This assumption may be tenable for some studies. However, from the standpoint of exposure assessment in which a chemical may biodegrade much more rapidly in sediment than in water, it is not tenable. This is because the chemical will disappear at a faster rate in shallower waters where its encounter with sediment will be more frequent. It is a hydrodynamic problem in assessing fate under these conditions; that is, what is the expected distribution of the chemical among compartments with varying degrees of biodegradation potential? The microcosm should not provide information on overall distribution; this is the predictive job of a mathematical model. Instead, the microcosm should provide a broad view of the biodegradation potential of each component and the possible interactions contained therein.

Further, biodegradation may be more rapid in and around rooted macrophytes such as those found in grass bed areas and adjoining salt marshes. Higher concentrations and activities of bacteria on the plant leaf surfaces, in the root rhizosphere and in the associated detrital material may contribute significantly to the biodegradation of certain chemicals. The exposure of the chemical to these areas would again be dependent on the hydrodynamics of the estuarine system, and the exposure can be substantial. A microcosm designed to model just the more geographically dominant neritic component of the estuarine system would ignore this contribution from the plants. If this type of microcosm were used to study the fate of a chemical in an estuary, an erroneous estimation of the chemical's fate could be obtained. Great caution, therefore, should be exercised in extrapolating microcosm results directly to the field. The same argument holds true in many cases for toxic effect studies as well.

THE ROLE OF MICROCOSMS IN QUANTITATIVE ASSESSMENTS

Given these limitations on extrapolability, how, in fact, is the microcosm effective in ^{2}waste assimilative capacity determinations? The key may be found in studying a microcosm within the same theoretical framework that has been formulated for studying

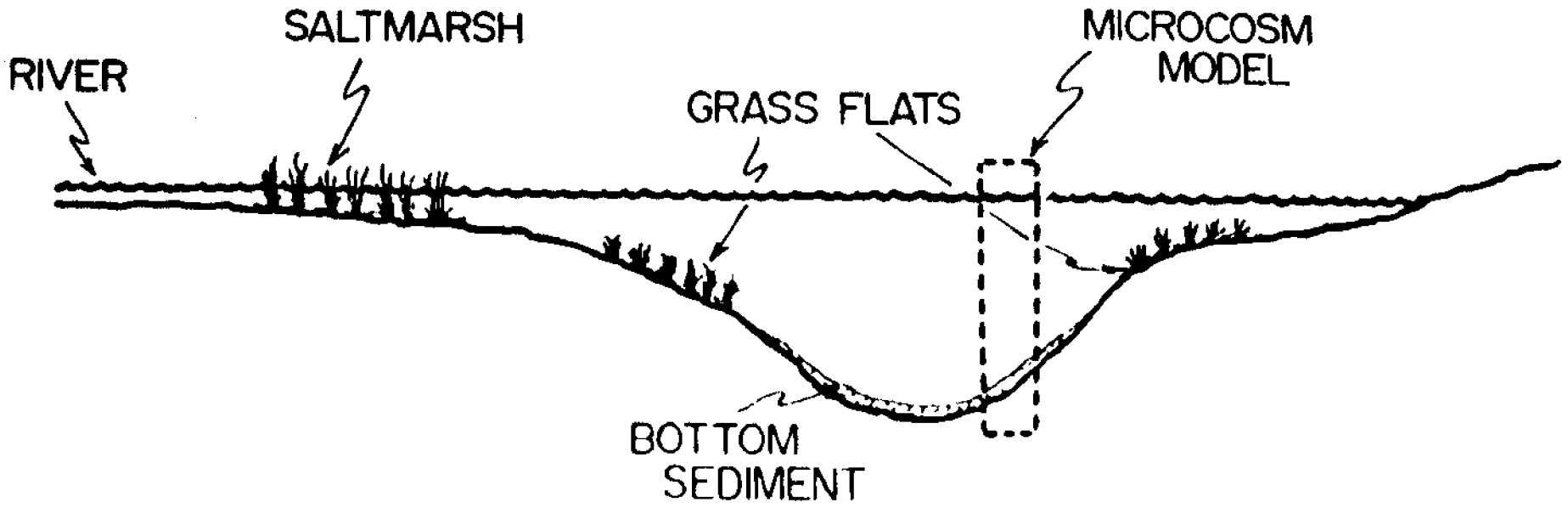

Figure 3. Diagrammatic cross section of a typical estuarine ecosystem. Dotted line box represents portion of the ecosystem that could be modeled in a microcosm.

a natural ecosystem, particularly in reference to the concept of studying ecology by combining holism with reductionism (Odum 1977). An ecosystem, according to Heath (1979) is an array of components interacting cooperatively to form a complex unit through which energy, matter and information may flow. The behaviors of the individual components of a system are constrained and coordinated through the network design of the system. Some of the properties of the system arise not from the components themselves but from the specific set of interactions within the system. Thus, it is not possible to characterize a system only from a knowledge of the component parts. Since a reductionist approach to ecology, by its very nature of analysis, causes a disruption of this network design or set of interactions, it must be combined with a study that preserves those interactions. As Schindler et al. (1980) stated, the attempt to understand the "state-of-the-whole" should thus be a central focus in all ecosystem studies. Only after this state has been assessed or measured should we attempt to fractionate the system into component subsystems. Indeed, the state of the whole should guide our definition of subsystems, rather than a priori definition of subsystems delimiting our recognition of properties of the whole.

Microcosms are considered by many investigators (Schindler et al. 1980; Hill and Weigert 1980; Heath 1979) to be a means of studying the complexities of natural ecosystems in the laboratory. Although the microcosm is itself a component of the ecosystem because of its uncoupling from the field, as noted above, it is nonetheless a complex system that will contain much of the "network design" and system-level interaction of a natural ecosystem.

For the purposes of extrapolation of laboratory data to the field the concepts of holism and reductionism must be combined. The state of the whole should be established in the laboratory, using a microcosm; then, through a process of manipulation and disassemblage of that whole into subsystems, the interworkings of the ecosystem can be understood. The microcosm then becomes a foundation around which to clarify the interactions and the rate-dependent processes of the subsystems. The subsystems are basically synonymous with the multispecies test represented in Figure 1. The relation of these subsystems to the whole is the critical experimental exercise because the subsystems most likely will: (a) generate new experimental questions and (b) produce insight into rates and system dynamics. The rates are crucial to extrapolation whether applied to toxic effects studies (rates of recruitment, rates of growth, reproductive rates, rates of metabolism, rates of grazing, rates of feeding, etc.) or to fate studies (rates of transport, rates of biodegradation, rates of photolysis, rates of bioaccumulation, rates of sorption, etc.). If rates can be studied, and the factors that control them investigated relative to the complex state of a microcosm state-of-the-whole, then predictive ecosys-

tem mathematical models can be developed and risk analysis performed. A specific fate study follows to illustrate how the microcosms can be manipulated and disassembled into subsystems (multispecies tests) to provide quantitative information on the factors controlling the fate of a chemical in an aquatic system, and subsequently to provide data for a mathematical model.

EXAMPLES OF THE QUANTITATIVE USE OF MICROCOSMS

The dosing with the toxicant p-cresol of several man-made stream channels (the channels are located in Monticello, Minn., and are operated by the EPA Environmental Research Lab at Duluth, Minn., and Michigan State University [Cooper et al. 1981]) provided the opportunity to determine how accurately the fate of p-cresol could be predicted in the field streams. A microcosm study involving the fate of p-cresol was conducted to provide a picture of the "state-of-the-whole." The microcosm design is shown in Figure 4. Intact portions of the riffle area and pool area in the streams were transported to the microcosms in trays (36 cm diam, 10 cm high). A recirculation device completely mixed the water column (80 L) and produced turbulence similar to that in the stream. The stream channel, which was dosed with 8 ppm p-cresol, was actually a closed recirculating loop; consequently the microcosms, as a riffle-pool pair, could be maintained as closed systems. Since the stream was autotrophically based, diurnal dissolved oxygen profiles were used to calibrate the microcosm with the field. Invertebrate populations in the rocks and sediment were also used as calibration points. In both cases good simulation of the stream was obtained in the microcosm (Pritchard, unpublished results).

The microcosms (riffle-pool pair) were spiked with 8 ppm p-cresol, and the disappearance of the parent compound was followed, using high pressure liquid chromatograph analysis. The result (Figure 5) is representative of the integration and interaction of fate processes in the system; the rate of disappearance of p-cresol is controlled by some unknown number of factors whose integration produces the microcosm result. No loss of p-cresol occurred in azoic microcosms.

It is important to point out that this microcosm result has little predictive value of its own. Since the microcosm is not a duplicate of the stream, the results have no quantitative bearing on the rate of disappearance of p-cresol in the stream channel; therefore, only a qualitative judgment can be made of its fate in the stream. However, a quantitative extrapolation of laboratory data to the field is possible if the controlling variables which affect the compound's fate in the microcosm can be demonstrated. For this particular chemical, kinetic information about its biodegradation (specifically, rate constants) is needed.

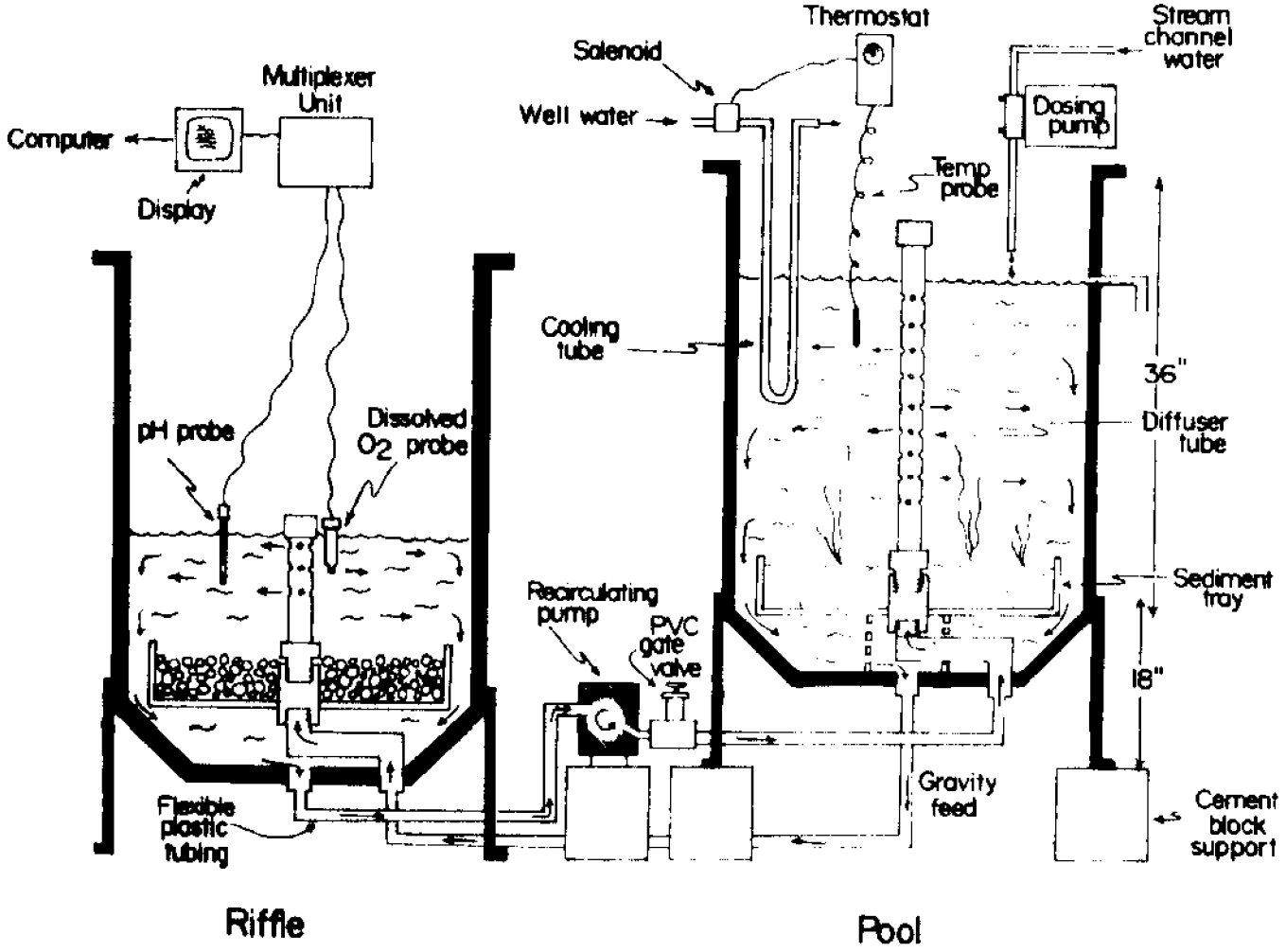

Figure 4. Schematic diagram of microcosms used to model the riffle and pool sections of the Monticello stream channels.

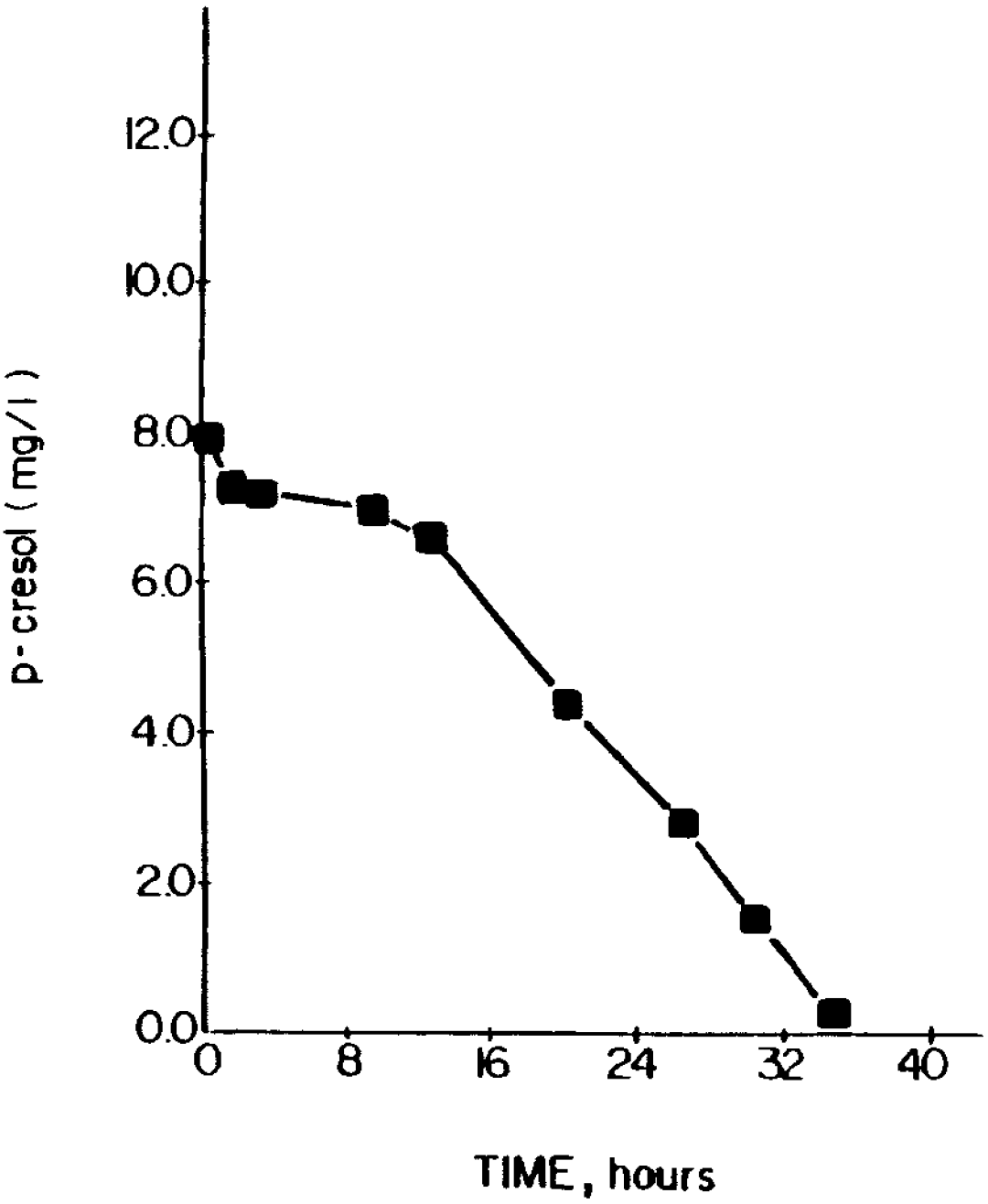

Figure 5. Rate of disappearance of p-cresol in a riffle-pool microcosm. Microcosm was operated as closed, continuously mixed reactor.

The manipulation of the microcosm to understand more clearly the biological factors controlling the fate of p-cresol were undertaken. Our study compared the rate of degradation of p-cresol in microcosms containing only riffles and only pools. Figure 6 shows that the all-pool microcosm degraded p-cresol much slower than the all-riffle microcosm. The degradation rate in the pool-riffle microcosm was intermediate. Clearly, the manipulation indicated that the stream riffle areas were quite important in the biodegradation of p-cresol. Since respiking of the microcosms produced similar degradation rates in all systems (Figure 6), it would appear that the contribution from the riffles is important in initiating degradation of p-cresol.

It was necessary to disassemble the state-of-the-whole into subsystems or multispecies tests to obtain more quantitative details. In microbial systems, mixed microbial populations associated with water, sediment, rock surfaces and plant surfaces can be readily separated from the microcosm and studied in shake flasks to determine their p-cresol biodegradation potential. Figure 7 shows the degradation rates of p-cresol in shake flasks containing components from the stream. Bacteria associated with rock surfaces were the most active degraders, whereas bacteria associated with the water were the least active.

The important consequence of these subsystem experiments is that degradation per unit mass, or rate constants, can be obtained; that is, rates per liter of water, rates per rock, rates per gram of sediment, etc. The individual contribution of each of these rates can be established by determining the combination and integration required to simulate the microcosm results. If the approximate mass is known for each component in the field stream, and this rate information can be integrated, the rate of disappearance of p-cresol in the streams can be predicted. A mathematical model greatly facilitates this exercise. Preliminary efforts in this regard for the p-cresol studies have shown that the subsystem or multispecies test information is sufficient to predict results in the stream (as verified by its sufficiency to predict fate in the microcosms), and that the key controlling variables for the fate of p-cresol can be dissected out by examination of subsystems.

It is interesting that knowledge of the activities of pure cultures (single species) of p-cresol-degrading bacteria, although readily isolated from the stream or microcosm, was insufficient to predict the rate of biodegradation of p-cresol in the stream. These isolated organisms behave much differently when separated from their natural assemblages. Invaluable qualitative insight into microbial biodegradation mechanisms of p-cresol and the relationship of chemical structure to biodegradability, however, has been gained using pure cultures of bacteria (Dagley and Patel 1957; Hopper and Taylor 1975; Buswell 1975).

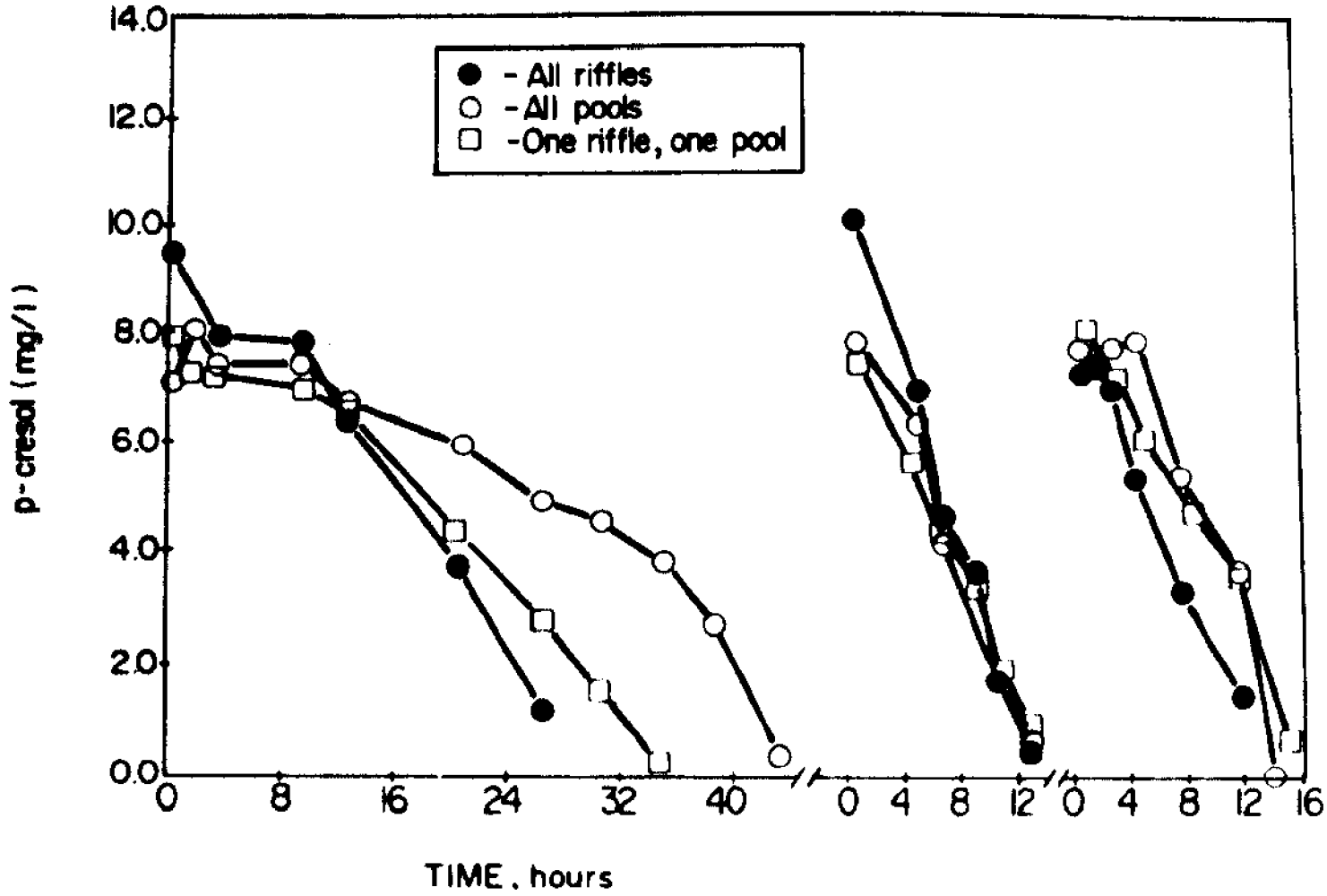

Figure 6. Rate of disappearance of p-cresol from three types of microcosm sets: all riffles (●); all pools (o); one riffle/one pool (□). At hours 45 and 60 all three microcosms were respiked with p-cresol.

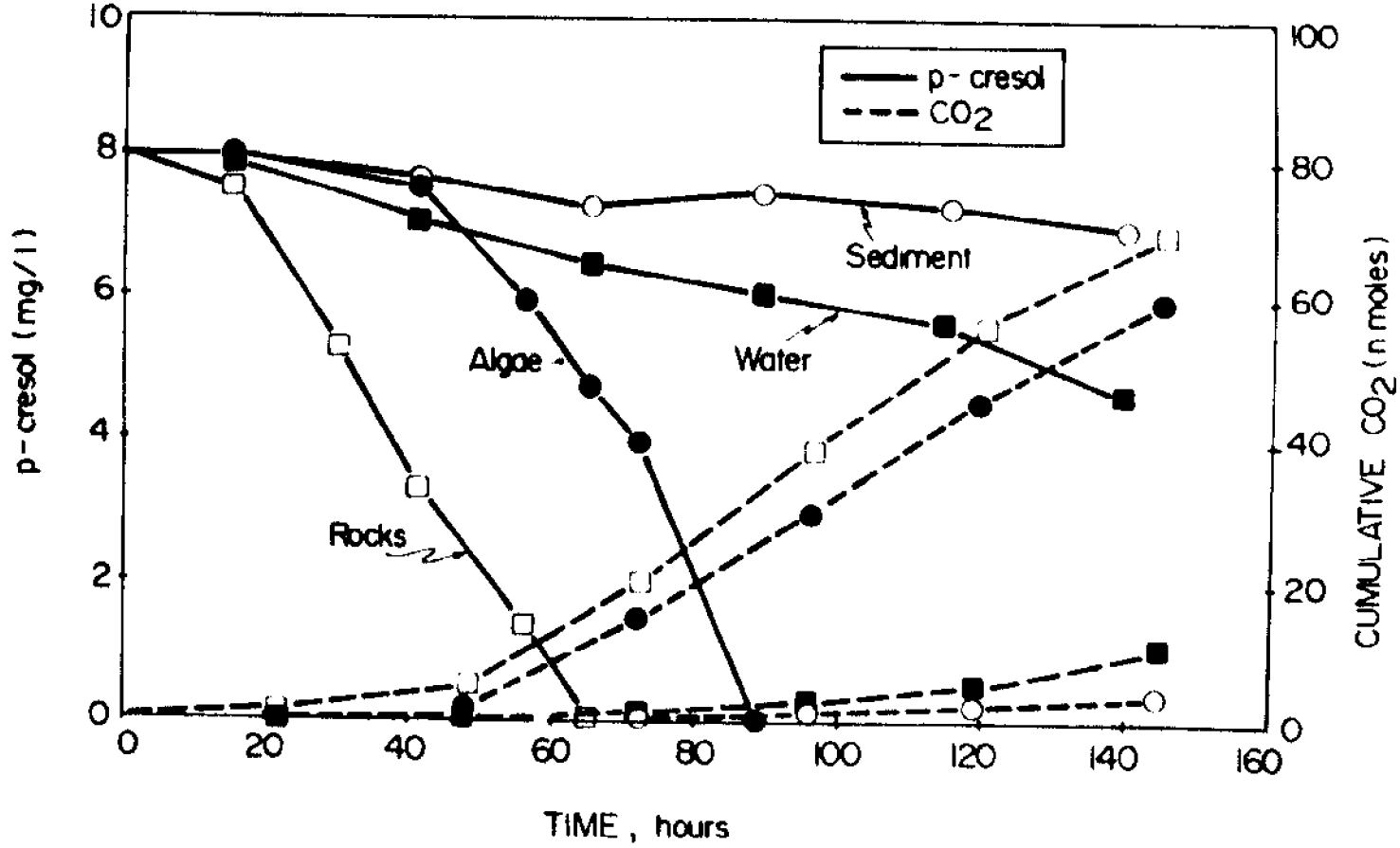

Figure 7. Disappearance (solid lines) and mineralization (dotted lines) rates of p-cresol in shake flask systems containing different components from the stream channels. For algae (Elodea 162 mg wet wt. per liter), sediment (340 mg wet wt. per liter) and rocks (seven, 1.4 mg dry wt. of periphyton per rock), components were washed three times with sterile stream water and then incubated in sterile stream water supplemented with p-cresol.

Single-species tests are excellent and invaluable qualitative measures of toxic effects, but provide inadequate sources of information on which to predict dynamic consequences of toxicant stress, particularly at sublethal concentrations. An approach as outlined for a fate assessment, that is, starting with the "state-of-the-whole" and working toward simpler subsystems, should be undertaken to develop quantitative measures of toxic effects as required for determinations of waste assimilative capacity. The ease of working with natural assemblages of microorganisms in the laboratory obviously makes this approach useful. Although it is more difficult to perform toxicological studies on natural assemblages of plants and animals in the laboratory, it is nonetheless an effort that should be seriously considered in the future.

A potential example of this approach to effect studies can be projected with the benthic community settling system described by Hansen and Tagatz (1980). By working with field colonized sediment, the same experimental system can be developed as a microcosm. Structural and functional aspects of the benthic community can be measured and calibrated with the field. Good simulation in community structure has been shown by Flint et al. (1982). This laboratory microcosm thus provides a "state-of-the-whole" which can be perturbed with a toxicant. Manipulation of the microcosm and the development of subsystems can be carried out to determine the population dynamics of the benthic community and perhaps to pinpoint the toxicant response both at the structural and functional levels. Experiments that examine variations in organic loading, recruitment, functional compensation, resilience to toxicant stress, changes in dominance patterns, etc., can all be attempted to understand the dynamic aspects which lead to the "state-of-the-whole." Cores could also be taken from the microcosms to develop subsystems for further testing, particularly functional measures such as dissolved oxygen patterns and Eh profiles (Flint et al. 1982). Although the cost and the problem of trying to study complex ecosystem processes in small containers are initially greater than single-species tests, the microcosm approach and acquisition of kinetic information about fate and effect responses should ultimately lead us to a more reliable determination of waste assimilative capacity.

REFERENCES

Ausmus, B.S., G.J. Dodson and D.R. Jackson. 1978. Behavior of heavy metals in forest microcosms. III. Effects on litter-soil carbon metabolism. Water Air Soil Pollut. 10: 19-26.

Bourquin, A.W. and P.H. Pritchard. 1979. Proceedings of the Workshop: Microbial Degradation of Pollutants in Marine Environments, Pensacola Beach, Florida, April 9-14, 1978. EPA-600/9-79-012, U.S. Environmental Protection Agency, Gulf Breeze, Fla.

Branson, D.R. 1978. Predicting the fate of chemicals in the aquatic environment from laboratory data. In: J. Cairns, Jr., K.L. Dickson and A.W. Maki (eds.), Estimating the Hazard of Chemical Substances to Aquatic Life, pp. 55-70. ASTM STP 657, American Society for Testing and Materials, Philadelphia, Pa.

Buswell, J.A. 1975. Metabolism of phenol and cresols by Bacillus stearothermophilus. J. Bacteriol. 124: 1077-1083.

Cairns, J. 1981. Biological Monitoring. Part VI - Future Needs. Water Res. 15: 941-952.

Campbell, I.C. 1981. A critique of assimilative capacity. J. Water Poll. Control Fed., 53: 604-606.

Cooper, D.C. 1973. Enhancement of net primary productivity by herbivore grazing in aquatic laboratory microcosms. Limnol. Oceanogr. 18: 31-37.

Cooper, W., R. Boling and J. Stout. 1981. Evaluation of models for a toxicant stressed stream ecosystem. Annual Report to EPA Grant No. R807555010, Environmental Research Laboratory, Duluth, Minn.

Dagley, S. and M.D. Patel. 1957. Oxidation of p-cresol and related compounds by a Pseudomonas. Biochem. J. 66: 227-233.

Federal Register. 1979. Environmental Protection Agency, Toxic Substances Control: Discussion of premanufacture testing policy and technical issues--request for comment. Fed. Regist. 44(53): 19240.

Federal Register. 1980. Guidelines for deriving water quality criteria for the protection of aquatic life and its uses. Appendix B. Fed. Regist. 45(231): 79341-79349.

Flint, R.W. and C.R. Goldman. 1975. The effects of a benthic grazer on the primary productivity of the littoral zone of Lake Tahoe. Limnol. Oceanogr. 20: 935-944.

Flint, R.W., T.W. Duke and R.D. Kalke. 1982. Benthos investigations: Sediment boxes or natural bottom. Bull. Environ. Contam. Toxicol. 28: 257-265.

Giddings, J. and G.K. Eddlemon. 1978. Photosynthesis/respiration ratios in aquatic microcosms under arsenic stress. Water Air Soil Pollut. 9: 107-212.

Gillett, J.W. and J.D. Gile. 1976. Pesticide fate in terrestrial laboratory ecosystems. Int. J. Environ. Stud. 10: 15-22.

Goldberg, E. 1979. Assimilative capacity of U.S. Coastal Waters for pollutants. Proceeding of a workshop. Crystal Mountain, Wa., 7/29-8/3, 1979 NOAA Environ. Res. Lab., Boulder, Colo.

Hansen, D.J. and M.E. Tagatz. 1980. A laboratory test for assessing impacts on developing communities of benthic estuarine organisms. In: J.G. Eaton, P.R. Parrish and A.C. Hendricks (eds.), Aquatic Toxicology, pp. 40-57. ASTM STP 707, American Society for Testing and Materials, Philadelphia, Pa.

Haque, R. 1980. Transport and fate of toxic chemicals in the environment. U.S. EPA Workshop Proceedings, Norfolk, Virginia, Dec. 17-10, 1980. U.S. Environmental Protection Agency, Washington, D.C.

Hargrave, B.T. 1970. The effect of a deposit-feeding amphipod on the metabolism of benthic microflora. Limnol. Oceanogr. 15: 21-30.

Harris, W.F. 1980. Microcosms as potential screening tools for evaluating transport and effects of toxic substances. EPA-600/3-80-092, U.S. Environmental Protection Agency, Washington, D.C.

Heath, R.T. 1979. Holistic study of an aquatic microcosm: Theoretical and practical implications. Int. J. Environ. Stud. 13: 87-93.

Hill, J. and R.G. Wiegert. 1980. Microcosms in ecological modeling. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp. 138-163. (Conf-781101, National Technical Information Service, Springfield, Va.)

Hopper, D.J. and D.G. Taylor. 1975. Pathways for the degradation of m-cresol and p-cresol by Pseudomonas putida. J. Bacteriol. 126: 1-6.

Isensee, A.R., P.C. Kearney, E.A. Woolson, G.E. Jones and V.P. Williams. 1973. Distribution of alkyl arsenicals in model ecosystem. Environ. Sci. Technol. 7: 841-845.

Jackson, D.R., C.D. Washburne and B.S. Ausmus. 1977. Loss of Ca and NO_3-N from terrestrial microcosms as an indicator of soil pollution. Water Air Soil Pollut. 8: 279-284.

Jackson, D.R., W.J. Selvidge and B. Ausmus. 1978. Behavior of heavy metals in forest microcosms: II. Effects on nutrient cycling processes. Air, Water Soil Pollut. 10: 13-18.

Jassby, A., M. Dudzik, J. Rees, E. Lapan, D. Levy and J. Harte. 1977. Production cycles in aquatic microcosms. Research Reporting Series, EPA 600/7-77-077, U.S. Environmental Protection Agency, Washington, D.C.

Levin, S.A. 1982. New Perspectives in Ecotoxicology Workshop Report. Ecosystems Research Center, Cornell University, Ithaca, N.Y.

Maki, A.W., K.L. Dickson and J. Cairns. 1980. Biotransformation and the fate of chemicals in the aquatic environment. In: Workshop Proceedings, Pellston, Mich., Aug. 14-18, 1980. American Society of Microbiology, Washington, D.C.

Metcalf, R.L., G.K. Sangha and I.P. Kapoor. 1971. Model ecosystem for the evaluation of pesticide biodegradability and ecological magnification. Environ. Sci. Technol. 5: 709-713.

Odum, E.P. 1977. The emergence of ecology as a new integrative discipline. Science 195: 1289-1293.

Pritchard, P.H. 1981. Model ecosystems. In: R.A. Conway (ed.), Environmental Risk Analysis for Chemicals, pp. 257-353. Van Nostrand Reinhold Co., New York.

Schindler, J.E., J.B. Waide, M.C. Waldron, J.J. Hains, S.P. Schreiner, M.L. Freedman, S.L. Benz, D.R. Pattigrew, L.A. Schissel and P.J. Clark. 1980. A microcosm approach to the study of biogeochemical systems: Theoretical rationale. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp. 192-203. (CONF-781101, National Technical Information Service, Springfield, Va.)

Van Voris, P., R.V. O'Neill, W.R. Emanuel and H.H. Shugart. 1980. Functional complexity and ecosystem stability. Ecology 61: 1352-1360.

Waide, J.B., J.E. Schindler, M.C. Waldron, J.J. Hains, S.P. Schreiner, M.L. Freedman, S.L. Benz, D.R. Pettigrew, L.A. Schissel and J.P. Clark. 1980. A microcosm approach to the study of biogeochemical systems. 2. Responses of aquatic laboratory microcosms to physical, chemical and biological perturbations. In: J.B. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp. 204-233. (CONF-781101, National Technical Information Service, Springfield, Va.)

Weiss, P.A. 1971. The basic concept of hierarchic systems. In: P.A. Weiss (ed.), Hierarchically Organized Systems in Theory and Practice, pp. 1-43. Hafner Press, New York.

The Use of Self-Selected, Generic Aquatic Microcosms for Pollution Effects Assessment

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INTRODUCTION

Microcosms have been the subject of much discussion, especially in terms of assessing ecosystem-level effects of toxic chemicals (Giesy 1980; Hammons 1981b). Chemical induced alteration of properties such as autotrophic and heterotrophic activity, biogeochemical cycling and taxonomic composition generally have been neglected in most chemical assessment tests. While such testing could be done most realistically on intact natural ecosystems, it is not economically and logistically feasible to field test even 10% of the more than 45,000 chemicals currently produced in this country. Microcosms offer advantages of short time scales, small physical size, replicability, reproducibility and no contamination of the environment. They are relatively inexpensive to use and may be standardized to produce qualitative predictions regarding potential environmental impacts.

Microcosms have been defined as "small living models of ecosystem processes" (Leffler 1980). This definition is most applicable to "generic" microcosms. The major advantage of such microcosms is not that they simulate any particular ecosystem, but that they provide insights into general properties characteristic of many ecosystems. It should be recognized that the concept of simulation refers to a continuum from very general to highly site-specific situations. Because of the size constraints inherent in a laboratory system, generic microcosms have been more successful as simulations of ecosystem functional properties rather than ecosystem structure. This philosophical approach to generic microcosms is typical of what has been termed "mixed flask cultures" (Hammons 1981b).

It is the most commonly used type of aquatic model ecosystem (Abbott 1966, 1967, 1969; Beyers 1962, 1963; Bryfogle and McDiffett 1979; Cooke 1967, 1977; Cooper 1973; Crouthamel 1977; Fraleigh 1971; Fraleigh and Dibert 1980; Gordon et al. 1969; Heath 1979; Hendrix et al. 1981; Kelly 1971; Kurihara 1978a, b; Leffler 1977, 1978, 1980, 1981; McConnell 1962, 1965; Neill 1972, 1975; Ollason 1977; Reed 1976; Thomas 1978; Waide et al. 1980). In most cases such microcosms are initiated by an inoculum derived from an existing natural source either directly or through a period of self-selection in the laboratory. Synthetic generic microcosms can also be developed from individually cultured species (Taub 1969a, b, c, 1976, 1982; Taub and Crow 1980). This paper will describe and evaluate self-selected, mixed flask microcosms in terms of their usefulness for assessing ecosystem-level pollution effects in aquatic systems.

MICROCOSM DESCRIPTION

Since the main reason for using microcosms is to focus on ecosystem-level pollution effects, the major goal of microcosm design is to develop a miniature ecosystem which responds quickly to perturbation. The emphasis is on ecosystem-level properties, although species-level attributes such as fecundity and mortality can also be monitored if desired. Generic microcosms are viewed as distinct ecosystems in their own right, rather than site-specific simulations of particular natural systems. They thus function as very generalized simulations of a large class of ecosystems.

Biotic Inoculation

Generic microcosms for which ecosystem effects protocols have been developed are small (1 to 4 liters), static, open, freshwater ecosystems (Leffler 1981). Since each microcosm is considered a unique ecosystem, intra-experiment replication among microcosms is enhanced by using defined chemical media and standard physical conditions (light, temperature, day length). In order to study ecosystem-level effects it is essential that the microcosms function as homeostatic, self-sustaining ecosystems capable of existing through time periods of at least a year. They should be independent of outside subsidies except for light and replacement of evaporated water. Well-developed interactive couplings of the organisms are essential to achieve these goals. In a self-selected microcosm the inocula used to initiate the systems are gathered from a variety of intact, existing communities where tightly coupled interactions among populations are assumed to exist. Ponds, old laboratory aquaria, hollow trees and cemetery urns are examples of inoculum sources. In each case the organisms obtained are

generally preselected to small bounded systems with a high ratio of surface area to water volume. These field sources are combined in a laboratory stock culture for 3 months prior to the inoculation of the test microcosms. This pretest period in the laboratory permits selection of organisms for the specific microcosm environment and enhances interactive couplings among populations. In the generic microcosm approach, the exact species composition is not important. However, certain minimal functional groups should be represented to ensure a homeostatic, self-sustaining ecosystem. Leffler's (1981) generic microcosm protocol suggests that the inoculum contain at least the following groups:

1. Two species of edible, single-celled green algae. These provide a food source for grazing invertebrates, and the presence of two or more species permits selective predation and interspecific competition.
2. A filamentous green alga.
3. A blue-green, nitrogen-fixing alga.
4. A grazing, free-swimming macroinvertebrate; eg. Daphnia spp.
5. A benthic, detritus-feeding macroinvertebrate; eg. ostracod spp.
6. Assorted bacteria and protozoa.

Once the organisms are inoculated into the chemical medium of the test microcosms, no test chemicals are added for 6 weeks. This time period permits the biota to become well established and a dominant factor in the functioning of the ecosystems. Replication among microcosms is enhanced by crossinoculation during this period. This 6 week period also ensures that all microcosms to be used in the pollutant testing are good replicates of each other. Individual microcosms which deviate significantly from the majority of replicates based on the standard parameters described below are excluded from the assay.

The rationale of the self-selected inoculation method arises from the desire to develop a self-sustaining, homeostatic microecosystem. Organisms are collected that are adapted or acclimated to interacting with each other. Many interactive pathways already exist, and whole communities are sampled to ensure that all relationships necessary for a functional ecosystem are present. The naturally derived inocula are placed in stock holding tanks to allow the organisms to adjust to the physical-chemical conditions of the laboratory environment. Some species may go extinct and others will change in dominance. Generally a homeostatic, self-perpetuating community will develop in about 90 days. A similar adjustment is permitted when the test microcosms are inoculated from the stock cultures. This procedure allows the establishment of interactive pathways through natural processes. The experi-

menter is relieved of concern over whether such pathways would exist in systems created by combining organisms from single species cultures for short periods of coexistence. Although the total number of species from all taxonomic groups and the numbers of individuals of each species can never be fully known, a diversity of organisms is ensured by this inoculating procedure. The purpose of these microcosms is to evaluate pollutant effects on the functional integrity of ecosystems, not on all of their individual components.

Boundaries

Most natural ecosystems lack sharp, closed boundaries. Distinctions between ecosystems generally take the form of gradients which are open to the interchange of energy and matter. The boundaries of a microcosm are very well defined and permit little or no movement of chemicals into or out of the immediate system. In addition the walls of a microcosm vessel create a high ratio of surface area to water volume. For pelagic ecosystems this lessens the realism of a microcosm simulation. In light of the "unique ecosystem" philosophy associated with generic microcosms, this is of little consequence. As in the case of lighting and temperature regimes, the boundaries are considered unique environmental constraints associated with this particular type of ecosystem.

Since the boundaries of the microcosms permit very little movement of organisms into or out of the systems, reinoculation from stock cultures is important. Effects of tested chemicals are evaluated in terms of the microcosm's resilience as well as resistance (Leffler 1977; Webster 1975). In order to enhance a realistic response to chemical stress, it is essential that biotic invasion be possible. Microcosms are generally reinoculated from stock cultures on a weekly basis as water evaporates. This procedure ensures that genetic diversity is maintained and adds to the realism of the microcosm.

Parameters

The emphasis of microcosm studies is on ecosystem-level impacts rather than individual species effects. As a result, microcosm behavior generally is monitored by integrative parameters above the population level of organization. Other parameters such as single species responses may also be measured with a slight increase in expense. A workshop which studied methods for measuring effects of chemicals on aquatic ecosystem properties suggested five major classes of properties which should be monitored in lentic aquatic microcosms (Hammons 1981a):

1. Autotrophy—^{14}C uptake, diel oxygen changes, diel CO_2 changes, chlorophyll, fluorescence.

2. Heterotrophy—$^{14}CO_2$ release from labeled substrate, diel oxygen changes, microorganism enumeration.
3. Chemical-physical properties—pH, pE, turbidity.
4. Nutrient concentrations and fluxes—nitrogen fixation rates, NO_3, NH_4, ortho-P, total C, DOC.
5. Taxonomic composition—relative proportions of the dominant species.

Advantages of Self-Selected Generic Microcosms

The generic microcosms described here permit general assessment of pollutant induced ecosystem-level responses because they are ecosystems in their own right. They are self perpetuating and homeostatic. Because of the inoculation procedure of such microcosms, established, interactive pathways among the species are assumed to exist. Unlike many single species bioassays, microcosms are self sustaining with a minimum of external subsidiaries. Self-selected microcosms are relatively inexpensive to establish and monitor since all the organisms come from a single self-maintaining stock culture. There is no need to maintain cultures of individual species. This lowers the expense and lessens the technical expertise required. Because the microcosms are self-sustaining ecosystems, both short-term and long-term (60 days or more), acute and chronic (depending on dosing procedures) impacts of pollutants can be assessed.

EXPERIMENTAL DESIGN

Physical Design

Replication among microcosms is attained through the initial inoculation and several cross-seeding inoculations during the first 3 weeks of establishment. The degree of replication varies for different parameters. Coefficients of variation are generally less than 30% for production and respiration measured by diel oxygen changes, less than 30% for Eh measurements, and less than 10% for pH measurements (Leffler 1981). A randomized block design is used for placement of the microcosms within a growth chamber. The microcosms are also moved among the shelves of the chambers twice weekly. These procedures minimize the intrachamber gradients.

Replicate microcosms receiving a particular concentration of a test chemical are compared against the behavior of undosed, control, replicate microcosms by means of a t-test. This procedure accounts for normal variability among undosed microcosms and for the precision of measurements possible for each parameter.

Hypothesis

The null hypothesis being tested is that a particular test chemical has no effect on a model ecosystem, the microcosm. The alternative hypothesis is that the test chemical does cause an effect on the microcosm. The data gathered from the study can be analyzed in several ways. The values for each parameter at each sampling time could be plotted for both treatments and control and the resulting graphs examined for general trends. While feasible, this approach can be difficult to interpret because of the amount of data generated from several parameters, a variety of exposure level treatments and a number of sampling periods.

As an alternative the data can be analyzed by stability analysis, a statistically based method which compares confidence bounded trajectories of treatment and control microcosms (Webster 1975; Leffler 1977, 1978, 1981). Three forms of stability are illustrated in Figure 1. Resistance stability is a measure of the maximum deflection of pollutant dosed microcosms from the nominal trajectories of control microcosms. It can be used to calculate effective concentration (EC20 for example) values for the test chemical. Resilience stability measures the time required for dosed microcosms to recover from the acute impact of the test chemical. It can provide information about the removal of the test chemical from a toxic condition, due either to physical processes such as adsorption or to biological degradation. Total relative stability analysis combines both resistance and resilience to yield a measure of the total impact of the test chemical on a monitored property of the microcosms. It is calculated by integrating the differences between the statistically defined trajectories of the dosed microcosms and the control microcosms. Effective concentration values can also be calculated from total relative stability measures.

Each of these stability measures provides a single number for the impact of the chemical on each of the monitored properties. These numbers are most useful for ranking the impact of different chemicals in terms of the same parameter. Effective concentration values calculated from either resistance or total relative stability measures can provide more definitive, quantitative measures of impact and may be directly compared with results from single species bioassays. Lethal concentration (LC20 or LC50) values are not appropriate since ecosystem "death" is ill defined and because most monitored ecosystem parameters are continuously rather than discretely distributed. A Relative Effects Index can be used to combine the total relative stability analyses of all measured parameters into a single number useful for ranking the impact of different treatments or test chemicals (Leffler 1981). While this approach simplifies interpretation of a large amount of data, its major disadvantage is the loss of information which results from reducing the data to one number. One cannot determine what part of the system is affected or at what time after introduction of the chemical the greatest impact occurs.

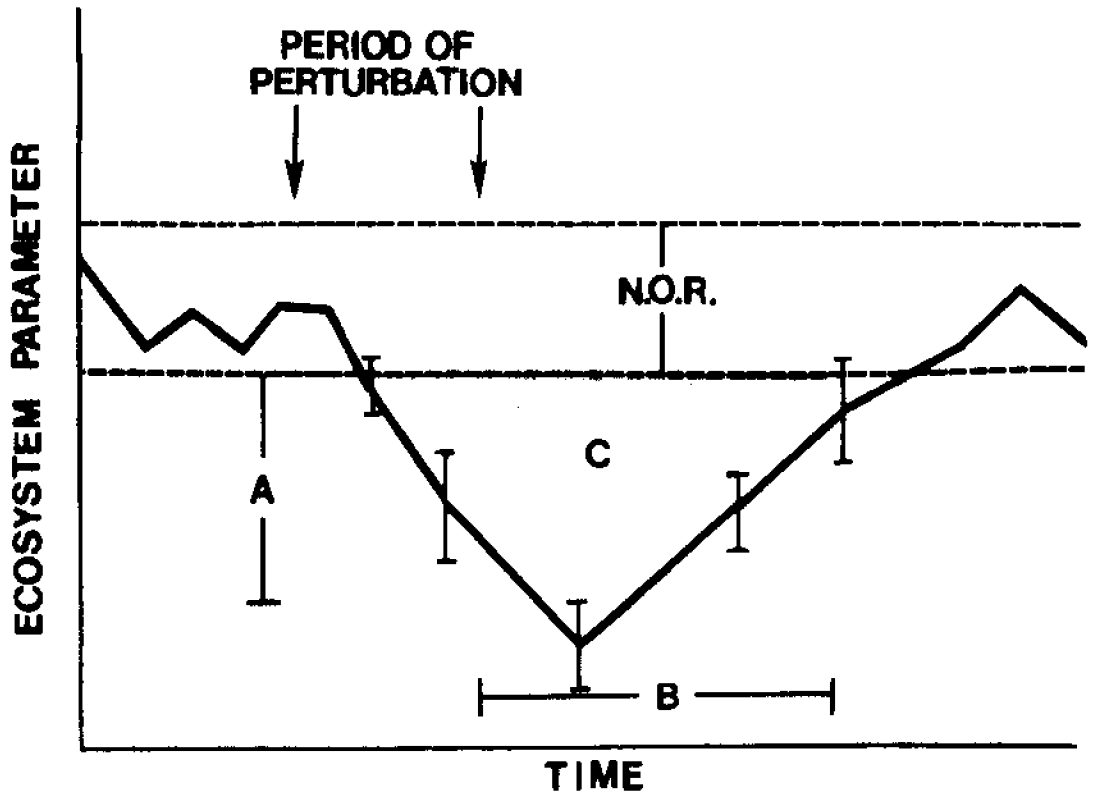

Figure 1. Hypothetical response curve illustrating the empirical stability indices for an ecosystem parameter before and after a perturbation such as exposure to a pollutant. Dashed lines are boundaries of the normal operating range (N.O.R.), which is defined as confidence bounds about the mean steady state value of the parameter. Resistance (A) is measured as the maximum significant deflection of the perturbed trajectory from the normal operating range relative to the mean steady state value. Resilience (B) is the time required for the system to return to the normal operating range after the perturbation. Total relative stability (C) is measured as the integral difference between the perturbed trajectory and the normal operating range.

Difficulties arise if replicate microcosms within the same treatment display the same behavior but are temporally out of phase. This time lag creates large variability within a treatment and obscures any significant differences from controls. Waide et al. (1980) tried to overcome this problem by plotting two parameters against each other in a timeless phase space. Although out-of-phase replicates will have identical trajectories in such a diagram, quantitative analysis of the deviation from control microcosms is difficult.

When the null hypothesis that a particular test chemical has no effect on the microcosms is evaluated it is statistically possible to make two kinds of error. A Type I error occurs if one concludes that a test chemical caused an ecosystem effect when in fact it did not. A Type II error occurs if one concludes that the test chemical causes no ecosystem effects when it really does. Both types of error can be reduced by increasing the sampling size. This is a reasonable option to consider when using microcosm assays. Alternatively, for the purpose of screening potentially hazardous chemicals it may be reasonable to increase the probability of making a Type I error in order to decrease the likelihood of a Type II error. As a result it might be reasonable to reduce the significance level in microcosm screening test analyses to 80% instead of the traditional 95%. This would create a more conservative test, at least from a regulator's viewpoint. It would increase the probability of concluding that the test chemical has an effect when in fact it does not and decrease the probability of concluding no effect when in fact the chemical does cause an impact. Decisions about such matters must be made prior to testing.

Assumptions

It is assumed that a microcosm is either a general representation of a class of natural ecosystems or is a site-specific simulation of a particular ecosystem. The logic of the generic microcosm approach assumes that by describing the impacts on the essential functional properties of one ecosystem it is possible to suggest qualitative descriptions of the response of similar properties in other ecosystems. Such an assumption can be evaluated by comparing microcosm results with those of field situations. Since generic microcosms are not designed to mimic any other specific natural ecosystems, this is the key assumption for this entire approach to effects testing. The other critical assumption is that the exact species composition of a generic microcosm is unimportant for detecting ecosystem-level impacts as long as certain key functional groups are represented. This assumption can be tested by comparing how microcosms differing in species composition respond to a series of test chemicals.

The generic microcosms for which protocols currently exist are freshwater, static systems. It is assumed that these chemical-physical characteristics will not limit generality of their results. In order to increase between-microcosm replication an artificially constructed chemical medium is used. This medium was designed originally to promote growth of algae and macroinvertebrates, not to simulate naturally occurring water (Leffler 1981; Taub and Dollar 1964). Because the fate and effects of a pollutant are influenced by the chemical matrix of the water, use of a specific artificial medium might decrease the ease of extrapolating from a generic microcosm to a natural ecosystem. This limitation could be evaluated through a series of experiments in which different media are used in microcosms which are then compared with each other and with a variety of field studies. A component of the artificial medium, such as EDTA (which is required to hold micronutrient iron in solution), may also unrealistically chelate the test pollutant.

Two major assumptions are that ecosystems really exist and that they are worthy of concern in assessing pollution effects. Engelberg and Boyarsky (1979) recently questioned whether ecosystems are really functional units while Knight and Swaney (1981), McNaughton and Coughenour (1981), and C.F. Jordan (unpublished manuscript) argued in support of the ecosystem concept. The findings of the National Academy of Sciences' Committee to Review Methods for Ecotoxicology and a series of Environmental Protection Agency workshops held at Oak Ridge National Laboratory affirmed the concern over ecosystem-level pollution effects (NAS 1981; Hammons 1981b).

Finally, it must be assumed that the monitored parameters accurately and precisely reflect events or processes within the microcosms. At present, methodology needs to be refined to increase the sensitivity of detecting changes within the microcosms.

EXTRAPOLATION

The basic philosophy of the generic microcosm approach is that a microcosm is an ecosystem in its own right and therefore ecosystem-level impacts in a microcosm may be extrapolated to similar impacts in other natural ecosystems. As discussed in Hammons (1981b), however, extrapolation really has two components: realism and generality. These form opposite extremes of a continuum, and it is generally impossible to maximize both. Since the generic microcosm approach is based entirely on the assumption of generality, these systems must be used for making general statements about chemical impacts, not for direct prediction of site-specific impacts. The microcosms are useful for screening chemicals and indicating which ones require further testing to fully ascertain their impacts on freshwater aquatic ecosystems.

Significance of Monitored Properties

Generic microcosms are primarily useful for evaluating ecosystem-level impacts. How can such microcosm properties be extrapolated to natural ecosystems? An alteration of the autotrophic and heterotrophic components of an ecosystem could affect food chains and result in impact on species important to human commerce and recreation. According to the second law of thermodynamics energy is lost at each step of a food chain. A decrease in autotrophy at the base of a grazing food chain may ultimately lead to a reduction in game fish biomass several levels removed. Similarly an increase in heterotrophy, most notably by bacteria and fungi, may decrease the food supply passing to game fish at the top of a detrital food chain. An increase in either autotrophy or heterotrophy may result in algal or bacterial blooms. Both may adversely affect game fish by depleting the waters of dissolved oxygen. Bacterial and fungal metabolism is also closely related to the recycling of nutrients, thus influencing the productivity of the water body.

Alteration of chemical and physical parameters such as pH and pE may also have significant impacts on an aquatic ecosystem. Because of equilibrium phase state shifts in chemical speciation, threshold effects may be encountered which are far greater than small changes may indicate. The recent depletion of fish populations in certain Adirondack lakes due to acid precipitation is an example. Changes in the equilibrium of chemical species may alter the availability of nutrients to organisms or may directly affect the metabolism of organisms. An excess of nutrients can lead to algal blooms, resulting in lowered dissolved oxygen concentrations which stress game fish. Deficiencies of nutrients in the water reduce primary productivity and in turn lower the productivity of all higher tropic levels.

The relative abundance of the dominant species in a microcosm can also be monitored. Taxonomic changes may indicate possible changes in community structure, food chains, or key functional groups. All of the information provided by many single species bioassays could be obtained from microcosms, but with increased realism and improved extrapolation. Most economically important species cannot be included in a generic microcosm because of size limitations. For these harvestable species a pollutant's impact can only be inferred from microcosm results. These species should be tested separately. Similarly, possible predictions about human health effects are very limited. Some impacts to swimmers or human consumers of aquatic life might be inferred if bioconcentration were measured in various microcosm organisms. It should be remembered, however, that generic microcosms were designed as ecosystem-level effects tests, not as tests for harvestable species or humans.

Problems of Extrapolation

Because of the diversity and complexity of the environment it is unlikely that any of the properties monitored in a microcosm will show the same quantitative response in a given, site-specific, natural ecosystem. However, similar qualitative impacts may occur. The more toxic a chemical is, the more confident one can feel about extrapolating microcosm effects to the natural environment. One of the problems involved in extrapolating microcosm results, the effect of the chemical medium, has already been mentioned. Additional physical conditions such as the absence of currents and sediments, temperature cycles, wavelengths of light, light intensity and seasonal variability may cause a chemical's fate and impact in the natural environment to differ from its fate and impact in a microcosm. Some of the determining conditions such as sediments can be added to a simple microcosm for a particular assessment.

Extrapolation from microcosms directly to a site-specific field situation is possible only if all of the limitations are assessed. Microcosm results should be carefully interpreted in light of the test pollutant's chemical-physical properties. Of course, none of the problems encountered is unique to generic microcosms. They certainly apply even more strongly to single species bioassays. Mathematical models and site-specific microcosms also fail to incorporate fully the complexity of natural systems. In fact, when considering the economic costs involved in trying to simulate the actual complexity of nature, one might conclude that a generalized screening test is really the most practical assessment we can hope to achieve.

Validation

Research is essential to define the degree to which microcosm-derived generalizations are applicable to large classes of ecosystems. How can ecosystems be grouped into functionally different types of systems? How can generalizations be formulated for each of these ecosystem classes? A thorough literature review and analysis might help to answer the question concerning the level of generalization possible among different types of ecosystems. Ponds and enclosure studies should be used to examine the impact of chemicals and to evaluate the types of generalities which could be drawn from microcosm studies. Case studies of chemicals which have been purposefully or accidentally released into the environment should be evaluated, and effects should be compared with effects that the same chemicals produce in microcosms. Properties for which generalizations between field and microcosm ecosystems are possible should be defined. At present this type of validation effort is just starting on a small scale.

PERSPECTIVE

If generic microcosms are to become effective pollution assessment tools, their strengths and weaknesses must be evaluated with respect to other forms of testing. Major reviews of this question have recently been completed (Hammons 1981b; NAS 1981).

Single Species Bioassays

Single species tests have been the major form of environmental effects assessment in wide use. Protocols are well developed for a variety of species for both acute and chronic tests. Recently, however, the weaknesses of the single species approach have been discussed at length (NAS 1981). They have been criticized for their lack of realism and the difficulty involved in extrapolating from pure culture results to effects on the same species in nature.

Generic microcosms are subject to the same criticisms as single species tests, but the criticisms are less harsh. Generic microcosms are more realistic and probably more generalizable than are single species tests. Much of their improved realism results from their increased complexity and the fact that they are homeostatic and self sustaining. Microcosms can thus exist independently of highly arbitrary and artificial external support. Microcosms permit evaluation of pollutant effects on the functional properties of the ecosystem level of organization. Effects due to species interactions, trophic organization, and biogeochemical cycling can be assessed. Generic microcosms and single species bioassays are very similar in their pollution assessment uses and in the ability to extrapolate their results to field conditions. Both are most effective as screening tests to indicate chemicals requiring further study. Microcosms and single species tests are probably equivalent in expense over testing periods of equal length. Both are relatively inexpensive.

Microcosms have been shown to provide data not predicted by single species tests. Triethylene glycol is a chemical commonly used as a carrier compound for introducing test chemicals into single species bioassays. Leffler (1981) showed that triethylene glycol was severely deleterious in aquatic microcosms, probably by being degraded to oxylic or formic acid. These results have been confirmed independently in similar generic microcosms with different species composition (F.B. Taub, personal communication). Similarly Taub (1982) has reported malathion effects in microcosms significantly different from those which would be predicted from single species algal or Daphnia tests alone.

Single species tests do provide useful information which is not available from microcosm tests. Such data relate to specific organisms, particularly those of economic significance. Because of size limitations large organisms such as macrophytes and fish can-

not be sustained in a generic microcosm. Commercially important fish and shellfish should also be tested separately both because of the size limitations and because of the detailed information desired about these particular species.

Site-Specific Simulation Microcosms

An alternative approach to ecosystem effects testing has been the development of site-specific simulation microcosms (Harte et al. 1980; Perez 1981; Pilson et al. 1980; Tagatz 1976). These systems are designed to mimic the ecological behavior of a specific body of water. They are generally far more complex than generic microcosms, both in taxonomic structure and in their design and maintenance procedures. Site-specific microcosms possess greater ecological realism than generic microcosms. Both types of system are capable of making general predictions, but site-specific predictions can be derived only from simulation microcosms.

Generic microcosms are far less expensive than simulation microcosms, but also provide less specific information. Actually the two systems were developed for different purposes. The simulation microcosms are designed to predict the effects of a chemical in a specific type of ecosystem. Generic microcosms are designed as screening tools for rapidly evaluating the general environmental effects of a variety of chemicals. The choice of which type of microcosm to employ depends on the kind of information required for a particular situation. The two approaches are not contradictory, but should complement each other. Because of their cost, generic microcosms would usually be used first to screen chemicals which require further testing in site-specific simulation microcosms.

Field Evaluation Studies

Field testing of a potential pollutant is of course ideal for obtaining realistic data which are easily extrapolated. Both generic and site-specific microcosms possess far less ecological realism than a field study. However, field studies are proportionately very expensive and logistically difficult. In terms of evaluating large numbers of chemicals for environmental hazard, field studies are simply not possible from both the economic and habitat destruction perspectives. Field studies are most useful on a small scale for validating results obtained from laboratory microcosms.

CONCLUSION

Our current ability to evaluate the ecological effects of potentially polluting chemicals is still at an infant stage of development. Self-selected, generic microcosms may become an impor-

tant assessment tool despite their limitations. The basic assumption that functional ecosystem-level effects observed in a generic microcosm can be generalized to natural systems is testable through field validation studies. Generic microcosms are most useful as a means of screening chemicals for potential ecological effects. Single species bioassays lack sufficient ecological realism for this purpose, while site-specific simulation microcosms are generally too expensive. If justified, generic microcosms can be made more site specific by the use of locally derived inocula, the addition of local sediments, or alteration of chemical-physical parameters. None of these variations would alter the system's cost effectiveness.

At present it appears that the most feasible approach to screening chemicals for environmental effects is to use generic microcosms. They should be supplemented by single species toxicity tests for those organisms which are of particular commercial importance or are ecologically important but are too large for the microcosm. On the basis of generic microcosm results and economic considerations, certain chemicals should then be tested in site-specific simulation microcosms. All laboratory-derived information must be carefully evaluated in terms of baseline field validation studies, life histories of commercially important organisms, and the peculiarities of specific localities.

REFERENCES

Abbott, W. 1966. Microcosm studies on estuarine waters. I. The replicability of microcosms. J. Water Pollut. Control Fed. 38: 258-270.

Abbott, W. 1967. Microcosm studies on estuarine waters. II. The effects of single doses of nitrate and phosphate. J. Water Pollut. Control Fed. 39: 113-122.

Abbott, W. 1969. High levels of nitrate and phosphate in carboy microcosm studies. J. Water Pollut. Control Fed. 41: 1748-1751.

Beyers, R.J. 1962. Relationship between temperature and the metabolism of experimental ecosystems. Science 136: 980-982.

Beyers, R.J. 1963. The metabolism of twelve aquatic laboratory microecosystems. Ecol. Monogr. 33: 281-306.

Bryfogle, B.M. and W.F. McDiffett. 1979. Algal succession in laboratory microcosms as affected by an herbicide stress. Am. Midl. Nat. 101: 344-354.

Cooke, G.D. 1967. The pattern of autotrophic succession in laboratory microcosms. Bioscience 17: 717-721.

Cooke, G.D. 1977. Experimental aquatic laboratory ecosystems and communities. In: J. Cairns, Jr. (ed.), Aquatic Microbial Communities, Garland Reference Library of Service and Technology, Vol. 15, pp. 59-103. Garland Publ., New York.

Cooper, D.C. 1973. Enhancement of net primary productivity by herbivore grazing in aquatic laboratory microcosms. Limnol. Oceanogr. 18: 31-37.

Crouthamel, D.A. 1977. A microcosm approach to the effects of fish predation on aquatic community structure and function. Ph.D. Dissertation, Univ. of Georgia, Athens, Ga.

Engleberg, J. and L.L. Boyarsky. 1979. The noncybernetic nature of ecosystems. Am. Natur. 114: 317-324.

Fraleigh, P.C. 1971. Ecological succession in an aquatic microcosm and a thermal spring. Ph.D. Dissertation, Univ. of Georgia, Athens, Ga.

Fraleigh, P.C. and P.C. Dibert. 1980. Inorganic carbon limitation during ecological succession in aquatic microcosms. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp 369-401. (CONF-781101, National Technical Information Service, Springfield, Va.)

Giesy, J.P., Jr. (ed.). 1980. Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978. (CONF-781101, National Technical Information Service, Springfield, Va.)

Gorden R.W., R.J. Beyers, E.P. Odum and R.G. Eagon. 1969. Studies of a simple laboratory microecosystem: Bacterial activities in a heterotrophic succession. Ecology 50: 86-100.

Hammons, A.S. (ed.). 1981a. Ecotoxicological Test Systems: Proceedings of a Series of Workshops. ORNL-5709; EPA 560/6-81-004, Oak Ridge National Lab., Oak Ridge, Tenn.

Hammons, A.S. (ed.). 1981b. Methods for Ecological Toxicology: A Critical Review of Laboratory Multispecies Tests. ORNL-5708; EPA-560/11-80-026, Oak Ridge National Lab., Oak Ridge, Tenn.

Harte, J., D. Levy, J. Rees and E. Saegebarth. 1980. Making microcosms an effective assessment tool. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp 105-137. (CONF-781101, National Technical Information Service, Springfield, Va.)

Heath, R.T. 1979. Holistic study of an aquatic microcosm: Theoretical and practical implications. Int. J. Environ. Stud. 13: 87-93.

Hendrix, P.F., C.L. Langer, E.P. Odum and C.L. Thomas. 1981. Microcosms as Test Systems for the Ecological Effects of Toxic Substances: An Appraisal with Cadmium. Final Report EPA Grant No. R 805860020, U.S. EPA Environmental Research Lab., Athens, Ga.

Kelly, R.A. 1971. The effects of fluctuating temperature on the metabolism of freshwater microcosms. Ph.D. Dissertation, Univ. of North Carolina, Chapel Hill, N.C.

Knight, R.L. and D.P. Swaney. 1981. In defense of ecosystems. Am. Natur. 117: 991-992.

Kurihara, Y. 1978a. Studies of "succession" in a microcosm. Sci. Rep. Tohoku Univ. Ser. 4 (Biol.) 37: 151-160.

Kurihara, Y. 1978b. Studies of the interaction in a microcosm. Sci. Rep. Tohoku Univ. Ser. 4 (Biol.) 37: 161-178.

Leffler, J.W. 1977. A microcosm approach to an evaluation of the diversity-stability hypothesis. Ph.D. Dissertation, Univ. of Georgia, Athens, Ga.

Leffler, J.W. 1978. Ecosystem response to stress in aquatic microcosms. In: J.H. Thorp and J.W. Gibbons (eds.), Energy and Environmental Stress in Aquatic Systems. DOE Symposium Series 48, Augusta, Ga., Nov. 2-4, 1977, pp 102-119. (CONF-771114, National Technical Information Service, Springfield, Va.)

Leffler, J.W. 1980. Microcosmology: Theoretical applications of biological models. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp 14-29. (CONF-781101, National Technical Information Service, Springfield, Va.)

Leffler, J.W. 1981. Microcosms and Stress Criteria for Assessing Environmental Impact of Organic Chemicals. Final Report Subcontract No. T6411 (7197) 025, Battelle Columbus Labs., Columbus, Ohio. 79 pp

McConnell, W.J. 1962. Productivity relations in carboy microcosms. Limnol. Oceanogr. 7: 335-343.

McConnell, W.J. 1965. Relationship of herbivore growth to rate of gross photosynthesis in microcosms. Limnol. Oceanogr. 10: 539-543.

McNaughton, S.J. and M.B. Coughenour. 1981. The cybernetic nature of ecosystems. Am. Natur. 117: 985-990.

NAS (National Academy of Sciences). 1981. Testing for Effects of Chemicals on Ecosystems: A Report by the Committee to Review Methods for Ecotoxicology. National Academy Press, Washington, D.C.

Neill, W.E. 1972. Effects of size-selective predation on community structure in laboratory aquatic microcosms. Ph.D. Dissertation, Univ. of Texas, Austin, Tex.

Neill, W.E. 1975. Experimental studies of microcrustacean competition, community composition, and efficiency of resource utilization. Ecology. 56: 809-826.

Ollason, J.G. 1977. Freshwater microcosms in fluctuating environments. Oikos 28: 262-269.

Perez, K. 1981. Experimental Marine Microcosm Protocol: An Assessment of the Ecological Effects, Human Health Risks, and the Fate and Transport of Chemicals in a Site-Specific Marine Ecosystem. Draft Protocol, U.S. EPA Environmental Research Lab., Narragansett, R.I.

Pilson, M.E.Q., C.A. Oviatt, and S.W. Nixon. 1980. Annual nutrient cycles in a marine microcosm. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp 753-778. (CONF-781101, National Technical Information Service, Springfield, Va.)

Reed, C.C. 1976. Species diversity in aquatic microecosystems. Ph.D. Dissertation, Univ. of Northern Colorado, Greeley, Colo.

Tagatz, M.E. 1976. Effect of mirex on predator-prey interaction in an experimental estuarine ecosystem. Trans. Am. Fish. Soc. 105: 546-549.

Taub, F.B. 1969a. A biological model of a freshwater community: A gnotobiotic ecosystem. Limnol. Oceanogr. 14: 136-142.

Taub, F.B. 1969b. A Continuous gnotobiotic (species defined) ecosystem. In: J. Cairns, Jr. (ed.), The Structure and Function of Freshwater Microbial Communities, pp. 101-120. Research Monograph 3, Virginia Polytechnic Inst. and State Univ., Blacksburg, Va.

Taub, F.B. 1969c. Gnotobiotic models of freshwater communities. Verh. Int. Ver. Limnol. 17: 485-496.

Taub, F.B. 1976. Demonstration of pollution effects in aquatic microcosms. Int. J. Environ. Stud. 10: 23-33.

Taub, F.B. 1982. Synthetic microcosms as biological models of algal communities. In: L.E. Shubert (ed.), Algae as Biological Indicators, Academic Press, New York.

Taub, F.B. and M.E. Crow. 1980. Synthesizing aquatic microcosms. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp 69-104. (CONF-781101, National Technical Information Service, Springfield, Va.)

Taub, F.B. and A.M. Dollar. 1964. A Chlorella-daphnia food chain study: The design of a compatible chemically defined culture medium. Limnol. Oceanogr. 9: 61-74.

Thomas, C.L. 1978. A microcosm study of the interactions of nutrients and cadmium in aquatic systems. M.S. Thesis, Univ. of Georgia, Athens, Ga.

Waide, J.B., J.E. Schindler, M.C. Waldron, J.J. Hains, S.P. Schreiner, M.. Freedman, S.L. Benz, D.R. Pettigrew, L.A. Schissel and P.J. Clark. 1980. A microcosm approach to the study of biogeochemical systems. 2. Responses of aquatic laboratory microcosms to physical, chemical, and biological perturbations. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp 204-223. (CONF-781101, National Technical Information Service, Springfield, Va.)

Webster, J.R. 1975. Analysis of potassium and calcium dynamics in steam ecosystems on three southern Appalachian watersheds of contrasting vegetagion. Ph.D. Dissertation, Univ. of Georgia, Athens, Ga.

Measurement of Pollution in Standardized Aquatic Microcosms

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INTRODUCTION

The use of microcosms in pollution studies follows the use of microcosms by ecologists to control extraneous variables as a means of gaining a better understanding of ecological properties or to pretest potential field studies; much of the early literature has been reviewed by Cooke (1977). Microcosms were evaluated as testing systems for the environmental transport of toxic substances by Witherspoon et al. (1976), and as potential screening tools for evaluating the effects and transport of toxic substances by Harris (1980). Microcosms have also been compared with other test systems for deriving ecotoxicological evaluations (Hammons 1981). A recent symposium provides a review of the diverse efforts that are encompassed within the title "Microcosms," and most are pertinent to our understanding of the potential effects of pollution (Giesey 1980).

The microcosms discussed here are often termed "model ecosystems" or "synthetic microcosms," and allow the investigator to control variables such as the initial organism species assemblage and relative abundances, the nature of the liquid medium, either a chemically defined solution or treated natural water, and the temperature, light intensity and cycle. A test chemical is added, and the systems are monitored for chemical fate and/or ecological effects. It should be noted that the investigator controls the initial conditions, but the biological components soon modify the system in complex ways. Replicate systems can be established simultaneously, and randomly assigned to treatment groups.

Synthetic aquatic microcosms constitute moderately complex assemblages of organisms that fulfill important ecological roles in

aquatic communities. Although the first experiment termed a "synthetic microcosm" was assembled from different species that had been isolated from a single, naturally occurring community (Nixon 1969), most synthetic microcosms have been synthesized from convenient laboratory stocks of single species cultures. Some synthetic microcosms have been gnotobiotic (i.e., the total species assemblage including bacteria was defined), for example, Taub (1969a, b, c); Nixon (1969); and Sugiura et al. (1982). However, most synthetic microcosms have undefined microbial communities.

Many synthetic microcosms have been used to examine the environmental fate of chemicals, with little or no documentation of effects on the biota or ecosystem-level variables. The fine series of studies done at Robert Metcalf's laboratory to explore the relative biodegradability and bioaccumulation of chemicals and their daughter products showed the utility of using easily synthesized ecological communities (Metcalf et al. 1971, 1972 and 1973). These studies proved useful in determining the fate of the chemicals, and their findings appear to predict or confirm the movement and transformations of chemicals in the natural environment. One may argue that those studies do not predict the rate or pathway of degradation of the chemical in a specific site, but Metcalf's simple microcosm seems complex enough to assure that most of the ecologically important chemical transformations were included. The communities were usually composed of the terrestrial plant Sorghum halpense, the caterpillar Estigmene acrea, the alga Oedogonium, unidentified diatoms and other phytoplankton, the snail Physa, the water flea Daphnia, the mosquito larva Culex pipiens quinquefasciatus, and the fish Gambusia affinis. The entire community was contained in an aquarium 10 x 12 x 20 in. (ca. 25 x 30 x 50 cm). The terrestrial community was supported by sand, and the liquid medium was 7 L of a standard reference water (Freeman 1953). The experiments were run for 30 days before samples were taken and the fish introduced; the entire experiment was sampled and terminated at day 33. If organisms died during the course of the experiment, replacements were added to assure the presence of the ecological and chemical functional capabilities. Radioactively labeled treatment chemicals were added at field application rates, and the parent and daughter compounds assessed (Metcalf et al. 1971).

There are innumerable studies where algae-Daphnia and algae-Daphnia-fish assemblages have been studied to explore bioaccumulation of chemicals. For chemical fate studies, researchers have often used less than acutely toxic levels, and have not documented ecological effects such as mortality, reduced growth rates or trophic interactions. For example, the accumulation of dieldrin was studied in a Scenedesmus, Daphnia magna, and guppy (Poecilia reticulata) system by Reinert (1972).

Ringelberg and Kersting developed a multicompartmental mi-

crocosm to isolate the autotrophic, herbivore and decomposer units so that these components could be measured in semi-isolation (Ringelberg and Kersting 1978; Ringelberg 1977). Although they initiated their autotrophic community with Chlorella and Scenedesmus, they reported that other algal species became established after a short time. The herbivore used was Daphnia magna. The medium was filtered (0.45 μm) lake water. This microcosm design was used to explore the effects and fate of the herbicide dichlobenil (Kersting 1978).

INSIGHTS INTO POLLUTION MEASUREMENTS DURING THE DEVELOPMENT OF THE AQUATIC MICROCOSM BIOASSAY

Our laboratory had been engaged in designing aquatic microcosms that would allow us to analyze trophic interactions (Taub and Dollar 1968; Taub 1969a, b, and c; Taub and McKenzie 1973; Taub 1973; Taub 1977) and responses to toxic substances (Taub 1976). Since 1976, our laboratory has worked toward the development of a multitrophic, ecosystem bioassay that could be standardized between laboratories; that is, different researchers could obtain similar results if they performed similar experiments independent of the site or season of the bioassay. The research has not only served to refine the Standardized Aquatic Microcosm Protocol (Report to U.S. FDA on Contract No. 223-80-2352), but has also served to indicate various ways that aquatic communities respond to toxic substances. Although the data shown here are from freshwater microcosms, a similar marine microcosm was developed, but never used by us to test responses to toxic chemicals (Taub 1976). There are no reasons to think the principal findings would be different in marine ecosystems.

As a step toward developing a microcosm for displaying the ecological effects of chemicals, a mathematical model was used to explore the likely responses to mortality induced in a single species (Crow and Taub 1979; Taub and Crow 1980). The model contained three species of algae and either one or two grazers. In the absence of a second grazer, when we imposed an induced 20% mortality on Grazer #1 (who fed mostly on Alga #1, slightly on Alga #2 and hardly at all on Alga #3), the induced mortality had relatively little effect other than to displace the abundance cycles of Grazer #1 relative to the control ecosystem. However, when Grazer #2 (who fed mostly on Alga #2, slightly on Alga #1 and hardly at all on Alga #3) was added to the model, the same 20% mortality induced on Grazer #1 caused Grazer #1 to become rare, Grazer #2 to increase, and Alga #2 to become rare. Thus in spite of relatively little feeding overlap between the two grazers, the presence of a competitor that did not suffer from the increased mortality

changed the communities' responses to a stress. From this we concluded that communities of different structure could be expected to show different responses to a similar induced mortality. Therefore, we should not expect that a microcosm bioassay would yield reproducible results between different experiments if the community structure was not constant. It also suggests that different natural communities, even at the same site but at different seasons, should be expected to respond differently to the same toxic stress. Evidence for this is provided in the section titled "Effects of Copper Sulfate."

It will be necessary to briefly describe the aquatic microcosm protocol and the control results so that the following data can be interpreted. Although the technique has been modified slightly over the course of the experiments, modifications are detailed only if necessary for this discussion. The rationale and general techniques have been described in other publications (Taub and Crow 1978; Taub et al. 1981; Taub et al. 1982; Taub 1982; a formal protocol is in preparation). The algal community consists of 11 species that have been selected to represent three different taxonomic groups, and include many species that are traditionally used for bioassays. The algae include a range of sizes from small to large filamentous forms that would be suitable as food either for the Daphnia or for amphipods. The animals include the grazers Daphnia (a commonly used bioassay organism), which feed on small and moderate sized particles; protozoa and rotifers, which feed on small algae and bacteria; amphipods, which feed on benthic detritus and filamentous algae; and ostracods, which are detritivores. The containers are 1 gallon glass jars containing a simple, reproducible sediment and 3 L of medium. Thirty microcosms are initiated, and 24 are used for the experiment; these can easily fit on a laboratory table under a pair of 8 ft lights.

The concentration of chelate in the chemically defined medium has an important effect on the responses of the bioassay to copper; we have designed a variety of media and differing chelation potentials. The pH buffering capacity could also be varied, but we have not yet done this. The protocol steps and the variables monitored are summarized in Tables 1 and 2. A typical experimental design might consist of six replicates in each of four treatment groups: (1) control, (2) a single addition of a low concentration, (3) a single addition of a high concentration and (4) a repeated addition of low concentration. If a solvent is used, one of the treatment groups should be a solvent control.

The microcosms have been designed to begin as a spring, or nutrient rich, aquatic environment where algae have an opportunity to grow prior to heavy grazing and to develop into a summer community in which the algae are heavily grazed and their growth depends on nutrient recycling. The ecological interactions of nutrient depletion (Figure 1), primary production and accumulation of algal biomass (Figure 2), and secondary production with the estab-

Table 1. Summary of aquatic microcosm protocol.

PREPARATION
Purchase supplies, obtain cultures.
Make master solutions, activate cultures.
Prepare bottles with sediment and medium (minus phosphate, silicate, iron, trace minerals and vitamins), autoclave, cool.
Add sterile medium components not previously added.
Cap with clear Petri-dish lids; place on light table.

DAY	
0	Analyze initial chemical concentrations. Inoculate algae.
4	Analyze chemical concentrations. Enumerate algae. Inoculate animals.
7	Full variable measurements. Cull 30 microcosms to 24. Randomly assign to treatment groups. ADD TEST CHEMICALS.

MEASUREMENTS TWICE WEEKLY TO DAY 63.
Enumerate each algal species.
Enumerate each animal species.
Oxygen concentrations day/night.
In-vivo fluorescence.
pH.

MEASUREMENTS TWICE WEEKLY FOR 3 OR 4 WEEKS, ONCE WEEKLY THEREAFTER TO DAY 63.
Carbon uptake.
Total inorganic carbon.
Extracted chlorophyll and phaeopigments.
Algal nutrients.

Table 1. Continued

POST-EXPERIMENT ACTIVITIES
Keypunching of data*
Statistical analyses comparing each treatment with the control for each variable (about 2 weeks).
Preparation of graphics (another 2 weeks).
Interpretation of results.
Report completed 60 days after experiment completion.

*Most of the data are keypunched as the experiment progresses. Data through day 28 are analyzed statistically for the purpose of determining quality of the experiment and the data handling package. The time required for each step represents the realistic time requirements.

Table 2. List of variables measured with units.

COMMUNITY VARIABLES			UNITS
DO1	=	Dissolved Oxygen, Morning	ppm = mg L^{-1}
DO2	=	Dissolved Oxygen, Night	"
DO3	=	Dissolved Oxygen, Morning	"
DDOAM	=	Dissolved Oxygen Gain (AM)	"
DDOPM	=	Dissolved Oxygen Loss (PM)	"
DELDO	=	Dissolved Oxygen 24 Hour Change	"
RATIO	=	Net Photosynthesis - Respiration Ratio	"
pH			
PO4	=	Phosphate	µg at L^{-1} = µM
NO3	=	Nitrate	"
NO2	=	Nitrite	"
NH3	=	Ammonia	"

PRIMARY PRODUCTION VARIABLES			UNITS
SELAN	=	Selenastrum	cells ml^{-1}
CHLAM	=	Chlamydomonas	"
SCENE	=	Scenedesmus	"
ANKIS	=	Ankistrodesmus	"
CHLOR	=	Chlorella	"
D213	=	Nitzschia (Diatom 213)	"
D216	=	Gomphonema (Diatom 216)	"
ANABE	=	Anabaena	"
LYNGB	=	Lyngbya	"
STIG	=	Stigeoclonium	"
ULOTH	=	Ulothrix	"
AVALG	=	Available Algae	mg per 100 ml
ABIOM	=	Algal Biomass	"
CHLAS	=	Chlorophyll-a, (spectrographic)	µg L^{-1}
CHLAFD	=	Chlorophyll-a, (fluor, dark filter)	"
CHLAFL	=	Chlorophyll-a, (fluor, light filter)	"
PHAEOS	=	Phaeopigments (spectrographic)	"
PHAEOFD	=	Phaeopigments (fluor, dark filter)	"
PHAEOFL	=	Phaeopigments (fluor, light filter)	"
C14L	=	C14 Light Uptake	µg C L^{-1} hr^{-1}
C14D	=	C14 Dark Uptake	"
C14	=	C14 Net Uptake	"
ALK	=	Alkalinity (Estimate of Inorganic Carbon)	no units

Table 2. List of variables with units (continued).

SECONDARY PRODUCTION VARIABLES			UNITS
SDAPH	=	Small Daphnia	ind. per 100 ml
MDAPH	=	Medium Daphnia	"
LDAPH	=	Large Daphnia	"
DAPHN	=	Daphnia, all sizes	"
PSDAP	=	Ratio of Small to Total Daphnia	"
MDSIZ	=	Mean Daphnia Length	"
DBIOMA	=	Daphnia Biomass	"
SAMPH	=	Small Amphipods	"
LAMPH	=	Large Amphipods	"
AMPH	=	Amphipods	"
OSTRA	=	Ostracods	"
COPEP	=	Copepods (no longer added)	"
EPHIP	=	Ephippia	present/absent
PHILO	=	Philodina	ind. per 100 ml
ROTIF	=	Small Rotifers	"
HYPO	=	Hypotrichs	"
MISCEL	=	Miscellaneous Protozoans	"
VORT	=	Vorticella	"
NEMER	=	Nemertines	"
BODO	=	Bodo	"
AELOS	=	Aeolosoma (no longer added)	"
CLARI	=	Water Clarity	%

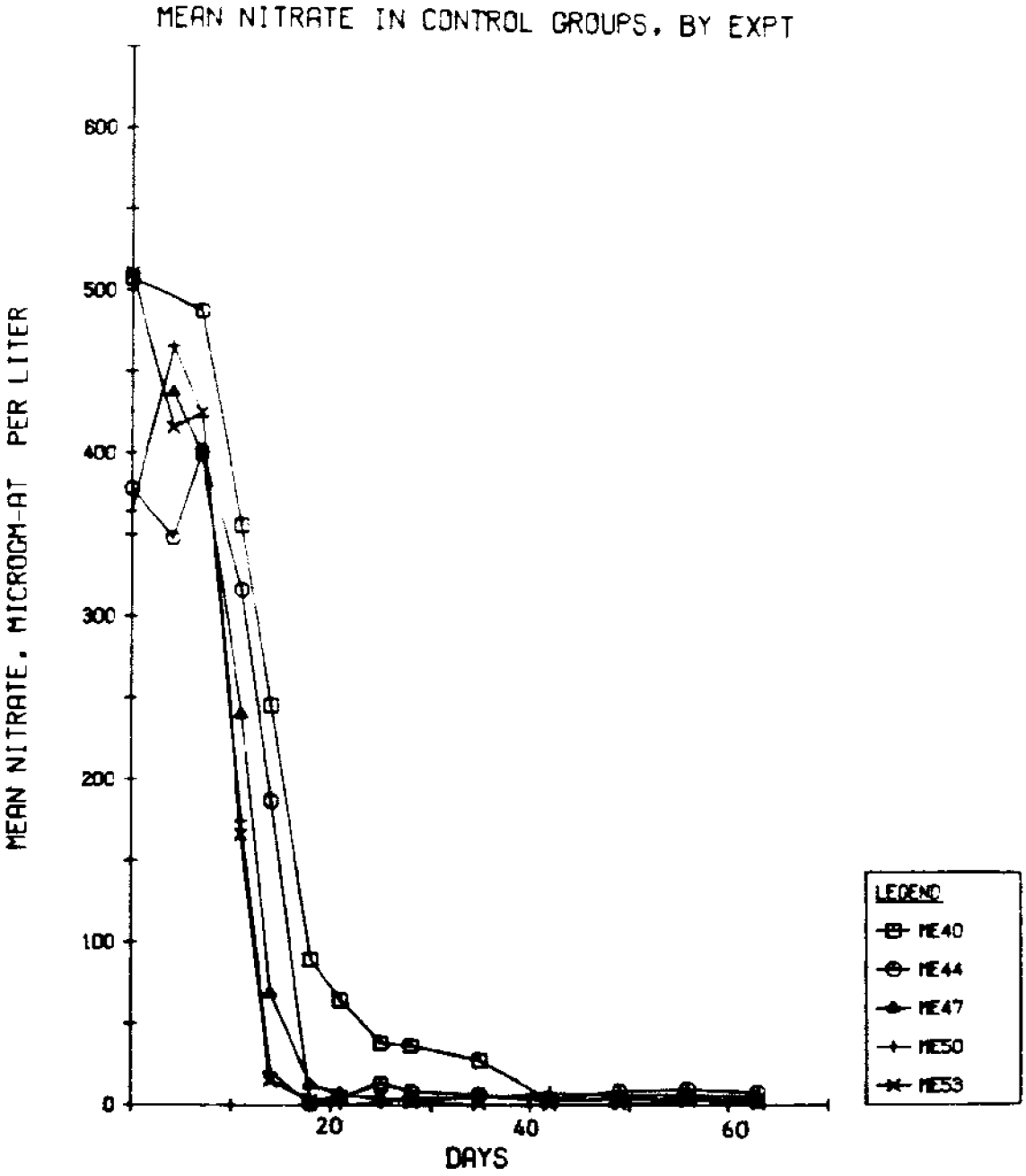

Figure 1. Nitrate depletion in control microcosms. Each line and symbol represents the mean of six replicates in the control group of an experiment. The units are μg at L^{-1} or μM. The separate experiments can be identified by the ME number. For ME 40, the control group (NEW) represents the "new" species assemblage and medium used in the other experiments shown in this graphic.

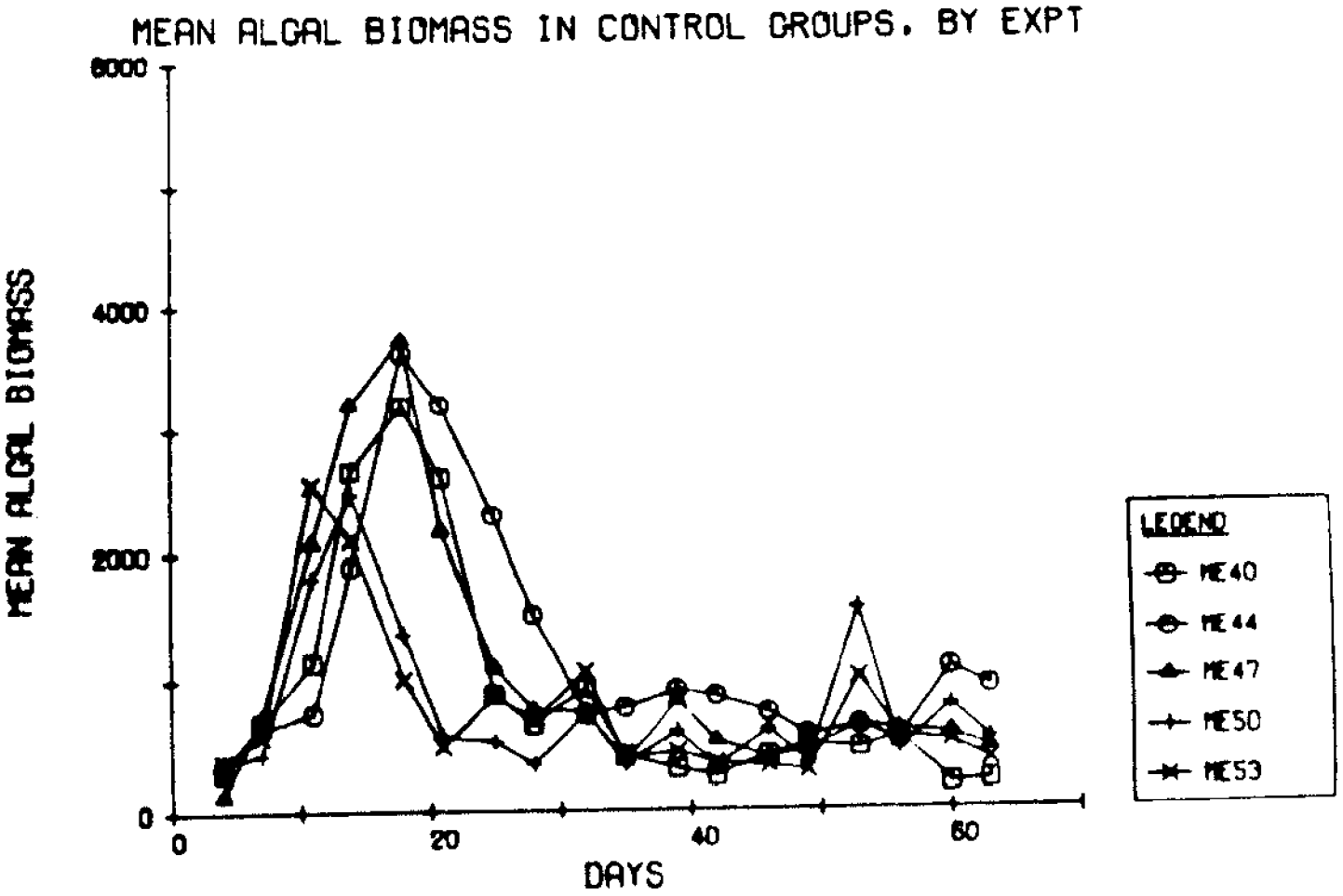

Figure 2. Algal biomass in control microcosms (symbols as in Figure 1). The units are volumes of algae, 10^4 μm^3 ml^{-1}. Note the increase in algal biomass concurred with nitrate depletion.

lishment of grazer populations (Figure 3) are demonstrated. The microcosm behavior is forced by the inoculation of small populations of 11 species of algae into a rich medium (0.5 mM NO_3 is the limiting nutrient) that meets all of their nutritional requirements, and the addition of grazers four days later. Because the initial medium contains only trace amounts of organics, the algae must grow if food is to be available for the grazers. The algal species compete among themselves for the initial algal nutrients and later for the recycled nutrients. The algae and grazers, especially the Daphnia, strongly interact. The nature of the interaction clearly shows the depletion of the initial nitrate supply concurrent with the increase in algal biomass; the algal biomass is reduced by the rapidly increasing Daphnia population. Eventually the Daphnia overgraze the algae, and the Daphnia population decreases to a more stable level; the algae may show a subsequent increase. Note that the initial conditions of the experiment force the nature of the responses. The community is structured to allow shifts in algal dominance so that some of the algal species support a planktonic grazer (Daphnia) while others support a benthic food chain (amphipods). If the grazers are removed by a toxicant, the level of algal nutrients allows an algal bloom to develop, as will be shown. If the primary production is reduced, the effects of the lower food supply can be reflected in eventually lowered grazer populations.

The protocol allows for the frequent sampling of 24 microcosms for nutrients, organism abundances and metabolic properties at high frequency (twice a week) and low cost (relative to field studies). For primary and secondary production, virtually all of the ecological properties that can be measured on natural ecosystems can be measured on microcosms.

The data illustrations and probability tables are generated from the computerized data handling system. The computer also provides tables of data for each microcosm, including means, standard deviations and coefficients of variance. The computer output provides some data in multiple formats to allow the human user to scan the data for different kinds of effect, for example, treatment effects or interactions within a microcosm. The computer package also includes a "comment sheet" on which all laboratory problems and extra observations are entered; this sheet is printed each time data are received from the computer.

The computer also performs numerous calculations to convert the organism counts (numbers and volume measured) into consistent units, totals the small, medium and large Daphnia into total Daphnia as well as estimates of Daphnia biomass, etc., and converts the three oxygen measurements into oxygen gain, loss, P/R ratio and change over 24 h. Treating the microcosm as a research system, we were able to explore a large number of alternate expressions of the data; treating it as a standard bioassay, we would reduce the number of variables handled.

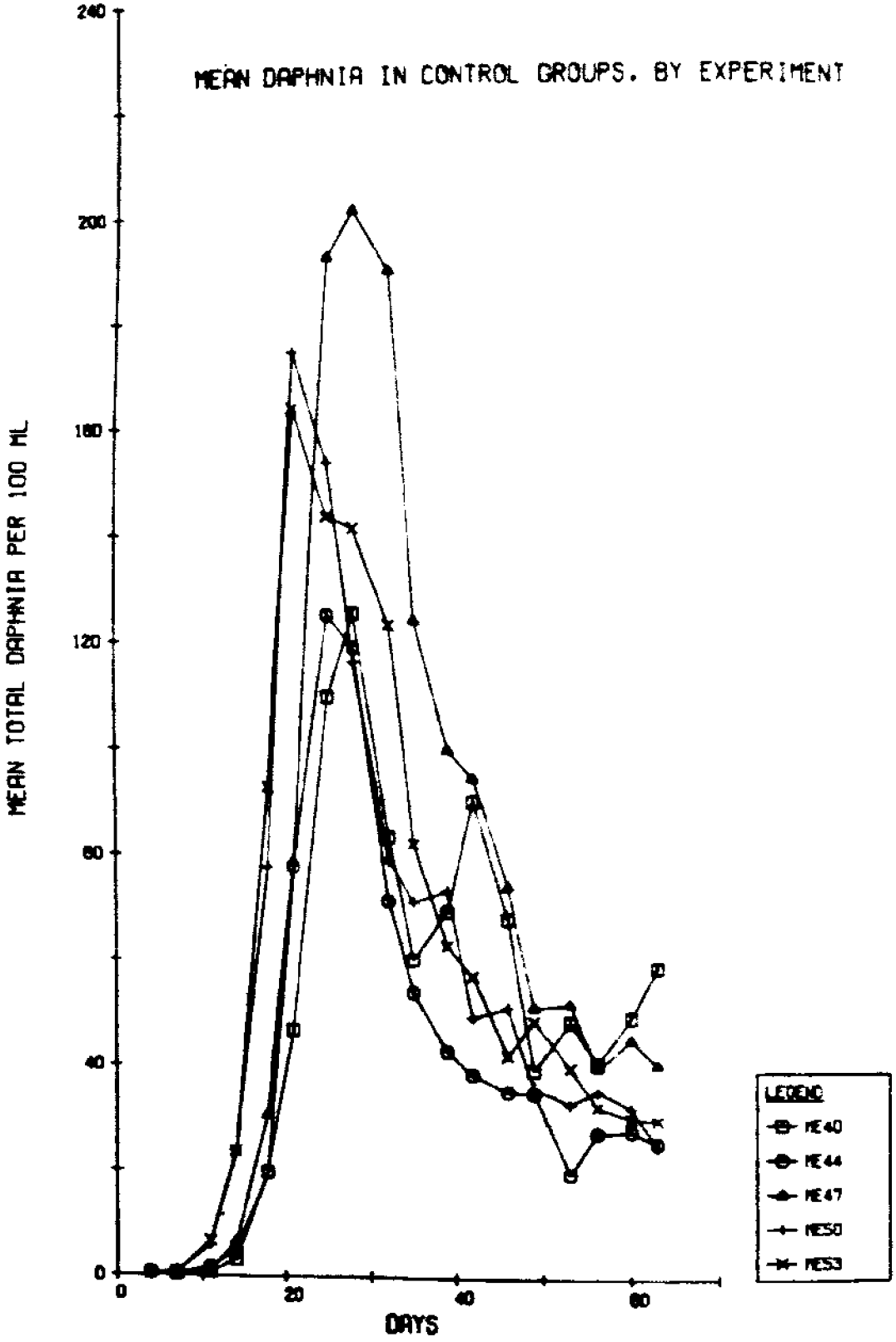

Figure 3. Daphnia density in control microcosms (symbols as in Figures 1 and 2). The units are the number of animals per 100 ml. Note the reduction in algal biomass (Figure 2), concurrent with the increase in Daphnia.

Automation of the data handling is possible only because the organisms are standardized between experiments; the data sheets allow for the reporting of a limited number of organisms that may result from contamination, but most of the organisms and all of the chemical-physical variables are entirely standard. It is the automation of the data handling that allows results to be interpreted quickly during the experiment (we recommend that the data through day 28 be examined and that the experiment be discontinued if seriously flawed) and at the end of the experiment.

From studying such microcosm experiments, we have been able to explore our ability to detect ecological responses to a variety of environmental stresses. Most of the problems we encountered are not unique to microcosms but are properties of natural ecosystems as well, for example, data collection over time from the same system, which compromises the statistical independence of the data; behavior of replicate systems in a similar manner but slightly out of phase with each other, which causes superficially large variances when the data are examined for a specific day; noisiness of species enumeration data.

The only problems that seem unique to small, batch microcosms are the depletion of microcosm volume by sample removal and the increased chance of random extinction of a species. The latter problem we meet by the weekly reinoculation of very small numbers of each species of alga and reinoculation of the animals if the counts indicate that the species is extinct. The reinoculations are made after the enumeration and are of such small numbers that the reinoculated organisms are fewer than our limits of detectability and do not influence our results unless they reproduce significantly. This ensures against random extinctions causing replicates to diverge or prevents extinctions caused by temporarily toxic conditions from having a permanent effect. In earlier experiments when we did not use reinoculation, any extinction was permanent. Random extinction of Daphnia in microcosms was shown to influence virtually all other properties, and to compromise our ability to detect treatment effects (Taub and Crow 1978). The reinoculation provides the ecological equivalent of immigration in natural ecosystems.

Demonstrating known mortalities was more difficult than originally anticipated. The first induced mortality experiments in the microcosms involved heat killing known proportions of the total microcosms or of the algae and other microrganisms or of the large grazers, and returning the killed material to the microcosms (Taub et al. 1980). In spite of the large magnitude of the mortalities (60% of the total, 45% of the algae and microorganisms, or 45% of the large grazers) the damage was repaired surprisingly quickly, usually within 10 days or fewer. If all trophic levels were damaged simultaneously, and early in the experiment when algal nutrients were available, the reduced grazing pressure allowed the algal

populations to recover rapidly. Effects on the Daphnia were greater if the algae were killed than if the Daphnia population itself was impacted.

It was surprisingly difficult to demonstrate statistical differences between control and treatment groups for many variables even immediately after the induced mortalities because slight variations between replicates, combined with a small sample size (four replicates per treatment in this experiment), created a wide acceptance region around the control mean. Upon examining the sources of variation between replicates, it became obvious that the replicates were behaving very similarly and sampling variations within microcosms were small. Increasing the number of replicates was therefore the most practical way to improve the power of the statistical test (Crow and Taub 1979).

The relationships between the variance among replicates, the sample size, the probability level of the Type I error, and the probability of statistically detecting an effect of a given magnitude have been described by Conquest (1983). Examination of the relationships shows that numbers of replicates less than five will rarely show statistical differences unless the variances (expressed as coefficient of variation, the standard deviation/mean) are very small, or differences between treatment groups very large. In some oligotrophic communities, the biological abundances may not be able to match the value needed to demonstrate a statistical difference. These relationships are often overlooked in field experiments when expenses dictate that only two or three replicates be used per treatment.

The ability of these microcosms to yield reproducible results between experiments is shown by means of five different experiments that were initiated at different times over 14 months (Figures 1, 2 and 3). Each line represents the mean of six replicate microcosms; only the controls are compared because each experiment included different toxicant treatments. To obtain this degree of reproducibility it was necessary to standardize the pre-inoculum algal culture condition and the initial algal density because differences affected subsequent community development (Taub et al. 1983). The differences between the experiments indicate that each experiment must have its own control group for statistical comparison with the treatment group.

RESULTS OF TOXIC CHEMICAL ADDITIONS TO AQUATIC MICROCOSMS

The real power of the aquatic microcosm bioassay is its ability to demonstrate the ecological effects of chemicals by comparing a treatment group with a control group within an experiment. Only a few examples can be presented here for illustrative purposes, and

the publications or contract reports (identified by ME numbers; see note appended to Acknowledgments) must be consulted for supporting data. To display the agreement among replicates within treatment groups, the six replicates for each of two treatment groups are shown for experiment ME 40 in Figures 4–7. These data represent half of the treatments of ME 40 and are for a species assemblage and medium similar to those used in streptomycin experiments ME 22 and ME 31. The other treatment groups in ME 40 used a slightly modified "new" species assemblage and medium that was used in subsequent ME experiments. The means in Figures 1, 2 and 3 are from the "new" assemblage and are therefore not the means of the treatment groups shown for Figures 4–7. The change in the medium (higher silicate) and the "new" assemblage (two diatom species added and a second blue-green species added, with the deletion of algal species that did not grow successfully in competition) improved our ability to show the effects of the chemicals, and did not alter any of the conclusions concerning the effects of streptomycin. The replicate data for the effects of streptomycin on "new" assemblages are in press (Taub et al. 1983). The arrow diagrams displaying the statistical differences (Figure 8) represent the entire experiment of 24 replicates.

Effects of Streptomycin

Streptomycin, a naturally occurring antibiotic used in three experiments (ME 22, 31 and 40) proved to be a selective algicide, causing algal dominance changes and an alteration of the normal successional sequence observed in controls. At 32 ppm, all variables associated with primary production were reduced temporarily, for example, dissolved oxygen gain during the lighted period (an index of photosynthesis) (Figure 4). Other measures of primary production, such as rates of carbon uptake and chlorophyll-a concentrations, were significantly depressed within 1 week of streptomycin treatment and remained so for 5 weeks in ME 40. Nutrient depletion was also delayed. An early elimination of the blue-green species was observed, and Ankistrodesmus was significantly reduced and the more resistant alga Scenedesmus was increased. Despite the fact that active streptomycin concentrations as determined by Bacillus subtilis assay decreased to undetectable levels within 3 weeks of addition, these shifts in algal species abundance persisted throughout the experiments. In some cases, shifts in individual species abundances were not apparent until after active streptomycin concentrations became undetectable. The volume of total algae (algal biomass) was reduced for a shorter period than were species shifts (Figure 5).

Decreased Daphnia populations were associated with the reduction in measures of primary production and standing crop (Figure 6). Although streptomycin did not prevent the increase in Daphnia, peak populations were smaller, presumably via a reduced food sup-

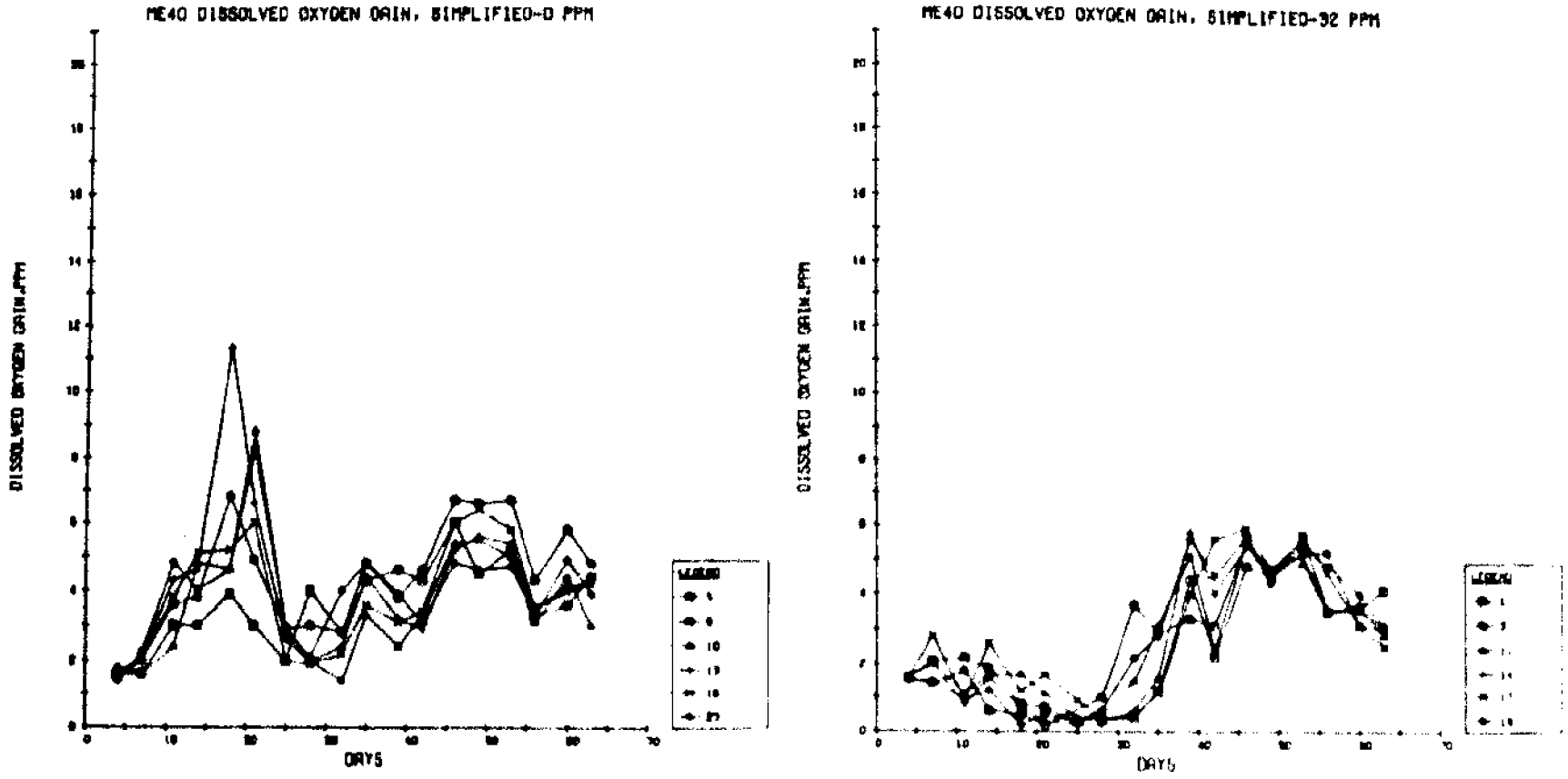

Figure 4. Dissolved oxygen gain during the lighted period (an estimate of net photosynthesis) for (SIMPLIFIED) control and 32 mg L^{-1} streptomycin treatment groups. Each line and symbol represents an individual microcosm. These microcosms constitute half of the ME 40 experiment (using an older species assemblage and medium) than the microcosms whose means are shown in Figures 1-3. The SIMPLIFIED COMMUNITY was similar to that used in the prior microcosm experiments with streptomycin.

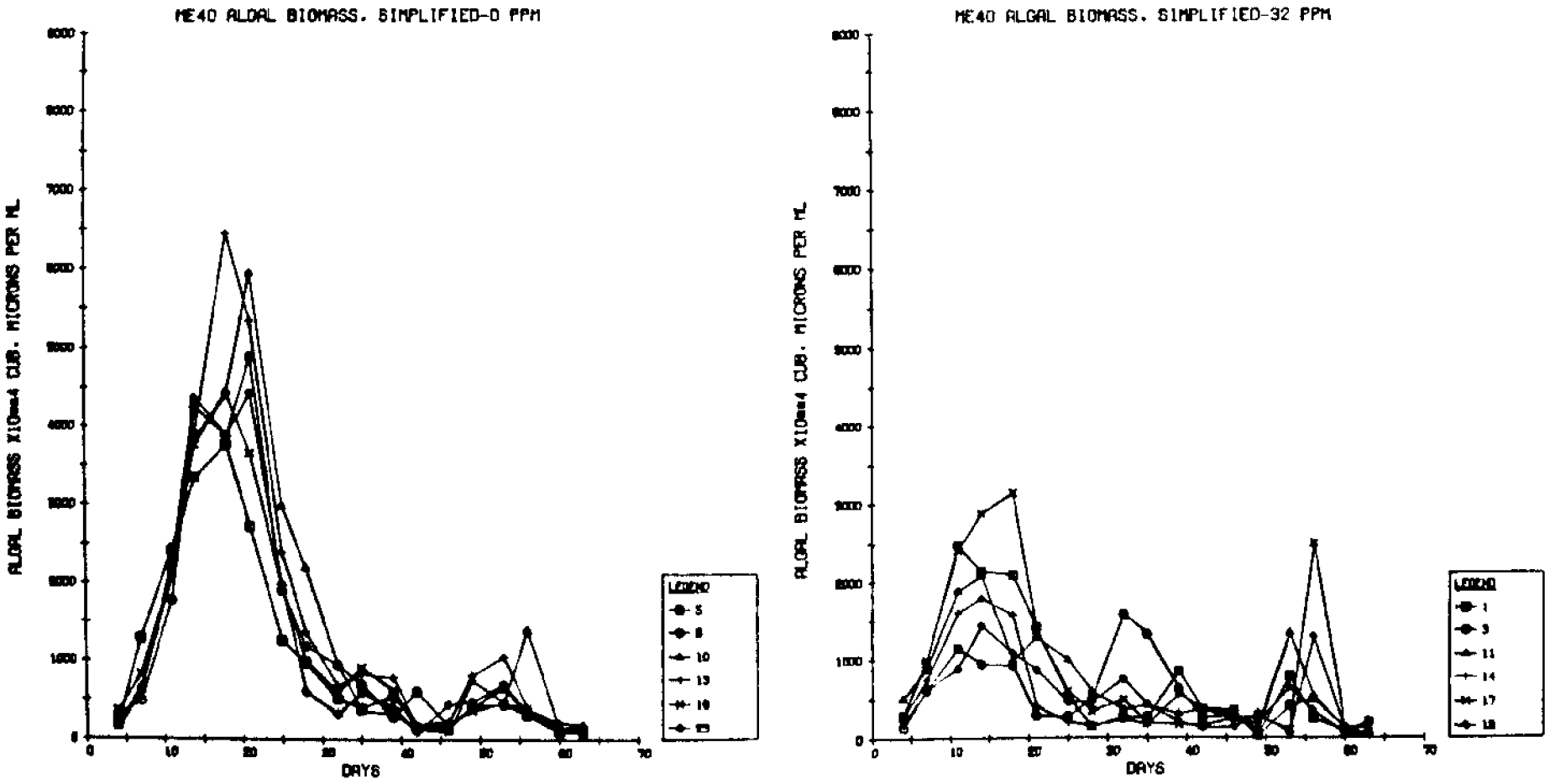

Figure 5. Algal biomass in (SIMPLIFIED) control and 32 mg L^{-1} streptomycin treated microcosms (symbols as in Figure 4). The units are volumes of alga, 10^4 μm^3 ml^{-1}.

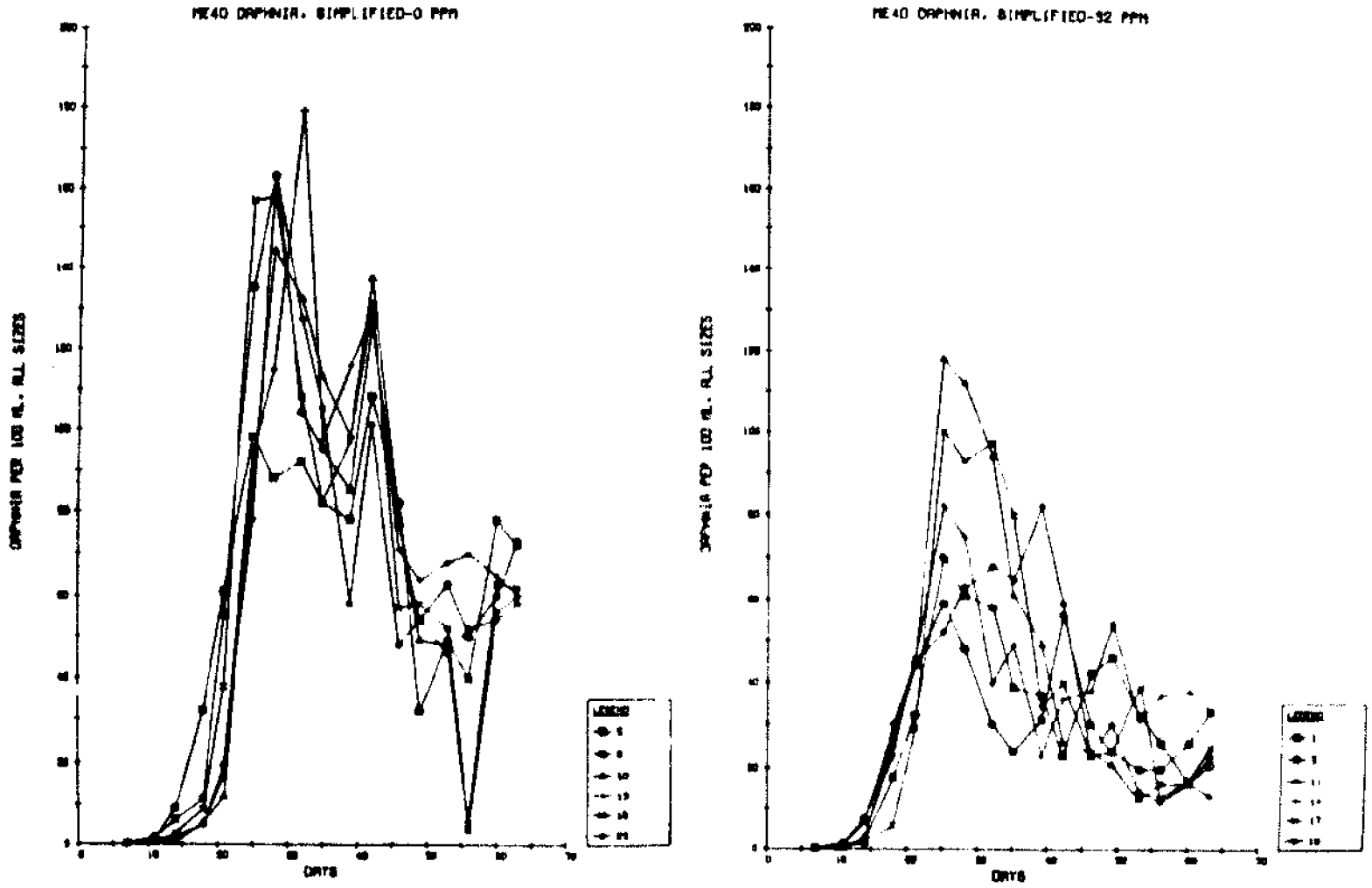

Figure 6. Daphnia density in (SIMPLIFIED) control and 32 mg L^{-1} streptomycin treated microcosm (symbols as in Figure 4). The units are the numbers of Daphnia per 100 ml.

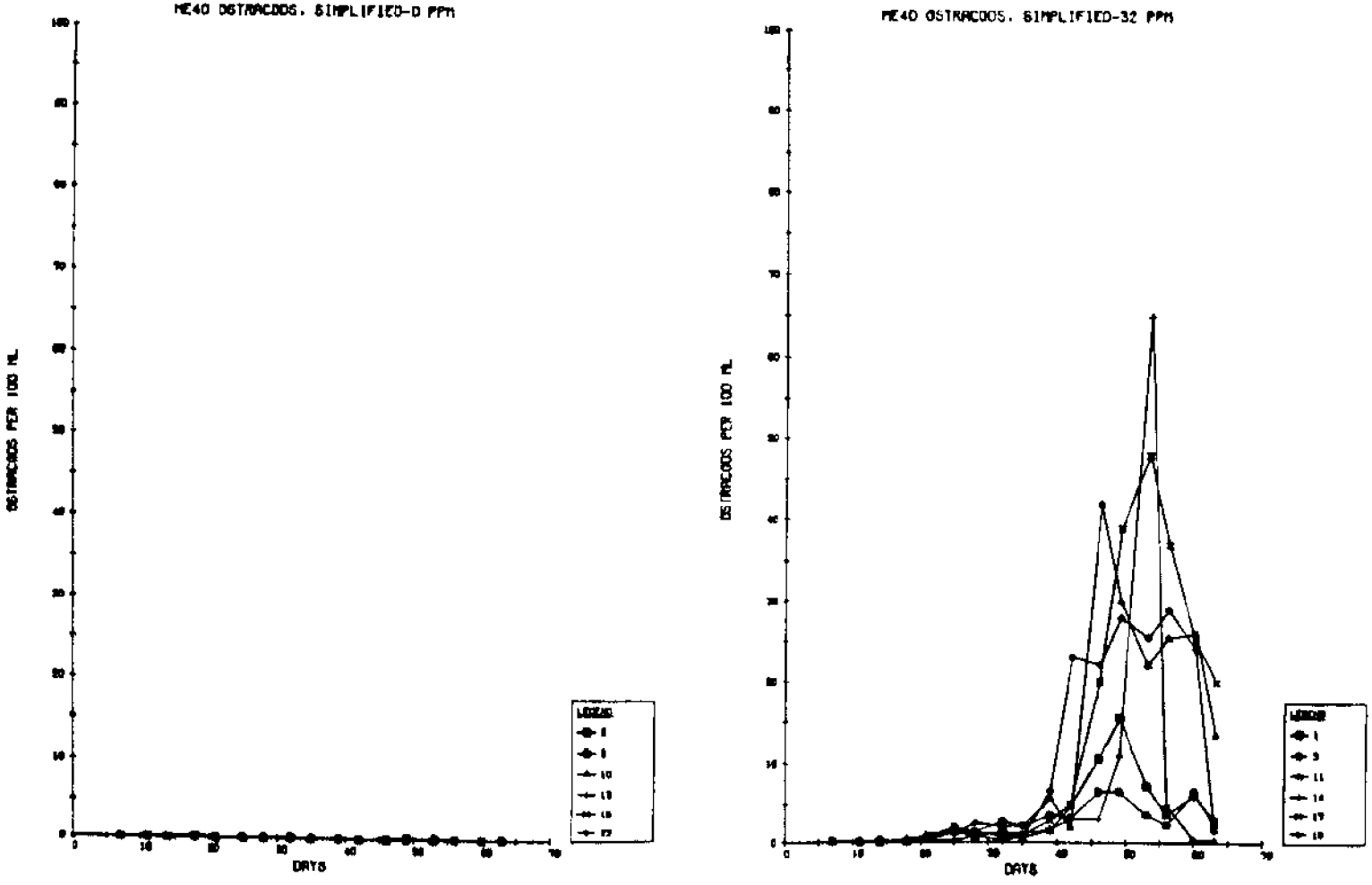

Figure 7. Ostracod density in (SIMPLIFIED) control and 32 mg L^{-1} treated microcosm (symbols as in Figure 4). Units are ostracods per 100 ml.

ply. Cyprinotus, an ostracod that most likely fills a detritivore role in the microcosms, vastly increased in density, a result that could not have been predicted from single species assay data (Figure 7). Some of these data have been published (Taub et al. 1981) and the complete data are in Reports ME 22, 31 and 40. The effects of treating microcosms with streptomycin early or late in their development has been explored (Kindig 1982).

The graphical presentation of data in Figures 4-7 display the agreement between replicate microcosms. The differences between control and treatment groups are obvious. As a means of summarizing the differences between the controls and treatment groups, the results of the analyses of variance are presented in arrow diagrams (Figure 8). These are linear contrasts between the controls and a treatment group. The number of arrows indicates the probability (one arrow is $P < 0.05$ and two arrows indicate $P < 0.01$); the direction of the arrow indicates whether the mean was greater than the control (up arrow), or lower than the control (down arrow). The arrow diagrams provide an indication of the important effects, but the means must also be considered in the biological interpretation. We have been able to avoid the need to transform discrete counts by using an inverse sampling technique.

We have not located literature on the effects of streptomycin on natural aquatic ecosystems, but have initiated some studies of our own. Samples taken from Green Lake (Seattle, Wash.)--a lake notorious for its noxious blooms of blue-green algae--were incubated in the laboratory with streptomycin. These studies validated the shifts in algal dominance with elimination of blue-greens (unpublished results, Univ. of Washington Graduate School Research Fund). Outdoor tanks have been filled with water from Lake Washington (Seattle, Wash.) and treated with streptomycin; results are still being analyzed. Visual observations confirmed a temporary reduction in algal standing crop (F.J. Hardy personal communication).

Effects of Malathion

Malathion was directly toxic to the grazers but not to the algae in single species tests. The main effect of a 10 ppb Malathion treatment in the microcosms was to eliminate most of the Daphnia for approximately 2 weeks, allowing a tremendous algal bloom to develop. During the period of Daphnia extinction, all algal community parameters were significantly increased, including Ankistrodesmus, Scenedesmus, Selenastrum and Anabaena densities, O_2 gain, chlorophyll-a concentration, rate of carbon uptake, algae available (to the Daphnia) and algal biomass. Following hydrolysis of the Malathion, Daphnia populations were reestablished (by weekly reinoculation) and rose sharply to peak densities comparable with those observed earlier in controls. The rapid increase in

LINEAR CONTRAST 2 STREPTOMYCIN MAIN EFFECT (0 VS. 32 PPM)

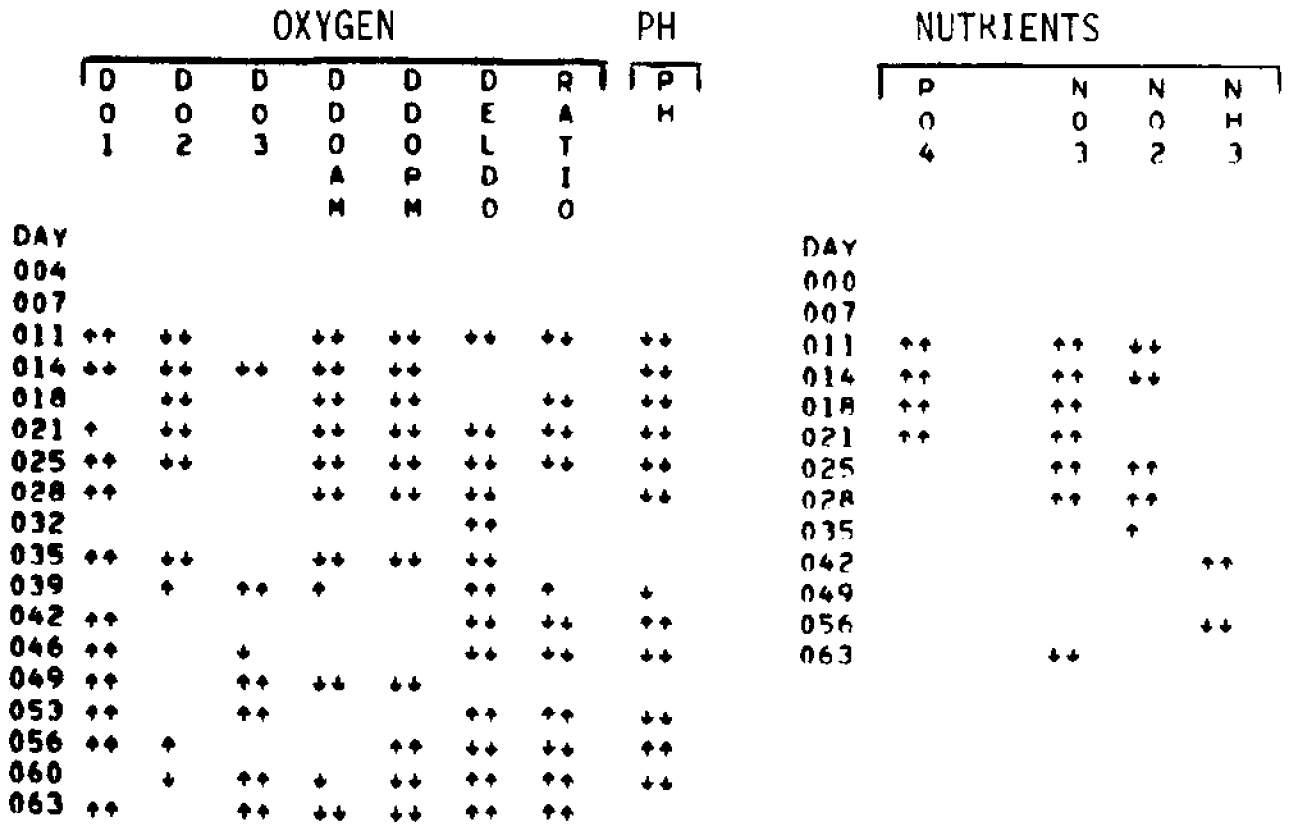

Figure 8a. Community variables. See text for explanation of symbols and Table 2 for variable names.

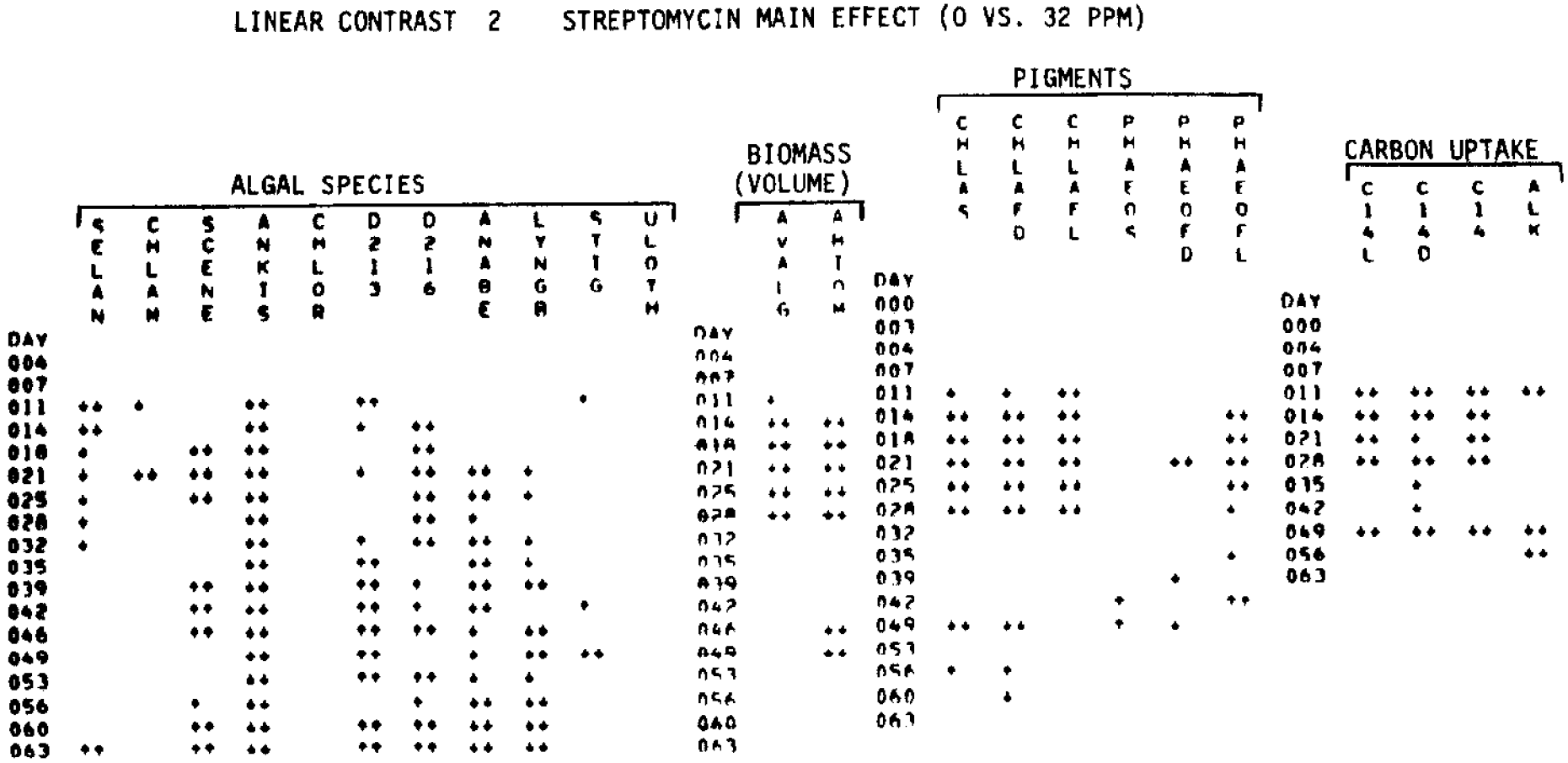

Figure 8b. Primary production variables. See text for explanation of symbols and Table 2 for variable names.

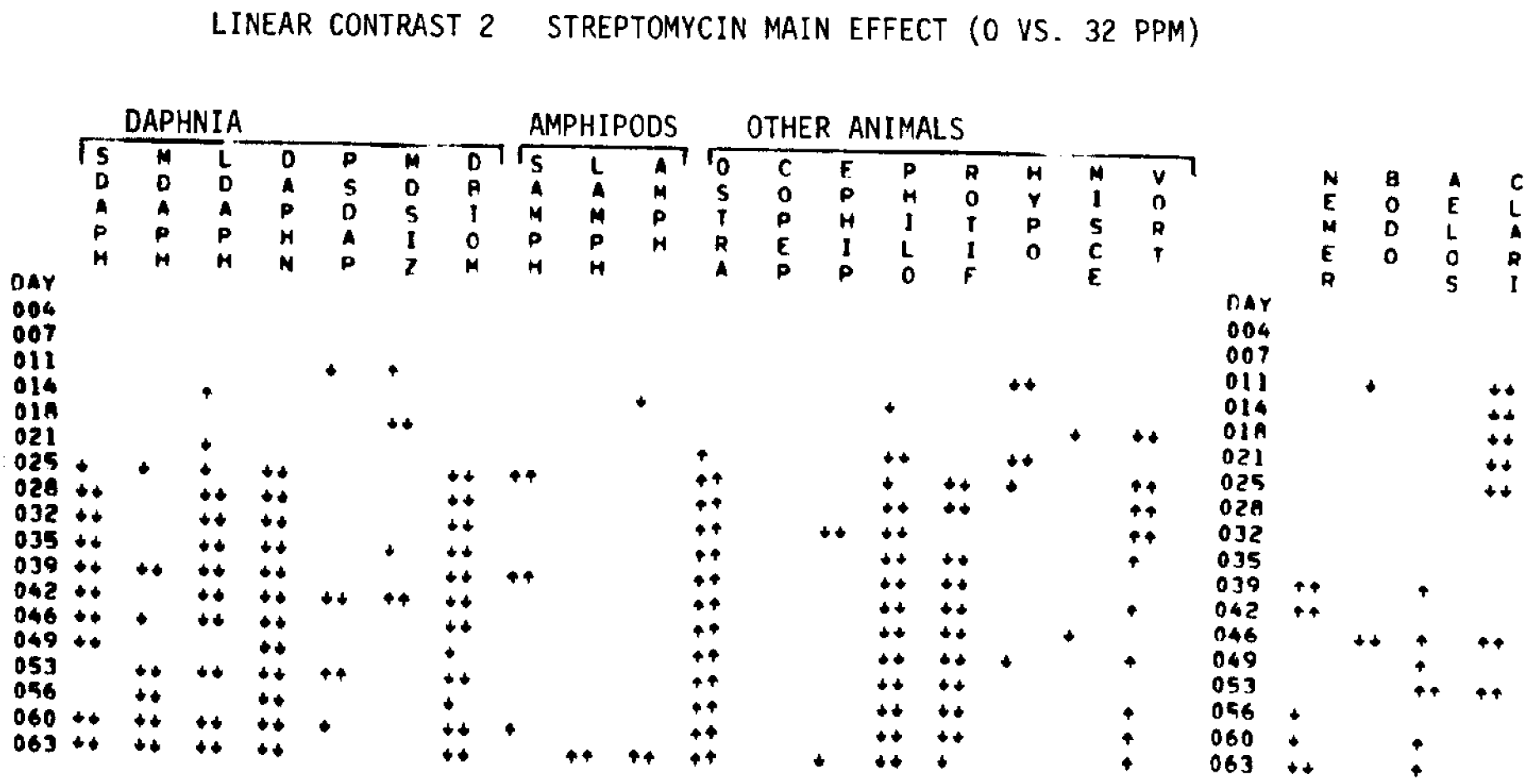

Figure 8c. Secondary production variables. See text for explanation of symbols and Table 2 for variable names.

grazing eliminated the algal bloom and sharply reduced all algal variables. Development of the filamentous algal community, which is less vulnerable to Daphnia grazing than are smaller algal cells, was delayed by Malathion treatment; significant reductions were observed in the densities of Lyngbya, Stigioclonium and Ulothrix. These may have been due to the persistence of the small green species such as Selenastrum, Chlorella and Chlamydomonas in the ungrazed, Malathion treated microcosms; these species could well be superior competitors to the filamentous species in the absence of grazing. Portions of these data have been published (Taub et al. 1981) and the complete data are in Report ME 44.

Although Malathion has been used extensively in aquatic environments, few ecosystem-level studies have attempted to evaluate its effects on all trophic levels. Mulla and Mian (1981) recently summarized much of the available information. They reported that Malathion in single species bioassays was not toxic to algae at the concentrations we used, and in the natural communities that were treated, daphnids or other invertebrates were often reported as killed, but no information was provided on the effects on the algae, either positive or negative. In pond studies the use of a similar insecticide, Dursban, was reported to cause algal blooms by the elimination of grazers (Hurlbert et al. 1972). Hurlbert et al. document other studies that have demonstrated algal blooms as the result of insecticide induced elimination of grazers. They have also reported Daphnia-rotifer competition interactions similar to those observed in the microcosms. Field studies confirmed our findings that Malathion did not remain active for long periods and that the reduction in invertebrates was temporary.

Effects of Copper Sulfate

Copper sulfate, depending upon the concentration used, was toxic to both the algal and animal components of the microcosms, but the latter were far more sensitive. We have been exploring the effects of chelation concentration on copper toxicity. With moderate concentrations of EDTA (0.026 mM) the effect of 2.5 ppm copper treatment (on day 21) was to eliminate the Daphnia populations for periods of 18 or more days. As with Malathion, this resulted in massive algal blooms, and the same species tended to increase in the absence of grazing; these included Ankistrodesmus, Scenedesmus, Chlorella and Selenastrum. Nitzschia, normally a dominant species, decreased. Rates of carbon uptake, chlorophyll-a concentration, and pH increased significantly during the period of active copper toxicity to the Daphnia. Following reestablishment of the Daphnia populations, Daphnia densities rose to peak values similar to those observed earlier in controls, and the algal bloom conditions were eliminated. Reduction of the EDTA concentration in the medium has resulted in a more sensitive bioassay microcosm.

These data, not yet published, are documented in Reports ME 53, ME 57 and ME 58.

Applications of copper in ponds and lakes for algal control have highly variable results. Applications for algal control may be at levels as high as 3 ppm or greater (McIntosh and Kevern 1974). McKnight (1981) noted the major effect of complexation with humic acids on copper speciation and suggested it was responsible for some of the differences between single species and field tests of algae. Ten days after treatment of a reservoir with 0.05 ppm copper, algal biomass and primary productivity returned to pre-treatment levels (McKnight 1981), although longer-lasting changes in phyto- and zooplankton composition were noted. Crance (1963) reported that addition of 0.05-0.08 ppm led to a reduction of the target alga Microcystis in fish ponds for periods of at most 25 days. Sawyer (1970) found that changes in the zooplankton community of a New Hampshire lake were the most persistent result of two applications of 0.035 ppm copper sulfate; primary production increased, then decreased briefly, and finally increased again 4 days after treatment. McIntosh and Kevern (1974) found that in one pond, addition of 3 ppm copper sulfate caused depression of cladoceran and rotifer populations, but the same treatment led to no effects in a second pond. A third pond treated with 1 ppm also showed no effects.

The effects of copper in the CEPEX marine enclosures (Thomas et al. 1977) depended upon the community structure. Copper added to communities in September resulted in increases in algal standing crop associated with the inhibition of zooplankton and reduced grazing pressure on the algal crops. These responses are similar to those observed in our microcosms. In contrast, when copper was added to communities established in June-July, phytoplankton crops, photosynthesis and growth rates were all initially inhibited. By the end of the 27 day experiment, treated systems had recovered to control conditions.

In spite of the reputation of copper sulfate as an algicide, it is rarely effective for long. Although it often changes algal dominance, it allows less sensitive species to achieve pre-treatment levels of primary production, and often has a longer and more dramatic effect of reducing sensitive species of zooplankton. The concentration of copper needed to be effective is related to the chemical properties of the water, usually expressed as hardness. Certainly, the microcosm results are consistent with at least some of the field results; additional research with variable concentrations of chelate may explain why different natural communities behave differently.

Effects of Dimilin

Dimilin was similar in effect to both Malathion and copper. Treatment with 100 ppb Dimilin resulted in the extinction of Daphnia for periods of up to 40 days. Amphipod and ostracod populations were also reduced, but to a lesser extent. The release of grazing pressure resulted in an algal bloom in which the same species tended to predominate as in previous experiments during ungrazed periods. As with Malathion treatment, Anabaena populations were significantly increased during the lengthy period of Daphnia extinction. Dimilin was eventually degraded, the Daphnia populations reestablished, and the algal community parameters reduced.

Our findings that Dimilin eliminated grazers and thereby caused algal blooms is similar to the finding in the microcosms treated with Malathion, and may be verified by analogy to the Dursban effects reported above (Hurlbert et al. 1972). Field investigations verify that Dimilin is more toxic to Daphnia than to amphipods and ostracods (Apperson et al. 1978; Ali and Mulla 1978; Farlow et al. 1978).

Effects of Triethylene Glycol

Triethylene glycol (TEG) was not demonstrated to be actively toxic to either algae or animals in single species tests, and initial development of TEG treated microcosms was not appreciably different from controls. A gradual and persistent lowering pH did become evident, however, in both TEG treatment groups, and was associated with reductions in primary production parameters, small sized species of algae which are the food source for Daphnia, eventual extinction of Daphnia, and an increase in rotifer density. It appears probable that the delay in response and observed effects was due to the accumulation of an acidic degradation product of TEG.

There is no a priori reason other laboratories cannot establish similar microcosms, but the possibility of their doing so has not been tested adequately. We are encouraged that our results with TEG confirmed the earlier findings of John Leffler's laboratory that TEG caused similar reductions in pH, with serious consequences to the algal communities and elimination of the Daphnia. Although he was using different organisms, his medium was very similar to the one we used; both have limited pH buffering capacity. Also, different variables were measured in the two systems, with only a few overlaps.

Although we have found no field literature on TEG, the effects demonstrated in the microcosm after acidification occurred are similar to those reported in lakes that have been acidified. Phytoplankton species dominance is often altered, Daphnia populations reduced or eliminated, and acid tolerant rotifers increased (Haines 1981).

COMPARISON OF HYPOTHESES BETWEEN MICROCOSM RESEARCHERS

Specifically there are three hypotheses representing differences between our approach and that of Leffler which can be experimentally tested: (1) that the organisms in the community will not affect the ecosystem-level responses, (2) that a single index of damage can be calculated on the basis of ecosystem-level responses, and (3) that microcosms should be allowed to reach stability before the treatment is initiated. Many of the data are available to test these hypotheses.

The results in our laboratory have shown that synthetic microcosms are feasible to establish, that replicates show similar behavior, and that it is possible to use simultaneous controls; that is, it is not necessary to consider each microcosm a unique entity that must serve as its own control. Rather, the use of simultaneous controls allows standard statistical comparisons to be made with acceptable rigor. The microcosm communities display interactions within trophic levels (competition), and between trophic levels (primary and secondary production). They also display effects not predicted from single species bioassays, for example, increases in populations from increased food supply and removal of competitors or predators (grazers).

RELATIONSHIP BETWEEN MICROCOSMS AND NATURAL COMMUNITIES

Caution must always be exercised in extrapolations between different communities, even if different natural communities are involved in the extrapolation. Water quality parameters such as nutrient level, pH buffering and chelation capacity must be considered in extrapolation from one community to another. It is well known that soft water communities have different vulnerabilities than hard water communities.

Differences in community structure and controlling processes can also be expected to limit the degree to which responses in one natural system will be predictive of changes in another natural system. For example, pond communities in which algal abundance is controlled by herbivores showed algal blooms when treated with the insecticide Dursban (Hurlbert et al. 1972); in contrast, a natural community whose grazer populations were at low density because of fish or invertebrate predation would not be likely to have an algal bloom as a result of an insecticide application (Hurlbert and Mulla 1981). The presence of competitors of differing sensitivities would also be expected to modify the responses of a specific community to a toxicant as in the mathematical model cited in the introduction. Also, the example was given of June and September

communities having different responses to the same concentration of copper: when the June community was dosed, the algal variables were reduced; when the September community was dosed, the algal variables were increased concurrent with the reduction in grazers (Thomas et al. 1977). Thus natural communities, although they share trophic level relationships, differ in their responses depending on the controlling factors.

Within the context of the papers in this volume, it seems most appropriate to ask what insights the microcosm results would supply for approaching the more difficult issue of measuring pollution effects in natural communities. The aquatic microcosm protocol had been designed to screen new chemicals and their degradation products for the effects they might have on ecologically important processes of primary and secondary production and nutrient recycling; and it was not designed to simulate a specific natural community.

However, it is natural to ask to what extent the microcosm results can be extrapolated to predicting the effects of a chemical on an aquatic community. At least the same precautions must be observed in extrapolating between microcosms and natural environments as between different natural environments. On the basis of agreement between the microcosm results and published field studies or our modest attempts at field validation, I hypothesize the following:

1. If a test chemical decreases primary production and alters algal species dominance, it is almost certain to have similar effects in a natural community, but it is unlikely that the microcosm results will predict the species that will become the new competitive dominant. The microcosm contains only a small subset of all possible species, and the outcome of competitive dominance depends on the species present and the balance of many complex relationships. The microcosm results may be more predictive of the taxonomic group of the species that will be most suppressed; for example, streptomycin inhibits blue-green algae (Cyanophyta or Cyanobacteria) to a greater extent than green algae in the microcosms and in samples from natural communities.
2. If the test chemical has little direct effect on primary production, but is selectively toxic to grazers, it will probably behave in a similar manner in natural systems, but again, the microcosms will not be able to predict which species will dominate. They are more likely to indicate which taxonomic groups may be most sensitive and therefore most reduced. Daphnia may be an adequate representative of Cladocera, but a poor representative of Copepoda.

3. Indirect effects that are observed in the microcosms, for example, algal blooms if grazers are eliminated, are also likely to occur in natural communities. However, given the uncertainties of predicting the species dominance of the direct effects of the chemical, the exact species involved in the indirect effects are not likely to be predicted.

Note that extrapolation is more complex than seeking a scaling factor that would convert the microcosm results to predict the effects in various real environments.

It is often asked if microcosms are likely to be better predictors than single species bioassays of natural ecosystem responses to chemicals. Because single species bioassays can rarely indicate responses other than shortened life span and reduced reproductive rate, they tend to predict reductions in population. Most single species bioassays also test primarily for the effects of the parent compound on the test organisms, and therefore are likely to miss toxic properties of degradation compounds. Because acute or chronic single species tests generally do not allow chelation or degradation of the parent compound, or replacement of killed organisms, they tend to predict permanent alterations of the biological community.

In contrast, microcosm results can indicate either reductions from direct toxicity or increases if the food supply is increased, competition reduced, or predators reduced. The potential for the removal of the parent compound and the accumulation of degradation compounds allows temporary toxicity and recovery to be demonstrated, or alternatively, more toxic conditions to become apparent. Some of the changes that have been observed could not have been predicted at all from the single species results.

In summary, these specialized types of microcosms (1) utilize a valid experimental design, (2) represent natural responses which could be validated by testing natural communities with similar water quality and community organization, and (3) include the measurement of significant parameters and processes in that they include important ecosystem functions, although they do not include harvestable species or measure risks to human health.

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Requests for Protocol or ME## reports on FDA Contracts 223-76-8348 and 223-80-2352 should be directed to the Project Officers, Dr. Buzz L. Hoffmann, Chief, Env. Impact Section, Bureau of

Foods, HFF-27, Rm. 6415, Food and Drug Administration, 200 C Street, S.W., Washington, DC 20204 or Mr. John C. Matheson III, Bureau of Veterinary Medicine, HFV-310, Rm. 7-89, Food and Drug Administration, 5600 Fishers Lane, Rockville, Md. 20857.

REFERENCES

Ali, A. and M.S. Mulla. 1978. Impact of the insect growth regulator Diflubenzuron on invertebrates in a residential-recreational lake. Arch. Environ. Contam. Toxicol. 7: 483-491.

Apperson, C.S., C.H. Schaffer, A.E. Colwell, G.H. Werner, N.L. Anderson, E.F. Dupras, Jr. and D.R. Longanecker. 1978. Effects of Diflubenzuron on Chaoborus astictopus and nontarget organisms and persistence of Diflubenzuron in lentic habitats. J. Econ. Entomol. 71(3): 521-527.

Conquest, L.L. 1983. Assessing the statistical effectiveness of ecological experiments. Int. J. Environ. Stud. (in press).

Cooke, G.D. 1977. Experimental aquatic laboratory ecosystems and communities. In: J. Cairns, Jr. (ed.), Aquatic Microbial Communities, pp. 59-103. Garland Reference Library of Science and Technology, Vol. 15, Garland Publ., New York.

Crance, J.H. 1963. The effects of copper sulfate on Microcystis and zooplankton in ponds. Prog. Fish-Cult. (1963): 198-202.

Crow, M.E. and F.B. Taub. 1979. Designing a microcosm bioassay to detect ecosystem level effects. Int. J. Environ. Stud. 13: 141-147.

Farlow, J.E., T.P. Breaud, C.D. Steelman and P.E. Schilling. 1978. Effects of the insect growth regulator Diflubenzuron on non-target aquatic populations in a Louisiana intermediate marsh. Environ. Entomol. 7(2): 199-204.

Freeman, L. 1953. A standardized method for determining toxicity of pure compounds to fish. Sewage Ind. Wastes 25: 845, 1331.

Giesy, J.P., Jr. (ed.) 1980. Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978. (CONF. 781101, National Technical Information Service, Springfield, Va.)

Haines, T.A. 1981. Acidic precipitation and its consequences for aquatic ecosystems: A review. Trans. Am. Fish. Soc. 110: 669-707.

Hammons, A.S. (ed.). 1981. Ecotoxicological Test Systems: Proceedings of a Series of Workshops. ORNL-5709; EPA 560/6-81-004, Oak Ridge National Lab., Oak Ridge, Tenn.

Harris, W.F. 1980. Microcosms as Potential Screening Tools for Evaluating Transport and Effects of Toxic Substances. ORNL/EPA-4; EPA-600-80-042, Oak Ridge National Lab., Oak Ridge, Tenn.

Hurlbert, S.H. and M.S. Mulla. 1981. Impacts of mosquitofish (Gambusia affinis) predation on plankton communities. Hydrobiologia 83: 125-151.

Hurlbert, S.H., M.S. Mulla and H.R. Willson. 1972. Effects of an organophosphorus insecticide on the phytoplankton, zooplankton, and insect populations of fresh-water ponds. Ecol. Monogr. 42: 269-299.

Kersting, K. 1975. The use of microsystems for the evaluation of the effect of toxicants. Hydrobiol. Bull. 9: 102-108.

Kersting, K. 1978. Experiments with dichlobenil in a micro-ecosystem. Proceedings EWRS 5th Symposium on Aquatic Weeds. European Weed Research Society, Wageningen, Netherlands.

Kindig, A.C. 1982. Interactions between algal nutrient availability and an algicide in aquatic microcosm communities. Ph.D. Dissertation, Univ. of Washington, Seattle, Wash.

Leffler, J. 1982. Development of an ecosystem level microcosm test for screening the effects of organic chemicals. Presented at the Society of Environmental Toxicology and Chemistry (SETAC), 2nd Annual Meeting, Nov. 22-25, 1981, Arlington, Va.

McIntosh, A.W. and N.R. Kevern. 1974. Toxicity of copper to zooplankton. J. Environ. Qual. 3(2): 166-170.

McKnight, D. 1981. Chemical and biological processes controlling the response of a freshwater ecosystem to copper stress: A field study of the $CuSO_4$ treatment of Mill Pond Reservoir, Burlington, Massachusetts. Limnol. Oceanogr. 26(3): 518-531.

Metcalf, L., K. Sangrha and I.P. Kapoor. 1971. Model ecosystem for the evaluation of pesticide biodegradability and ecological magnification. Environ. Sci. Technol. 5(8): 709-713.

Metcalf, R.L., I.P. Kapoor and A.S. Hirwe. 1972. Development of persistent biodegradable insecticides related to DDT. In: Degradation of Synthetic Organic Molecules in the Biosphere, pp. 244-259. National Academy of Sciences, Washington, D.C.

Metcalf, R.L., G.M. Booth, C.K. Schuth, D.J. Hansen and Po-Yung Lu. 1973. Uptake and fate of di-2-ethylhexyl phthalate in aquatic organisms and in a model ecosystem. Environ. Health Perspect. 4: 27-34.

Mulla, M.S. and L.S. Mian. 1981. Biological and environmental impacts of the insecticides Malathion and Parathion on nontarget biota in aquatic ecosystems. Residue Rev. 78: 101-135.

Nixon, S.W. 1969. A synthetic microcosm. Limnol. Oceanogr. 14: 142-145.

Reinert, R.E. 1972. Accumulation of dieldrin in an alga (Scenedesmus obliquus), Daphnia magna and the guppy (Poecilia reticulata). J. Fish. Res. Board Can. 29(10): 1413-1418.

Ringelberg, J. 1977. Properties of an aquatic microecosystem. II. Steady-state phenomena in the autotrophic subsystem. Helgol. Wiss. Meeresunters. 30: 134-143.

Ringelberg, J. and K. Kersting. 1978. Properties of an aquatic microecosystem. I. General introduction to the prototypes. Arch. Hydrobiol. 83: 47-68.

Sawyer, P.J. 1970. The effects of copper sulfate on certain algae and zooplankters in Winnisquam Lake, New Hampshire. Water Resources Research Center Rep. WWR-2, Univ. of New Hampshire, Durham, N.H.

Sugiura, K., M. Goto and Y. Kurihara. 1982. Effect of Cu^{2+} stress on an aquatic microcosms: A holistic study. Environ. Res. 27: 307-315.

Taub, F.B. 1969a. A continuous gnotobiotic (species defined) ecosystem. In: J. Cairns, Jr. (ed.), The Structure and Function of Freshwater Microbial Communities, pp. 101-120. Research Monograph 3, Virginia Polytechnic Inst. and State Univ., Blacksburg, Va.

Taub, F.B. 1969b. Gnotobiotic models of freshwater communities. Verh. Int. Ver. Limnol. 17: 485-496.

Taub, F.B. 1969c. A biological model of a freshwater community: A gnotobiotic ecosystem. Limnol. Oceanogr. 14(1): 136-142.

Taub, F.B. 1973. Biological models of freshwater communities. EPA 660/3-73-008, Washington, D.C.

Taub, F.B. 1976. Demonstration of pollution effects in aquatic microcosms. Int. J. Environ. Stud. 10(4): 23-33.

Taub, F.B. 1977. A continuous gnotobiotic (species-defined) ecosystem. In: J. Cairns, Jr. (ed.), Aquatic Microbial Communities, pp. 105-138. Garland Reference Library of Sciences and Technology, Vol. 15, Garland Publ., New York. (Reprint of 1971 article.)

Taub, F.B. 1982. Direct and indirect effects of test chemicals displayed by synthetic, aquatic microcosms. Presented at the Society of Environmental Toxicology and Chemistry (SETAC), 2nd Annual Meeting, Nov. 22-25, 1981, Arlington, Va.

Taub, F.B. and M.E. Crow. 1978. Loss of a critical species in a model (laboratory) ecosystem. Verh. Int. Ver. Limnol. 20: 1270-1276.

Taub. F.B. and M.E. Crow. 1980. Synthesizing aquatic microcosms. In: J.P. Giesy, Jr. (ed.). Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp. 69-104. (CONF-781101, National Technical Information Service, Springfield, Va.)

Taub, F.B. and A.M. Dollar. 1968. The nutritional inadequacy of Chlorella and Chlamydomonas as food for Daphnia pulex. Limnol. Oceanogr. 13(4): 607-617.

Taub, F.B. and D.H. McKenzie. 1973. Continuous cultures of an alga and its grazer. Bull. Ecol. Res. Comm. (Stockholm) 17: 371-377.

Taub, F.B., M.E. Crow and H.J. Hartmann. 1980. Responses of aquatic microcosm to acute mortality. In: J.P. Giesy, Jr. (ed.). Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp. 513-535. (CONF-781101, National Technical Information Service, Springfield, Va.)

Taub, F.B., P.L. Read, A.C. Kindig, M.C. Harrass, H.J. Hartmann, L.L. Conquest, F.J. Hardy and P.T. Munro. 1983. Demonstration of the ecological effects of streptomycin and Malathion on synthetic aquatic microcosms. Presented at the 6th ASTM symposium on Aquatic Toxicology, Oct. 12-13, 1981, St. Louis, Mo.

Thomas, W.H., O. Holm-Hansen, D.L.R. Seibert, F. Azam, R. Hodson and M. Takahashi. 1977. Effects of copper on phytoplankton standing crop and productivity: Controlled ecosystem and experiment. Bull. Mar. Sci. 27: 34-43.

Witherspoon, J.P., E.A. Bondietti, S. Draggan, F.B. Taub, N. Pearson and J.R. Trabalka. 1976. State-of-the-Art and Proposed Testing for Environmental Transport of Toxic Substances--Final Report. EPA-560/5-76-001; EPA 40-404-73, Oak Ridge National Lab, Tenn. GPO, Wash. D.C.

Chapter 3. Community Parameters and Measures of Community Impact

A Framework for Evaluating Community Measures of Marine Pollution

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INTRODUCTION

Both theoretical and empirical results in ecology indicate that community composition, as expressed in the abundance of individuals of different species, is strongly affected by the physical and chemical properties of the area that the community occupies. From this general result one might reasonably argue that the effect of marine pollutants could be measured by the change in community composition. This paper presents a systematic approach to investigating the practical usefulness of pollution indicators based on the community composition.

There is a large literature on the indices of community composition; Green's (1979) recent book presents a good review of the literature. A series of volumes on statistical ecology (Patil 1979), particularly volumes 6, 7, 10 and 11, contains many papers dealing with community properties and pollution. Using community composition to indicate the effect of marine pollution is a common strategy in environmental assessment. The best documented case history of this kind of strategy is found in the literature evaluating a small oil spill in West Falmouth, Mass. (Sanders et al. 1980). A quick review of all this literature shows that making the connection in the real world between changes in community structure and pollution is not as straightforward as one might hope.

Rather than review this literature again we will develop a general setting in which community indicators of pollution effects are used, and a general method of evaluating the efficacy of these measures. We then evaluate the effectiveness of two community measures, similarity and diversity, as indicators of pollution.

COMMUNITY INDICATORS

This section presents, in a formal setting, the problems of finding effective community-based measures of marine pollution. This should be thought of largely as a conceptual framework, rather than as a starting point for the analysis of data from surveys.

We can think of the relationship between pollution and the community as a very complicated nonlinear regression problem. Let $\underline{y}$ denote a k-dimensional vector that describes the properties of the community at some point, t, in either time or space. The i-th component of the vector $\underline{y}$, y_i, might denote the mean number of individuals of species i in a square meter of sediment. If we knew the vector of pollutant concentrations at the point t, $\underline{z}_t$, and the vector of natural physical and chemical parameters at the point t denoted by $\underline{x}_t$, then we might be able to predict the mean community vector $\underline{y}$:

$$\underline{y} = \underline{f}(\underline{x}_t, \underline{z}_t).$$

The mean community vector, $\underline{y}$, is not observed directly, but rather is estimated by sampling the community. Let $\underline{Y}$ denote the observed community vector:

$$\underline{Y} = \underline{y} + \underline{e}.$$

The error term, $\underline{e}$, incorporates the random effects of local variation and sampling error.

At this point we have defined an impossible problem. We have both an unknown k-dimensional vector, $\underline{y}$, and an unknown k-dimensional function, $\underline{f}$. It is clear that the number of observations in time and space will be too limited to estimate the functional relationship between $\underline{x}$, $\underline{z}$ and $\underline{y}$.

The key to the solution of this problem is to reduce the dimensionality so that we can begin to investigate the relationship between the pollutant and the community. The dimension-reducing problem is carried out in two steps. The first step is to define an environmental effect function, E, that measures the mean effect of the pollutant on an important community parameter G. G could represent productivity, biomass numbers or presence of important species. Let us suppose that the important community parameter, G, is a function of the community vector alone, $G(\underline{y})$. The effect function, E, is then the contrast between areas with pollutant at zero and at $\underline{z}$.

$$E(\underline{z}; \underline{x}) = G(f(\underline{z}; \underline{x})) - G(f(o; \underline{x})). \qquad (1)$$

This is all very well if one can control for $\underline{x}$, the natural variable. In field studies and studies of natural events in the ocean the variability due to sampling and local variation in $\underline{x}$ may be large enough to make estimation of E impossible from the typical set of environmental field data.

Figure 1 illustrates the above equations for the case when there is a single pollutant variable, for example, the concentration of mercury, and a single natural variable, $\underline{x}$. The contours of the function G, for example, biomass, are plotted in the figure in solid lines. The dashed lines parallel to the $\underline{x}$ axis are the effect contours represented by eq. (1).

Since the natural variation in $\underline{x}$ is often much larger than the effects due to pollution, the second dimension-reducing step is to find a community index that is a function of $\underline{y}$, say I ($\underline{y}$), that has two properties. The first property is that it is relatively insensitive to variation in the natural parameters. The second is that the index is relatively sensitive to the pollutant effect, E. In other words, we need an index that is highly correlated with the pollutant effect, E, over a range of natural conditions, $\underline{x}$. In terms of Figure 1, our index, I ($\underline{y}$), should have the property that its contour lines, indicated by the dotted lines in Figure 1, are more nearly parallel to the x-axis.

Figure 1 can be used to illustrate some of the problems with using field observation programs to detect effects of pollutants. For example suppose only G and the pollutant level, $\underline{z}$, are measured in a line transect survey. The observed relationship between G and $\underline{z}$ will depend on orientation of the line transect. If the transect is parallel to the G contours then there will be no decrease in G as the pollutant levels increase. On the other hand, if the transect were perpendicular to the G contours the effect of the pollutant would be overestimated. In both cases the hypothetical community index, I ($\underline{y}$), would give a more reliable indication of the pollutant's effect.

Finding a good community based indicator of pollution is not merely a statistical problem, but rather a theoretical and applied ecological problem. From what is known about interaction within communities and the effects of a pollutant on a community, ecologists should be able to devise a number of indices that at least in theory have the properties outlined above.

The statistical problem is then relatively straightforward: to design experiments to evaluate a relatively small number of competing community indices. This could be done using controlled ecosystems experiments, such as MERL (Marine Ecosystems Research Laboratory at the University of Rhode Island) and CEPEX (Controlled Ecosystem Pollution Experiment).

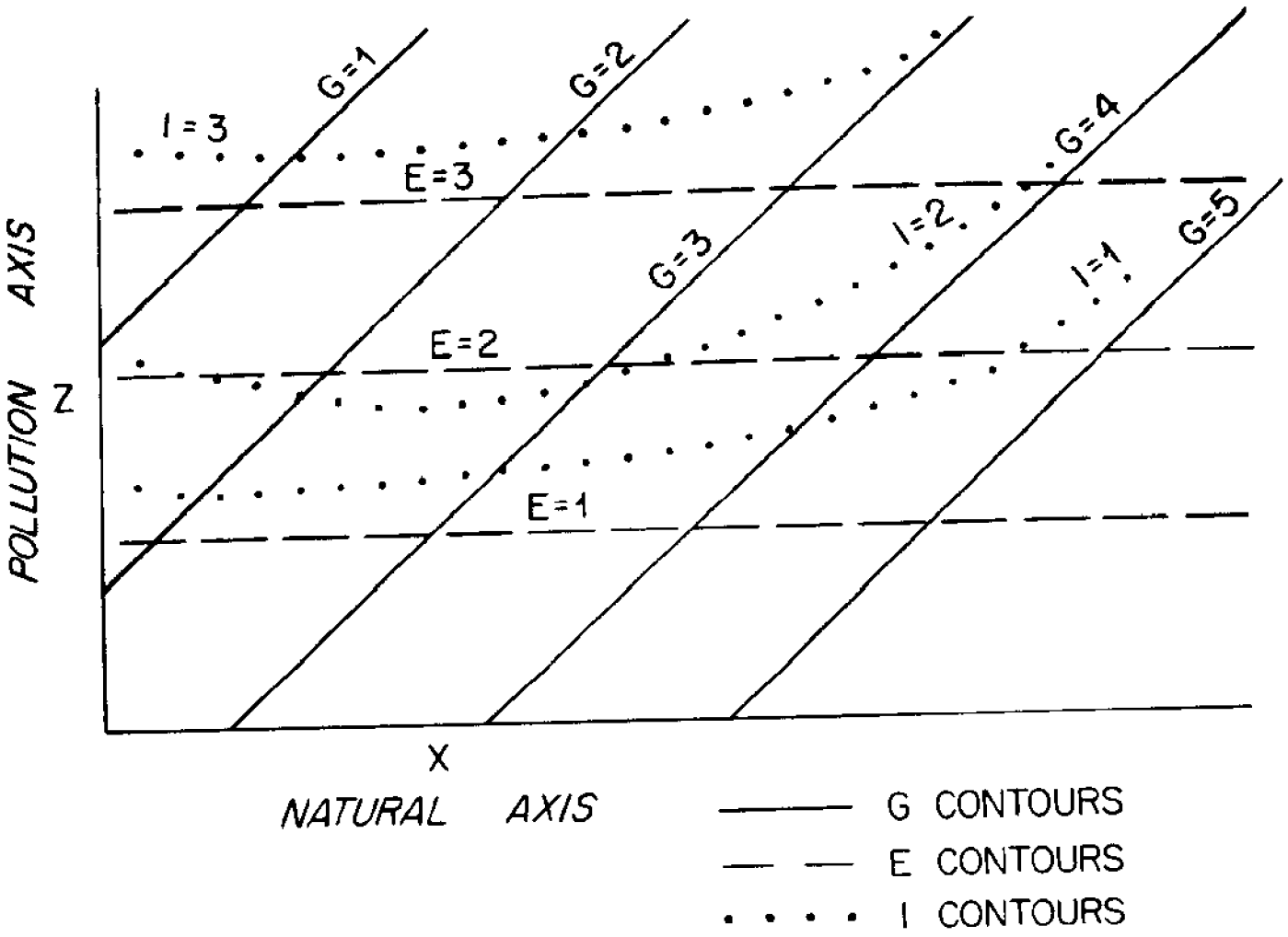

Figure 1. Response surface contours.

EMPIRICAL EVALUATION OF COMMUNITY INDICES

The properties of a good community index of pollution may be obvious. Constructing an index with these properties is more difficult. In this section we investigate diversity and similarity measures as possible indices of pollution.

Diversity Indices

A number of theoretical arguments indicate that community diversity will decrease with decreasing environmental predictability (Dennis and Patil 1979; Sanders 1968). Thus, diversity indices appear to be natural candidates for community-based measures of marine pollution.

How would we seek to evaluate a diversity index? A minimum criterion is that a diversity index should be correlated with known concentrations of pollutant under controlled experimental conditions. The measure should also have good sampling or statistical properties and should be relatively insensitive to changes over time in the natural environment.

Figure 2 shows the results of such an evaluation using benthic community data from MERL. Hurlbert's (1971) expected species index of species diversity is used to compare MERL oiled and control tanks. While all tanks were experiencing considerable changes with season, etc., as well as changes in the community due to the effects of the oil on the benthic community, there was little change in the community diversity index. Our experience with both MERL and CEPEX and the work of others seems to indicate that these results are fairly common.

In summary, diversity measures, like all indices, provide a way to reduce a complex multivariate time series of changes in species abundance to a single univariate time series; that is, to changes in diversity over time. The statistical properties of diversity indices have been well studied (Smith and Grassle 1977; Good 1953; Bowman et al. 1971) and present no particular difficulties. The results in the studies cited above seem to indicate that diversity is not sensitive to short term fluctuations caused by either natural or pollution events.

Similarity Indices

Another widely used approach is to compare communities in affected and relatively unaffected areas by using a measure of similarity that gives a numerical indicator of the difference between the two communities' species composition. All similarity measures have a value of one if the proportion of individuals from each species in both communities is the same, and the similarity measure is zero if the two communities have no species in common. There exists a large literature on similarity measures (Goodman and Kruskal 1980; Orloci et al. 1979).

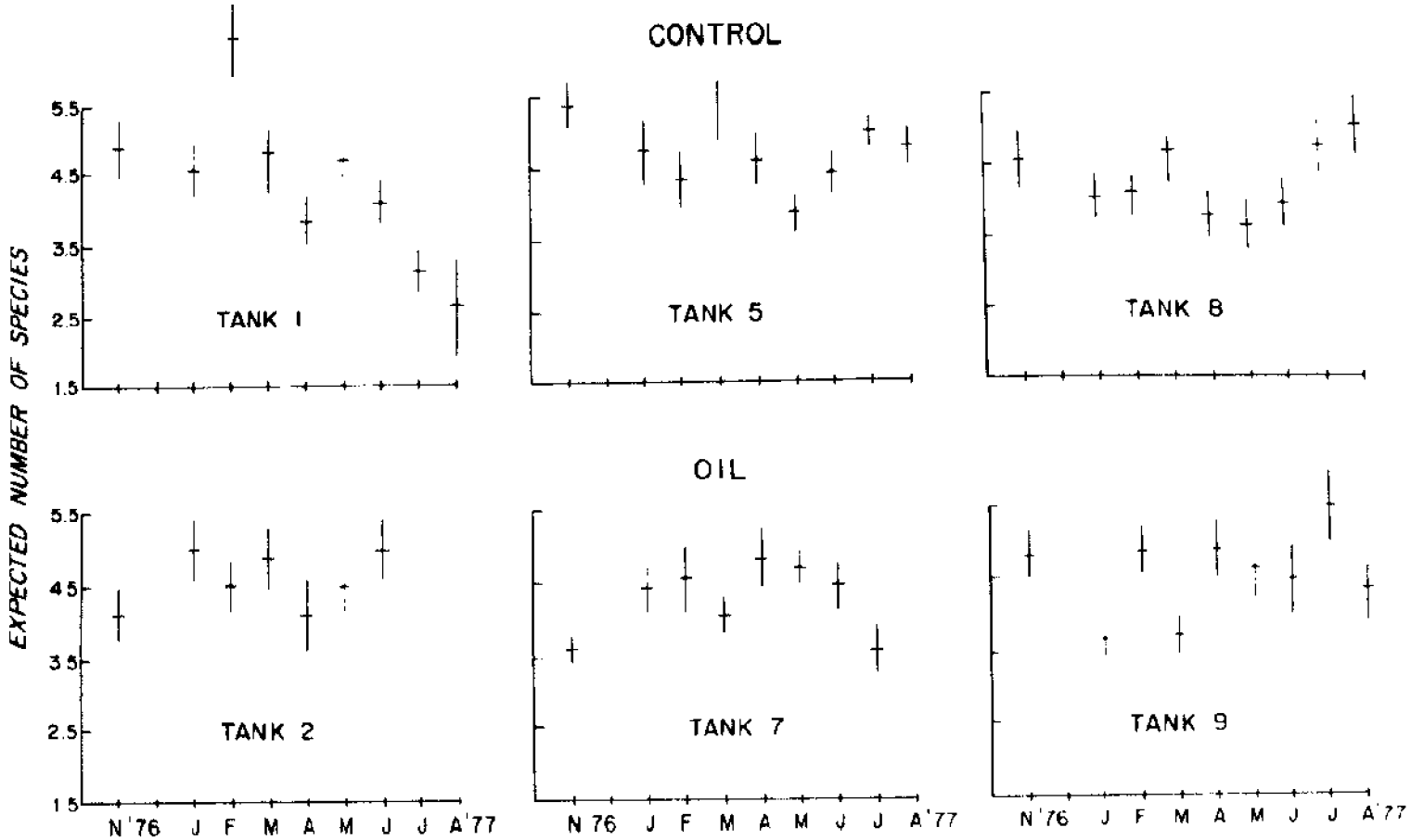

Figure 2. Hurlbert's estimate of the expected number of species in a random sample of size 20 drawn from the community in MERL. Oil was added to the experimental tanks after the February sampling date (Smith et al. 1979).

The motivation for using similarity measures as indicators of response to a pollutant is that the community species composition should change, with sensitive species dropping out and more robust species increasing their relative importance as pollution increases. Thus, a community based indicator of pollution effect is the change in community composition as measured by a similarity index.

Before we discuss some of the difficulties with using this index let us look at an example of its application in a controlled ecosystem setting. Figure 3 summarizes the results of the 1978 MERL experiment, which was almost identical to the 1977 experiment described above except that the dosage level of No. 2 fuel oil was about one-half that in the 1977 experiment. Here the similarity measure used is information overlap (Horn 1966). Individuals in each benthic community were classified to taxa rather than to species. The six controlled tanks are compared with the three experimental tanks at each date. Figure 3 plots the dissimilarity measure which can be thought of as one minus the similarity: 0 indicates similar species composition, 1 indicates the communities share no species in common. The three solid lines represent the three experimental tanks compared with the pooled control. The bars represent the range of the dissimilarity when the controls were compared with a pooled community consisting of the remaining five controls. The treatment time series is the total saturated hydrocarbons in the sediment.

It would appear that similarity measures based on community composition might be a meaningful way to assess impact of pollutants. Sampling properties of similarity measures have been investigated (Smith et al. 1980; Goodman and Kruskal 1979). Both theoretical and empirical results indicate that species or taxa mix will change as communities are affected by a pollutant. Also, over a fairly broad range of natural variation the species mix changes relatively slowly.

Unfortunately many of the applications of similarity measures to environmental problems have been flawed by a lack of understanding of some important technical problems. The first problem is that many of the most common similarity measures, percent similarity, Canberra metric, and all presence/absence indices, have large biases that are sample size dependent (Grassle and Smith 1976). For fixed sample sizes this statistical bias increases as the taxonomic classification becomes finer. Thus, similarity indices based on taxa or other broad functional groups will have less bias than those based on finer species categories.

The second problem is that the interpretation of changes in species composition as reflected in changes in similarity measures has been made much more difficult by the indiscriminate use of clustering programs, and the tree diagrams they produce, to present the similarity measures. There is a natural use for these cluster diagrams in numerical taxonomy. Using cluster diagrams in envi-

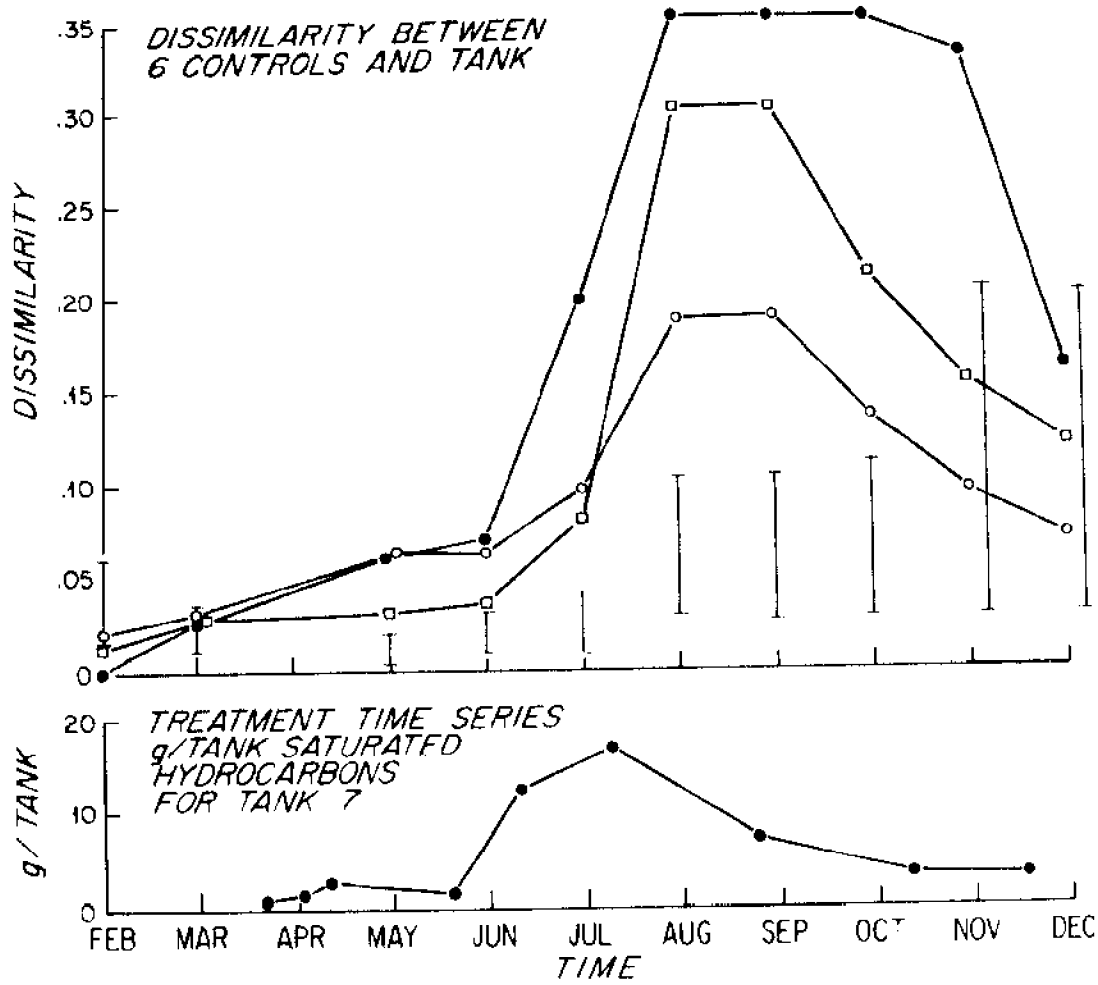

Figure 3. Horn's measure of dissimilarity between 6 control tanks and three tanks treated with No. 2 fuel oil in MERL, 1978. The bars represent the range of dissimilarities between pairs of control tanks (Smith et al. 1982).

ronmental studies may only obscure the natural independent variables of time and space.

Analysis of environmental field data is part classical statistical analysis of survey data and part careful presentation of multivariate data. The former problem is well understood and for the most part carefully handled in marine work. The presentation of multivariate data on community structure, pollutants and natural environmental variables has received less attention from ecologists and environmental regulators.

REFERENCES

Bowman, K.O., I. Hutcheson, E.P. Odum and R.L. Shenton. 1971. Comment on the distribution of indices of diversity. In: G.P. Patil, E.C. Pielou and W.E. Waters (eds.), Statistical Ecology, Vol. 3, pp. 315-359. Pennsylvania State University Press, University Park, Pa.

Dennis, B. and G.P. Patil. 1979. Species abundance, diversity and environmental predictability. In: J.F. Grassle, G.P. Patil, W.K. Smith and C. Taillie (eds.), Ecological Diversity in Theory and Practice, pp. 93-114. Statistical Ecology Series Vol. 6. International Co-operative Publ. House, Fairland, Md.

Good, I.J. 1953. The population frequencies of species and the estimation of population parameters. Biometricka 40: 237-264.

Goodman, L.A. and W.H. Kruskal. 1980. Measures of Association for Cross Classifications. Springer Series in Statistics, Vol. 1, Springer-Verlag, N.Y.

Grassle, J.F. and W. Smith. 1976. A similarity measure sensitive to the contribution of rare species and its use in investigation of variation in marine benthic communities. Oecologia 25: 13-22.

Green, R. H. 1979. Sampling Design and Statistical Methods for Environmental Biologists. John Wiley and Sons, New York.

Horn, H. 1966. Measurement of overlap in comparative ecological studies. Am. Nat. 100: 419-424.

Hurlbert, S.H. 1971. The nonconcept of species diversity: A critique and alternate parameters. Ecology 54: 577-586.

Kruskal, W. and L.A. Goodman. 1979. Measures of Association for Cross Classifications. Springer-Verlag, New York.

Orloci, L., C.R. Rao and W.M. Stiteler. 1979. Multivariate Methods in Ecological Work. Statistical Ecology Series Vol. 7. International Co-operative Publ. House, Fairland, Md.

Patil, G.P. 1979. Statistical Ecology Series. International Co-operative Publ. House, Fairland, Md.

Sanders, H.L. 1968. Marine benthic diversity: A comparative study. Amer. Nat. 102: 243-282.

Sanders, H.L., J.F. Grassle, G.R. Hampson, L.S. Morse, S. Garner-Price and C.C. Jones. 1980. Anatomy of an oil spill: Long-term effects from the grounding of the barge Florida off West Falmouth, Massachusetts. Mar. Res. 38: 265-380.

Smith, W. and J.F. Grassle. 1977. Sampling properties of a family of diversity measures. Biometrics 33: 283-292.

Smith, W., V.R. Gibson, L.S. Brown-Leger and J.F. Grassle. 1979. Diversity as an indicator of pollution: Cautionary results from microcosm experiments. In: J.F. Grassle, G.P. Patil, W.K. Smith and C. Taillie (eds.), Ecological Diversity in Theory and Practice, pp. 269-277. Statistical Ecology Series Vol. 6, International Co-operative Publ. House, Fairland, Md.

Smith, W., V.R. Gibson and J.F. Grassle. 1982. Replication in controlled marine systems: Presenting the evidence. In: G.D. Grice and M. R. Reeve (eds.), Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems, pp. 216-225. Springer-Verlag, New York.

Biochemical Stability in Planktonic Communities

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THE PROBLEM

If the concerns of a State Department Conference on Biological Diversity are true, one million species will become extinct by the end of the century (Norman 1981). This is a staggering loss of genetic diversity of the world's pool of biological resources, not to mention the pathetic epitaph it symbolizes for environmental work in general. We will not know where and how it was that insidious effects eventually culminated in death. There were too many species, and too much to be learned about each one, for us to have understood what was going on, even if research had increased a hundred fold.

In a broader sense these problems emphasize the futility of environmental science. Examples abound. Habitat alteration in the Middle Atlantic was widespread during the World War I period. The eminent malacologist Thurlow Nelson liked to tell a story about driving a nail into a Raritan Bay oyster: a short time later he removed it copper plated! Environmental insults to the New York Bight have not resulted in widespread extinctions. The effect has been more a matter of altered ranges and abundances (Jeffries and Johnson 1976). But even among commercially valuable species, no one could tell you what any one suffered from, let alone offer a prognosis on its future.

Again it has been a problem of too many species with too many sets of physiological attributes and behavioral patterns. Furthermore, no species acts alone in nature. Predictions have to incorporate interactions among populations and all the environmental factors controlling distribution. Also we need to account for changing abundances in time and space. The sum of all this exceeds a reasonable level of support for any environmental strategy.

We need to base decisions on something better than a compendium of cause-effect responses gleaned from organisms held in a laboratory jug, an outdoor tank, or as populations distributed along an environmental gradient. Current paradigms fall short in providing a comprehensive rationale. As Woodwell (1974) put it: "There is no way of determining thresholds for the myriad of substances that can be developed. . .no way of monitoring for the substances, and little basis for belief that thresholds for effect on natural ecosystems exist."

A SOLUTION

Background

We must find new measures revealing dynamically the integrity of natural systems. This is the substance of ecological stability theory, a subject equally divided between fact and mystique. A cogent approach is outlined by Orians (1975) and May (1975), who describe stable domains of entire systems in parameter space. The theory itself may defy practical application, but I believe the direction indicated is the right one, if we can find sets of attributes in nature that serve as convenient estimators of these domains.

To estimate stability in the classical manner, one determines equilibrium populations by setting all growth rates equal to zero and then analyzes the effects of perturbation around the equilibrium. The stable points of a system—to which it will return if perturbed—are located within a limited portion of parameter space defined by interactions of species with one another and with environmental factors. Outside this range of the stable domain, the equations describe a collapsing ecosystem. Mapping the equations is accomplished topologically. The resultant surface, drawn in two or three dimensions, has important heuristic, if not practical, value at present.

Consider, for example, a dynamical landscape in parameter space shaped like a volcano. The status of a system is represented by a point. For parameter values near the center of the volcano, the system returns to equilibrium after perturbation, but on the lip, perturbation may have catastrophic consequences.

By passing a plane perpendicular to the volcano-shaped landscape, we obtain a section of the stable domain. May (1975) claims that a simple community has a large, dynamically robust stable domain, whereas a complex community yields a small, dynamically fragile domain.

Successive points within the stable domain describe a trajectory of normality, revealing most vitally the system's condition as a definable yet ever-changing entity in nature. Moreover this trajectory predicts the future. A trajectory drifting toward the limit would be foreboding, a warning that something is wrong and close inspection should be taken.

We need to know where our coastal ecosystems are at any given time and place in a mosaic of stable relations, but how would the interaction coefficients be obtained? The task seems impractical if not impossible. Furthermore present theory deals with variations in time, not space. "The analytical problems in dealing with both time and space are formidable" (Orians 1975).

Example

I advanced an idea that the mathematically described stable domain has a correlate expressed by the monomeric composition of entire communities (Jeffries 1979). The monomers selected were fatty acids, because their structure is flexible, allowing modifications that are environmentally induced, perhaps to a degree that for marine organisms is unique. The central importance of fatty acids as indicators of ecological state is in part energetic. Storage of nutritional reserves is a critical problem. The strategy developed by planktonic species seems to rely on modification and selective utilization of the all-important lipid fraction, rather than depot storage in specialized organs. Thus as communities change, or are about to change, so do their patterns of fatty acid composition.

Biochemical correlates are rooted in homeostasis, not at the individual organism level but at the community level, which for some critics requires a leap in faith. One objective of this paper is to address that problem. I am not ready to point to a specific abnormality brought about by specific cause. First it must be established that stress is an internal condition borne by homeostasis, from which we can hypothesize that there exist generalized responses among monomers, an ever-changing state mediated naturally but confounded by pollutants in systems ranging from cells to communities. What emerges in patterns of monomeric composition are clear signals on the health of the whole system. The actual factors regulating distribution and abundance are left for the future and the identification of specific syndromes. Here, however, is holistic insight, not like the reductionist compartments of the ecosystem modeler, nor the physiologist's so-called controlled experiment; rather this is a glimpse of reality unencumbered by assumption.

SOME RESULTS

Temporal Domains

Zooplankton were sampled over two annual cycles in three coastal habitats representing a sequence of increasing environmental variability: Rhode Island Sound, Narragansett Bay, and Green Hill Pond. I tested the hypothesis that environmental instabilities culminated in seasonal succession of biogeographic species assemblages, and that these birth-to-death processes at the com-

munity level were uniquely portrayed by fatty acid patterns in the plankton of each habitat. If patterns changed due to natural (seasonal) death, then there should be another pattern that accompanied abnormal (pollutant) death. The experiment, then, consisted of comparisons along and between natural gradients.

The erratic pond habitat supported the most variable standing crop, but Narragansett Bay, a midpoint in the range of physical (temperature-salinity) variation, was the least variable biologically. Moreover productivity appeared greatest in the bay, as species populations waxed and waned seasonally in smooth succession.

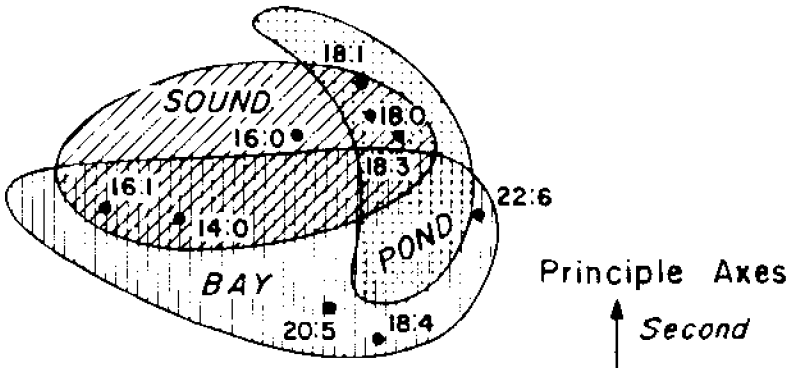
Behavior of the ten major fatty acids in microzooplankton (73-153 μm) and in macrozooplankton (> 240 μm) was examined as a trajectory of sample change in multidimensional phase space defined by the ten fatty acid variables. Thus for samples taken monthly over two annual cycles, there were 24 sample points in 10-dimensional space. By correspondence analysis, dimensionality was reduced to two principal axes, from which each community's trajectory in time was cast. Correspondence analysis also showed the opposite relation: the arrangement of variables in sample space. Interrelated variables clustered; nearness of a variable to a cluster of sample points represented strength of association among these samples and the variable (Jeffries and Lambert 1980).

In Figure 1, annual sample patterns on the first two axes of correspondence analysis are compared. These chemical domains were drawn as areas encompassing general limits of annual cycles. Statistically they represented the parameter space within which each habitat's planktonic community operated metabolically over an annual cycle. Monounsaturated 16 and 18 carbon fatty acids (16:1 and 18:1) characterized winter-spring (boreal) plankton, whereas the 14-18 carbon saturates (14:0, 16:0, 18:0) along with the long-chain polyunsaturate 22:6, typified summer-fall (temperate) assemblages. Sample change from one biogeographic species group to the next occurred around the pivotal position of 20:5. Thus the patterns were seasonally distinct regardless of habitat type; this is the first biochemical correlate.

Narragansett Bay had the largest sample domain in fatty acid space. Change took place consistently here, not erratically as in the sound, or in the pond. These comparatively small sample domains in the pond and sound meant that their macrozooplankton assemblages were spatially lacking in a biochemical sense. In a temporal sense so were their respective species assemblages, particularly in the unstable pond habitat, where the boreal (winter) component was all but lacking. The pond's annual sample domain was in the summer-fall portion of biochemical space. So was its plankton production, led by the temperate copepod Acartia tonsa. In the sound, a comparatively rich species complement occurred, but crops were low and erratically produced. Population dynamics indicated that this area supported admixtures from inner and outer

CHEMICAL DOMAINS OF ZOOPLANKTON COMMUNITIES IN THREE DISSIMILAR HABITATS

MICROZOOPLANKTON



MACROZOOPLANKTON

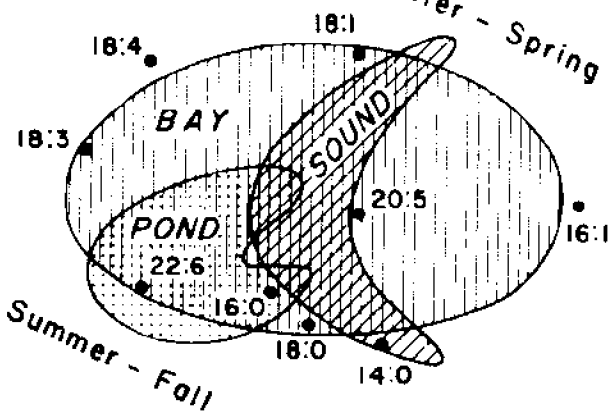


Figure 1. Correspondence analysis of fatty acids from plankton taken monthly in sound, bay, and pond habitats; simplified representation of sample domains on the first two principal axes, showing relative areas; also indicated are locations of fatty acid variables (no. n-carbon atoms:no. double bonds) in relation to sample domains.

coastal areas rather than a consistently functioning fauna of its own. Corresponding to its biochemical domain extending but weakly into summer and winter realms, the sound's respective biogeographic components developed poorly. Thus the more stable assemblage, the larger was its chemical domain; this is the second biochemical correlate.

Annual trajectories in the phytoplankton-microzooplankton were more erratic than in the holoplankton. Because the former was an ill-defined assortment of mixed taxonomic and ecological representation, this pattern again suggested that consistency of biochemical pattern was a direct correlate of productive organization in plankton communities.

Spatial Domains

Plankton samples were taken biweekly during two summers, at six stations extending from Rhode Island Sound, through Narragansett Bay and into its polluted tributary, the Providence River. I tested the hypothesis that a planktonic assemblage could be located accurately to position on this gradient from its fatty acid composition. The 10 major fatty acids (2% of total) at all six stations were subjected to multiple discriminant analysis, which identified each sample's habitat of origin (sound, bay or river) with 90-100% accuracy (Jeffries and Lambert 1981). Thus each assemblage had a biochemical identity mediated by the environment; this is the third biochemical correlate.

A factor responsible for the relation between fatty acid composition and its habitat may be the amount of suspended detrital material, both natural and from sewage, that is eventually incorporated into the tissues of grazing animals. Detritus is rich in stearic acid (18:0) and other saturates relative to the characteristically marine polyunsaturated fatty acids occurring abundantly in living particulate organic matter. This detrital pattern is simply passed on from food to consumer (Jeffries 1972a). Since detrital load increases in a landward direction, the more estuarine a sample, the higher its stearic acid content.

For further comparison a discriminant scale was calibrated over the range of biological conditions occurring during summer in Rhode Island waters; upon this scale additional positions calculated from published data were superimposed; also included were unpublished results from an investigation of two rias on the Atlantic coast of northwest Spain (Jeffries and Corral 1979). For simplicity and for further examination of trophic factors, only stearic acid and one polyunsaturate (docasahexaneoic acid, 22:6) were incorporated in the discriminant function. As shown in Figure 2, all European zooplankton came out on the estuarine side of R_o, the midpoint between Rhode Island's river and sound conditions (Jeffries and Lambert 1982). A sample from Newfoundland fell on the opposite side of R_o, corresponding to the Rhode Island Sound condition.

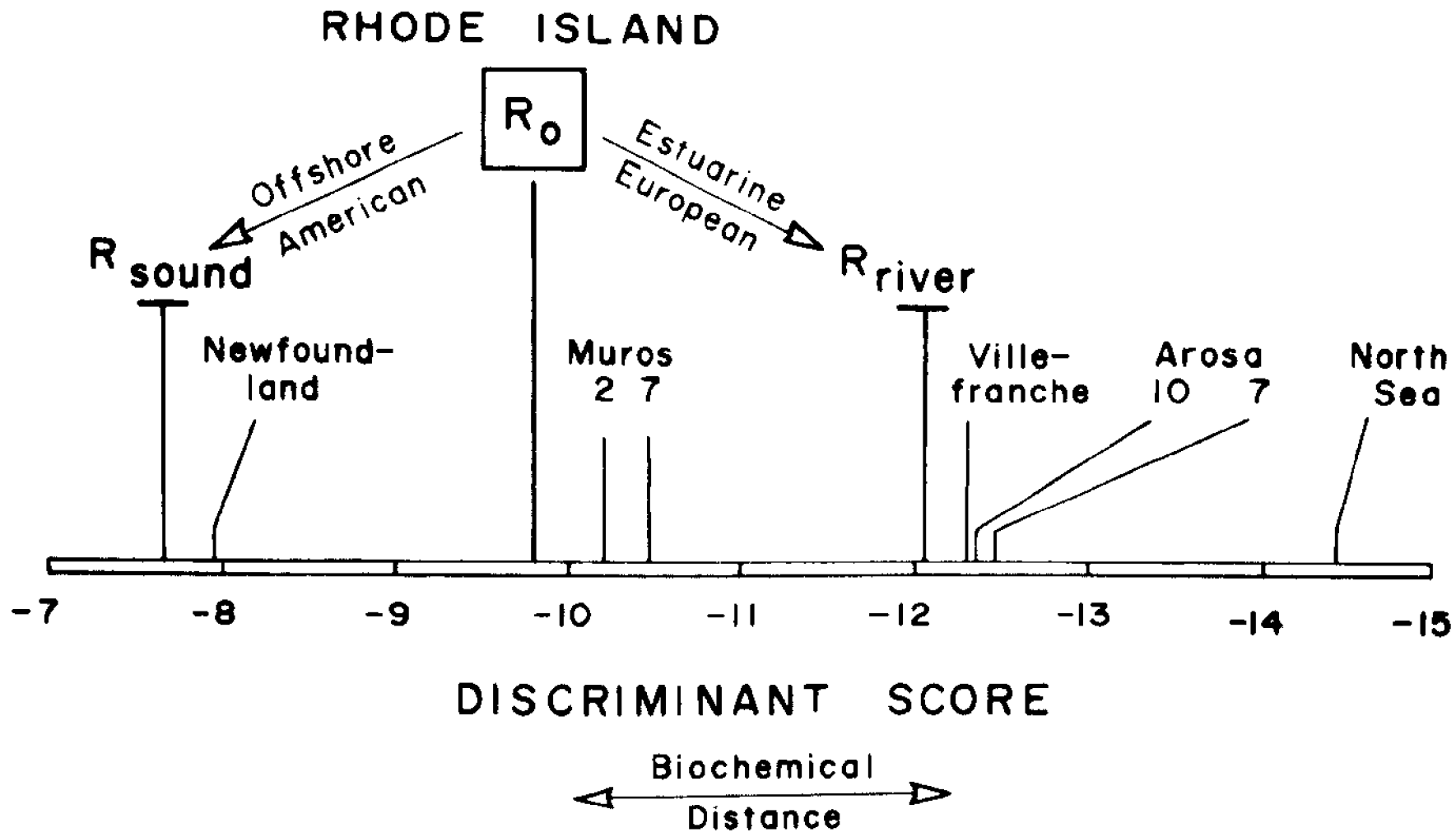


Figure 2. Ordination of zooplankton on a fatty acid scale calibrated for the range of variation occurring in Rhode Island waters during two consecutive summers.

Several explanations seem plausible for the estuarine affinity of European zooplankton. On either side of the North Atlantic, rates governing the production and diagenesis of organic matter differ significantly, because residence times of coastal waters differ, as a result of obvious differences in oceanic circulation, runoff patterns and geomorphology. Nevertheless, certain cases do seem extreme, for example, the North Sea (immediately outside the Wadden Sea) and Villefranche Bay in the Mediterranean. However, samples are few and the scope is broad, so the general pattern and its causes require verification.

If spatial domains in fatty acid space do in fact portray ranges of conditions over which a community is stable, there also should be a relation between relative size of a community's domain and its species diversity. Furthermore biochemical stability should be inversely related to taxonomic species diversity, because mathematically a simple community has a larger, more dynamically robust stable domain than a diverse and complex community.

To test this idea, I compared biochemical stabilities along the environmental gradient, from Rhode Island Sound to Providence River (during both summers of the investigation), with copepod species diversity, which decreased in a landward direction. The accompanying fatty acid matrices defined volumes in n-dimensional vector space representing stability of the community at each station. Changes in volume along the environmental gradient were described by the determinants of the respective matrices. The natural logarithms of these determinants (arc sin transformed data in radians) expressed chemical robustness at each station for the period of the investigation (Jeffries and Lambert 1981).

A strong relation between chemical robustness and species diversity emerged from this analysis. The relation was negative, in agreement with what would be predicted for mathematical stabilities of communities representing a sequence ranging from relatively complex (dynamically fragile, offshore) to simple (dynamically robust, riverine):

$$S_b = 4.11 - 0.93\, E(1K)$$

where S_b is biochemical stability and E(1K) is Hurlbert's (1971) rarefaction index (no. copepod species expected per 1000 individuals). With the station at the mouth of the bay excluded (a geographic intermediate between bay and sound having features common to both depending on phase of the tide), species diversity statistically accounted for 78% of the variation in chemical robustness from inner estuarine to offshore communities. Thus stability of monomeric composition was inversely related to species diversity; this is the fourth biochemical correlate. Departure from this relation might indicate environmental abnormality, simply and early in the process.

The fact that the polluted, Providence River plankton were clearly part of the overall relation attested to high secondary productivity here. Neither species representation, population dynamics, nor predatory influences by the ctenophore Mnemiopsis leidyii gave indication of adverse effect resulting from sewage inputs ranging up to 60 mgd (227,100 m^3 day^{-1}). It follows that energetically rich compounds in pollution may exert a stabilizing effect on production; this is the fifth biochemical correlate.

SYNTHESIS

Any number of factors essential for an organism's survival may become lethal if supplied at too high a level. A factor or resource that is a normal part of a pristine environment thereby emerges in artificialities arising from man's activities. Phosphate is essential for algal growth, but in extraordinary concentrations due to heavy pollution, an entirely different species assemblage from that usually expected may result. The organism's role shown in Figure 3 is to integrate factors from both sources, natural and artificial. The message going to homeostatic systems has a switch: what was normal environmental background in one instance flips over to the environmental stressor side if the intensity of application becomes too high.

Mammalian physiologists have shown that the adrenal-pituitary system produces stereotyped responses to all manner of external factors. Although stressors differ, a mammal's pattern of homeostatic response to any one is essentially the same. In Selye's (1949) general adaptive syndrome, a non-specific action results in stimulation of the hypothalamus, which increases production of ACTH. This hormone stimulates the adrenal cortex, which in turn releases glucocorticoids that mobilize glucose to meet demands imposed by disruption of homeostasis.

Invertebrates lack the above form of endocrine regulation, but a generalized response to external stress, taking an altogether different form, is still a reasonable proposition. For example, the hard clam (Mercenaria mercenaria) maintains a free amino acid pool in which the molar ratio of taurine to glycine is usually less than 3.0. In response to disease or to laboratory conditions, the clam's taurine:glycine ratio may go as high as 6.6 (Jeffries 1972b). Further indication that this response is general can be found in the edible mussel (Mytilus edulis). Mussels high in the intertidal zone, near the landward edge of their distribution, have a higher taurine:glycine ratio than those in deeper waters (Bannister 1974).

Success and failure of homeostatic responses affect population growth rates, but at the community level, represented in Figure 3 as benthos-plankton-nekton, a prediction based on lower levels of organization has virtually no chance of being correct. "The self-

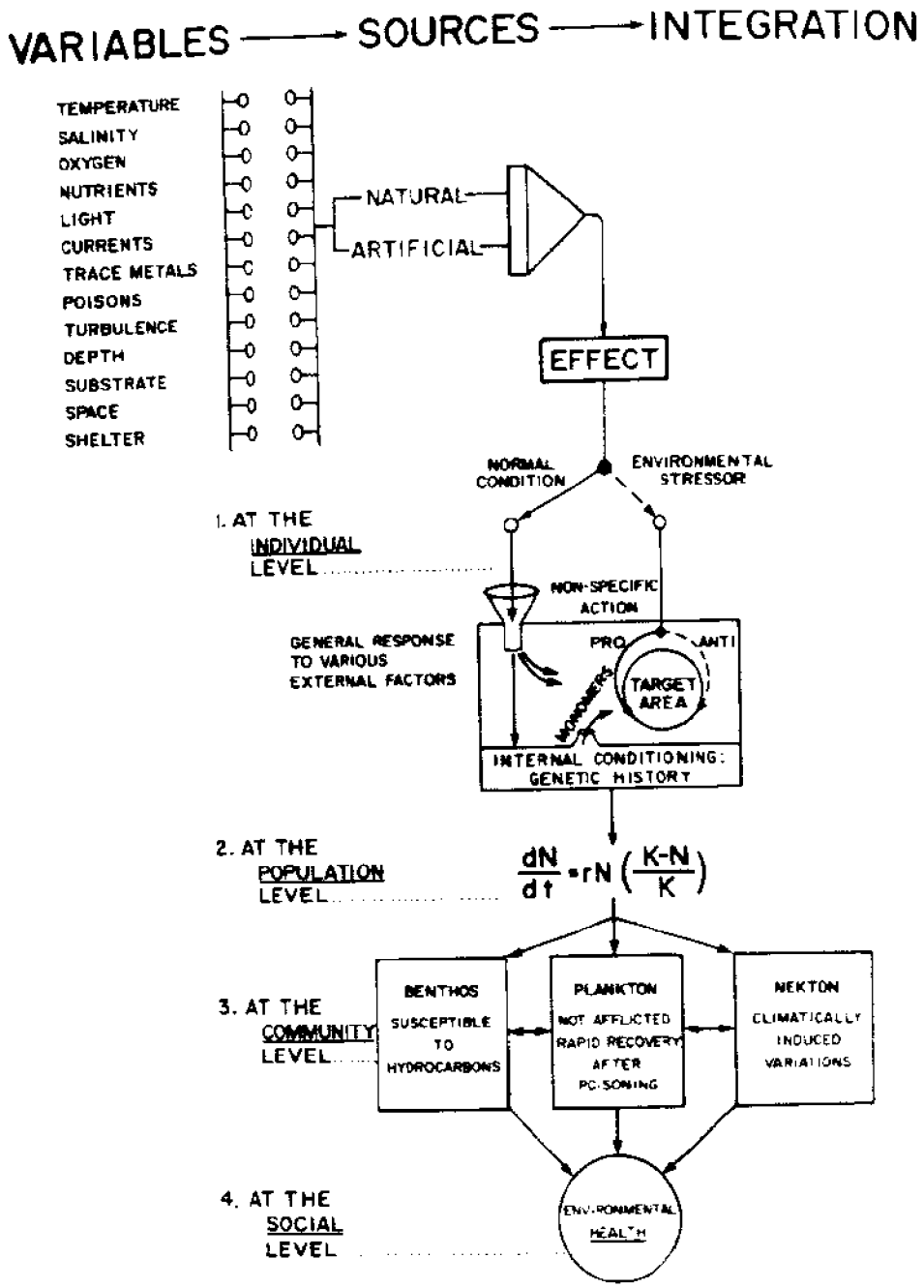


Figure 3. A flow chart for response, starting with environmental factor and ending with society. Figured at the individual level is a general adaptive syndrome adapted heavily from Selye (1978). For each environmental stressor there is a nonspecific action that may have opposite effects depending on intensity and the other factors represented. Aspects may be found in the hard clam's syndrome of responses along a gradient of pollutants (Jeffries 1972b). The target area in this case is the amoebocytes, which are mobilized greatly, presumably in response to a dissolved, hydrocarbon irritant. At the community level examples of response are from Jeffries and Johnson (1976).

limiting, self-regulating properties of such a collection of species when they happen to constitute a biotic community must therefore be properties of the community itself and characteristic of that level of organization. Any characteristic pattern of change found in such a collection, whether evolutionary or cyclical, is quite unpredictable on the basis of the characteristics of the individual species alone. In this sense, therefore, the existence of biotic communities as entities with a new level of organization is indisputable" (MacFadyen 1963).

Thus we must be concerned with the theory of community structure if prediction is our goal. Despite practical problems with a mathematically based theory of stability, this is really what we are after, no matter what the particular investigator's approach may be. More directly, I advance the idea that monomeric responses, as revealed here by statistical patterns of fatty acid composition, are generalized, in the sense of a modified "stress concept."

No one physiological measure or set of ecological measures on a single species would suffice as a predictor for an entire community. But when ecological state is set dynamically in a statistical framework, it represents a slice through the totality of processes responsible for the group's existence. The cut taken here goes through the whole, through common sets of properties behaving in shared ways, by a multitude of organisms, each affected by a complex of environmental stressors interacting with natural factors. Holistic insight from biochemical correlates is now but a glimpse, yet it is the approach to an ideal, if not the ideal itself, that we should seek.

In measuring biochemical responses to changing environments, one attempts to perceive by way of trajectories in time and space the world's topological shape and size. Numbers alone do not suffice: "Anyone who thinks that numbers constitute the real world. . . is under an illusion. . . . It could be argued, indeed, that quantification is simply the result of certain defects in the human nervous system that do not permit us to form complex images of topological structures" (Boulding 1980).

In this paper I have tried to map numbers that allow us to view metabolic responses that appear to be part of the real world's topological structure. Five biochemical correlates emerged. The test will come when the properties of species assemblages are predicted over conditions ranging from normal change to mass mortality.

ACKNOWLEDGMENTS

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REFERENCES

Bannister, M.L. 1972. Metabolic correlates of exposure in intertidal aggregations of the blue mussel, Mytilus edulis Linne. M.S. Thesis, Univ. of Rhode Island, Kingston, R.I.

Boulding, K.E. 1980. Science: Our common heritage. Science 207: 831-836.

Hurlbert, S.H. 1971. The nonconcept of species diversity: A critique and alternative parameters. Ecology 52: 577-586.

Jeffries, H.P. 1972a. Fatty acid ecology of a tidal marsh. Limnol. Oceanogr. 17: 443-440.

Jeffries, H.P. 1972b. A stress syndrome in the hard clam, Mercenaria mercenaria. J. Invert. Pathol. 20: 242-251.

Jeffries, H.P. 1979. Biochemical correlates of seasonal change in marine communities. Am. Nat. 113: 643-658.

Jeffries, H.P. and J. Corral. 1979. A comparison of zooplankton distributions in two estuaries (rias) of northern Spain. Unpublished Manuscript.

Jeffries, H.P. and W.C. Johnson. 1976. Petroleum, temperature, and toxicants: Examples of suspected responses by plankton and benthos on the Continental Shelf. In: B. Manowitz (ed.), Effects of Energy-Related Activities on the Atlantic Continental Shelf, pp. 96-108. BNL 50484, Brookhaven National Lab., Brookhaven, N.Y.

Jeffries, H.P. and R.M. Lambert. 1980. Biochemical correlates of complexity and change in plankton communities. C.M. 1980/L:68, International Council for the Exploration of the Sea, Copenhagen, Denmark.

Jeffries, H.P. and R.M. Lambert. 1981. Environmental gradients on a coastal megalopolis. I. Biochemical correlates of spatial heterogeneity in zooplankton communities. C.M. 1981/L:2, International Council for the Exploration of the Sea, Copenhagen, Denmark.

Jeffries, H.P. and R.M. Lambert. 1982. Biochemical correlates of structure and stability in divergent plankton communities. pp. 91-101. In: Kennedy, V.S. (ed.), Estuarine Comparisons, pp. 99-101. Academic Press, New York.

MacFadyen, A. 1963. Animal Ecology--Aims and Methods. Pittman, London.

May, R.M. 1975. Stability in ecosystems: Some comments. In: V.H. Van Dobben and R.H. Lowe-McConnell (eds.), Unifying Concepts in Ecology, pp. 161-168. Junk, The Hague, Netherlands.

Norman, C. 1981. The threat to one million species. Science 214: 1105-1107.

Orians, G. H. 1975. Diversity, stability and maturity in natural ecosystems. In: V.H. VanDobben and R.H. Lowe-McConnell (eds.), Unifying Concepts in Ecology, pp. 139-150. Junk, The Hague, Netherlands.

Selye, H. 1949. Textbook of Endocrinology, 2nd ed. Acta Endocrinology, Montreal, Canada.

Selye, H. 1978. The Stress of Life, 2nd ed. McGraw-Hill, New York.

Woodwell, G.M. 1974. Success, succession and Adam Smith. BioScience 24: 81-87.

Comparative Evaluation of Some Diversity Measures for Assessing Environmental Changes in an Estuarine Community

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BACKGROUND

An ecological study of diversity is a study of the variation in the numbers of different groups (usually taxa) under different ecological circumstances. It is well known, for example, that there generally are more species present in tropical than in temperate latitudes. Thus, the total number of species present is a simple measure of diversity; however, total numbers do not take into account any evenness component of the species. Many measures (diversity and related indices) have been developed in an attempt to account for relative abundance and the number of species.

It is now generally recognized that any index number involves an inevitable loss of information compared with data from which it has been derived. Ecologists therefore suggest that diversity indices should be used in conjunction with careful sampling designs and multivariate or other population analyses. This is sound and reasonable advice for comprehensive studies.

On the other hand, we suggest that diversity indices alone may have utility for certain applied ecological studies. Some of the current criticism of diversity indices stems from uncritical applications without regard for the assumptions implicit in the formulae and/or the bias in their estimation. Our application of diversity indices is not intended to replace more comprehensive studies if time and funds permit. Instead, we demonstrate that careful choice and use of diversity indices can be a valid preliminary guide for further critical work.

There seems to be a substantial recent commitment toward monitoring, assessing and ultimately minimizing man's impact on living communities. One outcome of this commitment is the development of a science of biomonitoring and assessment (Cairns et al. 1979). In this newly developing science, some investigators attempt to evaluate changes in organism state variables caused by pollutants or environmental disturbances. One of these state variables is the joint abundances of collections of species or identifiable groups from communities (not necessarily at a species level).

The statistical tools for studying joint abundances of species without regard to identity are: (a) probability distributions fitted to abundance data by species, and (b) diversity indices which condense data on abundance into a single number. Many measures of diversity have been developed and applied, resulting in a rich literature on this subject. See, for example, Dennis and Patil (1977, 1979), Dennis et al. (1979), May (1975), Patil and Taillie (1979), Smith et al. (1979) and the entire review volume edited by Grassle et al. (1979) describing recent progress and reviews of this subject.

We recognize at the outset that the use of a single number to represent data on a community results in some loss of information about the community. Hamilton (1975), Hurlbert (1971), Whittaker (1972) and others have clearly enunciated some of the limitations of diversity indices. They suggest that diversity indices confound the number of species and the evenness within which organisms are distributed among species, as well as not permitting valid regional comparisons. However, not all of the above criticisms are necessarily disadvantageous, and we further suggest that some of the deprecation of diversity indices may be the result of a lack of a full appreciation of the properties and limitations of the indices. Continued use of diversity indices seems justified because they are easily used, explicit techniques for the condensation of long taxonomic lists. In many areas of applied ecology, when results of environmental surveys are to be utilized by non-ecologists, it seems highly desirable to make such summaries in a generally comprehensible manner.

The theoretical basis for several indices of diversity has been discussed in detail by Pielou (1975) and she has demonstrated that the index proposed by Brillouin (1962) seems the most acceptable for applied ecological problems. Laxton (1978) has reached a similar conclusion based on set theoretic arguments. Brillouin's index (H), where

$$H = \frac{1}{N} \ln \frac{N}{N_1!, N_2!, \ldots N_s!},$$

is considered useful and does not require unrealistic assumptions about the sampled populations. Kaesler et al. (1978) have also demonstrated the practical value of this index by showing that

small replicated samples provide better discrimination than a single large sample, that diversities at a generic level are almost as effective as those at a species level and save time and money, and that hierarchical diversities of classifications based on functional morphology and trophic group analysis using this index seem very promising. Finally, Smith et al. (1979) have modified Brillouin's index to enhance its statistical properties which permit simple estimation of the sampling variance of the index.

We conclude from the above that Brillouin's index has desirable properties for applied ecology. However, it has been fairly common to utilize more than one index of diversity or similarity, but it has become increasingly apparent (Solomon 1979) that the use of two or more such indices may result in contradictory statements about evenness or diversity. In order to help reconcile this problem Solomon (1979) has developed, on theoretical grounds, the concept of partial ordering (majorization) for sets of species abundance vectors. The use of partial ordering admits the possibility that a pair of communities may have different species abundance patterns but may not be comparable with respect to diversity.

This application and investigation of joint abundances without regard to identity is directed toward demonstrating more effective but simple tools, while pointing out limitations so that the user and the decision maker can utilize summary data effectively. Specifically, we apply the concept of majorization and an equitability index to species abundance vectors from two sets of samples of macroinvertebrates from Narragansett Bay and compare the results with the conventional Brillouin diversity index.

The data sets come from a single survey of the Quonset Point area where it was proposed to extend existing dredged channels and piers (CRC 1977, Figure 1). One grab sample was taken at each station (0.1 m^2 Smith-McIntyre), and a relatively coarse 1.0 mm sieve was used to remove organisms. An assumption was made that the results from this small number of samples could be extended to subareas of known bottom type. Although there had been discharges of metal-plating wastes, sewage treatment plant effluents, spilled oil, and vessel wastes in the general area, the most noticeable impacts were those associated with the dredging of channels and turning basins. The natural bottoms are 3-7 m deep and range from fine sand to silty sand. The dredged areas are 10 m deep and contain 95% silt/clay sediments and are potential depositional areas for pollutants. For this analysis 14 samples from undredged bottoms are compared with 8 samples from dredged areas. Reconnaissance cruise samples (CRC 1977) and samples from a study of the Davisville area (Pratt 1980) aid in the biological interpretation of 22 samples considered here. Species counts are given in Table 1.

Table 1. Numbers of benthic invertebrates recovered in 0.1 m^2 samples.

	NATURAL AREAS														DREDGED AREAS							
	7	8	9	10	13	14	18	21	22	23	29	30	31	32	11	12	17	19	20	24	25	28
POLYCHAETA																						
Harmothoe extenuata	.	1	.	.	.	.	.	1	.	.	.	.	1	.	.	.	.	.	.	.	.	.
Pholoe minuta	7	5	22	3	.	.	.	.	.	2	21	.	.	3	.	1	1	.	.	1	5	1
Phyllodoce arenae	5	6	5	.	.	2	1	.	.	4	.	13	2	1	.	.	.	.	.	.	.	.
Eumida sanguinea	7	15	.	.	.	.	.	1	2	1	.	1	3	2	.	.	.	.	.	.	.	.
Glycera americana	16	26	18	14	6	11	13	12	24	11	8	25	25	12	.	3	3	2	.	1	.	2
Glycinde solitaria	1	.	4	2	3	1	6	1	.	2	1	1	.	4	3	.	.	.	.	.	.	1
Nephtys incisa	.	.	.	1	.	.	.	.	.	.	2	.	1	1	2	7	8	2	2	2	5	7
Exogone verugera	1	6	.	.	.	.	.	2	1	2	.	.	.	26	.	.	.	.	.	.	.	.
Podarke obscura	.	1	.	.	.	.	.	.	2	.	1	.	.	1	.	.	.	1	.	.	.	1
Mediomastus ambyseta	2	1	11	1	3	1	6	3	6	2	7	.	3	37	4	48	6	3	.	3	.	7
Clymenella torquata	20	4	5	46	1	.	11	9	6	.	25	6	8	1	.	3	.	1	1	.	.	1
Maldanopsis elongata	.	1	22	2	1	.	1	9	6	1	30	11	19	5	.	6	2	.	.	.	5	15
Streblospio benedicti	.	.	.	.	.	.	1	.	.	.	.	.	.	1	3	1	.	.	.	.	.	.
Polydora ligni	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Chaetopterus variopedatus	4	.	.	1	.	.	.	.	.	.	.	.	2	1	.	.	.	.	.	.	.	.
Spiochaetopterus oculatus	772	554	49	82	12	11	292	47	125	77	8	545	168	96	5	2	.	2	.	.	6	.
Lumbrinereis tenuis	7	1	.	4	.	4	1	6	2	13	.	.	.	6	.	.	.	.	.	1	.	.
Ninoe nigripes	.	.	.	.	.	.	.	.	2	.	4	.	2	.	.	1	.	.	.	.	.	1
Arabella iricolor	.	1	.	.	.	.	.	.	.	1	.	.	.	1	.	.	.	.	.	.	.	.
Drilonereis longa	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
Polycirrus sp.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	.
Pectinaria gouldii	23	34	3	33	4	4	.	6	6	12	30	20	18	26	21	18	6	2	2	.	1	5
Scoloplos acutus	1	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.
Tharyx acutus	.	.	1	2	.	.	1	.	.	.	.	.	.	.	1	5	.	1	.	.	.	.
Pherusa affinis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	1	.	.	.	1	.
Sabella microphthalma	.	1	.	.	.	.	.	.	.	.	.	.	1	1	.	.	.	.	.	.	.	.
MOLLUSCA																						
Solemya velum	23	4	2	.	2	1	3	3	.	4	.	2	2	5	.	.	.	.	.	.	.	.
Nucula annulata	47	79	459	3	16	1	3	1	89	18	315	16	211	286	27	123	274	274	25	106	395	311
Yoldia limatula	1	7	11	1	3	1	2	.	5	2	26	.	1	.	7	6	14	39	21	31	16	11
Mytilus edulis	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Mercenaria mercenaria	5	14	2	9	4	.	8	.	6	.	1	9	.	.	.	.	.	.	.	.	.	.
Pitar morrhuana	1	6	7	1	3	.	4	.	3	2	11	1	.	.	.	13	9	4	3	2	1	5
Mulinia lateralis	21	67	27	35	4	.	6	10	23	1	9	7	2	.	96	59	22	9	2	79	3	10
Tellina agilis	18	13	6	16	24	24	2	9	23	8	12	11	7	.	1	2	.	16	11	18	.	2
Macoma tenta	83	508	283	129	124	144	56	.	118	10	528	72	101	48	9	71	14	32	19	10	31	121
Ensis directus	4	.	.	.	.	.	1	2	2	.	.	.	.	.	.	.	.	.	.	.	.	.
Lyonsia hyalina	.	5	1	1	.	.	.	2	1	.	.	.	.	.	.	1	.	.	.	.	.	.
Pandora gouldiana	.	2	2	6	.	.	.	.	1	.	1	.	.	1	.	1	.	.	.	1	.	.
Petricola pholadiformis	.	2	5	2	.	1	1	.	.	.	1	.	.	.	.	.	.	1	.	.	.	.
Periploma papyratium	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.
Bivalve R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	1	2	.	2	.	.	.

Bivalve T	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Crepidula fornicata	2	2	.	15	1	.	2	.	.	.	.	2	.	.	.	.	.	.	.	.	.
Crepidula plana	.	5	.	37	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
Turbonilla interrupta	.	.	26	14	.	.	.	.	3	1	80	10	68	.	.	19	48	.	.	.	4	44
Epitonium sp.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.
Mitrella lunata	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.
Rupleura caudata	1	2	1	.	3	.	.	1	2	2	1	1	.	1	.	.	.	.	.	.	1	2
Polinices hetos	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	1	.	.	1	.
Naerarius trivittatus	.	1	6	.	5	1	.	3	1	3	8	1	2	4	1	2	6	4	3	2	.	3
Acteocina canaliculata	21	31	26	10	106	34	20	129	85	166	12	83	114	129	4	4	7	.	3	5	25	8
Acteon punctostriatus	2	2	1	.	6	2	2	2	3	.	.	1	10	2	4	7	4	2	1	1	8	3
Cylichnella oryza	.	.	2	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1
CRUSTACEA																						
Diastylis sculpta	1	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	1	.	.	.	.
Leucon americana	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
Edotea montosa	.	.	4	.	.	.	.	4	.	23	.	1	4	1	1	.	.	1	.	.	.	1
Idotea baltica	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.
Ampelisca abdita	216	112	34	1	1	41	8	70	19	216	2	40	63	99	6	11	9	15	15	2	.	5
Ampelisca verrilli	.	.	.	.	.	.	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.
Unciola irrorata	1	.	2	.	.	.	.	2	1	2	3	.	4	.	.	.	4	.	.	.	.	.
Corophium sp.	.	.	.	.	.	.	.	2	.	1	.	.	3	2	.	.	.	.	.	.	.	.
Paraphoxus spinosus	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Listriella barnardi	.	.	.	.	.	.	.	2	.	5	.	.	1	.	.	.	.	.	.	.	.	.
Elasmopus levis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.
Jassa falcata	.	.	.	.	.	.	1	.	.	.	.	.	.	1	1	.	.	.	.	1	.	1
Amphipod A	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
Amphipod B	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.
Neomysis americana	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Crangon septemspinosa	.	.	.	.	.	.	.	.	2	.	1	.	.	.	1	.	.	.	.	.	.	.
Pagurus longicarpus	2	.	.	1	.	.	.	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.
Upogebia affinis	.	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.
Libinia dubia	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Neopanope sayi	.	1	.	1	.	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.
Pinnixa chaetopterana	.	2	.	.	.	.	1	4	.	.	.	.	.	2	.	.	.	.	.	.	.	.
Pinnotheres ostreum	.	1	.	.	.	.	.	.	.	.	.	.	1	2	.	.	.	.	.	.	.	.
OTHER																						
Ceriantheopsis americanus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	6	2	.	.	3	.	.
Edwardsia elpunculoides	9	14	17	16	3	2	.	5	5	1	.	.	.	.	.	2	1	.	.	.	.	.
Stylochus ellipticus	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.	.
Rhynchocoela spp.	6	1	1	2	1	1	1	1	1	5	.	.	.	.	1	1	1	1	.	1	.	.
Phoronis sp.	.	2	.	2	.	.	.	.	.	6	.	.	.	.	.	5	.	.	.	1	.	.
Pentamera pulcherrima	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

METHODOLOGY

The material which follows is largely taken from Solomon (1979) and is designed to help the reader understand the concept of majorization.

The species abundance characteristics of an s-species community can be described by a probability vector $\vec{p} = (p_1, p_2 \ldots p_s)$, where p_i is the relative abundance of the i^{th} species.[1] We[2] now define a partial order on the set of ordered probability vectors of dimension s. Let

$$P \equiv \{ p \in R^s \mid 1 \geq p_1 \geq \cdots p_s \geq 0; \Sigma p_i = 1 \}.$$

Suppose that $\vec{p} = (p_1, p_2 \ldots p_s)$ and $\vec{q} = (q_1, q_2 \ldots q_s)$ are two vectors which belong to P.[1] This can be denoted by $p, q \in P$.[2] If

$$\sum_{i=1}^{j} p_i \geq \sum_{i=1}^{j} q_j \text{ for each } j = 1,2 \ldots, s-1,$$

then we can state that $\vec{p}$ majorizes $\vec{q}$, which is written as

$$\vec{p} > \vec{q}.$$

It can be shown that if $\vec{p} > \vec{q}$ and $\vec{q} > \vec{r}$, then $\vec{p} > \vec{r}$. As Solomon (1979) points out, $\vec{p} > \vec{q}$ can be interpreted as follows: "An s-species community with species abundance vector $\vec{p}$ is more dominated than one with vector $\vec{q}$."

Accepting majorization as a common basis for the conventional diversity indices, Solomon suggested that if $\vec{p} > \vec{q}$, a diversity distance might be measured by:

$$|| \vec{p} - \vec{q} || \equiv \sum_{j=1}^{s-1} \left(\sum_{i=1}^{j} p_i - \sum_{i=1}^{j} q_i \right) = \ldots \sum_{i=1}^{s} iq_i - \sum_{i=1}^{s} ip_i$$

This suggests that $R \equiv \sum_{i=1}^{s} i_{pi}$ is a numerical diversity index.

That is, for a highly dominated community, the numerical value of R should be small whereas for an even community the value of R will be larger. Thus, R is an evenness index. The range of this index for given s is

$$1 \leq \sum_{i=1}^{s} ip_i \leq (s+1)/2,$$

and this index can be standardized for s by forming

$$I = \frac{\sum_{i=1}^{s} i p_i - 1}{\frac{s+1}{2} - 1}$$

so that $0 \leq I \leq 1$ for all s.

Solomon (1979) has shown that all of the common diversity indices are S-concave. For example, Brillouin's index and many others are an important sub-class of S-concave functions which include all functions of the form

$$f(p_1 \cdots p_s) = \sum_{i=1}^{s} \phi(p_i),$$

where ϕ is a concave (in the usual sense) function of a single variable. It should be noted that $\sum_{i=1}^{s} i p_i$ is S-concave (as is I), but not of the form $\sum_{i=1}^{s} \phi(p_i)$ for some concave function ϕ as are the commonly used diversity indices, such as the Brillouin index.

A computer program (Fortran IV) was written to compute relative measures of diversity and to partially order (majorize) the species lists from the 22 stations. The listing of this is provided in the appendix.

RESULTS

Calculated values of the Brillouin diversity index with confidence limits for the 14 stations from natural sediments, compared with those found for the 8 disturbed stations, are shown in Table 2. Also shown in Table 2 is similar information on Solomon's evenness index. The two environments (natural versus disturbed) were compared using all data by means of the "t" test. Using this test we could not reject at $\alpha = 0.10$ the hypothesis that no differences exist between the natural and disturbed communities using both indices. For both cases the Behrens-Fisher test was calculated according to Snedecor and Cochran (1978).

Table 2. Calculated values for the Brillouin diversity index and the evenness index of Solomon from all Quonset and Davisville survey data.

Index	Sample Size	Sample Location	95% Conf. Int.	90% Conf. Int.	$\bar{X}$	$\hat{\sigma}$
Brillouin	14	Natural	1.6998-2.0463	1.733-2.0156	1.8734	0.3010
Brillouin	8	Disturbed	1.3462-1.9874	1.4099-1.9237	1.6668	0.3830
Solomon	14	Natural	0.0548-0.0779	0.0568-0.0758	0.0663	0.0200
Solomon	8	Disturbed	0.0352-0.0749	0.0392-0.0709	0.0550	0.0237

It is clear from the above that neither the Brillouin index nor Solomon's evenness index was able to discriminate between the two sets of samples even at an 0.10 probability level. Let us now apply the concept of majorization to both indices and compare the results with those obtained above.

By using the technique of Solomon described above, which involves an evenness index and the majorization technique, it is possible to distinguish four stations within each of the environments (perturbed and unperturbed) which are comparable. The basis for this selection is as follows. The technique of majorization requires that the species abundances within each station be sorted in descending order of numerical abundance. The abundance vectors developed in this manner for each station are then standardized by dividing each abundance by the total number of organisms within the station. Station pairs are then compared by creating two new vectors, $\vec{q}_1$ and $\vec{q}_2$, which contain the running sums of the probabilities within vectors $\vec{p}_1$ and $\vec{p}_2$. Thus, for example, the third element of $\vec{q}_1$ is the sum of the first three probabilities in vector $\vec{p}_1$. Station No. 1 is said to majorize station No. 2 if all of the elements of $\vec{q}_2$ are greater than or equal to the corresponding elements of $\vec{q}_1$. The two stations are not comparable if majorization is not present (i.e., $\vec{q}_{1_2} > \vec{q}_{2_3}$ and $\vec{q}_{1_4} < \vec{q}_{2_4}$).

The four stations in each environment, the calculated index values and the rank order of the indices are shown in Table 3. Note that the ranking procedure used for both the Solomon and Brillouin indices orders the data in an identical way. With these rank ordered data it is now possible to use the Wilcoxon sum procedure for

a non-parametric test of the hypothesis of no difference between the two environments (Hollander and Wolfe 1973), as follows:

$$W = \sum_{j=1}^{n} R_j = 26$$

where R_j refers to the rank of Y_j in an ordering from least to greatest of N observations where $N = m + n$ observations $X_1, \ldots, X_m$ and $Y_1, \ldots, Y_n$. W is the sum of the ranks assigned to the Y's. We reject H_o if $W \geq w(\alpha, m, m)$, if $26 \geq w(\alpha, 4, 4)$. We find that $w(0.014, 4, 4,) = 26$ and we reject the hypothesis of no difference at $\alpha = 0.014$.

Clearly, both the Brillouin index and the evenness index of Solomon provide identical results. These results indicate statistically significant differences between areas in spite of the fact that sample sizes were relatively small, that is, four samples per environment.

Table 3. Station types, station numbers, the calculated evenness index, Brillouin's diversity index, and rank order for O between survey sampling stations.

Environment	Station No.	Solomon's Index	Rank	Brillouin's Index	Rank
Natural	10	0.106795	8	2.413034	8
Natural	22	0.092461	7	2.263429	7
Natural	31	0.081229	6	2.213370	6
Natural	32	0.069067	5	2.052837	5
Disturbed	19	0.035145	2	1.318216	2
Disturbed	24	0.050687	4	1.733086	4
Disturbed	25	0.029247	1	1.064712	1
Disturbed	28	0.043058	3	1.530373	3

In an assessment of the Quonset area (CRC 1977), it was noted that samples from the dredged areas generally had low numbers of species and individuals compared with samples from undredged bottoms. The dominant species in dredged areas were similar to those in natural deep, soft-bottomed parts of Narrangansett Bay. The four stations selected by majorization had benthic communities very similar to these natural areas. The excluded samples included two (11, 20) with very low numbers of individuals and species from areas near pollutant sources. These impoverished stations did not have populations of disturbance-indicating species, with the possible exception of Mulinia lateralis at station 11. The other excluded samples were from more cohesive sediments where species richness was increased by the presence of bivalves adapted for soft bottom (M. lateralis, Pitar morrhuana) and of deposit feeding polychaetes requiring some substrate stability, such as Pectinaria gouldii.

Samples from the undredged areas contained larger numbers of species, individuals, crustacean species, suspension feeders, and tube and burrow dwellers than did samples from the dredged areas. Gradients in depth, wave exposure, sediment grain size, organic detritus load and biogenic modifications were reflected by changes in indicator species. The four samples selected by majorization are typical of silty sands in Narragansett Bay. Samples with relatively high densities dominated by a single species are excluded. Many excluded samples had species in common with selected samples at abundances within the expected large range in variation. It was not possible to see the difference between these and selected samples by inspection.

The lack of a larger number of comparable samples from "natural" bottoms illustrates the inadequacy of 14 samples to resolve the gradients within the study area. Even within depth-grain size strata there is significant patchiness of density. Two key tube-building species (the amphipod Ampelisca and the polychaete Spiochaetopterus) have the ability to form very dense colonies which result in log normal patterns of relative species abundance.

There are many cases where less than the desired resources are available for an environmental survey program. The small effort expended on the benthos survey at Quonset Point was a result of its being only one part of a complex impact assessment study. For the Quonset Point data base, species identifications were important in determining the distribution of species with known substrate requirement, of those important to man and of those indicating disturbance. Selection of samples by majorization made it possible to show that the diversity index values of two subareas were statistically different. This difference helps to justify predictions of changes which would take place if additional natural bottom were deepened. Five samples taken in 1980 (Pratt 1980) in the northern dredged area yielded only one to five species per sample, confirming the identification of an area under stress.

This attempt to select comparable samples from Quonset Point survey data has illustrated the uniformity in community makeup imposed by sedimentation in the deepened areas and the great variation elsewhere. Attempts are often made to judge environmental impact by deviations from an expected species number, diversity value or canonical distribution of relative abundance. In this study area, groups selected by majorization provided useful starting places for examining trends within preselected strata.

REFERENCES

Brillouin, C. 1962. Science and Information Theory, 2nd ed. Academic Press, New York.

Cairns, J., Jr., G.P. Patil and W.E. Waters. 1979. Environmental Biomonitoring, Assessment, Prediction and Management--Certain Case Studies and Related Quantitative Isues. Statistical Ecology Series Vol 11, International Co-operative Publ. House, Fairland, Md.

CRC (Coastal Resources Center). 1977. The Redevelopment of Quonset/Davisville: An Environmental Assessment. Mar. Tech. Rep. 55, Coastal Resources Center, Univ. of Rhode Island, Kingston, R.I.

Dennis, B. and G.P. Patil. 1977. The use of community diversity indices for monitoring trends in water pollution impacts. Trop. Ecol. 18: 36-51.

Dennis, B. and G.P. Patil. 1979. Species abundances, diversity, and environmental predictability. In: J.F. Grassle, G.P. Patil, W.K. Smith, and C. Taillie (eds.), Ecological Diversity in Theory and Practice, pp. 93-114. Statistical Ecology Series Vol. 6, International Co-operative Publ. House, Fairland, Md.

Dennis, B., G.P. Patil and O. Rossi. 1979. The sensitivity of ecological diversity indices to the presence of pollutants in aquatic communities. In: J. Cairns, G.P. Patil and W.E. Waters (eds), Environmental Biomonitoring, Assessment Prediction and Management--Certain Case Studies and Related Quantitative Issues, pp. 379-413. Statistical Ecology Series Vol. 11, International Co-operative Publ. House, Fairland, Md.

Grassle, J.F., G.P. Patil, W. Smith and C. Taillie (eds.). 1979. Ecological Diversity in Theory and Practice. Statistical Ecology Series Vol. 6, International Co-operative Publishing House, Fairland, Md.

Hamilton, M.H. 1975. Indexes of diversity and redundancy. J. Water Pollut. Control Fed. 47: 630-632.

Hollander, M. and D.A. Wolfe. 1973. Nonparametric Statistical Methods. John Wiley and Sons, New York.

Hurlbert, S.H. 1971. The nonconcept of species diversity: A critique and alternative parameters. Ecology 52: 577-586.

Kaesler, R.C., E.E. Herricks and J.S. Crossman. 1978. Use of indices of diversity and hierarchical diversity in stream surveys. In: K.L. Dickson, J. Cairns, Jr. and R.J. Livingston (eds.), Biological Data in Water Pollution Assessment: Quantitative and Statistical Analyses, pp. 92-112. ASTM STP 652, American Society for Testing Materials, Philadelphia, Pa.

Laxton, R.R. 1978. The measure of diversity. J. of Theor. Biol. 70: 51-67.

May R.M. 1975. Patterns of species abundance and diversity. In: M.C. Cody and J.M. Diamond (eds.), Ecology and Evaluation of Communities, Belknap Press, Cambridge, Mass.

Patil, G.P. and C. Taillie. 1979. An overview of diversity. In: J.F. Grassle, G.P. Patil, W.K. Smith and C. Taillie (eds.), Ecological Diversity in Theory and Practice, pp. 3-27. International Co-operative Publ. House, Fairland, Md.

Pielou, E.C. 1975. Ecological Diversity. John Wiley and Sons, New York.

Pratt, S.C. 1980. Effects of development at Davisville, Rhode Island on the marine environment. Report to the Rhode Island Dept. of Economic Development, Coastal Resources Center, Univ. of Rhode Island, Kingston, R.I.

Smith, W., J.F. Grassle and D. Kravitz. 1979. Measures of diversity and unbiased estimators. In: J.F. Grassle, G.P. Patil, W. Smith and C. Taillie (eds.), Ecological Diversity in Theory and Practice, pp. 177-191. Statistical Ecology Series Vol. 6, International Co-operative Publ. House, Fairland, Md.

Snedecor, G.W. and W.G. Cochran. 1978. Statistical Methods. Iowa State Univ. Press, Ames, Iowa.

Solomon, D.L. 1979. A comparative approach to species diversity. In: J.F. Grassle, G.P. Patil, W. Smith and C. Taillie (eds.), Ecological Diversity in Theory and Practice, pp. 29-35. Statistical Ecology Series Vol. 6, International Co-operative Publ. House, Fairland, Md.

Whittaker, R.H. 1972. Evolution and measurement of species diversity. Taxon 21: 213-251.

Appendix 1: Fortran IV Computer Program

```
C
C         The first 2 data points of the data file should be the number
C         of rows and columns (e.g. # of species and stations) .
C
C        ABUN   -- MATRIX OF SPECIES ABUNDANCES
C        BINDEX -- VECTOR OF VALUES FOR BRILLOUIN'S INDEX OF DIVERSITY
C        INDEX  -- VECTOR OF VALUES FOR NEW DIVERSITY INDEX
C        PROB   -- MATRIX OF SPECIES ABUNDANCE PROBABILITITES BY STATION
C        PSUM   -- MATRIX OF RUNNING SUM OF SPECIES PROBABILIES
C        SPEC   -- NUMBER OF SPECIES (ROWS IN ABUNDANCE MATRIX 'ABUN')
C        STAT   -- NUMBER OF STATIONS (COLUMNS IN ABUNDANCE MAT. 'ABUN')
C
      INTEGER     SPEC, STAT
      REAL*8      ABUN(100,25), PROB(100,25), PSUM(100,25)
      REAL*8      REALI, SUM, SWITCH
      REAL*8      INDEX(25), BINDEX(25)
      LOGICAL     TEST, COMP1, COMP2
C
      COMMON      BINDEX, ABUN, SPEC, STAT
C
      READ (5,*) SPEC, STAT
C
      DO 10 I=1,SPEC
        READ (5,*) (ABUN(I,J), J=1,STAT)
   10 CONTINUE
C
C         Sort each column of the matrix ABUN so that each column is
C         in decreasing order (e.g. highest abundance first) .
C
      DO 20 K=1,STAT
        ND = SPEC
        NM = SPEC - 1
         DO 30 I=1,NM
           ND = ND - 1
            DO 40 J=1,ND
              IF (ABUN(J,K) .GE. ABUN(J+1,K)) GO TO 40
              SWITCH = ABUN(J,K)
              ABUN(J,K) = ABUN(J+1,K)
              ABUN(J+1,K) = SWITCH
   40        CONTINUE
30         CONTINUE
   20 CONTINUE
C
C         WRITE OUT SORTED SPECIES ABUNDANCE MATRIX 'ABUN'
C
      WRITE (6,1006) SPEC, STAT
      DO 45 I=1,SPEC
        WRITE (6,1004) (ABUN(I,J),J=1,STAT)
   45 CONTINUE
C
      DO 50 J=1,STAT
        SUM = 0.0D0
         DO 60 I=1,SPEC
           SUM = SUM + ABUN(I,J)
60       CONTINUE
C
         DO 70 I=1,SPEC
           PROB(I,J) = ABUN(I,J) / SUM
70       CONTINUE
   50 CONTINUE
C
C         WRITE OUT MATRIX OF SPECIES ABUNDANCE PROBABILITIES 'PROB'
C
      WRITE (6,1007)
      DO 75 I=1,SPEC
```

```
        WRITE (6,1005) (PROB(I,J),J=1,STAT)
   75 CONTINUE
C
      DO 76 J=1,STAT
        SUM = 0.0
        DO 77 I=1,SPEC
          SUM = SUM + PROB(I,J)
          PSUM(I,J) = SUM
   77   CONTINUE
   76 CONTINUE
C
C       WRITE OUT RUNNING SUMS OF PROBABILITIES FROM 'PROB' AS 'PSUM'
C
      WRITE (6,1008)
      DO 78 I=1,SPEC
        WRITE (6,1005) (PSUM(I,J),J=1,STAT)
   78 CONTINUE
      WRITE (6,1009)
C
      LIMSTT = STAT - 1
      LIMSPC = SPEC - 1
      DO 80 I=1,LIMSTT
        IPLUS1 = I + 1
        DO 90 J=IPLUS1,STAT
          TEST = .FALSE.
          DO 95 K=1,LIMSPC
            IF (PSUM(K,I) .EQ. PSUM(K,J)) GO TO 92
            IF (TEST) GO TO 93
            TEST = .TRUE.
            COMP1 = (PSUM(K,I) .GE. PSUM(K,J))
            GO TO 95
   93       COMP2 = (PSUM(K,I) .GE. PSUM(K,J))
            IF ((COMP1.OR.COMP2) .AND. (.NOT.COMP1.OR..NOT.COMP2))
     1          GO TO 97
   92       IF (PSUM(K,I).GT.0.9999999) GO TO 94
            IF (PSUM(K,J).GT.0.9999999) GO TO 94
   95     CONTINUE
   94     IF (.NOT.COMP1) GO TO 96
          WRITE (6,1000) I, J
          GO TO 90
   96     WRITE (6,1001) I, J
          GO TO 90
   97     WRITE (6,1002) I, J
C         WRITE (6,1010) K, PSUM(K,I), PSUM(K,J)
   90   CONTINUE
   80 CONTINUE
C
C       CALCULATE SOLOMON'S DIVERSITY INDEX
C
      WRITE (6,1009)
      DO 100 J=1,STAT
        INDEX(J) = 0.
        DO 110 I=1,SPEC
          REALI = I
          INDEX(J) = INDEX(J) + REALI * PROB(I,J)
  110   CONTINUE
        INDEX(J) = (INDEX(J) - 1) / (((SPEC+1)/2.)-1)
        WRITE (6,1003) J, INDEX(J)
  100 CONTINUE
C
C       CALL SUBROUTINE BRILL TO CALCULATE THE BRILLOUIN INDEX
C          FOR THE MATRIX ABUN AND STORE THE VALUES IN BINDEX
C
        CALL BRILL
      DO 120 I=1,STAT
```

```
      WRITE (6,1011) I, BINDEX(I)
  120 CONTINUE
      STOP
 1000 FORMAT (2X,'STATION ',I2,' IS MORE DIVERSE THAN STATION ',I2)
 1001 FORMAT (2X,'STATION ',I2,' IS LESS DIVERSE THAN STATION ',I2)
 1002 FORMAT (2X,'STATION ',I2,' AND STATION ',I2,' ARE NOT COMPARABLE')
 1003 FORMAT (2X,'STATION ',I2,' HAS A DIVERSITY INDEX OF ',F10.6)
 1004 FORMAT (8(2X,F8.3))
 1005 FORMAT (8(2X,F8.6))
 1006 FORMAT (2X,'THERE ARE ',I2,' SPECIES AND ',I2,' STATIONS',/)
 1007 FORMAT (/,2X,'MATRIX OF SPECIES ABUNDANCE PROBABILITISS',/)
 1008 FORMAT (/,2X,'RUNNING SUM OF SPECIES ABUNDANCE PROBABILITIES',/)
 1009 FORMAT (//)
 1010 FORMAT (2X,I3,2(2X,F14.9))
 1011 FORMAT (2X,'THE BRILLOUIN INDEX FOR STATION ',I2,' IS ',F10.6)
      END
C
      SUBROUTINE BRILL
C
      REAL*8        ABUN(100,25), BINDEX(25), FREQ(25), FACT(25)
      REAL*8        AFAC, BFAC, CFAC, ZERO, ONE
      INTEGER       IABUN(100,25), SPEC, STAT, LOW, HIGH, KFAC
      COMMON        BINDEX, ABUN, SPEC, STAT
C
      ZERO = 0.0D0
      ONE  = 1.0D0
C
      DO 10 J=1,STAT
        FREQ(J) = ZERO
         DO 20 I=1,SPEC
           IABUN(I,J) = ABUN(I,J)
           FREQ(J) = FREQ(J) + ABUN(I,J)
   20     CONTINUE
   10 CONTINUE
C
      DO 30 J=1,STAT
        FACT(J) = ZERO
        AFAC = ONE
        BFAC = ONE
        LOW  = 1
        HIGH = 1
         DO 40 I=1,SPEC
           K = SPEC - I + 1
           IF (IABUN(K,J) .EQ. 0) GO TO 40
           IF (IABUN(K,J) .EQ. HIGH) GO TO 45
           HIGH = ABUN(K,J)
            DO 50 L=LOW,HIGH
              AFAC = AFAC * L
   50       CONTINUE
   45       FACT(J) = FACT(J) + DLOG(AFAC)
            LOW = ABUN(K,J) + 1
   40     CONTINUE
         HIGH = FREQ(J)
          DO 35 L=1,HIGH
            BFAC = BFAC * L
   35     CONTINUE
         BFAC = DLOG(BFAC)
         CFAC = BFAC - FACT(J)
         BINDEX(J) = CFAC / HIGH
   30 CONTINUE
      RETURN
 1001 FORMAT (2X,F15.5,2X,F15.5,2X,F15.5)
 1002 FORMAT (2X,I10)
 1003 FORMAT (2X,F16.4)
      END
```

Statistical Analysis of Sampling Data to Assess Impact in Marine Environments

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INTRODUCTION

Field sampling studies have been and will continue to be emphasized as a proper and reasonable means of evaluating environmental impacts. Whether these studies are "before and after" or "reference area vs. impact area" studies, the goals are the same: to determine if significant changes in community structure have occurred as a result of some suspected source of impact. Since these studies are correlative, one can expect only indirect proof that an impact has or has not taken place.

The most commonly used measure of environmental impact has been the diversity index in one of its various forms. Its practical shortcomings are by now well documented (e.g., Livingston 1975; Logan and Maurer 1975; Smith et al. 1979) and the original theoretical basis for using diversity as a meaningful measure of community organization is questionable (May 1973; Goodman 1975). Nevertheless, diversity will most often decrease in cases of severe stress from pollution, although one can demonstrate negative impacts in other ways as well. Diversity indices can provide valuable information on the effects of pollution, but it is clear that they do not provide easy answers to many questions about pollution effects.

In cases where the effects of a suspected source of pollution may be subtle or when other confounding factors make the resolution of individual effects difficult, other methods of analyzing community data will usually be more valuable than calculating diversity indices. The following discussion considers some common sense methods of assessing impact and discusses their strengths and weaknesses. They differ most from earlier work in the emphasis placed on study design and interpretation of results, although our general approach relies on well-known analytical procedures.

METHODS

An effective sampling program must match the particular impacts and taxa of interest because no one design can detect all possible modes of impact. Our premise is that changes in the abundances or species composition of the taxa would represent the major biological impacts of a suspected pollutant. In order to detect such changes, sampling programs must take place in all seasons at both impact and reference stations, and the sampling frequency should be less than the mean generation time of the target organisms so that taxa cannot colonize and become extinct between sampling dates. Physiological and behavioral changes--movement out of affected areas, changes in feeding rates, etc.--represent a type of impact that is best studied by sampling designs that concentrate on the most stressful periods.

Change in Abundance or Biomass

Given a biologically meaningful frequency, a rationale for assessing potential changes in abundance between reference and impact stations can be established. The analysis is methodologically similar to the standard use of ANOVA (analysis of variance) in experimental settings. However, since the comparison of field sites is not a true experiment, our approach uses ANOVA in an exploratory technique that measures variation between sites in abundance (or biomass) of the target species. If proximity to the potential source of impact explains little variability in the ANOVA, this would suggest that the potential impact had little to do with patterns of abundance. Finding that significant variation is attributable to location would suggest that significant impact had occurred. It would not, however, in and of itself prove anything about impact, since we do not allocate treatments independently of other sources of variation. It would indicate that impact is one likely cause of differences in abundance of biomass among stations, and it would also invite further analysis of the factors responsible for location differences. Such analyses should attempt to evaluate the relative importance of natural and impact-related variation in physical and chemical factors among locations because they may explain differences in abundance or biomass.

By recognizing the differences between this approach and the standard use of ANOVA, we can avoid misinterpretation and can improve our sampling designs and analyses. The primary difference is that our approach cannot randomly allocate "treatments" (presence or absence of pollutant) to experimental units (Eberhardt 1976; Green 1979; Small et al. 1979). Thus, we can never single out the pollutant as the cause of the pattern of abundance; any other location related factor could be responsible.

A second difference is our inability to control variation among experimental units in field studies; different sites within the im-

pact and reference areas will have large natural environmental heterogeneity. Because of these differences, analysis of environmental variation becomes as important as the impact-reference comparison in interpreting the observed patterns.

A third consideration in using ANOVA in field comparisons is temporal and spatial correlation between samples. This creates dependence between closely spaced samples (in time or space) and is especially critical in defining replicates. In practice, the lack of independence among samples means that most repeated samples cannot be treated as replicates. The correlation may be due to the exogenous influence of other factors; for example, seasonal variation forces temporal correlation and substrate variation may force spatial correlation. These influences can be analyzed as independent variables or covariates. For example, seasonality can be treated as a discrete, class variable (if only a few dates are sampled) or as a sinusoid covariate. Depending on the application, different frequencies may be modeled (e.g., annual, diel or tidal). However, the correlation may be due to exogenous factors that cannot be modeled or to endogenous correlation. For example, patches of benthic organisms may be depleted by schools of fish, so that densities of samples within the patches are dependent. Local growth and division of phytoplankton may create patches for short periods of time; chlorophyll measurements taken in several places within the patch or at several times within the life of the patch would be dependent. In these cases, knowledge of the natural history of the organisms or autocorrelation analysis may indicate the temporal and spatial scales of correlation. Samples taken much farther apart than these scales may be considered independent, while more closely spaced samples should be combined.

A number of techniques can be used to analyze environmental variation. Multiple regression can be used to correlate abundance with environmental variation. ANOVA modeling of the environmental variables and other multivariate techniques can be used to analyze the spatial and correlation structure of the variation. For any such analysis, the sampling sites should include the natural range of variability within the impact and reference areas. Restriction of sampling sites to reduce the observed variation within the impact and reference areas prevents the analysis of the variation and the comparison of impact and reference areas; any remaining environmental differences between the impact and control areas are confounded with the main comparison. Furthermore, the reduction of within-area variation will decrease the power of techniques like ANOVA to detect correlation between the environmental factors and abundances.

Even when the ANOVA model is used in this exploratory role, its assumptions should be met as nearly as possible. Data transformations can stabilize variances and normalize the distributions of abundances. In designing a sampling strategy one may rely on

classical sampling theory (Cochran 1977) to determine a desired sampling effort, and this approach has received increasing attention in impact analysis (Saila et al. 1976; Cuff and Coleman 1979; Downing 1979). Although this approach may be useful for defining minimum detectable differences, the interpretation of these calculations is subject to the same caveats expressed for the ANOVA model.

The following discussion explores the use of the techniques discussed above in evaluating the effects of several potential sources of impact on the benthos in a mesohaline subestuary of the Chesapeake Bay. The objective of the study was to investigate impacts on fish and benthos of the effluents from a once-through cooling system of a power plant. In addition to having typical estuarine gradients in temperature and salinity, the area had been dredged and it received a number of industrial effluents. Thus, the study addresses several of the problems mentioned above, including confounding factors and tremendous natural heterogeneity.

Monthly or twice-monthly samples of fish (one trawl sample) and benthos (three grab samples per station) were taken from a grid of five transects with three stations each (Figure 1). By sampling over a large part of the subestuary, we were able to detect significant environmental variability and relate it to the distribution of organisms. Salinity, dissolved oxygen and temperature were recorded for all samples. Sediment core samples were taken with the benthic samples, and the particle size distribution, organic fraction and concentrations of six metals were measured in these cores. ANOVA of the ln-transformed catch per unit effort for the major species and higher taxonomic groups indicated very strong data and location effects for the benthos, and strong date and occasional location effects for the fishes (location-date interactions were significant for some groups). The abundances of the benthic groups showed clear longitudinal gradients; most species were most common down-estuary, and a few polychaetes were more common up-estuary. In addition, most groups and species were more abundant at the main impact station than the model predicted, as evidenced by the fact that the largest cell deviations were those at the impact station. Fish abundances were more variable; despite this, the stations nearest the discharge often had high abundances. These results display the strengths and weaknesses of the ANOVA approach—location effects are clearly indicated, but the relationships between these effects and the impact and other sources of environmental variability are unclear.

These patterns were clarified by analyses of environmental variation, which suggested that the spatial patterns of benthic abundance were primarily related to sediment characteristics not related to the power plant, while the more mobile fish were responding to more transient environmental conditions, including thermal effects of the plant.

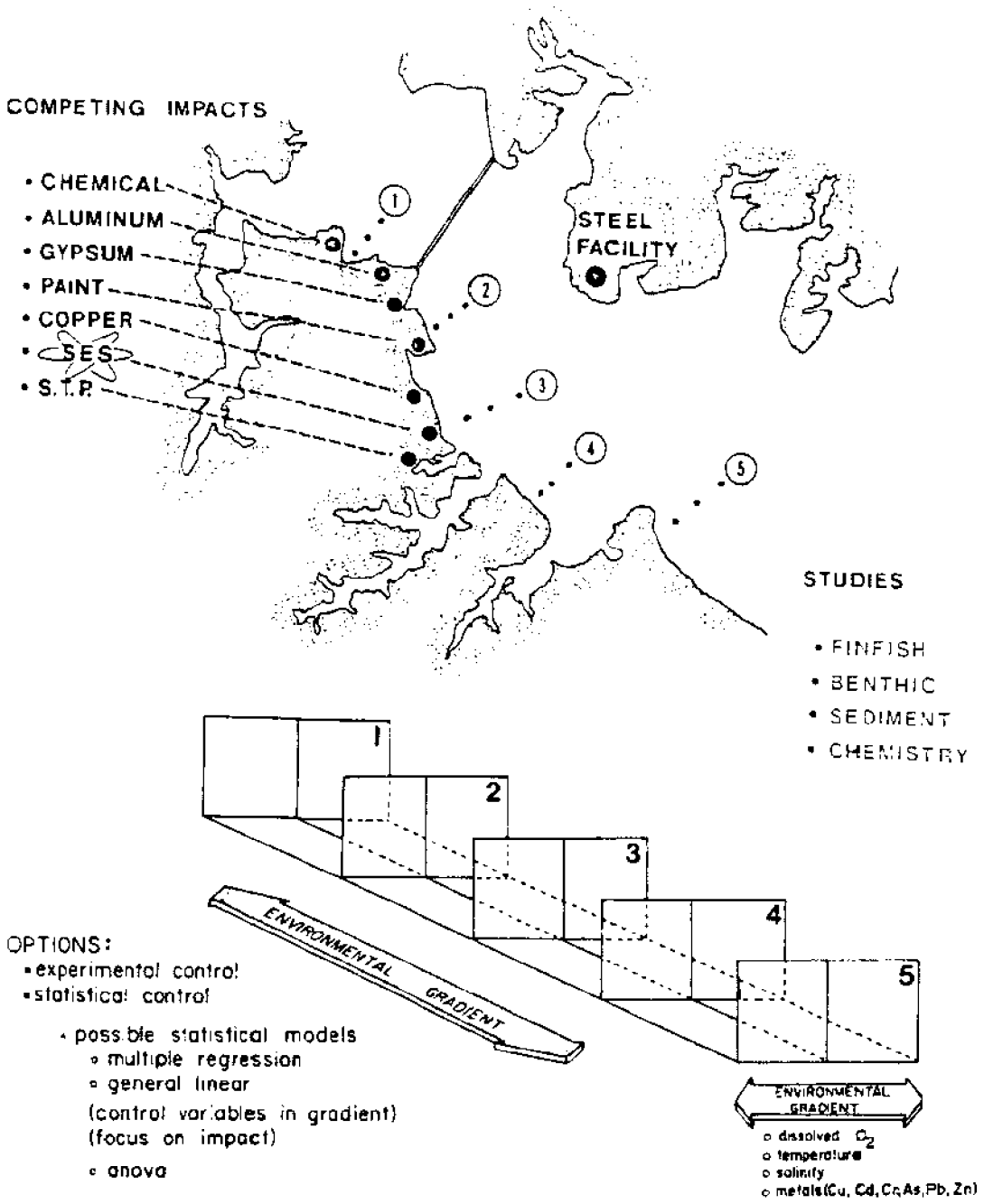

Figure 1. Map of the study area showing the location of stations and of potential sources of impact on the aquatic biota of Baltimore's Outer Harbor. Below the map is a conceptual description of the study area and a list of the options to evaluate the impact of the steam electric station (SES).

The large number of sediment variables was unwieldy for detailed analysis; since many of these were expected to be correlated, principal components analysis was used to describe patterns of covariance. Two components accounted for 0.66 of the total variation (Table 1). The first was clearly related to a gradient in sediment characteristics ranging from "polluted" (high silt-clay fraction, high proportion of organics, high concentrations of metals) to "unpolluted" (high sand fraction, low organic fraction and low metal concentrations). The second component clearly related to seasonal effects and was highly correlated with temperature, salinity and dissolved oxygen. Plots of these components and ANOVA of the components with transect, station and season showed the strong geographical patterning of the sediments, with the "polluted" sediments occurring up-estuary, near the dredging and industrial outfalls. The down-estuary sites, as well as the impact stations, had cleaner sediments. The influence of this gradient on benthic abundance was indicated by multiple regression of the abundances against the two principal components: all higher taxonomic groups and most dominant single species were highly correlated with the first (sediment) component. This pattern of correlation was consistent with the ecology of the groups: bivalves, amphipods and some polychaetes were most abundant in the sandier sediments, while a few polychaetes were most abundant in finer sediments. No relationship was found between abundance and thermal loading.

Fish abundances showed a very different relationship to environmental variation. Use of temperature, salinity, depth and dissolved oxygen as covariates in ANOVA indicated frequent positive associations between temperature and abundance at each site. Thermal loading was a major component in the temperature variation, although natural longitudinal and depth gradients were also present. Other factors were not consistently correlated with abundances. No relationship was found between fish abundance and the benthic principal components (sediment or season) or between fish and benthic abundance.

Thus, the sampling program emphasized direct measurement of environmental factors and extensive sampling over a large area to include the full range of environmental variation. By analyzing spatial and temporal patterns of abundance and environmental variation, covariance between environmental factors, correlation of abundance with environmental factors, and by considering the biology of the study organisms, a strong argument was made for plant-related and plant-independent factors as significant determinants of fish and benthic abundances, respectively.

Table 1. Unrotated factor loadings for first three principal components.

Components	Factor 1	Factor 2	Factor 3
Water temperature	-0.019	0.971	0.057
Bottom DO	-0.013	-0.793	0.405
Bottom salinity	0.141	-0.904	-0.146
Silt-clay fraction	0.689	0.041	0.018
Sediment temperature	-0.010	0.961	0.040
Mean temperature deviation	0.050	0.035	0.913
Volatile solids	0.881	-0.192	0.101
As	0.472	-0.340	-0.375
Cd	0.671	0.212	-0.123
Cu	0.934	0.048	0.078
Cr	0.867	0.009	0.228
Pb	0.902	0.121	-0.026
Zn	0.807	0.174	-0.133
Cumulative proportion of variance explained	.39	.66	.76

Similarity in Species Composition

In many cases, impacts on communities may be most easily detected by changes in the abundances of the most common species. However, since rare species may be of special interest or because changes in species composition may occur without major changes in abundance, it is desirable to evaluate similarity in species composition quantitatively in studies of impacts on communities.

Many measures of similarity have been applied to species abundance data, and several reviews of the properties of these indices exist (e.g., Cheetham and Hazel 1969; Sneath and Sokal 1973; Baroni-Urbani and Buser 1976). Unfortunately, measures of similarity between samples depend on sample size and are biased estimates of the similarity between parent populations; the sampling theory depends on the total number of species in the communities or the distribution of species abundances, which are unknown in practice. As a result, similarity has been used most often for descriptive purposes, rather than for testing hypotheses of similarity of species composition. Recently, Hendrickson (1978) has discussed the use of Cochran's Q and M statistics to test for heterogeneity in species occurrence (presence or absence) among a set of sites and has developed a more exact distributional theory and associated test statistic for this situation (J.A. Hendrickson, Jr. personal communication). Using simulations, Baroni-Urbani and Buser (1976) have developed a similarity index with associated probabilities to test for dissimilarity between a pair of samples, although there are questions concerning the generality of their results (Simberloff and Connor 1979; J.A. Hendrickson, Jr. personal communication).

Accepting the problem of sample-size dependence, Smith and Grassle (1977) and Connor and Simberloff (1978) have developed indices that incorporate the sample size dependency: the expected species similarity (ESS) between two communities for samples of m individuals is estimated as the expected number of species in common between samples of m individuals from each of the two community samples. This is normalized (producing the index NESS) to the average of the expected species similarities within two pairs of two samples of m individuals from the parent populations. Smith and Grassle (1977) derive the measure for relative abundance data; Connor and Simberloff (1978) define the analog for presence-absence data. The variance in number of shared species can be used to determine a confidence interval for similarity; although this is an underestimate of the true length of the confidence interval, it can be used to suggest lack of difference in similarity between pairs of samples. Use of these indices requires the assumption that the distribution of species abundances is multinomial, and, of course, it assumes random sampling. To the extent that these assumptions are violated, the meaning of the estimates of similarity will be unclear.

These techniques may be illustrated by an analysis of the species compositions of the benthic samples described above. For each date, the normalized expected species similarities between all pairs of stations were calculated. The basic patterns were similar for a wide range of sample sizes; for convenience, we will discuss the results for a subsample size of 10 individuals although results for up to 50 individuals (the minimum number in any of the samples) were all quite similar. These similarities followed the basic pattern of individual species abundances. There was a pronounced up-estuary--down-estuary gradient in similarities, and secondary clustering by depth. However, for several dates these indices suggest spatial patterns not revealed by the analyses of abundance. Data for the stations near the plant discharge clustered together, and tended to be very dissimilar from data for almost all other stations except, occasionally, for some of the stations farthest up-estuary (Figure 2a). This similarity between species composition of discharge and up-estuary stations is not seen in abundance or substrate similarity: the up-estuary stations have very low abundances while the abundances at the discharge are moderately high, and the discharge substrate is mixed silt/sand while up-estuary sediment is silty. The similarity could reflect temperature effects--the discharge and up-estuary stations are the warmest. Further investigations of this pattern are under way.

For comparison, the similarities between sites were calculated using the Jacard coefficient and the vectors of relative species abundance. Although these indices showed the basic longitudinal gradient of community structure, they did not identify the clusters of discharge sites as clearly as the NESS (Figure 2b). Because of its sample size dependence, the Jacard index performed erratically for stations with low diversities and sample sizes.

DISCUSSION

The techniques considered here have been used by us to estimate environmental impact in a number of studies over the past few years. We believe that the applications as described contain a proper mix of analytical rigor and healthy skepticism. The use of statistical methods has, in our opinion, swung from underutilization in the early years of impact studies to uncritical overutilization in the past decade; we hope that increased understanding of the limits of the techniques will lead to more proper use.

It is important to remember that most aquatic field impact studies are observational and not experimental in the classical sense. Therefore, the goal of a field impact study is to establish correlation, and not causation as is the goal of a properly executed experimental study. To apply ANOVA to sampling data as a definitive way of testing for impact is only to fool oneself with superfi-

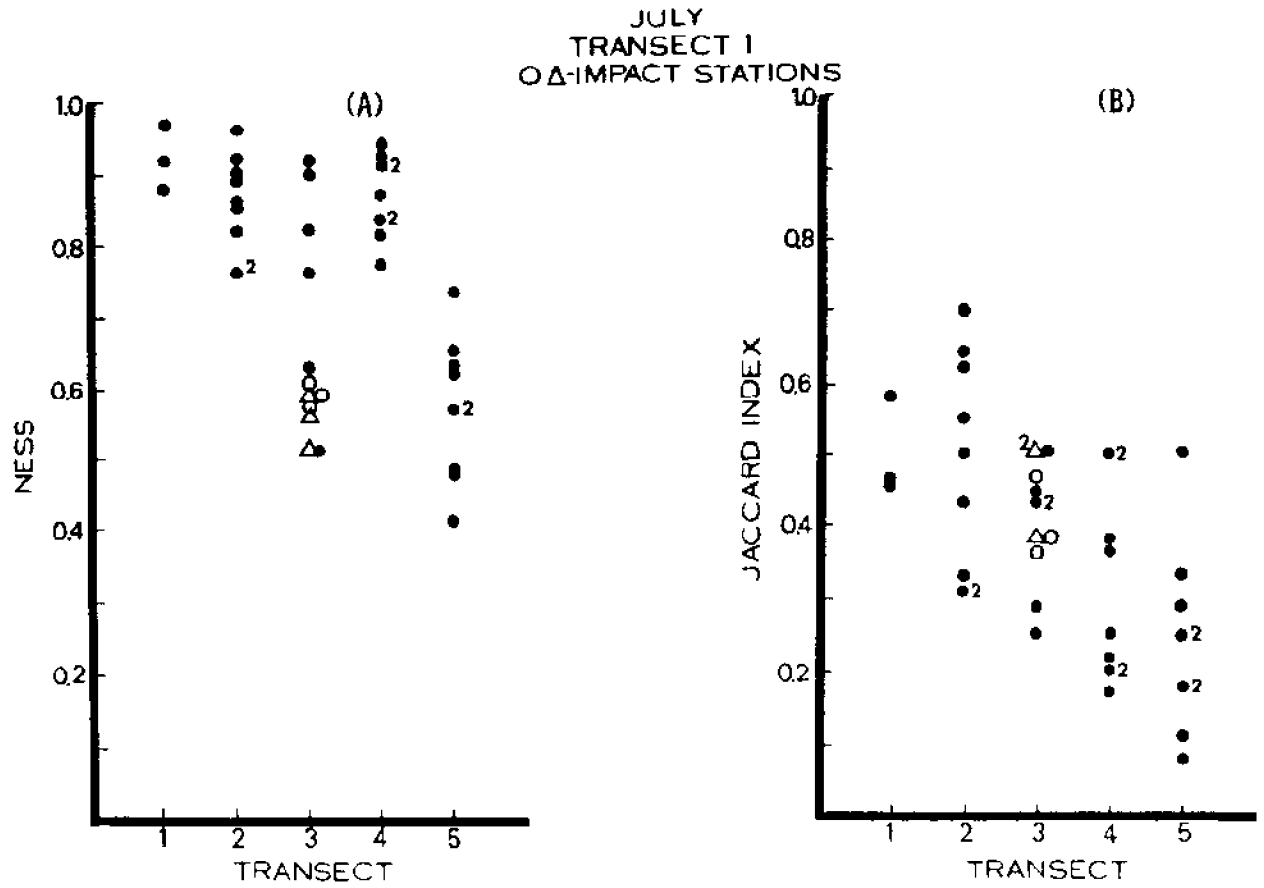

Figure 2. Plot of comparisons of stations on transect 1 with stations on each of the other transects in the study area, using the normalized expected species similarity (NESS) in (A) and the Jacard index in (B). Stations receiving thermal effluents are designated impact stations and are indicated by triangles and open circles on the figures.

cial sophistication. The use of ANOVA as an exploratory technique to identify variables for further investigation, however, is useful and proper, especially when followed by regression, principal components analysis, or discriminant analysis designed to identify consistency of association rather than causality.

The conclusions of a study of community structure will also typically depend on the indices of diversity and similarity that are used. The development of a sampling theory for similarity and diversity has led to more informative indices. We think that indices based on rarefaction are very promising, especially for comparing samples of very different sizes. As with any statistic, interpretation depends on the validity of the underlying assumptions. In particular, the dispersion of individuals in the community may create dependence between the identities of individuals within samples; a sampling design based on the spatial pattern of the community must be designed to minimize this problem.

In summary, we believe that future studies must rely on quantitative methods of assessing impact, but also suggest that only by using statistical techniques with a proper understanding of their limitations will significant progress be made in improving the quality of environmental science.

REFERENCES

Baroni-Urbani, C. and M.W. Buser. 1976. Similarity of binary data. Syst. Zool. 25:251-259.

Cheetham, A.H. and J.E. Hazel. 1969. Binary (presence-absence) similarity coefficients. J. Paleontol. 43:1130-1136.

Cochran, W.G. 1977. Sampling Techniques. John Wiley and Sons, New York.

Connor, E.F. and D.S. Simberloff. 1978. Species number and compositional similarity of the Galapagos flora and avifauna. Ecol. Monogr. 48:219-248.

Cuff, W. and N. Coleman. 1979. Optimal survey design: Lessons from a stratified random sample of macrobenthos. J. Fish. Res. Board Can. 36:351-361.

Downing, J.A. 1979. Aggregation, transformation, and the design of benthos sampling programs. J. Fish. Res. Board Can. 36:1454-1463.

Eberhardt, L.L. 1976. Quantitative ecology and impact assessment. J. Environ. Manage. 4:27-70.

Goodman, D. 1975. The theory of diversity-stability relationships in ecology. Quart. Rev. Biol. 50:237-266.

Green, R.M. 1979. Sampling Design and Statistical Methods for Environmental Biologists. John Wiley and Sons, New York.

Hendrickson, J.A., Jr. 1978. Statistical analysis of the presence-absence component of species composition data. In: K.L. Dickson, J. Cairns, Jr. and R.J. Livingston (eds.), Biological Data in Water Pollution Assessment: Quantitative and Statistical Analyses, pp. 113-124. ASTM STP 652, American Society for Testing and Materials, Philadelphia, Pa.

Livingston, R.J. 1975. Impact of kraft pulp-mill effluents on estuarine and coastal fishes in Apalachee Bay, Fla. Mar. Biol. 32: 19-48.

Logan, D.T. and D. Maurer. 1975. Diversity of marine invertebrates in a thermal effluent. J. Water Pollut. Contr. Fed. 47: 515-523.

May, R.M. 1973. Stability and Complexity in Model Ecosystems. Population Biology Monographs No. 6, Princeton Univ. Press, Princeton, N.J.

Saila, S.B., R.A. Pikanowski and D.S. Vaughan. 1976. Optimum allocation strategies for sampling benthos in the New York Bight. Estuar. Coast. Mar. Sci. 4: 119-128.

Simberloff, D.A. and E.F. Connor. 1979. Q-mode and R-mode analyses of biogeographic distributions: Null hypotheses based on random colonization. In: G.P. Patil and M. Rosenweig (eds.), Contemporary Quantitative Ecology and Related Ecometrics, pp. 123-138. International Co-operative Publ. House, Fairland, Md.

Small, R.D., T. Capizzi and L.E. Sage. 1979. Statistical design and analysis of power plant entrainment studies. In: H.T. Davis, R.R. Pairie and T. Truett (eds.), 1978 DOE Statistical Symposium, pp. 66-89. CONF-781108, Oak Ridge National Lab., Oak Ridge, Tenn.

Smith, W. and J.F. Grassle. 1977. Sampling properties of a family of diversity measures. Biometrics 33: 283-292.

Smith, W., V.R. Gibson, L.S. Brown-Leger and J.F. Grassle. 1979. Diversity as an indicator of pollution: Cautionary results from microcosm experiments. In: J.F. Grassle, G.P. Patil, W. Smith and C. Taillie (eds.), Ecological Diversity in Theory and Practice, pp. 269-277. Statistical Ecology Series Vol. 6, International Co-operative Publ. House, Fairland, Md.

Sneath, P.H.A. and R.R. Sokal. 1973. Numerical Taxonomy. W.H. Freeman and Co., San Francisco, Calif.

Chapter 4.
Bioaccumulation Tests

Introduction

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Efforts to evaluate the potential impact of a chemical in the marine environment invariably include an estimate of the chemical's propensity for bioaccumulation in the tissues of resident or representative marine species, most commonly fish or bivalves. This estimate can be made under experimental conditions in the laboratory or by the descriptive technique involving analysis of field-collected samples.

In the laboratory, bioaccumulation is assessed by directly exposing a group of organisms to the chemical mixed in saltwater of the appropriate salinity and then measuring the concentration of the chemical in the tissues of the exposed organisms (body burden) as a function of the duration of the exposure period. The results of this laboratory test may be expressed as a bioaccumulation rate--the rate at which the body burden increases--or a bioconcentration factor--the unitless quotient of the equilibrium body burden divided by the average aqueous exposure concentration. In some cases, exposure may be to chemical-containing food or sediments rather than water.

Field survey or monitoring programs involve the collection of samples of indigenous populations and the measurement of chemical residues in the tissues of these organisms. Measurements of the body burdens detected in samples collected from different areas or from the same area during some prolonged sampling period are compared in an attempt to elucidate the trends in chemical contamination of the environment.

Unfortunately, a consideration conspicuously absent from many of these studies is an evaluation of the consequence of the chemical body burden to the individual organism, the population of which it is a part, the ecological community in which it resides and the higher trophic level consumer that feeds on it. That is, mere presence of a chemical, be it a naturally occurring one such as a trace

element, or an anthropogenic one such as a plasticizer, does not necessarily indicate imminent harmful effects. Since few studies have attempted to correlate body burden with adverse effects on the organism, conclusions of the biological significance of measurable chemical residues in tissues are based on comparisons of retrospective data, which have generally indicated that chemicals with a great propensity for bioaccumulation tend to be implicated in damage to organisms and general environmental degradation. In addition, relatively few bioaccumulation studies have attempted to determine the elimination rates of accumulated chemicals from tissues upon cessation of exposure. Therefore, the longevity of the body burden and any associated harmful effects are unknown.

The papers in this chapter were solicited from individuals in several different environmental arenas. These included federal and state government regulatory agencies, academic institutions and industrial consulting companies. Each individual has had extensive and intensive experience in the collection, analysis and interpretation of data relating to the bioaccumulation of chemicals by marine organisms.

Collectively the chapter discusses:

1. Conceptual concerns in the development, interpretation and use of bioaccumulation data for hazard evaluations and other regulatory purposes.
2. Significance of chemical residues in the tissues of marine organisms.
3. Importance of specific sites of chemical bioaccumulation on the physiological processes of detoxification and subsequent elimination of residues from the body.
4. Utility of laboratory bioaccumulation data to predict body burdens in field-collected organisms and to indicate the significance of these residues.
5. Utility of sentinel organisms and field monitoring programs in describing temporal and spatial trends of chemical fates in the marine environment.

The papers that follow provide important information, pose thoughtful questions and suggest various approaches for evaluating the effects of chemicals in the marine environment.

What Is the Meaning of Bioacculumlation as a Measure of Marine Pollution Effects?

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INTRODUCTION

This paper is presented to stimulate discussion of the role of bioaccumulation as a meaningful measure of effects of marine pollution. The term "bioaccumulation" is used in a very general sense to include all measurements of contaminants in tissues of organisms, without regard to source or mechanism of introduction to the organism. The paper is argumentative in that its aims are to stimulate active discussion of bioaccumulation as a meaningful measure of demonstrably adverse effects of marine pollution.

MOTIVATION FOR MEASURING BIOACCUMULATION

When ecological awareness blossomed in the general public in the late sixties and early seventies, marine pollution became a topic of widespread interest. Every university, college and community college in the country felt obligated to offer courses or degrees in marine ecology and the aquatic environment. This produced an eruption of professors and students who had to find research topics in which to invest their new-found interest. The result was a mad rush to investigate anything and everything hinting of aquatic ecology. Bioaccumulation was a very appealing topic in this context, for reasons entirely unrelated to demonstrating adverse effects of marine pollution. The availability of subject matter was inexhaustible; bioaccumulation could be studied in every species and subspecies in the marine environment. Moreover, the availability of unique subject matter for student investigation and funding support could be increased even more by studying bioaccumulation in every

tissue and organ of every marine species. Another expansion of subject matter was realized by studying bioaccumulation of an almost endless array of elements, compounds and degradation products in every tissue of every species. The possibilities were endless, and attention to bioaccumulation expanded to fill them.

Bioaccumulation was also an appealing subject because it was partly a chemical problem as well as a biological one. Thus, it could support faculty and students in more than one department and could be touted as an example of "interdisciplinary research." It was also easy to add to almost any other kind of study. The only necessary component, an organism to analyze, could be obtained as an ancillary to any sort of other project ranging from field surveys of community structure, to laboratory bioassays, to taxonomic collections. Even museum specimens could be used. Thus, no one had to make expensive special trips to the field. It was not necessary to know much about the biology of the animals to measure contaminants in their tissues. Interpretation of the ecological meaning of these data, however, was usually ignored.

Obviously, not all bioaccumulation studies were motivated primarily by these considerations, and a number of serious and valid scientific investigations have been conducted (e.g., Kimerle et al. 1981; Macek et al. 1979; Schimmel et al. 1979; Veith et al. 1979). However, bioaccumulation rapidly became ingrained in the ecological consciousness as a standard item to be measured in every possible study, just as everyone always measured temperature and salinity whether or not they were likely to fluctuate abnormally during the study or would have any biological consequence if they did. Only after masses of data had been collected, often largely motivated by the above underlying reasons, did a retrospective consideration of their meaning reveal that bioaccumulation was an appropriate and useful, even critical, topic of investigation in some cases.

APPLICATION OF BIOACCUMULATION MEASUREMENTS

By the time the question of the meaning of bioaccumulation was asked, the measuring of it had already become a standard activity. The answer was usually that bioaccumulation was measured "to protect the environment" in some undefined sense. Gradually, it came to be measured for two general reasons. The first was to determine existing conditions and to monitor changes in them (Stainken and Rollwagen 1979; Grieg et al. 1977; Livingston et al. 1978; Wenzloff et al. 1979; Stout 1980; Young et al. 1981). The most elementary form of this reason for measuring bioaccumulation was simply that it was measurable; the more refined form was the stated need to establish baselines and monitor deviations from them. Little thought was given to whether the baseline conditions

were so low that even a substantial increase might pose no threat, or so high that even maintaining the baseline would produce, or might have already produced, unacceptable effects. The idea was primarily to establish a baseline and applaud if the trend declined below it and wring our hands if bioaccumulation climbed above the baseline. Action in response to the changing trend almost never resulted.

Measuring bioaccumulation to protect the environment was also related to testing specific discharges. Such studies are required by the criteria implementing the Ocean Dumping Act (U.S EPA 1977; Peddicord and Hansen 1982), and have been conducted for years in relation to specific compounds that may be found in particular discharges (Kimerle et al. 1981; Schimmel et al. 1979; Hansen et al. 1974). In this kind of application, a new component was added in that a decision based on the findings was implied. Animals were exposed to a sample of the material to be discharged and the resulting bioaccumulation determined. This was assumed to be a prediction of the bioaccumulation that would occur in the field if such a discharge were allowed. Thus, the certainty of monitoring historical events was replaced by the uncertainty of predictive evaluations. This uncertainty was recognized but paid only lip service by most people. If an increase in bioaccumulation was detected in the lab, it was treated as a foregone conclusion that a similar or greater change would occur if the discharge took place, and further, that the change would result in an environmentally adverse effect. This brought a public outcry to "do something!" about the impending environmental degradation. This pressure for action obscured the facts that the predicted change might have no environmental effect even if it occurred, and that the prediction was often so uncertain that a change of lesser or greater magnitude was about as likely to occur in the field as the one predicted (Clark 1977, 1980).

In the above manner one application of bioaccumulation to protect the environment led to the collection of data that were put to little use, and the other led to more action than the data could support. Both generated much study, but the amount of real environmental protection resulting from either approach is questionable.

The second reason for measuring bioaccumulation is that the law requires it. This is really just an institutionalization of the view that it is measured to protect the environment. However, those who measure bioaccumulation to protect the environment tend to be eager advocates of the measurement, while those who are motivated by the legal requirement are characterized by reluctant acceptance of the measurement.

The Marine Protection, Research, and Sanctuaries Act (Ocean Dumping Act) and the Clean Water Act both require that before proposed discharges can be permitted, they must be evaluated under criteria that consider, among other things, "...the effect of

disposal on...concentration...through...biological...processes," which clearly means bioaccumulation. The legal requirement to measure bioaccumulation institutionalizes its application to environmental protection by predicting effects of proposed discharges, although there is also some legal emphasis on monitoring with a view toward stopping discharges if some ill-defined impact level is exceeded.

The underlying original impetus for legal institutionalization of bioaccumulation measurement lies in the general public awareness and clamor for environmental action described earlier. It was established as a good thing in the common mind of the environmental lay public, and therefore was advocated to, and readily accepted by, Congress. Unfortunately, institutionalizing it did not automatically increase the quality of the data, the accuracy of the predictions, or the ability to correlate given levels of bioaccumulation with meaningful environmental effects. What it did was demand that decisions about allowing particular discharges be made on the basis of inadequate data. In effect, the legislation was and remains beyond the technical state of the art to implement (Peddicord and Hansen 1982; Engler 1980).

Prior to the attempt to implement this legislation, the advocates of bioaccumulation took the "better to err on the safe side" approach to interpreting bioaccumulation data. The underlying assumption was that since we generally do not know the effects of particular levels of bioaccumulation, we must assume any level is bad. Further, since we cannot predict precisely how much uptake will occur, just to be on the safe side we must assume any indication of bioaccumulation means that adverse effects will inevitably occur. Since this was the common attitude among advocates of bioaccumulation, who prior to legislation were the only ones interested in the topic, this became the underlying conceptual basis for the interpretation of bioaccumulation data.

However, legislation added a new twist, in that it imposed a consequence of erring in either direction. Previously, the consequence of perceived adverse environmental impact if bioaccumulation occurred led to the attitude that no risk of any bioaccumulation should be allowed. Legislation added a consequence to this side of the "err on the safe side" approach by imposing an economic burden on anyone whose discharge was denied because it might cause bioaccumulation. This left regulators with a certain lack of enthusiasm about trying to implement an approach beyond the state of scientific capability when faced with threats of suits from one group if decisions were deemed to allow too much bioaccumulation, and another group if decisions were deemed too cautious. The regulatory system was designed to work within this system of checks and balances. However, it is not unreasonable to hope that the regulatory system not demand more precise evaluations of likely impacts than the present state of scientific practice warrants.

MEANING OF BIOACCUMULATION

If we are to determine specific levels of bioaccumulation causing particular ecological effects in given species, it will be necessary to distinguish between change and effect. Since environmental consequences of particular levels of bioaccumulation cannot be determined, the "just to be on the safe side" approach to data interpretation has viewed any bioaccumulation as unacceptable. This approach, implicit to varying degrees in many bioaccumulation studies, is not satisfactory for at least two basic reasons. First, it makes effect dependent on both the chemical state of the art and the statistical sample size. If change and effect are synonymous, then "effects" will be seen at ever-declining levels as chemical detection capabilities continually improve. "Effect" can also be prevented by using a smaller sample size so that no change is statistically detectable, and thus no "effect" has occurred. This is ludicrous; effect and change are simply different concepts and cannot be equated. Second, since we do not know the biological consequences of particular levels of bioaccumulation, we have no way of judging the acceptability of the pre-change baseline conditions. It is conceptually possible that the levels against which change is determined may be sufficient to cause, or already to have caused, adverse environmental impact. In such a case, no change relative to baseline certainly would not mean no effect. It is equally possible, and I suggest far more common, that the baseline is so far below levels having any adverse consequence that a relatively small increase above present conditions would be environmentally irrelevant. To be meaningful in measuring effects of marine pollution, consequences of bioaccumulation are going to have to be stated in terms of demonstrably adverse biological responses to specified levels of bioaccumulation.

PURPOSE OF BIOACCUMULATION MEASUREMENTS

The scientific state of the art is generally not capable at present of precisely quantifying the consequences of particular levels of bioaccumulation. One reason is that, in the rush to measure, no one has defined clearly just what the measurement is supposed to tell us, nor in what sense it is to be used to protect the environment. The application of the concept continues to be plagued by confusion over just what it is supposed to achieve.

Bioaccumulation data have been applied to the protection of human health in relation to levels considered acceptable in fish and shellfish for human consumption by the U.S. Food and Drug Administration (FDA) or other similar national or international bodies (Wenzloff et al. 1979; Stout 1980; Bebbington et al. 1977; Hildebrand et al. 1980). These levels are established in an at least

superficially logical and objective manner, and more importantly in some regards, are legally institutionalized. There is general agreement that protection of human health is a legitimate application of bioaccumulation, and most people are satisfied that the FDA limits are an acceptable interpretive vehicle to achieve this. However, some people believe this is as much as can be obtained from bioaccumulation at present and that attempts to get more out of it are unfounded. The fact that FDA limits for fish and shellfish exist for a mere handful of contaminants is seen as confirmation that bioaccumulation is not a problem except in a few cases. Others claim that if contaminants can affect human health they must affect the health of marine organisms also. Further, since almost everything is toxic at high enough concentrations, almost every contaminant at lower concentrations may well cause some sort of adverse effect to organisms. Therefore, any bioaccumulation of any contaminant is viewed as conceptually bad.

While a certain amount of logic in the latter view is obvious, a difficulty arises when it confronts the narrower one in a regulatory context. One view assumes that the purpose of bioaccumulation measurements is to protect human health and concludes that a discharge is acceptable if it does not cause bioaccumulation above FDA limits. The other view assumes that the purpose of measuring bioaccumulation is to protect aquatic organisms against risk of adverse effects, and concludes that a discharge is unacceptable if a chance of measurable bioaccumulation of some chemicals is indicated by the data.

Unfortunately, the latter view lacks an objective quantitative basis for implementation. There exist no values analogous to the FDA limits against which regulators can compare bioaccumulation data to determine whether adverse effects on marine organisms are indicated. Of the vast number of possible species-contaminant combinations that exist in the marine environment, there are less than a handful in which science can say that a particular level of bioaccumulation results in a specific, demonstrably adverse effect. Thus, those who believe the purpose of measuring bioaccumulation is broadscale environmental protection are left to fall back to the "just to be on the safe side" approach to data interpretation. This inevitably places them in confrontation with those who believe their discharge is being unduly restricted without adequate indication that it would cause demonstrable damage to the environment.

It is obvious that one of the major practical obstacles to more effective use of bioaccumulation measurements in the marine pollution field is lack of agreement on what those measurements can and should be used for. This is partly a scientific problem and partly a communications problem. The latter can be minimized early in any study by a clear description of what the measurements are intended to achieve and the conceptual perspective from which the data are to be interpreted.

PRESENT STATUS OF BIOACCUMULATION

The underlying aspect of the problem is the scientific one. I recognize that the field is rapidly developing and that not only do scientists hold a variety of views, but those views are constantly changing. However, I do suggest that bioaccumulation conceptually does pose a threat to the marine environment and can be a meaningful measure of an ecologically adverse effect of marine pollution. I suggest further that we have overreacted to this conceptual truth, and that in actuality bioaccumulation is a relatively rare problem. At present we are unable, with very few exceptions, to define conditions under which it becomes a real environmental problem. Almost without exception we are unable to define, even approximately, the environmental consequences of a particular level of bioaccumulation in a given species. We cannot quantitatively define the levels of bioaccumulation producing the conceptually apparent environmental consequences, nor can we determine the conditions under which these consequences will be manifested in particular species. Therefore, I suggest that at present the only defensible application of bioaccumulation data to measurement of effects of marine pollution is to the protection of human health in relation to FDA limits.

FUTURE USEFULNESS

If bioaccumulation measurements are ever to be useful in predictive regulation of ecological effects as well as human health effects, a data base quantitatively relating bioaccumulation to ecological effect must be developed. I suggest that the following will be necessary considerations in developing that data base.

- Change must not be equated with effect. To develop data relating bioaccumulation to enzyme activity, for example, is a necessary first step, but in itself is inadequate. It is also essential to determine how much change in enzyme activity, etc., is within the normal adaptive capability of the organism and at what level it begins to cause a persistent, demonstrably adverse effect. The data base must go beyond correlating bioaccumulation with change in a particular parameter to evaluating the importance of that change to the organism, population and ecosystem.

- Use in a regulatory program means that the effects measured must be ones whose importance is obvious to administrators, judges and the public as well as scientists. A regulatory evaluation lacking this characteristic invites challenge from the environmental activists on one hand or the

environmental inactivists on the other. For this reason, I suggest that studies relating bioaccumulation to survival and reproduction are much more useful than studies relating bioaccumulation to respiration, for example. The latter relationship may hold as much or perhaps more scientific interest, but because its overall ecological consequence is not readily demonstrable it will be much less useful in a regulatory program. Therefore, it will contribute little to environmental protection and in that sense cannot be considered a truly meaningful measure of marine pollution effects.

- To be useful in a regulatory program, effects must be reliably and quantitatively predictable before the discharge takes place. To relate bioaccumulation to effect in an after-the-fact monitoring program is of limited utility except as it might provide feedback to help refine the accuracy of predictive techniques.

- The accuracy of predictive techniques must be verified by field studies. Approaches such as caging experiments, microcosms and macrocosms, which are beyond the scope of this paper, may be useful intermediate steps in the verification process under certain circumstances. However, a carefully designed study of an actual discharge operation is required for full verification of predictive techniques.

- We must not only develop verified techniques for predicting ecologically important effects. We must also develop a sound regulatory protocol for applying those techniques and interpreting the resulting data. A hazard assessment or risk evaluation approach is the logical way to achieve this. Not until this final step is taken will we have a system for truly meaningful predictive measurement of marine pollution effects.

Obviously, even partial achievement of all the preceding goals is well in the future. Moreover, regulatory decisions to allow or prohibit particular discharge operations will continue to be made daily in the absence of adequate data and fully satisfactory evaluative techniques. We must work toward the above goals while not pretending bioaccumulation can be used for purposes beyond the current state of the art. I believe this means the only defensible application of bioaccumulation to measurement of meaningful effects of marine pollution, at present, is to the protection of human health in relation to FDA limits.

CONCLUSION

In very cold realistic terms, the environment is not protected by scientific concern, workshops, supervision of graduate students, published papers or good research. The environment is protected through regulations. Regulators make major environmental decisions daily on the basis of information at hand. If we scientists do not understand the regulatory process and its needs, and do not help regulators in ways they can understand and relate to, they will make the decisions without us.

We can choose not to participate in those decisions because we know the limitations of our tests and we do not have all the answers. Or we can participate in those decisions by directing our research to give the regulators answers they need, and providing them our expertise, clearly labeled as opinion, not fact. This is the only way we will have really major or rapid influence on environmental protection. I suggest that by following this approach, 10% of the scientists working in this field have had more tangible influence on environmental protection in the last 5 years than the rest of us put together. The choice is ours. If we really want to have a noticeable influence on environmental protection, we must assist the regulators in terms of their needs.

REFERENCES

Bebbington, G.N., N.J. Mackay, R. Chvojka, R.J. Williams, A. Dunn and E.H. Auty. 1977. Heavy metals, selenium and arsenic in nine species of Australian commercial fish. Aust. J. Mar. Freshwater Res. 28: 277-286.

Clark, R.B. 1977. Sewage in the sea. Mar. Pollut. Bull. 8(12): 265-266.

Clark, R.B. 1980. Getting back to normal. Mar. Pollut. Bull. 11(1): 1-2.

Engler, R.M. 1980. Prediction of pollution potential through geochemical and biological procedures: Development of regulation guidelines and criteria for the discharge of dredged and fill material. In: R.A. Baker (ed.), Contaminants and Sediments, Vol. 1, pp. 143-169. Ann Arbor Science Publ., Ann Arbor, Mich.

Greig, R.A., D.R. Wenzloff, A. Adams, B. Nelson and C. Shelpuk. 1977. Trace metals in organisms from ocean disposal sites of the Middle Eastern United States. Arch. Environ. Contam. Toxicol. 6(4): 395-409.

Hansen, D.J., P.R. Parrish and J. Forester. 1974. Aroclor 1016: Toxicity to uptake by estuarine animals. Environ. Res. 7: 363-373.

Hildebrand, S.G., R.H. Strand and J.W. Huckabee. 1980. Mercury accumulation in fish and invertebrates of the North Fork Holston River, Virginia and Tennessee. J. Environ. Qual. 9(3): 393-400.

Kimerle, R.A., K.J. Macek, B.H. Sleight, III and M.E. Burrows. 1981. Bioconcentration of linear alkylbenzene sulfonate (LAS) in bluegill (Lepomis macrochirus). Water Res. 15: 251-256.

Livingston, R.J., N.P. Thompson and D.A. Meeter. 1978. Long-term variation of organochlorine residues and assemblages of epibenthic organisms in a shallow north Florida (USA) estuary. Mar. Biol. 46: 355-372.

Macek, K.J., S.R. Petrocelli and B.H. Sleight, III. 1979. Considerations in assessing the potential for and significance of biomagnification of chemical residues in aquatic food chains. In: L.L. Marking and R.A. Kimerle (eds.), Aquatic Toxicology, pp. 251-268. ASTM STP 667, American Society for Testing and Materials, Philadelphia, Pa.

Peddicord, R.K. and J.C. Hansen. 1983. Technical implementation of the regulations governing ocean disposal of dredged material. In: D.R. Kester, B.H. Ketchum I.W. Duedall and P.K. Park (eds.), Wastes in the Ocean, Vol. II; Dredged Material Disposal in the Ocean. John Wiley and Sons, New York.

Schimmel, S.C., J.M. Patrick, L.F. Faas, J.L. Oglesby and A.J. Wilson, Jr. 1979. Kepone toxicity and bioaccumulation in blue crabs. Estuaries 2(1): 9-15.

Stainken, D. and J. Rollwagen. 1979. PCB residues in bivalves and sediments of Raritan Bay. Bull. Environ. Contam. Toxicol. 23: 690-697.

Stout, V.F. 1980. Organochlorine residues in fishes from the northwest Atlantic Ocean and Gulf of Mexico. Fish. Bull. 78(1): 51-58.

U.S. EPA (Environmental Protection Agency). 1977. Ocean Dumping--Final Revision of Regulations and Criteria. U.S. Federal Register, 11 January 1977, Part VI, 42: 2462-2490.

Veith, G.D., D.L. DeFoe and B.V. Bergstedt. 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish. Res. Board Can. 36: 1040-1048.

Wenzloff, D.R., R.A. Greig, A.S. Merrell and J.W. Ropes. 1979. A survey of heavy metals in the surf clam, Spisula solidissima, and the ocean quahog, Arctica islandica, of the mid-Atlantic coast of the United States. Fish. Bull. 77(1): 280-285.

Young, D.R., M.D. Moore, T.K. Jan and R.P. Eganhouse. 1981. Metals in seafood organisms near a large California municipal outfall. Mar. Pollut. Bull. 12(4): 134-138.

Methods of Evaluating Pollutant Biomagnification in Marine Ecosystems

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INTRODUCTION

During the last three decades numerous studies have been conducted relative to marine pollution. In the 1950s and 1960s, the major topic of concern involved the injection of radioactive materials into ocean systems (NAS 1957, 1971; Schultz and Klement 1963; Nelson and Evans 1969). Beginning about 1970, emphasis shifted to problems involving the discharge, fate and effects of toxic trace elements, synthetic organics and petroleum hydrocarbons in the marine environment (FAO 1970; NSF 1972; SCCWRP 1973; Nelson-Smith 1973; Cox 1974; Church 1975; Goldberg 1976, 1979; Wiley 1978; Baker 1980).

These studies fell into two major categories--field surveys and laboratory toxicity tests (the latter often termed bioassays). Unfortunately, there usually was very little integration of these efforts. Most of the toxicity tests employed simple aqueous concentrations of the target constituents, often at unrealistically high (e.g., parts per million, milligram per liter) levels. In contrast, seawater concentrations of these materials now appear to be many orders of magnitude lower, often at the parts per trillion (nanogram per liter) level. Because of the extreme difficulty of reliably determining the physical/chemical states and corresponding concentrations at such levels, most environmental surveys have centered on documentation of mass emission rates of target constitutents in a few major sources (e.g., domestic and industrial wastewater, surface runoff, aerial deposition, vessel-related discharges) and on their distributions in bottom sediments and various "indicator" organisms (Young et al. 1973, 1975, 1978; Risebrough et al. 1974; McDermott et al. 1976; Eganhouse et al. 1978).

Thus, it usually has been difficult or impossible to apply the findings of the extensive toxicity literature to those of marine pollution surveys. Although innumerable contaminant elevations in sediments and the biota have been described, the toxicologists generally have not provided information that is useful in interpreting the ecological significance of such concentration elevations. In short, there has been no way to answer convincingly the "so what?" questions which continually plague regulatory agencies responsible for making environmental decisions.

One of the topics of greatest concern in pollution studies involves the accumulation of potentially toxic substances in organisms. Over the years a variety of terms have been used to describe this phenomenon. Recently, Brungs and Mount (1978) proposed a clarification of three of the most commonly used terms, summarized by Macek et al. (1979) as follows:

> Bioconcentration refers to that process whereby chemical substances enter aquatic organisms through the gills or epithelial tissue directly from the water; bioaccumulation is a broader term referring to a process which includes bioconcentration but also any uptake of chemical residues from dietary sources. Finally, biomagnification refers to a process by which the tissue concentrations of bioaccumulated chemical residues increase as these materials pass up the food chain through two or more trophic levels. Historically implicit in the use of this term is the connotation that residue concentration at successively higher trophic levels increases by multiples of whole numbers (for example, x 2, x 5, x 10).

As already mentioned, the literature contains a vast number of reports which describe abnormal accumulations of "pollutants" in the marine biota. A companion paper in this chapter by Jenkins and Brown discusses the implications of such bioaccumulation for selected trace metals and synthetic and petroleum hydrocarbons. Therefore, here I discuss bioaccumulation only as it relates to the process of biomagnification, which has caused so much concern to the general public and environmental managers over the years.

FOOD WEB STRUCTURE

Biological Assessment

Traditionally, the passage of contaminants through food webs has been considered either from the point of view of specific predator-prey relationships, or more generally in the context of average trophic (i.e., feeding) levels. An example of a simplified food web containing five trophic levels (I-V), taken from Mearns et al. (1981), is shown below:

V	Tertiary Carnivores (feed on IV)
IV	Secondary Carnivores (feed on III)
III	Primary Carnivores (feed on II)
II	Herbivores (feed on I)
I	Primary Producers

There are at least two major problems with this approach. First, it clearly is a gross oversimplification of the actual situation observed for marine food webs by ecologists over at least the last 60 years (e.g., Hardy 1924). Second, because it is qualitative rather than quantitative, it is not amenable to statistical analysis or mathematical modeling efforts directed toward describing the degree to which a specific pollutant would be expected to biomagnify in a given system.

Over the years, attempts have been made to modify this system to permit intermediate assignments of trophic level (Lindeman 1942; Odum 1971, ch. 4). More recently, Mearns and his colleagues have developed a method of making quantitative estimates of average intermediate trophic level position. In this approach trophic levels are assigned to prey items and some simplifying assumptions are made about the feeding habits of several kinds of organisms (e.g., diatoms and dinoflagellates are primary producers at Trophic Level 1.0; certain small crustacea are herbivores at Trophic Level 2.0). Then a weighted average prey trophic level is computed and the predator is assigned to the next higher full trophic level. To weight the prey data, Mearns used an index developed by Pinkas et al. (1971), the Index of Relative Importance:

$$IRI = \%F\ (\%N + \%V)$$

where %F is the percent frequency of prey item, %N is the percent by numerical abundance of prey item and %V is the percent by weight or volume of prey item. The specific weighting factor for a given prey item (already given a numerical assignment for its average trophic level as described above) is simply the percentage of the total IRI obtained for the predator's list of prey items. Although this approach is also characterized by certain arbitrary and simplifying assumptions (as readily admitted by its authors), it does appear to provide a useful method of obtaining a numerical assignment for a predator's average trophic level position, which Mearns et al. (1981) have termed the Trophic Level Assignment (TLA).

Chemical Assessment

Although some method of quantifying average trophic level position is necessary in any serious evaluation of marine biomagnification, this alone may not be sufficient. The reason is that feeding relationships in marine ecosystems often are so complex and/or variable that the assignment of single trophic level positions to the

organisms constituting a given web is a gross oversimplification. Isaacs (1972, 1973, 1976) has argued persuasively that certain marine food webs may be largely unstructured, that is, composed primarily of opportunistic feeders such that the multidirectional feeding relationships in effect "short-circuit" the potential for food chain magnification. In such cases, the failure to obtain a significant correlation between the concentration of a target substance in the biota and estimated trophic level position could be due to unrecognized homogenization, or mixing within the food web, rather than a general inability of the substance to biomagnify under any circumstance.

As a possible solution to this dilemma, Isaacs (1972) proposed that chemical indicators of structure be employed to test the biomagnification potential of a given food web. A major advantage of this approach is that chemical indicators should be more representative of average conditions than are the results from a specific stomach content analysis program.

One candidate chemical indicator is the ratio of two alkali metals, cesium (Cs) and potassium (K). Potassium, an essential electrolyte, must be maintained at fairly constant levels in tissues; this is not the case for cesium, which occurs only in trace quantities. Marine fish appear to obtain most of their cesium and potassium from their diet, rather than by direct uptake from seawater (Young 1970). Assuming that this is generally true for marine animals, fairly regular increases in the ratio of cesium to potassium over known food chain links or trophic level steps might be expected, because cesium has been found to have a biological half-life that is approximately two or three times that of potassium in a variety of animals (Anderson et al. 1957; McNeill and Trojan 1960; Green and Finn 1964; Pendleton 1964; Hanson et al. 1964; Pendleton et al. 1965; Hanson 1967; Gustafson 1967). Again assuming that this observation is valid for marine animals in general, the relative values of the Cs/K ratio in organisms constituting a given food web should provide an indication of the degree of trophic structure in that ecosystem, and thus indicate the potential for food chain increases of pollutant concentrations within the system.

The usefulness of this candidate chemical indicator of food web structure was tested in the quasi-marine ecosystem of the Salton Sea, a large saline lake in central southern California (Young 1970). Extensive studies by Walker and his colleagues (1961) had shown that this ecosystem contained a relatively simple, highly structured food web in the late 1950s. A year-long survey by Young in 1967 generally confirmed the persistence of these structured (i.e., constant) feeding relationships. The striped mullet (Mugil cephalis) fed almost exclusively on the dense phytoplankton populations of the sea, leading to a TLA of 2.0. In contrast, the threadfin shad (Dorosoma petenense) fed mostly on the herbivorous zooplankton (copepods and barnacle larvae), and thus had a TLA of 3.0. The

predominant diet of the sargo (Anistotremus davidsoni) and Gulf croaker (Bairdiella icistia) was the detrital-feeding worm, Neanthes succinea, whose estimated TLA value was 2.5; the corresponding TLA value for the two forage feeding fishes was 3.5. Similarly, their major predator the orangemouth corvina (Cynoscion xanthulus) had a TLA of 4.5.

A comparison between these TLAs and the average Cs/K ratios (n=6) measured by Young in muscle tissue of the five Salton Sea fishes is presented in Figure 1. This illustration suggests that the Cs/K ratio increased exponentially with TLA. To test this hypothesis, a correlation was sought between the (natural) logarithm of the mean value of Cs/K times 10^6 for a given fish species, and its corresponding TLA value (Young and Mearns, unpublished manuscript). The resulting correlation coefficient (0.94) was found to be highly significant ($p<0.001$), suggesting that the data are adequately related by the following straight line of best fit:

$$\ln (\text{Cs/K times } 10^6) = m(\text{TLA}) + b$$

where

$$m = 0.76$$
$$b = 0.58$$

It is easily shown that a relationship of the form

$$\ln C = m\,(\text{TLA}) + b$$

corresponds to a constant tissue concentration "amplification" factor per unit trophic level step, whose value is given by the expression e^m. Clearly, this is the simplest case of biomagnification; within the range of trophic levels sampled, the concentration (C_n) of a constituent in an organism at a given trophic level (n) is multiplied by a constant amplication factor (A.F.) to obtain the concentration at the next highest trophic level (n + 1). Thus:

$$\text{A.F.} \equiv \frac{C_{n+1}}{C_n} = e^m$$

However, for this simple case it is important to note that, although the amplification factor e^m is constant, it is not constrained either to be an integer or to have a value greater than 1.0. (Trophic level "amplification" factors of less than 1.0 sometimes are termed "discrimination" factors instead.)

In the example presented for the 1967 Salton Sea survey, the average amplification factor obtained for the Cs/K ratio is $e^{0.76} = 2.14$. This value is consistent both with specific predator/prey amplification factors for muscle tissue Cs/K ratios observed in the laboratory and field, and with the ratio of biological half-lives reported for these two alkali metals.

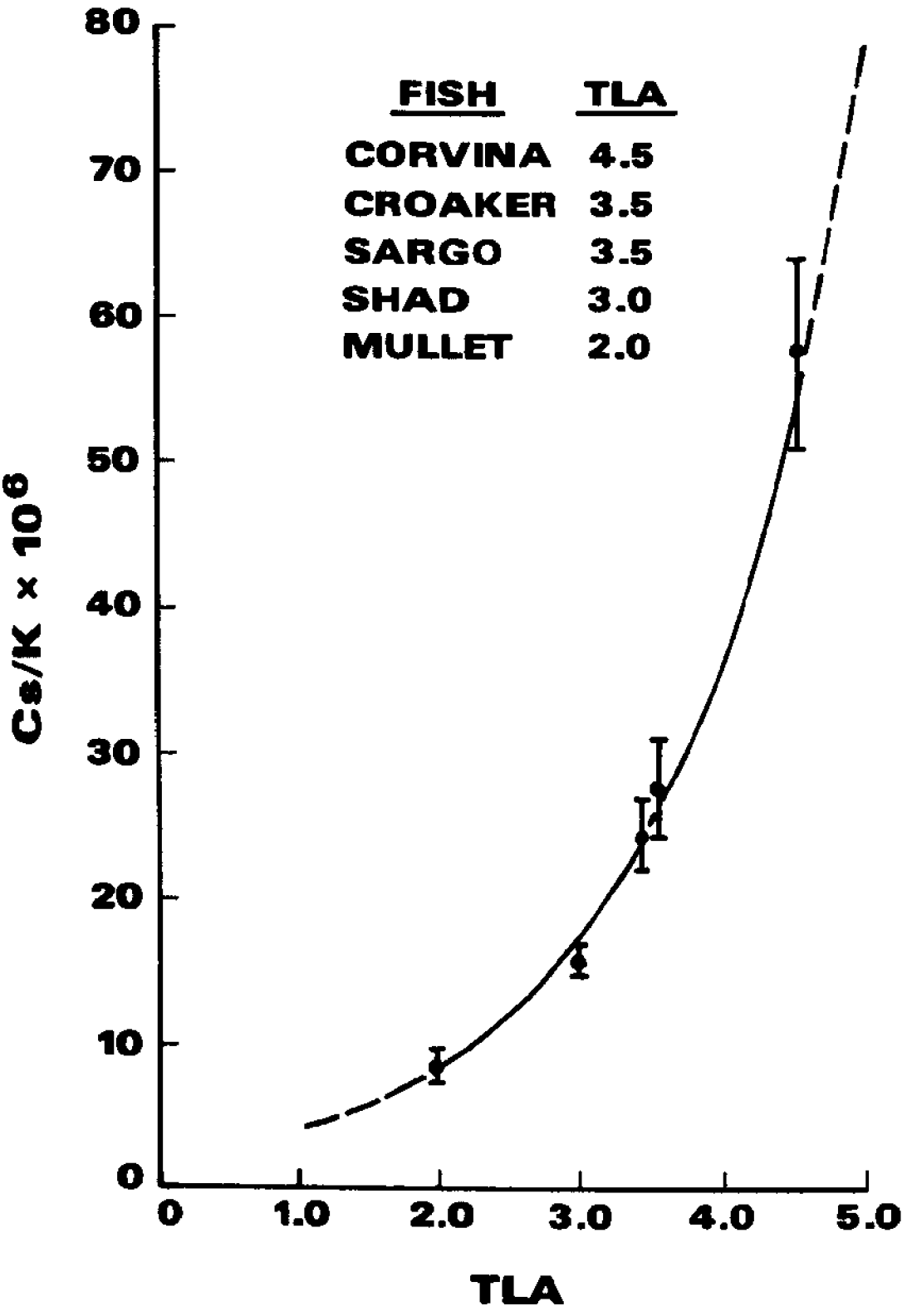

Figure 1. Average muscle tissue value (± std. error; n = 6) for cesium/potassium ratio vs. Trophic Level Assignment (TLA) for five fishes collected during 1967 from the Salton Sea. Also shown is the line for the corresponding equation of best fit: ln (Cs/K x 10^6) = 0.76 (TLA) + 0.58. (Data are from Young 1970).

This suggests that the Cs/K ratio is a useful index of the degree of structure, or biomagnification potential, in marine food webs. Other chemical constituents whose tissue concentrations increase with trophic level in a uniform manner also might be useful indices of food web structure. However, any constituent selected as an index of the pollutant biomagnification potential of a marine food web should not be a common component of anthropogenic inputs to the sea.

As indicated by the use of the term biomagnification potential, it should be noted that food web structure is a necessary but not sufficient condition for biomagnification of a given constituent to occur in that ecosystem. Other necessary conditions are that the constituent be biologically persistent, that is, have a relatively long biological half-life in the target organisms, and that the major route of uptake be through the foods of the organisms.

CASE STUDIES

A series of studies conducted between 1975 and 1981 in various marine ecosystems off southern California provide relevant examples of the application of the above-described approach (Young et al. 1980, 1981; Mearns and Young 1980; Mearns et al. 1981; Bascom 1982; Schafer et al. 1982). In seven of the nine studies, the Cs/K values were observed to increase exponentially with TLAs for the target organisms (Young and Mearns, unpublished manuscript). Corresponding average amplication factors ranged from about 1.4 to 1.9, suggesting the existence of reduced but measurable chemical structure, or potential for biomagnification of pollutants, in most of these systems. (In a completely homogenized food web, the average Cs/K amplification factor would be 1.0.) Concentrations of DDT, a pollutant reported to biomagnify in aquatic ecosystems (Woodwell et al. 1967; Macek et al. 1979), also fit the simple exponential model in a majority of the surveys analyzed to date.

One of the most consistent results of this investigation was the absence of trophic level increases for the target metals arsenic, cadmium, chromium, copper, iron, lead, manganese, nickel, silver and zinc. In six ecosystem surveys where a majority of these metals were measured, no exponential increase in concentration with TLA was observed. In contrast, approximately one-third of the results for a given metal and ecosystem indicated exponential decreases in concentration with TLA. The only exception was mercury; exponential increases in concentration with TLA generally were observed. This is very probably explained by the fact that when organic mercury also was analyzed, it usually was found to constitute most of the total mecury measured in these samples (Young et al. 1980; Schafer et al. 1982). As discussed above, substances with relatively long biological half-lives, such as organic

mercury compounds (Miettinen et al. 1970; Jarvenpaa et al. 1970; Tillander et al. 1970; Jernelov and Lann 1971; Cutshall et al. 1978), are the most likely to biomagnify in structured food webs.

There is one aspect of this finding that illustrates a critical point regarding biomagnification of trace metals. Although total mercury concentrations in seafood organisms from a highly contaminated municipal wastewater discharge zone off Los Angeles fit the simple exponential model, when these values were corrected by those for corresponding samples from control zones, the relationship disappeared. A visual evaluation of these "net" or "excess" values (outfall zone minus control zone) provided no indication that the anthropogenic fraction of the mercury measured in muscle of the seafood species increased with estimated trophic level position, despite the relatively high exposure of the organisms to mercury wastes around this submarine outfall system (Young et al. 1981). Related studies showed that almost all of the measurable mercury in surficial bottom sediments of this zone was inorganic (Eganhouse et al. 1978). Since inorganic mercurial compounds have considerably shorter biological half-lives than do the organic forms of mercury, the inorganic mercurials are less likely to undergo biomagnification. This may explain the significantly different behavior of the "gross" and "net" concentrations of mercury in this outfall zone foodweb.

Thus, it would appear to be very important in future biomagnification studies for trace metals, as well as other contaminants of concern, to include analysis of corresponding samples from valid control zones. Since trace elements are natural constituents of all organisms, it is the "excess" or "net" (rather than the "gross") tissue concentrations that should be compared with trophic level position in evaluating potential biomagnification problems. However, the difficulties in documenting the validity of a control zone are well recognized. In addition to being sufficiently removed from the source(s) of interest, the zone's food web should have characteristics similar to those of the test zone. It appears that the Cs/K ratio may be a useful parameter for evaluating the similarity of food web structure in the test and control zones.

LITERATURE REVIEW

In view of these recent findings made off California for metals of concern, which contradict the popular belief that pollutant biomagnification is a common (if not universal) phenomenon, it is instructive to consider the results of investigations made in various parts of the world during the last two decades on the marine biomagnification of trace elements.

One of the earlier studies, conducted off the mouth of the Columbia River, found that the radionuclides zirconium-95/nio-

bium-95 and cerium-141 were concentrated by primary producers and herbivores but not by carnivores, while chromium-51 was abundant only in the primary producers (Osterberg et al. 1964). Baptist and Lewis (1969), working with an experimental food chain, found that although zinc-65 and chromium-51 were transferred from the first to the fourth trophic levels, the equilibrium tissue concentrations of both nuclides generally declined up the food chain. Bowen et al. (1971) concluded that many elements, when studied with radionuclide tracers, appear to be subject to discrimination, that is, a reduction in concentration through each trophic level step. In a recent paper, Guary et al. (1982) concluded from the literature that most heavy metals in the marine environment generally show a decrease at higher trophic levels in a given food chain, and presented data suggesting that food chain magnification of plutonium by starfish does not occur in nature as had been previously hypothesized. In addition, Cheng et al. (1977) found that dry weight concentrations of both polonium and plutonium decreased by about a factor of 2 over a seaweed-kelpfly food chain link.

By loading brine shrimp with cadmium before feeding them to pink shrimp, Nimmo et al. (1977) measured the maximum transfer of cadmium (under the specified test conditions) from food to the next trophic level, obtaining consistent predator/prey concentration ratios of 0.02-0.03. Macek et al. (1979) also concluded from laboratory experiments that biomagnification of cadmium (and several synthetic organics other than DDT) within a freshwater food chain was insignificant compared with direct uptake from the water.

Burnett and Patterson (1980) have provided convincing evidence that, to be meaninful, analyses of lead in tissues of marine organisms must be conducted using ultraclean-room techniques. For example, under poorly controlled conditions the concentrations of lead measured in phytoplankton and muscle tissue of abalone, scallop, lobster and tuna ranged between about 80 and 800 nanograms per gram (fresh weight), with no convincing trend. However, analyses of these samples in a contamination-controlled laboratory provided a remarkably different story. Values dropped by factors of 10-1000, revealing a three-orders-of-magnitude decrease over the phytoplankton-macroinvertebrate-tuna food chain. Clearly, much of the published literature on lead in marine organisms is suspect owing to inadequate sample preparation and analytical techniques.

Klumpp and Peterson (1979) studied macrophytes and their molluscan predators in an English estuary, and concluded that there was no evidence for biomagnification of arsenic on an entire organism basis. Working in western Australia, Edmonds and Francesconi (1981) investigated a simple food chain, consisting of phytoplankton--detritus--detrital-feeding worm--whiting. Similar concentrations of arsenic were measured in the plant, worm and fish

samples, providing further evidence against the marine biomagnification of the element.

In the case of mercury, there are contradictory reports in the literature. Knauer and Martin (1972) measured total mercury in phytoplankton, zooplankton and anchovies off Monterey, California, finding no evidence of food chain amplification (i.e., biomagnification). Similarly, Williams and Weiss (1973) measured total mercury in a pelagic food chain off San Diego, California. They reported that the mercury content in almost all of the higher trophic level organisms collected at the greater depths of the survey were indistinguishable from the concentration of mercury in zooplankton at those depths. On the basis of such findings, Bryan (1979) concluded that there is not much evidence for the amplification of mercury in moving from invertebrates to small fish, but that there is a stronger argument when larger fish are considered. However, this conclusion may be influenced by the fact that, owing to the difficulty of cleanly dissecting tissues from small invertebrates, whole-body concentrations in such organisms often are compared with single-tissue (e.g., muscle) concentrations in larger organisms. Thus, whenever possible it would appear to be important in bioaccumulation and biomagnification studies to compare concentrations in similar tissues (e.g., muscle, liver, whole body), or at the least to point out that the comparisons made are for dissimilar types of tissues.

Data of numerous authors relating body mass or organism age and the concentration of mercury in the muscle tissue also are summarized by Bryan (1979). These results indicate that, in certain species, concentrations of mercury increase markedly with size or age. Since a marine organism usually moves higher in the food web as it grows, it is difficult to distinguish between the effects of age and trophic level in the case of constituents like mercury that appear to biomagnify; both factors probably are important.

CONCLUSIONS

1. Laboratory toxicity tests designed to provide information on the biological effects of elevated tissue (and sediment) concentrations of potential pollutants in marine ecosystems are needed, so that results of typical environmental surveys can be meaningfully evaluated.

2. The term "bioaccumulation" should be used to describe the uptake by an aquatic organism of a given constituent from water or

food. In contrast, the terms "biomagnification" or "bioamplification" should be used to describe that process by which tissue concentrations of a bioaccumulated constituent increase (or decrease) in a food web as the constituent passes from one trophic level to another.

3. A quantitative evaluation of the biomagnification of a given constituent in a given food web requires that a logical biological method of quantifying a member organism's average trophic level position be applied. An example of one such approach recently proposed (the Trophic Level Assignment) has been presented; however, methods are needed to evaluate both the accuracy and the precision of such biological assignments of trophic level positions.

4. The highly complicated and variable nature of most marine food webs leads to major uncertainties in biological assessments of average trophic level position in marine ecosystems. As a result, the absence of an apparent relationship between the concentration of a potential pollutant in a given organism and its assigned trophic position does not of itself negate the possibility that the substance might biomagnify in this (or a similar) food web sampled at another time or place. Thus, it appears useful in such studies also to apply an independent chemical method of quantifying the degree of "structure" or biomagnification potential of the target food web. Once the existence of this potential has been established, the hypothesis of biomagnification for specific pollutants of concern under these (or similar) conditions is disproved if trophic level increases of these pollutants are not observed. (It should be noted that a positive correlation between concentration and trophic position shows that the data are consistent with, but does not "prove," the hypothesis of biomagnification.)

5. An example of one chemical index (the cesium/potassium ratio) used in several recent marine pollution surveys has been presented. However, there remain a number of inadequately tested assumptions inherent in this type of approach. For example, (1) the index constituent(s) have a similar biological persistence in the target tissue of all the component organisms; (2) the major source of uptake is the organisms' food; and (3) there is generally a satisfactory approximation to equilibrium conditions. Thus, although chemical indices of food web "structure" appear to be potentially useful, such techniques need to be critically evaluated regarding their reliability and limitations in marine biomagnification studies.

6. Ideally, bioaccumulation and biomagnification investigations should be made on the same type of "tissue" (e.g., muscle, liver, whole body) for all species included in the study. When this is not possible, the existence of inconsistency, and the possible effects it might have on the results, should be discussed.

7. A survey of the literature on marine biomagnification of trace metals revealed that, with the possible exception of mercury, trophic level increases seldom have been observed for most metals of concern. This contradicts the widespread opinion (at least among environmental regulators and the general public) that all or most pollutants will biomagnify, often to potentially dangerous concentrations, in marine food webs.

8. For those constituents for which there is a natural background (e.g., trace metals such as mercury) or a measurable regional baseline (e.g., DDT), the essential question in pollutant biomagnification studies is whether or not there are measurable trophic level increases of the "excess" or "net" tissue concentrations (test zone minus control zone values) rather than increases in "total" or "gross" concentration of the constituent in the organisms from the area of interest. One study around a major submarine discharge of mercury-contaminated wastewater revealed no discernible trophic level increase of "excess" mercury. To date, very little attention has been paid to this concept in studies of pollutant biomagnification near point sources of concern.

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REFERENCES

Anderson, E.C., R.L. Schuch, W.R. Fisher and W. Langham. 1957. Radioactivity of people and foods. Science 125: 1273-1278.

Baker, R.A. (ed.). 1980. Contaminants and Sediments. Vol. 1. Fate and Transport, Case Studies, Modelling, Toxicity. Ann Arbor Science Publishers, Inc., Ann Arbor, Mich.

Baptist, J.P. and C.W. Lewis. 1969. Transfer to ^{65}Zn and ^{51}Cr through an estuarine food chain. In: D.J. Nelson and F.C. Evans (eds.), Proceedings of the Second National Symposium on Radioecology, Ann Arbor, Mich., May 15-17, 1967, pp. 420-430. CONF-670503, U.S. Atomic Energy Commission, Division of Biology and Medicine, Washington, D.C.

Bascom, W. 1982. The effects of waste disposal on the coastal waters of southern California. Environ. Sci. Technol. 16: 226A-236A.

Bowen, V.T., J.S. Olsen, C.L. Osterberg and J. Ravera. 1971. Ecological interactions of marine radioactivity. In: Radioactivity in the Marine Environment, pp. 200-222. National Academy of Sciences, National Research Council, Washington, D.C.

Brungs, W.A. and D.I. Mount. 1978. Introduction to a discussion of the use of aquatic toxicity tests for evaluation of the effects of toxic substances. In: J. Cairns, Jr., K.L. Dickson and A.W. Maki (eds.), Estimating the Hazard of Chemical Substances to Aquatic Life, pp. 15-26. ASTM STP 657, American Society for Testing and Materials, Philadelphia, Pa.

Bryan, G.W. 1979. Bioaccumulation of marine pollutants. Philos. Trans. R. Soc. Lond. B. 286: 482-505.

Burnett, M. and C.C. Patterson. 1980. Analysis of natural and industrial lead in marine ecosystems. In: M. Branica and Z. Konrad (eds.), Lead in the Environment. Proceedings of the International Experts Discussion on Lead Occurrence, Fate, and Pollution in the Marine Environment, Rovinj, Yugoslavia, Oct. 18-22, 1977, pp. 15-30. Pergamon Press, Oxford, England.

Cheng, L., V.F. Hodge and R.A. Lewin. 1977. Polonium and plutonium in an intertidal food chain. Nature 269: 795-796.

Church, T.M. (ed.). 1975. Marine Chemistry in the Coastal Environment. American Chemical Society, Washington, D.C.

Cox, G. (ed.). 1974. Marine Bioassays Workshop Proceedings. Marine Technological Society, Washington, D.C.

Cutshall, N. H., J. R. Naidu, and W. G. Pearcy. 1978. Mercury concentrations in Pacific hake, Merluccius productus (Ayres), as a function of length and latitude. Science 200:1489-1491.

Edmonds, J.S. and K.A. Francesconi. 1981. The origin and chemical form of arsenic in the school whiting. Mar. Pollut. Bull. 12: 92-96.

Eganhouse, R.P., D.R. Young and J.N. Johnson. 1978. Geochemistry of mercury in Palos Verdes sediments. Environ. Sci. Technol. 12: 1151-1157.

FAO (Food and Agriculture Organization). 1970. Proceedings of FAO Technical Conference on Marine Pollution and Its Effects on Living Resources and Fishing, Rome, Italy, Dec. 9-18, 1970. FIR:MP/70/R-9, United Nations, FAO, Rome.

Goldberg, E.D. (ed.). 1976. Strategies for Marine Pollution Monitoring. Wiley-Interscience, New York.

Goldberg, E.D. (ed.). 1979. Proceedings of a Workshop on Scientific Problems Relating to Ocean Pollution. Estes Park, Colorado, July 10-14, 1978. NOAA Environmental Research Laboratories, Boulder, Colo.

Green, R.M. and R.J. Finn. 1964. Loss of cesium-137 in the ashing of milk samples. Anal. Chem. 36: 692-693.

Guary, J.C., S.W. Fowler and T.M. Beasley. 1982. Routes of plutonium uptake and their relation to biomagnification in starfish. Mar. Poll. Bull. 13: 99-102.

Gustafson, P.A. 1967. Cesium-137 in freshwater fish during 1954-1965. In: D.J. Nelson and F.C. Evans (eds.), Proceedings of the Second National Symposium on Radioecology, Ann Arbor, Mich., May 15-17, 1967, 249-257, CONF-670503, U.S. Atomic Energy Commission, Division of Biology and Medicine, Washington, D.C.

Hanson, W.C. 1967. Cesium-137 in Alaskan lichens, caribou, and Eskimos. Health Phys. 13: 383-389.

Hanson, W.C., H.E. Palmer and B.I. Griffith. 1964. Radioactivity in northern Alaskan Eskimos and their food, summer 1962. Health Phys. 10: 421-429.

Hardy, A.C. 1924. The herring in relation to its animate environment. Part I. The food and feeding habits of the herring with special reference to the east coast of England. Ministry of Agriculture, and Fisheries, Fishery Invest., Ser. II, Vol. 7, No. 3.

Isaacs, J.D. 1972. Unstructured marine food webs and "pollutant analogues." Fish. Bull. 70: 1053-1059.

Isaacs, J.D.. 1973. Potential trophic biomasses and trace substance concentrations in unstructured marine food webs. Mar. Biol. 22: 97-104.

Isaacs, J.D. 1976. Reproductive products in marine food webs. Bull. South Calif. Acad. Sci. 75: 220-223.

Jarvenpaa, T., M. Tillander and J.K. Miettinen. 1970. Methyl mercury: Half-time of elimination in flounder, pike and eel. In: Proceedings of FAO Technical Conference on marine Pollution and Its Effects on Living Resources and Fishing, Rome, Italy, Dec. 9-18, 1970, p. 158. FIR: MP/70/R-9, United Nations, Food and Agriculture Organization, Rome.

Jernelov, A. and H. Lann. 1971. Mercury accumulation in food chains. Oikos 22: 403-406.

Klumpp, D.W. and P.J. Peterson. 1979. Arsenic and other trace elements in the waters and organisms of an estuary in SW England. Environ. Pollut. 19: 11-20.

Knauer, G.A. and J.H. Martin. 1972. Mercury in a marine pelagic food chain. Limnol. Oceanogr. 17: 868-876.

Lindeman, R.L. 1942. The trophic dynamic aspect of ecology. Ecology 23: 399-418.

Macek, K.J., S.R. Petrocelli and B.H. Sleight III. 1979. Considerations in assessing the potential for, and significance of, biomagnification of chemical residues in aquatic food chains. In: L.L. Markins and R.A. Kimmerle (eds.), Aquatic Toxicology, pp. 251-268 ASTM STP 667, American Society for Testing and materials, Philadelphia, Pa.

McDermott, D.J., D.R. Young and T.C. Heesen. 1976. PCB contamination of southern California marine organisms. In: Proceedings of the National Conference on Polychlorinated Biphenyls, Chicago, Ill., Nov. 19-21, 1975, pp. 199-208. EPA 560/6-75-004, U.S. Environmental Protection Agency, Washington, D.C.

McNeill, K.G. and O.A.D. Trojan. 1960. The cesium-potassium discrimination ratio. Health Phys. 4: 109-112.

Mearns, A.J. and D.R. Young. 1980. Trophic structure and pollutant flow in a harbor ecosystem. In: Biennial Report for 1978-1980, pp. 287-308. Southern California Coastal Water Research Project, Long Beach, Calif.

Mearns, A.J., D.R. Young, R.J. Olson and H.A. Schafer. 1981. Trophic structure and the cesium-potassium ratio in pelagic ecosystems. In: CalCOFI Report, Vol. XXII, pp. 99-110. California Cooperative Fisheries Investigation, La Jolla, Calif.

Miettinen, J.K., M. Heyraud and S. Keckes. 1970. Mercury as a hydrospheric pollutant. Part 2. Biological half-times of methyl mercury in four Mediterranean species: A fish, a crab, and two molluscs. In: Proceedings of FAO Technical Conference on Marine Pollution and Its Effects on Living Resources and Fishing, Rome, Italy, Dec. 9-18, 1970, p. 171. FIR:MP/70/R-9, United Nations, Food and Agriculture Organization, Rome.

NAS (National Academy of Sciences). 1957. The Effects of Atomic Radiation on Oceanography and Fisheries. NAS National Research Council, Washington, D.C.

NAS (National Academy of Sciences). 1971. Radioactivity in the Marine Environment. NAS National Research Council, Washington, D.C.

Nelson, D.J. and F.C. Evans (eds.). 1969. Proceedings of the Second National Symposium on Radioecology, Ann Arbor, Mich., May 15-17, 1967. CONF-670503, U.S. Atomic Energy Commission, Division of Biology and Medicine, Washington, D.C.

Nelson-Smith, A. 1973. Oil Pollution and Marine Ecology. Plenum Press, New York.

Nimmo, D.W., D.V. Lightner and L.H. Bahner. 1977. Effects of cadmium on the shrimps, Penaeus dirprari, Palaemonetes pugio, and Palaemontes vulgaris. In: Physiological Responses of Marine Biota to Pollutants, pp. 131-183. Academic Press, New York.

NSF (National Science Foundation). 1972. Baseline Studies of Pollutants in the Marine Environment: Background Papers for a Workshop, Brookhaven National Laboratory, Long Island, New York, May 24-26, 1972. NSF Office for the International Decade of Oceanography, Washington, D.C.

Odum, E.P. 1971. Fundamentals of Ecology, 3rd ed. W.B. Saunders Co., Philadelphia, Pa.

Osterberg, C., W.G. Pearcy and H. Curl, Jr. 1964. Radioactivity and its relationship to oceanic food chains. J. Mar. Res. 22: 2-12.

Pendleton, R.C. 1964. Accumulation of cesium-137 through the aquatic food web. In: R.A. Taft (ed.), Third Seminar on Biological Problems in Water Pollution, pp. 355-366. Sanitary Engineering Center, Cincinnati, Ohio.

Pendleton, R.C., C.W. Hays, R.D. Lloyd and B.W. Church. 1965. A trophic level effect on ^{137}Cs concentration. Health Phys. 11: 1503-1510.

Pinkas, L., M.S. Oliphant and I.L.K. Iverson. 1971. Food habits of albacore, bluefin tuna and bonito in California waters. Calif. Fish Game. 152: 1-105.

Risebrough, R., D.R. Young, T. Munson, M. Goodwin and R. Parrish. 1974. Contamination of marine resources for human consumption--synthetic organic compounds. In: Marine Bioassays Workshop Proceedings, 94-108. Marine Technological Society, Washington, D.C.

SCCWRP (Southern California Coastal Water Research Project). 1973. The Ecology of the Southern California Bight: Implications for Water Quality Management. Southern California Coastal Water Research Project, El Segundo, Calif.

Schafer, H.A., G.P. Hershelman, D.R. Young and A.J. Mearns. 1982. Contaminants in ocean food webs. In: Biennial Report for 1981-82, pp 17-28. Southern California Coastal Water Research Project, Long Beach, Calif.

Schultz, V. and A.W. Klement, Jr. (eds.). 1963. Proceedings of the First National Symposium on Radioecology, Fort Collins, Colo., Sept. 10-15, 1961. Reinhold, New York.

Tillander, M., K. Miettinen and I. Koivirto. 1970. Excretion rate of methyl mercury in the seal (Pusa hispida). In: Proceedings of FAO Technical Conference on Marine Pollution and Its Effects on Living Resources and Fishing, Rome, Italy, Dec. 9-18, 1970, p. 159. FIR:MP/70/R-9, United Nations, Food and Agriculture Organization, Rome.

Walker, B.W. (ed.). 1961. The ecology of the Salton Sea, California, in relation to the sportfishery. Calif. Fish Game. 113: 1-204.

Wiley, M.L. (ed.). 1978. Estuarine Interactions. Academic Press, New York.

Williams, P.M. and H.V. Weiss. 1973. Mercury in the marine environment: Concentration in sea water and in a pelagic food chain. J. Fish. Res. Board Can. 30: 293-295.

Woodwell, G.M., C.F. Wurster, Jr. and P.A. Isaacson. 1967. DDT residues in an east coast estuary: A case of biological concentration of a persistent insecticide. Science 156: 821-823.

Young, D.R. 1970. The distribution of cesium, rubidium, and potassium in the quasi-marine ecosystem of the Salton Sea. Ph.D. Dissertation, Univ. of California, San Diego, Calif.

Young, D.R., C.S. Young and G.E. Hlavka. 1973. Sources of trace metals from highly-urbanized southern California to the adjacent marine ecosystem. In: Cycling and Control of Metals, pp. 21-39. EPA National Environmental Research Center, Cincinnati, Ohio.

Young, D.R., D.J. McDermott, T.C. Heesen and T.K. Jan. 1975. Pollutant inputs and distributions off southern California. In: T.M. Church (ed.), Marine Chemistry in the Coastal Environment, pp. 424-439. American Chemical Society, Washington, D.C.

Young, D.R., T.K. Jan and T.C. Heesen. 1978. Cycling of trace metal and chlorinated hydrocarbon wastes in the Southern California Bight. In: M.L. Wiley (ed.), Estuarine Interactions, pp. 481-496. Academic Press, New York.

Young, D.R., A.J. Mearns, T.K. Jan, T.C. Heesen, M.D. Moore, R.P. Eganhouse, G.P. Hershelman and R.W. Gossett. 1980. Trophic structure and pollutant concentrations in marine ecosystems of southern California. In: CalCOFI Report, Vol. XXI, pp. 197-206. California Cooperative Fisheries Investigation, La Jolla, Calif.

Young, D.R., A.J. Mearns, T.K. Jan and R.P. Eganhouse. 1981. The cesium-potassium ratio and trace metal biomagnification in two contaminated marine food webs. In: Oceans 81 Conference Record, Vol. 1, pp. 570-574. Institute of Electrical and Electronic Engineers, Washington, D.C.

Kepone Uptake: A Comparison of Field and Laboratory Data

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INTRODUCTION

The ability to predict the environmental fate and effects of pollutants in the marine environment is of utmost importance in assessing the hazards posed by a compound's use and/or disposal. Most commonly utilized methods to establish potential environmental effects have involved an assessment with bioassays of a compound's acute and chronic toxicity.

For compounds that have the ability to bioaccumulate, the potential dangers from this process must also be determined. A "first cut" estimate can be made by determining the partition coefficients between an organic solvent, usually n-octanol, and water. If this coefficient exceeds 25,000, EPA requires a report of the potential hazard (under the Toxic Substances Control Act), and further study is required. The next step is to expose some likely target animal to the suspected contaminant through food and/or water.

Transfer by these routes is calculated as (1) a bioconcentration factor from water = concentration in the animal/concentration in water, and (2) a dietary accumulation factor from food = concentration in the animal/concentration in food. Further food chain accumulation studies are sometimes conducted in the laboratory if the importance of the compound indicates a need. Studies, both laboratory and field, are also performed to estimate the transfer of the contaminant from its usual reservoir in the field (i.e., sediment) to food organisms and water. With these data, an assessment can be made of the potential for a compound to accumulate in marine organisms.

The above procedures have been developed over the past ten years and have gained a general degree of acceptance in the discipline; however, their shortcoming lies in the lack of field verification. This is particularly true in the marine environment where field data on many aspects of the environmental compartmentalization are lacking.

The introduction of Kepone (1,1a,3,3a,4,5,5a,5b,6-decachloroctahydro-1,3,4-metheno-2H-cyclobuta (cd) pentalen-2-one) into the James River estuary in Virginia has resulted in a large number of investigations which provide in part the basis for an assessment, although an after-the-fact one, of how well the predictive system works in the marine environment.

The toxicity of Kepone to marine and estuarine life has been studied by Bookout et al. (1980), Bourquin et al. (1978), Couch et al. (1977), Hansen et al. (1977), Ninmo et al. (1977), Provenzano et al. (1978), Rubinstein (1979), Schimmel and Wilson (1977), Walsh et al. (1977), Fisher (1980), Roberts and Bendl (1982).

Studies on bioconcentration, bioaccumulation, depuration and transfer have been conducted by Bahner and Oglesby (1979), Van Veld (1980), Drifmeyer et al. (1980), Schimmel et al. (1979), Huggett and Bender (1980), Huggett et al. (1980), Slone and Bender (1980).

In addition to these investigations, various agencies of the Commonwealth of Virginia have been conducting monitoring studies on the river, biota, sediments and water.

METHODS

Samples of blue crabs (Callinectes sapidus), oysters (Crassostrea virginica) and two species of bottom-feeding fishes, croaker (Micropogon undulatus) and spot (Leiostomus xanthurus), were collected between 1976 and 1980 and analyzed for Kepone residues. Crabs were collected in the lower 10 km portion of the river, and oysters were collected from discrete "oyster rocks" shown in Figure 1. Fish were collected within the zones shown also in Figure 1.

Laboratory studies have shown maximum bioconcentration factors of 7,200 for fishes (Hansen et al. 1977). Transfer from food to fish is estimated to be on a 1:1 basis (Bahner et al. 1977; Van Veld 1980; Stehlik 1980). Uptake of Kepone from sediments by benthic food organisms occurs, and concentrations reached by the organisms approximate those of the sediments (U.S. EPA 1978). Estimates of the uptake of Kepone by fishes can be made by utilizing these data and field observations on the distribution of Kepone in the sediments, which serve as the reservoir for the aqueous phase.

Estimates of contamination levels in fishes and sediments for the period between 1976 and 1979 were made from the monitoring data of the Virginia State Water Control Board and the Virginia In-

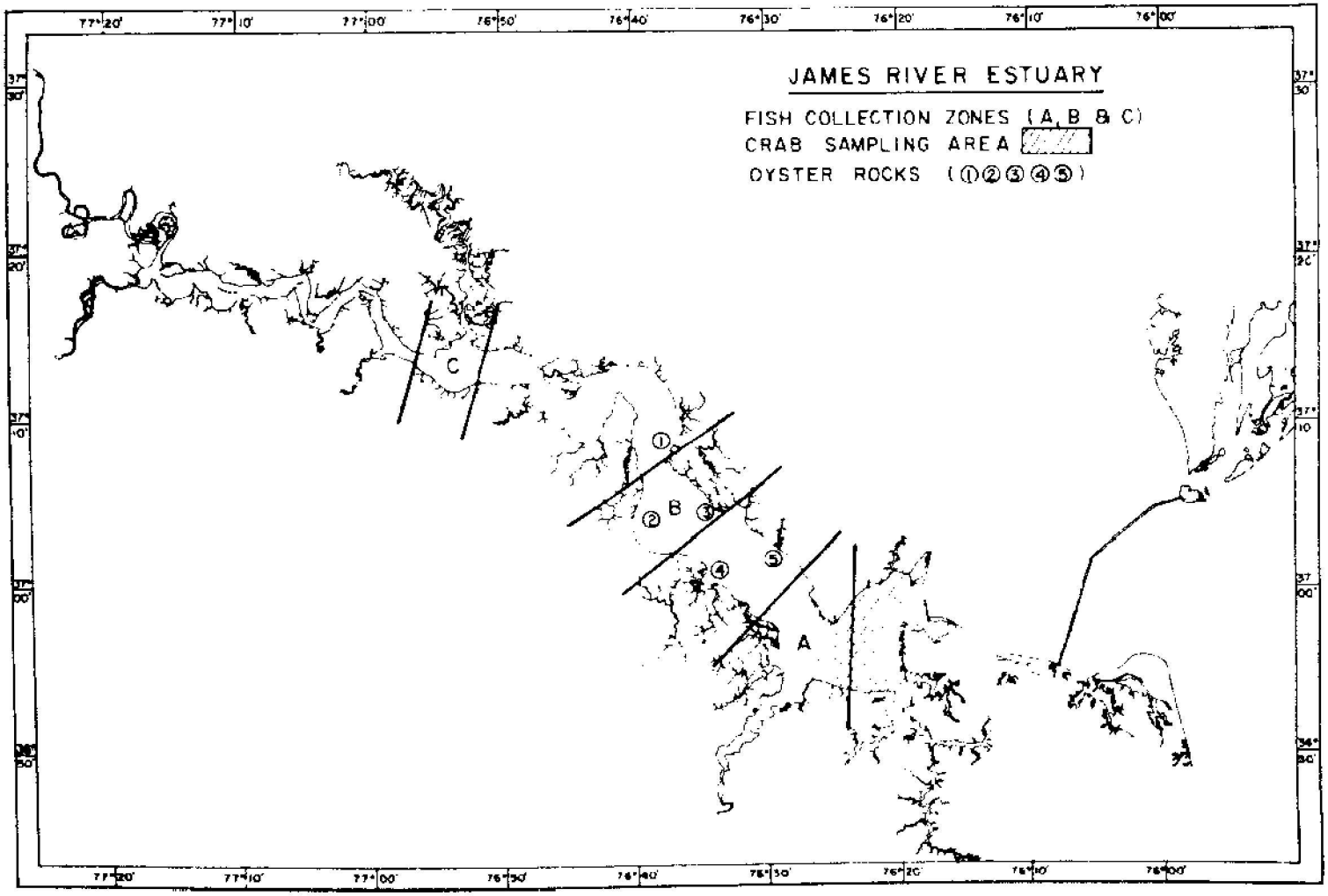

Figure 1. Sampling locations.

stitute of Marine Science. For the purposes of this study, we averaged the surface (upper 2 cm) sediment contamination levels over the zone in the river where the fish residue data were collected. Results from three zones are presented. Each zone is about 10 km long and the zones are approximately 20 km apart (Table 1). Dissolved Kepone levels were predicted by utilizing a partition coefficient between sediments and water of 5,000:1 (Strobel et al. 1981). Field data relating dissolved Kepone to sediment concentration are limited, but studies that have measured both parameters indicate that dissolved levels can be accurately estimated using this coefficient (Slone and Bender 1980).

RESULTS AND DISCUSSION

The residue levels of Kepone in fishes, predicted from laboratory exposures to contaminated water and food, are compared with field data in Figure 2. Significant deviations from the predicted values are apparent. There are several possible explanations for these differences:

1. The predicted bioconcentration factors are too low.
2. The actual levels of Kepone in the water are greater than predicted from the sediment-water partition coefficients.
3. There is greater accumulation through food than estimated from laboratory studies.

In the prediction, we utilized the maximum bioconcentration factor observed for fish (i.e., 7,200 vs. a mean value of 3,500 obtained from averaging the results of five different laboratory investigations). We believe, therefore, that the laboratory predictions used were not in error, at least on the low side.

Table 1. Mean Kepone in sediments (ng g^{-1}) and third quarter $\bar{x}$ for bottom feeding fish (spot and croaker) $\mu g\ g^{-1}$.

Year	Zone A		Zone B		Zone C	
	Seds.	Fish	Seds.	Fish	Seds.	Fish
1976	15	.72	67	1.6	117	-
1977	16	.47	56	1.8	144	-
1978	5	.36	19	0.9	37	-
1979	1	.35	16	-	65	0.6

Two studies have measured dissolved Kepone in the lower James. These investigations have shown levels of approximately 5 ng L^{-1}. Predictions from sediment concentrations at these sites indicate that the dissolved levels should have been approximately 7 ng L^{-1}. We feel confident, therefore, that actual levels of dissolved Kepone are not higher than the predictions and are, in fact, in remarkably good agreement.

If we are correct in our assessment of two possible explanations for error in the predictions, the most likely explanation is an underestimation in uptake through the food chain to account for the difference between predicted and measured amounts of dissolved Kepone.

Blue crabs bioaccumulate Kepone mainly from their food. Uptake from water is limited with a bioconcentration factor of only 8 (Schimmel and Wilson 977). Fisher (1980) determined the uptake of Kepone by juvenile blue crabs over a 2-month period. Utilizing his data, one can predict concentrations in crabs with the following equation:

$$C_a = DAF \times C_n (W_f/W_a)$$

where

C_a = predicted concentration in the crab
DAF = dietary accumulation factor = $C_e W_e / C_f W_f$
C_e = experimental equilibrium concentration in crabs
W_e = average weight of experimental crabs
C_f = experimental concentration in the food
W_f = weight of food consumed per day
C_n = concentration in natural food
W_a = average weight of crabs

The mean dietary accumulation factor derived from Fisher's studies is 30, when one utilizes the average weight of the crabs during his experiments. If we assume the blue crabs eat approximately 5% of their body weight per day (the ration used in Fisher's studies), then we can calculate the expected residue concentration in nature if we have an estimate of the level of food contamination.

Figure 3 compares field residue data collected from 1976 through 1980 with predictions based on the above equation. In this prediction we used, as an estimate of food contamination, the average residue level for all species of fish in the lower river. The agreement between laboratory predictions and the field residue data is very good for male crabs. Roberts and Leggett (1980) have shown that female crabs lose Kepone when they spawn, and since the predictive equation did not consider this loss, one could not expect agreement for females.

Oysters bioaccumulate Kepone from both dissolved and particulate phases. Uptake from particulate matter appears to be consid-

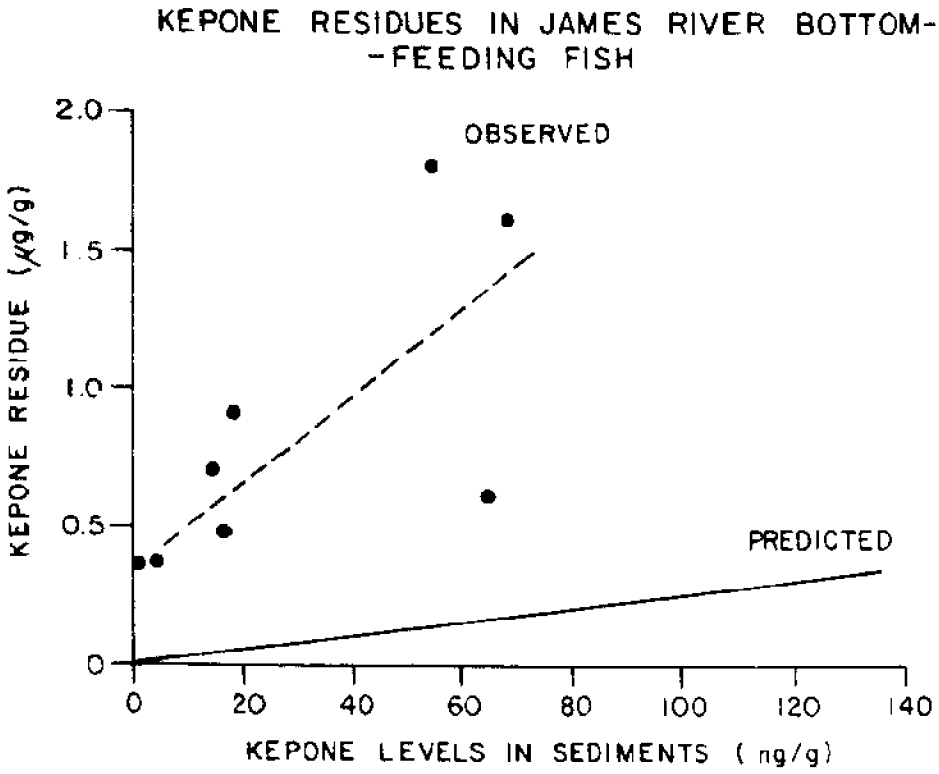

Figure 2. Kepone residues in fish vs. predicted uptake.

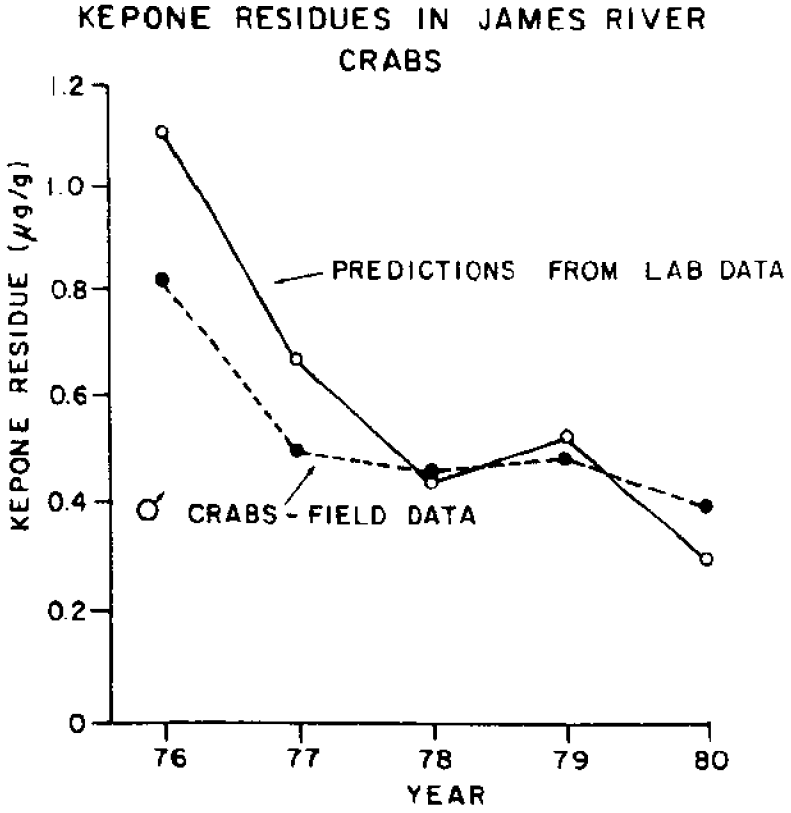

Figure 3. Kepone residues in blue crabs vs. predicted uptake.

erably less efficient than accumulation from the dissolved phase. Bahner et al. (1977) showed bioaccumulation factors from algae to oysters of 0.007, while Haven and Morales-Alamo (1977) demonstrated bioconcentration factors from sediments between 0.06 and 0.25. Bioconcentration factors from the dissolved phase averaged about 9,300 (Bahner et al. 1977).

Comparisons between laboratory uptake and field data for oysters are hampered to a certain degree by the oysters' natural variability as a function of season (Figure 4). However, if we normalize the field residue data for oysters to the water temperature at which the laboratory experiments were conducted, a reasonable fit is obtained (Figure 5). To construct this figure, we adjusted the observed residue levels for oysters at the time the sediment concentrations were determined to those that would be expected at 14°C. The predicted line was constructed utilizing a bioconcentration factor from the dissolved phase of 9,300.

Actual field observations of both water concentrations and oyster residues were made during 1979 when water temperatures approached 15°C. These values are shown on Figure 5. Unfortunately, our ability to measure dissolved Kepone was not developed until residues began to decline. Therefore, field confirmation is limited to the lower portion of the curve.

In the case of Kepone, our ability to predict residue levels in nature from laboratory data appears to be fairly good for crabs and oysters, while predictions for fish are poor. This is true not only for average residue predictions in migratory species but also for resident species. This limitation presents a serious practical problem in that fisheries are closed when residue levels of Kepone are excessive. The length of time such closures exist can of course have serious economic and legal consequences. In addition, evaluations of the cost versus benefit of cleanup must take into account the predicted length of closure with various cleanup alternatives including the no-action alternative. To answer questions of this nature for fisheries, it is clear that we need to develop a better understanding of what factors determine residue levels in the field.

Our ability to make predictions from laboratory data needs to be evaluated for other compounds; very few investigators have attempted to make comparisons of the kind discussed in this paper. We believe, at least for Kepone, that more attention needs to be given to uptake from food. Dietary accumulations may vary with the type of food consumed and/or with the actual rates of feeding in nature.

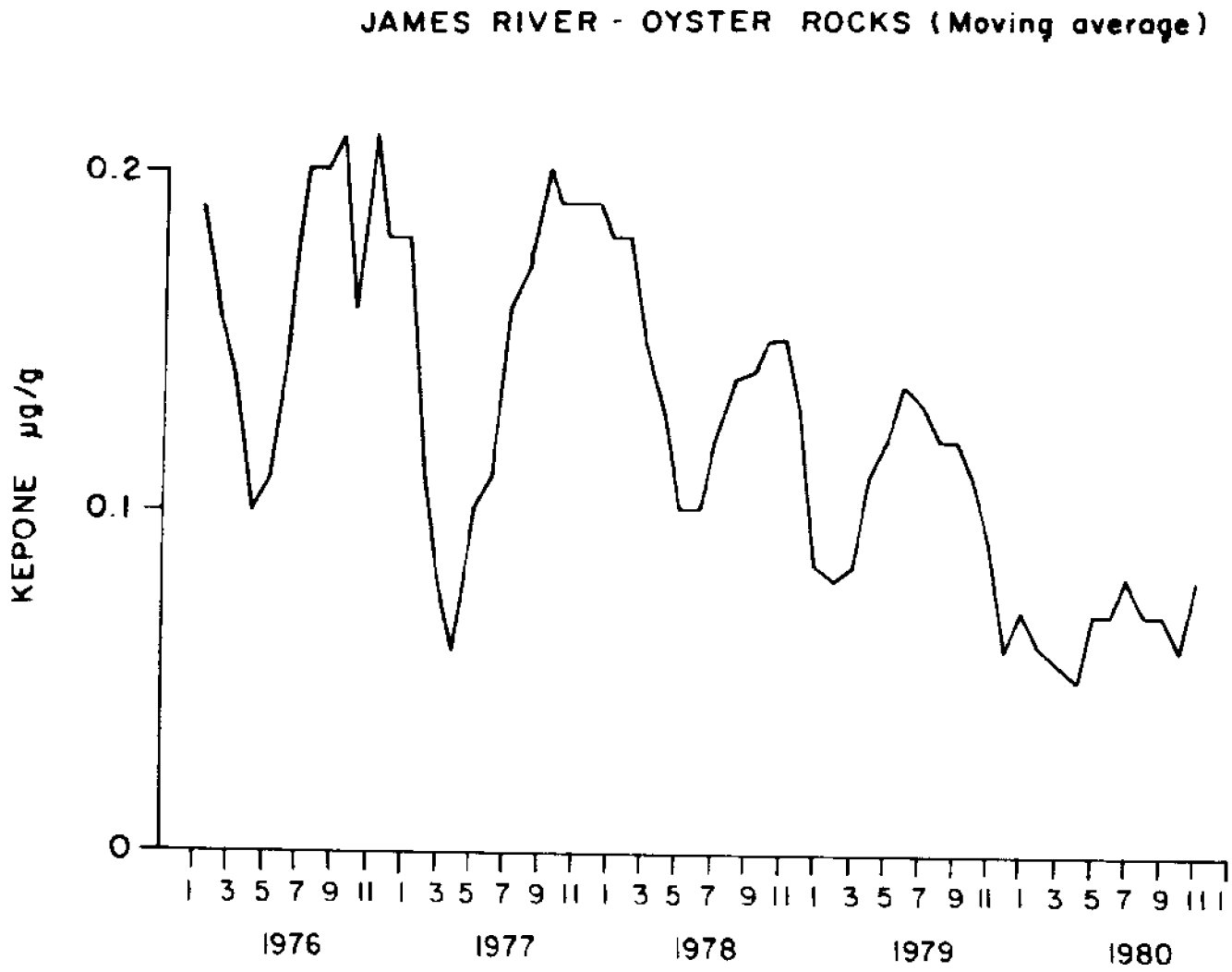

Figure 4. Kepone residues in oysters vs. time.

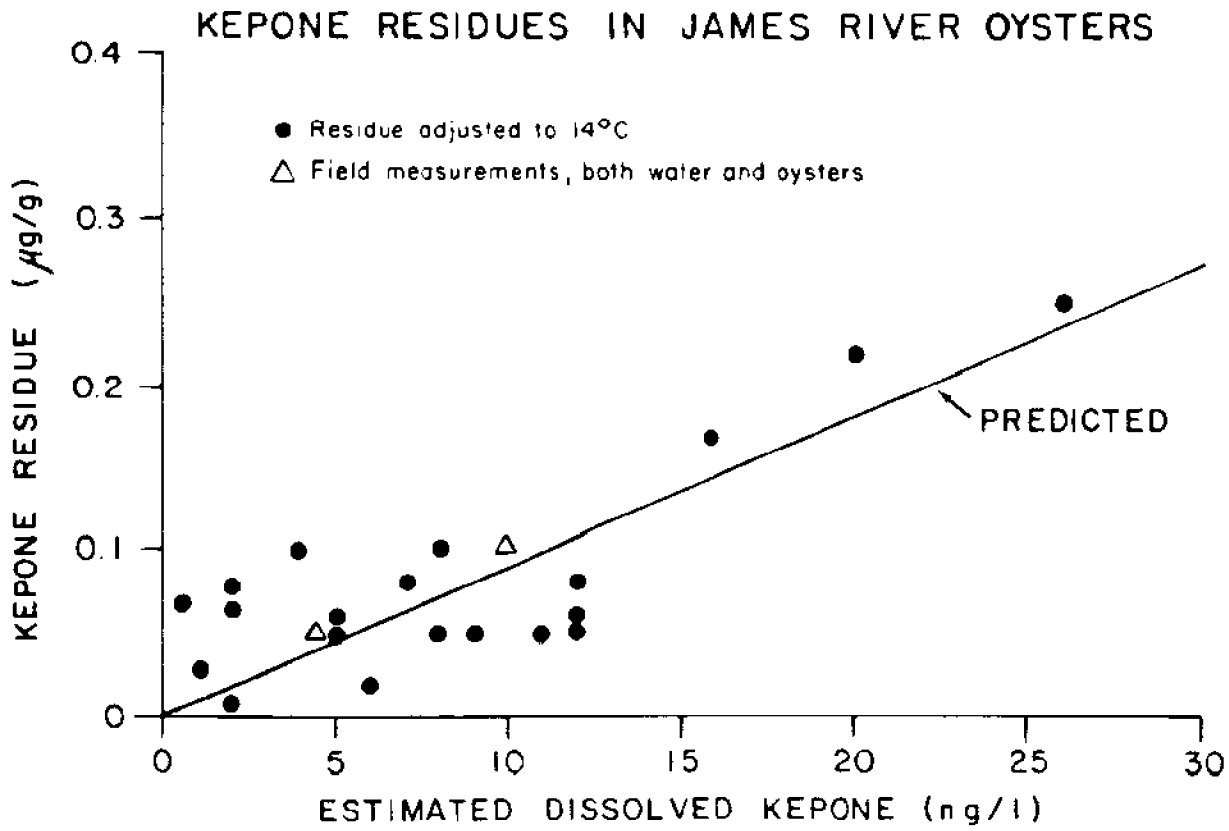

Figure 5. Kepone residues in oysters vs. predicted uptake.

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REFERENCES

Bahner, L.H. and J.L. Oglesby. 1979. Test of model for predicting Kepone accumulation in selected estuarine species. In: L.L. Marking and R.A. Kimerle (eds.), Aquatic Toxicology. ASTM STP 667, American Society for Testing and Materials, Philadelphia, Pa.

Bahner, L.A., A.J. Wilson, Jr., J.M. Sheppard, J.M. Patrick, L.R. Goodman and G.E. Walsh. 1977. Kepone® bioconcentration, accumulation, loss and transfer through estuarine food chains. Chesapeake Sci. 18: 299-308.

Bookhout, C.G., J.D. Costlow, Jr. and R. Monroe. 1980. Kepone effects on larval development of mud-crab and blue-crab. Water Air Soil Pollut. 13: 57.

Bourquin, A.W., P.H. Pritchard and W.R. Mahaffey. 1978. Effects of Kepone on estuarine microorganisms. Dev. Ind. Microbiol. 19: 489-497.

Couch, J.A., J.D. Winstead and L.R. Goodman. 1977. Kepone-induced scoliosis and its histological consequences in fish. Science 197: 585-587.

Drifmeyer, J.E., C.L. Rosenberg and M.A. Heywod. 1980. Chlordecone (Kepone) accumulation on estuarine detritus. Bull. Environ. Contam. Toxicol. 24: 364.

Fisher, D.J. 1980. Effects of ingestion of Kepone (at environmental levels) on juvenile blue crabs (Callinectes sapidus Rathbun). M.S. Thesis, Coll. of William and Mary, Williamsburg, Va.

Hansen, D.J., L.R. Goodman and A.J. Wilson. 1977. Kepone chronic effects on embryo, fry, juvenile and adult sheepshead minnows (Cyprinodon variegatus). Chesapeake Sci. 18: 227-232.

Haven, D.S. and R. Morales-Alamo. 1977. Uptake of Kepone from suspended sediments by oysters, Rangia and Macoma. U.S. EPA Annual Report, Gulf Breeze Environmental Research Laboratory.

Huggett, R.J. and M.E. Bender. 1980. Kepone in the James River. Environ. Sci. Technol. 14: 918-923.

Huggett, R.J., M.M. Nichols and M.E. Bender. 1980. Kepone contamination of the James River estuary. In: R.A. Baker (ed.), Contaminants and Sediments, Vol. 1, pp. 33-52. Ann Arbor Science Publ., Ann Arbor, Mich.

Nimmo, D.W.R., L.H. Bahner, R.A. Rigby, J.M. Sheppard and A.J. Wilson. 1977. Mysidopsis bahia: An estuarine species suitable for life-cycle toxicity tests to determine the effects of a pollutant. In: F.L. Mayer and J.L. Hamelink (eds.), Aquatic Toxicology and Hazard Evaluation. ASTM STP 634, American Society for Testing and Materials, Philadelphia, Pa.

Provenzano, A.J., K.B. Schmitz and M.A. Boston. 1978. Survival, duration of larval stages and size of post-larvae of grass shrimp, Palaemonetes pugio, reared from Kepone contaminated and uncontaminated populations in Chesapeake Bay. Estuaries 1: 239-244.

Roberts, M.H. and R.E. Bendl. 1982. Acute toxicity of Kepone to selected freshwater fishes. Estuaries.

Roberts, M.H. and A.T. Leggett. 1980. Egg extrusion as a Kepone-clearance route in the blue crab, Callinectes sapidus. Estuaries 3: 192-199.

Rubinstein, N.I. 1979. A benthic bioassay using time-lapse photography to measure toxicants on the feeding behavior of lugworms (Polychaeta: arenicolidae). In: W.B. Vernberg, F.P. Thurberg, A. Calabrese and F.J. Vernberg (eds.), Marine Pollution: Functional Responses, pp. 311-351. Academic Press, New York.

Schimmel, S.C. and A.J. Wilson, Jr. 1977. Acute toxicity of Kepone to four estuarine animals. Chesapeake Sci. 18: 244.

Schimmel, S.C., J.M. Patrick, Jr., L.F. Faas, J.L. Oglesby and A.J. Wilson, Jr. 1979. Toxicity and bioaccumulation in blue crabs. Estuaries 2: 8-15.

Slone, H.D. and M.E. Bender. 1980. Kepone monitoring at Skiffes Creek. DACW65-79-C-0027, U.S. Army Corp of Engineers, Norfolk District, Norfolk, Va.

Stehlik, L.L. 1980. Bioaccumulation and effects of Kepone on spot, Leiostomus xanthurus. M.S. Thesis, Coll. of William and Mary, Williamsburg, Va.

Strobel, C.J., R.E. Croonenberghs and R.J. Huggett. 1981. The suspended sediment-water partitioning coefficient for Kepone in the James River, Va. Environ. Pollut. (Ser. B). 2: 367-372.

U.S. Environmental Protection Agency. 1978. Mitigation feasibility for the Kepone-contaminated Hopewell/James River areas. Office of Water and Hazardous Materials Criteria and Standards Division, Washington, D.C.

VanVeld, P. 1980. Uptake, distribution, metabolism and clearance of Kepone by channel catfish (Ictalurus punctatus). M.S. Thesis, Coll. of William and Mary, Williamsburg, Va.

Walsh, G.E., K. Ainsworth and A.J. Wilson, Jr. 1977. Toxicity and uptake of Kepone in marine unicellular algae. Chesapeake Sci. 18: 222.

Mussel Watch Monitoring for the Assessment of Trace Toxic Constituents in California Marine Waters

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INTRODUCTION

This presentation summarizes the current research and monitoring efforts in California coastal marine waters which are employed by the state government to assess the impacts of trace toxic substances on marine organisms. Two things are being measured: trace toxic substance accumulation (trace elements, higher molecular weight petroleum hydrocarbons, synthetic halogenated hydrocarbons) and biological effects (physiological stress testing).

Bordered by over 1700 miles of coastline and several important estuaries, California has areas of urbanization in the Southern California Bight, Monterey Bay and San Francisco Bay, as well as other areas with low populations such as the Central Coast (Big Sur) and the North Coast. Wastes from the municipal and industrial sources are discharged into marine waters in the urbanized areas. The State Water Resources Control Board (SWRCB), the water quality regulatory body of the State of California, has operated a water quality surveillance and monitoring effort since 1977. The objective of this effort is to document and assess long term trends in selected indicators of the quality of coastal marine and estuarine waters. Initially in 1977, collections of intertidal mussels were taken along the coast of California at approximately 32 stations. In subsequent years, the number of coastal stations was reduced to 12 because of the uniformity in pollutant levels in mussels

from large portions of the coast. Monitoring in bays and estuaries was expanded to 24 stations in 12 harbors or estuaries, which had order-of-magnitude higher levels of many pollutants (Stephenson et al. 1980; Martin et al. 1980). The main purpose of chemical analysis of mussels was to document the identity, concentrations, and geographical distribution of mussel burdens of three major classes of environmental contaminants: higher molecular weight halogenated hydrocarbons, higher molecular weight petroleum hydrocarbons and trace elements.

A second phase of the California Mussel Watch Project has been physiological stress testing, referred to as the Biological Effects Assessment (BEA). The BEA study employs the study protocol of Bayne et al. (1979) and Widdows et al. (1981). The main purpose of this work is to document the cause, degree and geographical extent of physiological stress (measured as Scope for Growth) in Mytilus sp. from California waters. The first phase effort (FY 1980-1981) was to document the relationship between accumulated trace toxic materials and physiological stress at five sites in San Francisco Bay (and one reference site) and five sites from chemically influenced stations along the California coast (and one reference station).

To provide a critical review of the techniques employed to measure and assess the impacts of pollution on marine ecosystems, we examine our study design and objectives using the following criteria:

1. Does the technique utilize a valid experimental design?
2. Is the study applicable to the field or, conversely, to the laboratory?
3. How significant are the processes measured by the study, e.g., are they involved with commercially important species or human health?

TACTICS FOR ESTABLISHING MARINE CHEMICAL POLLUTANT SURVEILLANCE

Most toxic substances are difficult and expensive to analyze at concentrations encountered in waters of marine ecosystems. Many successful monitoring studies have been designed utilizing a variety of bioindicators or sentinel organisms such as mussels, other bivalves and species of fish as well as eggs of selected bird species (Goldberg 1981). The key to the successful completion of these studies has been the careful attention given to quality assurance among laboratories conducting the analysis, as well as field protocols that emphasize the collection of non-contaminated samples. The common features of successful studies have been uses of a bioconcentration organism, high quality analytical chemical procedures, adequate quality control and laboratory intercalibration, and

sample designs to account for such environmental influences as seasonality or marked environmental differences (such as temperature and salinity).

PREREQUISITES FOR SELECTION OF A CHEMICAL SURVEILLANCE TECHNIQUE—CALIFORNIA STATE MUSSEL WATCH

The initial intent of California's Mussel Watch project was to assess the general background levels and trends of persistent toxic substances in marine or estuarine waters. Environmental research had previously suggested that some bivalve organisms might be valuable as sentinel organisms for indicating levels of pollutants in coastal marine waters (Goldberg et al. 1978). Bivalves have been shown to accumulate certain potentially toxic substances to levels in excess of those found in seawater: trace elements, higher molecular weight petroleum hydrocarbon, and synthetic halogenated hydrocarbons. Phillips (1980) documented the usefulness of bivalves as indicators of trace element and trace organic pollutant sources and outlined the requirements for selection of appropriate species:

1. The species must integrate pollutants over time. That is, at higher concentrations, the pollutant burden increases; at lower concentrations, the pollutant burden decreases.*
2. The species should be sessile or sedentary to be representative of a particular geographic area.
3. The species should be common and abundant for ease of collection.
4. The species should be large enough to provide sufficient tissues for analysis.
5. The age (or size) should be sufficient to allow sampling of more than one year class.
6. The species should be tolerant of laboratory conditions.
7. The species should be tolerant of lower salinity and higher temperatures (estuarine adaptation).
8. There should be a correlation between water concentration and the organism body burden (i.e., bioconcentration factor).

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*We acknowledge that, upon exposure to certain pollutants, the concentration of another trace toxic substance could be decreased. The biological significance of such an interaction is not known.

9. The body burdens of the species should rapidly reach equilibrium and consistently conform to water concentrations, regardless of location or condition.

A model "biomonitoring" organism should fit these criteria; Goldberg et al. (1978) identified several unresolved scientific issues or problems with the use of bivalve and other species as sentinel or indicator organisms:

1. Determination of the minimum number of replicate samples to characterize a geographic area.
2. Determination of the influence of yearly biological cycles or sexual maturation (=gonad mass) on trace pollutant concentrations.
3. Identification of other seasonal patterns in trace pollutant concentrations.
4. Documentation of the sample size or numbers of replicates necessary to determine statistically relevant differences or similarities.
5. Documentation of the influence of other biological factors (e.g., age or sex) on the concentrations of trace toxic pollutants.

The State Mussel Watch has recognized these and other unresolved scientific issues related to the use of bivalve molluscs for trace chemical monitoring in marine waters (Stephenson 1979; Risebrough et al. 1980). The following questions were asked in regard to the sampling design:

Variation due to tissue sample and season

1. How do individual tissues (digestive gland) or gut contents influence the whole body concentrations of trace toxic substances in a pooled sample (= several individual mussels)?
2. How does the contribution of gonad tissue influence the total body trace element concentration during an annual cycle.

Variation due to geographic or field variability

3. What amount of variability can be attributed to the site of collection (i.e., tidal depth)?
4. Does the site of collection within a large population of mussels influence the trace element concentration?

Variation due to chemical analysis and statistical treatment

5. What differences in concentrations of selected synthetic organic pollutants, principally PCB's and DDT, can be detected and measured?
6. What is the analytical variation due to the chemical analytical procedures?

PREREQUISITES FOR SELECTING BIOLOGICALLY IMPORTANT RESPONSES—BIOLOGICAL EFFECTS ASSESSMENT (BEA)

Bayne et al. (1979) used a measurement of the effects of environmental stress (= Scope for Growth), which provided an integration of biochemical and cytological effects, and also evaluated the more general impacts upon the population. There are several other considerations or assumptions in the selection of an appropriate technique or pollutant stress response test that appear to be important:

1. The biological response indicator should be quantitatively influenced by toxic pollutants or other environmental stessors.
2. The biological response indicator should compensate for natural environmental stressors and thus respond only to stress induced by toxic pollutants.
3. The biological response indicator should have a significant biological or ecological meaning (survival, growth, recruitment, reproduction).
4. The biological response indicator should be a quantitative statement of sublethal or chronic impacts of pollution.
5. The biological response indicator should be reasonably easily measured in the field or laboratory.
6. If an adverse effect is measured at the organismal or population level, the biological response indicator should be interpretable at other levels of organization: subcellular, cellular and ecosystem.
7. The biological response indicator should be referable to historical biological and chemical information and data sets.

Questions addressing variation and statistical interpretation of the biological effects data were similar to those of the Chemical Surveillance and Monitoring Section.

Influence of sampling technique and statistics

1. Can the coefficients of variation of individual samples be best reduced by pooling individuals or by increasing the number of replicates?
2. What sample size is necessary to detect specified differences between the means of two samples?

Variation due to field sampling and season

3. Does the location in the intertidal zone influence the organism's scope for growth?

4. Does seasonality affect the scope for growth? Are the seasonal cycles (if present) of different populations similar or different?
5. Do physical and chemical factors (temperature, salinity, dissolved oxygen) influence the scope for growth?
6. How do laboratory conditions affect the scope for growth?

CRITERIA FOR CRITICAL EVALUATION OF CHEMICAL MONITORING TECHNIQUES

The purpose of this section is to examine and fully evaluate the existing California pollution monitoring projects (Mussel Watch and Biological Effects Assessment). The criteria address three general subjects: valid experimental design, integration or overlap of field and laboratory observations, and the general significance of the findings. The first of these criteria is, perhaps, most easily evaluated. The second criterion has been the subject of long term discussions, numerous study designs, and significant amounts of research. The third is somewhat an "eye-of-the-beholder" opinion, and we will share our view of the issue.

The immediate goal of the California Mussel Watch Program has been to provide an assessment of pollutant levels of trace toxic materials and their changes with time in California's marine waters. The data collected upon chemical body burdens can be used to evaluate representative organisms for compliance with known standards for the protection of marine resources as well as public health. Two questions arise: Are the toxic substances harmful to the organism itself? Is the contamination of the organism of public health significance? Our research has been directed to the first question.

At the initiation of this program, it was acknowledged that certain procedures and preanalyses had to be performed in order to optimize field collection procedures and define sample analytical variability. We believe these were necessary to permit collection of a statistically valid, yet cost effective, data base:

1. Preparation of reference stock "standards" for calibration of instrumentation (atomic absorption spectrophotometer).
2. Preparation of a mussel "standard" by pooling a large number of mussels from one site, followed by replicate preparation and analysis of the sample.
3. Participation in laboratory intercalibration exercise to determine the variability among laboratories doing mussel tissue evaluations for trace toxic substances.
4. Assessment of the contribution of digestive gland and gonadal tissues to overall total body trace elements concentration.

It was determined from intercalibrations, sample variability and field site variation analysis for trace elements (Stephenson et al. 1979, 1980) that the following collection and analytical programs would be consistent, reproducible and cost effective; they were therefore implemented:

1. A series of tissue standards (National Bureau of Standards) and mussel tissues were analyzed for trace elements. Results were within 10% of the mean of the certified values.
2. Purging (i.e., removal of gut contents of mussels) was judged not necessary. As a precautionary measure, aluminum and manganese analyses were made on all samples to indicate the general level of sediment contamination in the digestive gland.
3. Gonad tissues were excised on all samples because the preanalysis of the influence of gonad on whole body trace element concentration was inconclusive.
4. After a 2-year program of twice-yearly samples for trace elements and higher molecular weight hydrocarbons, the data were examined for consistent seasonal differences. No consistent seasonal differences were evident; we concluded that it was not necessary to sample during two periods of the year in order to assess the general statewide patterns in trace elements in the coastal zone.
5. The feasibility of the use of transplanted mussels (Mytilus californianus) was investigated (Stephenson et al. 1980). The purpose of using transplants was to understand the rate of tissue accumulation with time as well as to know the initial body burden of the mussel. The tissue burdens of transplanted mussels were compared with resident mussels (Mytilus edulis) in two bays and estuaries for a 6-month transplant period. The ability of the transplant mussel to "see" the same pattern for trace element depended upon the trace element (Figures 1 and 2). The purpose of these experiments was to determine when the transplanted mussels were in equilibrium (stabilized concentrations) with seawater, and to compare their body burdens with those of resident populations.
6. An experiment to determine the influence of collection site was performed. Micro-scale samples at 100 m intervals, two organism sizes (55-58 mm and 59-65 mm) and two tidal heights were analyzed for trace elements to determine the within- and between-site variations in concentration. For silver in Mytilus californianus, the collection locality and tidal height showed low variation and were judged unimportant. For cadmium, the opposite conclusion was reached. Tidal height greatly influenced the concentration; hence, for between-station comparisons samples

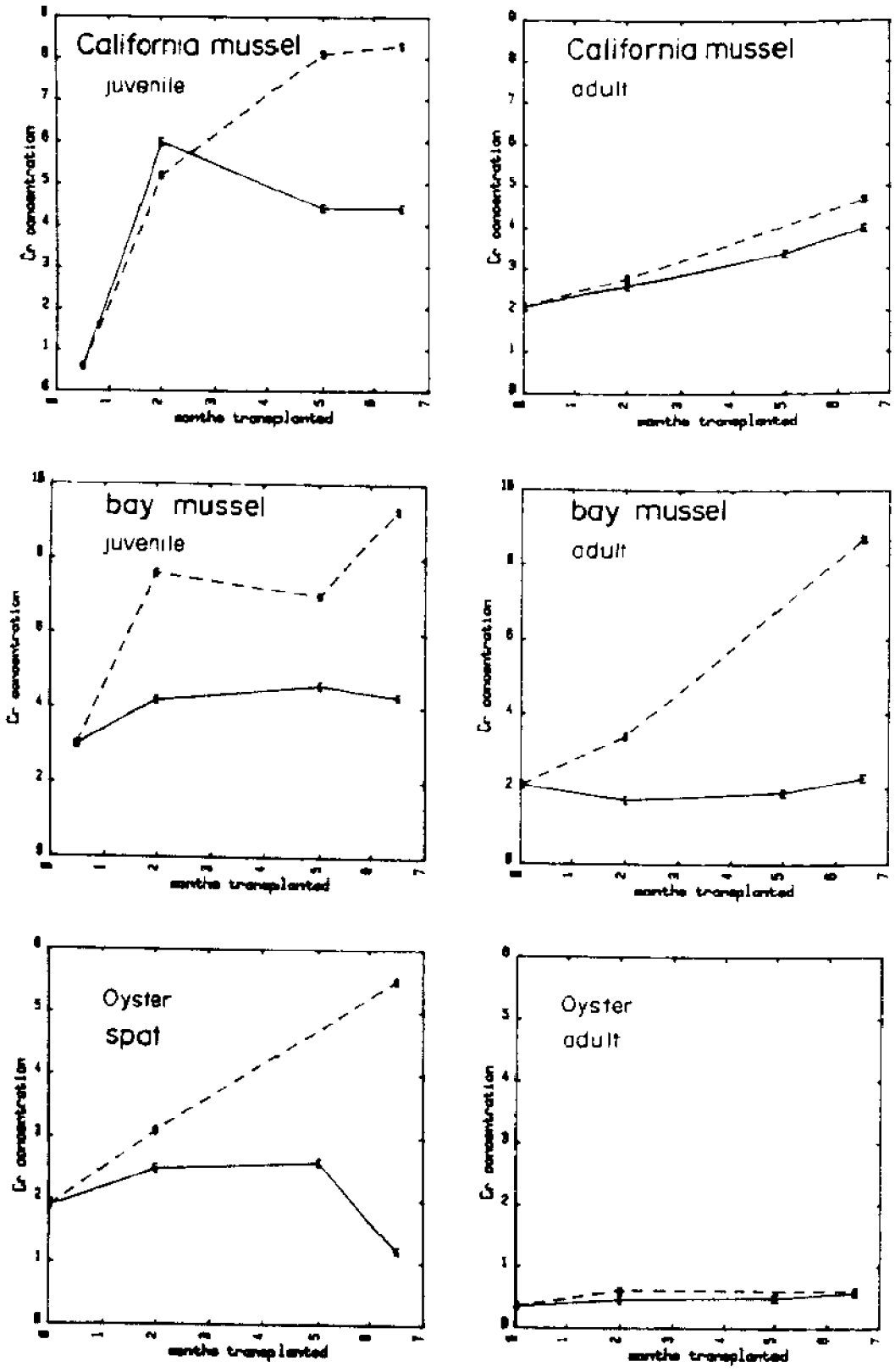

Figure 1. Tissue concentrations of chromium in three bivalve species from two size classes in San Francisco Bay and Elkhorn Slough for the 6 month transplant experiment. Dashed line = San Francisco; solid line = Elkhorn Slough (Stephenson et al. 1980).

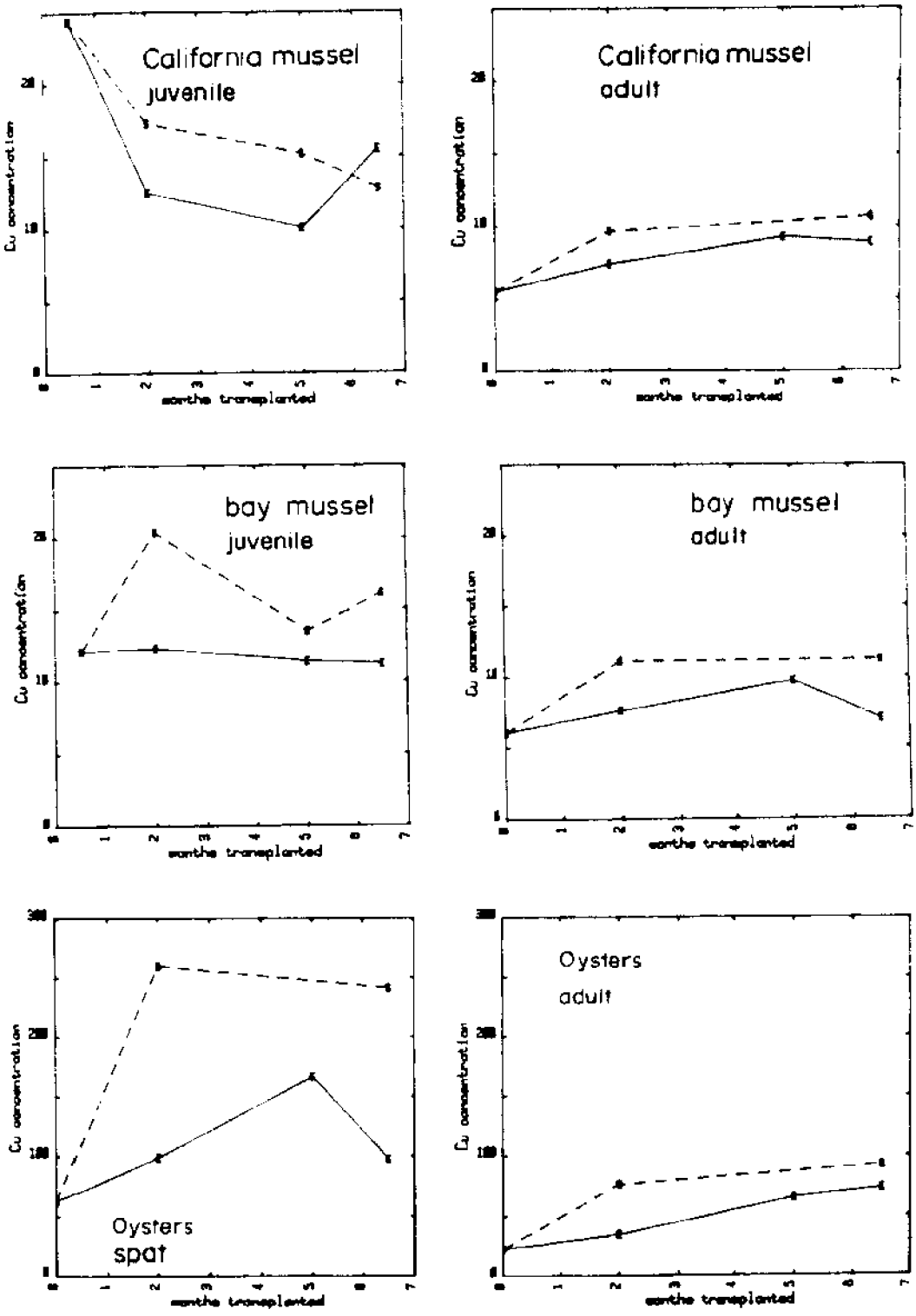

Figure 2. Tissue concentration of copper in three bivalve species from two size classes in San Francisco Bay and Elkhorn Slough for the 6 month transplant experiment. Dashed line = San Francisco; solid line = Elkhorn Slough (Stephenson et al. 1980).

collected from the same tidal height were mandatory (Figure 3).

The use of chemical surveillance and monitoring techniques in the marine environment has received little scientific review. Major questions remain, especially in relation to the analysis of trace quantities of synthetic chlorinated hydrocarbons, petroleum hydrocarbons and trace elements in seawater (see Goldberg et al. 1978). The state of the science is developed well enough at the present time to allow reproducible results for trace elements and selected synthetic chlorinated organic hydrocarbons. The identification and quantification of other compounds, such as many volatile organics and specific synthetic chlorinated hydrocarbons, as well as petroleum, are far from routine procedures in most laboratories.

CONFORMANCE OF STUDY DESIGN AND OBJECTIVES WITH STATED CRITERIA

Valid Experimental Design

The initial objective of mussel watch monitoring has been to provide an index to the general condition of bioaccumulative pollutants in coastal waters. While chemical monitoring is not "experimental" in the classical sense, it does contain elements of this approach. For each question, such as the level of detection required in chemical surveillance, we established a hypothesis: If there is a difference between samples of PCB compounds in mussel tissues, that difference is considered to exceed one order of magnitude. The purpose of monitoring was to determine the sensitivity of the surveillance system. Since we are dealing with natural populations, we have not been able to identify all variables that influence pollutant uptake and depuration. Sufficient information in the literature (Phillips 1980) has led us to the conclusion that mussels generally reflect trace toxic material conditions in the marine environment. The general assumption is made that, for water column associated pollutants, the mussel will "see" and accumulate the chemicals that are hazardous or deleterious to them or to humans. Several studies from the 1960s to the present have utilized this characteristic of bivalves (Butler 1973; Goldberg et al. 1978).

Trace Pollutant Bioaccumulation—A Natural Response

Phillips's (1980) summary of quantitative biological indicators of trace element and organochlorine pollution is the most complete for pollutant bioaccumulation in aquatic ecosystems available. The fact that trace elements, organohalogen and petroleum hydrocarbons accumulate in marine biota to levels far above those found in

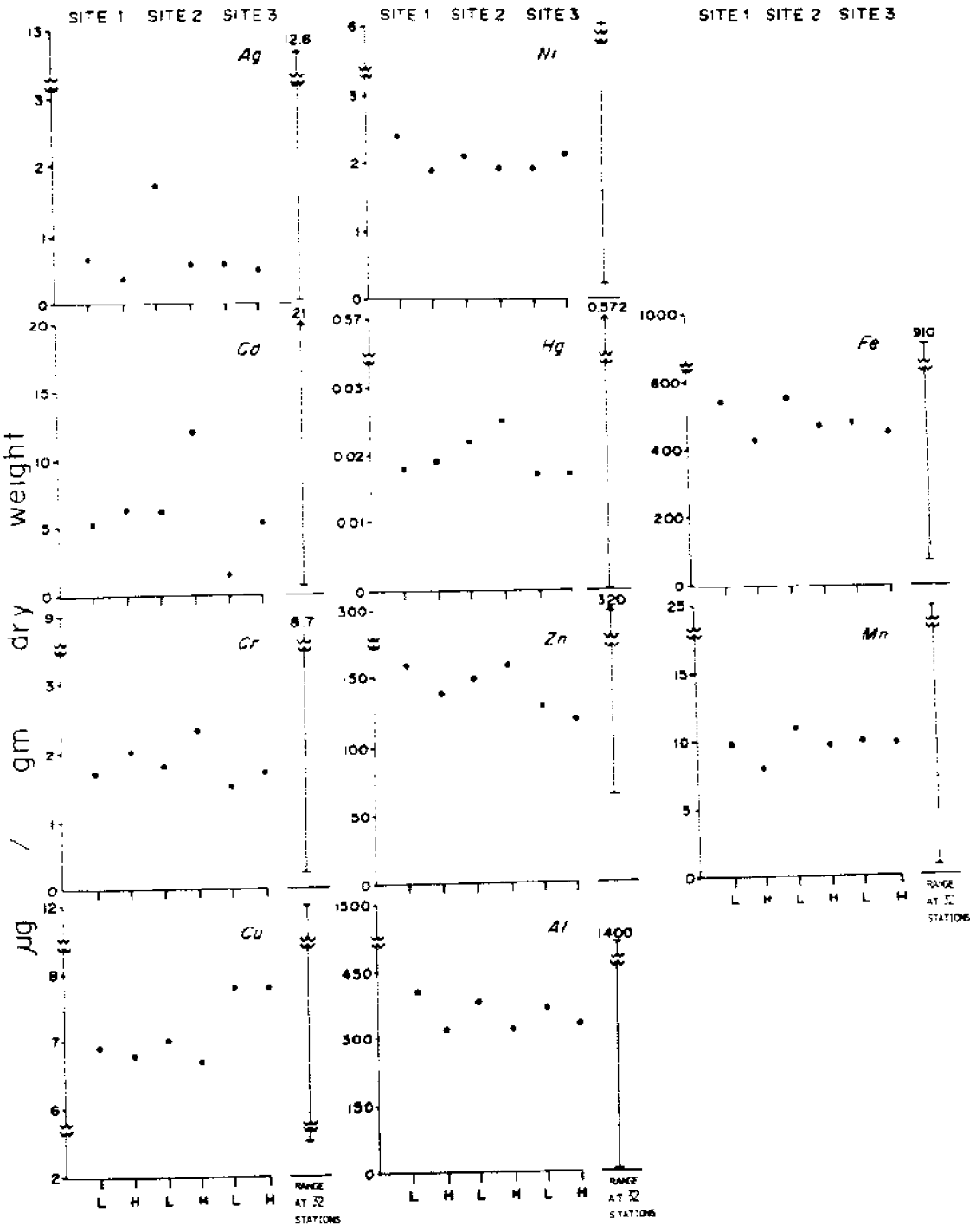

Figure 3. Within-site variation at three sites and two habitat elevations (L = low, and H = High) at Soberanes Point, Monterey County, California. Each data point is the mean of three samples. Range at 32 stations is the range of means of the 1977 data. Hg concentrations are $\mu g\ g^{-1}$ wet weight; all other trace element concentrations are $\mu g\ g^{-1}$ dry weight (Stephenson et al. 1979).

the surrounding water in laboratory experiments has been recognized by many investigators (Fowler et al. 1978; Phillips 1976). It should be emphasized that laboratory studies designed to estimate the kinetics of trace pollutant accumulation produce only order-of-magnitude estimations or approximations. The results produced from laboratory studies depend greatly on the route and length of exposure as well as biological factors (condition) of the experimental animals. Studies in the field have produced results that show temporal and spatial differences in pollutant concentrations (Goldberg et al. 1978; Martin et al. 1980; Stephenson et al. 1980). Although few studies have been conducted that relate ambient field concentrations of pollutants with those of organisms, de Lappe et al. (1980) have reported an association between particular higher molecular weight hydrocarbons (e.g., chlordane) and their concentrations in mussels. Clearly, there is need to further understand the relationship between elevated pollutant concentrations in the environment and concentrations in marine organisms.

General Significance of Findings

In a discussion of predicting the overall impact of pollutant discharge in the marine environment, Capuzzo (1981) suggested that one must understand the responses of individuals as well as populations of organisms to environmental perturbations. By her evaluation, responses to pollutants can be examined at five levels of organization: (1) biochemical and (2) cellular responses under simulated controlled conditions; (3) alterations in population dynamics under natural conditions; (4) alterations in community dynamics and (5) structure under natural conditions. Her theme is that, in order to manage pollutants in marine ecosystems, effects at each level should be evaluated. While all of these measures can be useful in understanding the impacts of pollutants on the marine environment, many measures from these five groups have been difficult to relate directly to anthropogenic pollutant events.

One of the deficiencies in most pollutant evaluation programs has been the lack of integration of findings from the various levels of organization, for example, cellular vs. organismal. To us, the most logical sequence would be to identify an impact at a level that is recognized as significant (some argument can be made that no consensus can be found for the term "significant"). The sequence of study would be to follow that perturbation into other levels of organization (organismal--cellular--subcellular). This process would enable us to understand how the pollutant affects the significant event and to predict future events in other locations, as well as to take corrective action if the event were interpreted as a pollution problem.

In the Southern California Bight, major municipal waste discharges have occurred since the 1940s. The impacts of these waste discharges have been to affect or change the marine communities

(plant and animal). There have been measurable changes in the abundance, diversity and structure, and an increase in benthic biomass in about 5% of the mainland shelf (Mearns 1981). There have been changes in the relative abundance of fish within 4 kilometers of the discharge sites. There has been an increase in chronic fin erosion in benthonic fish around two major outfalls. There has been contamination of fish, exceeding public health guidelines, by synthetic organic chemicals at several sites. There has been contamination of filter feeding invertebrates by trace element and synthetic organic compounds at several discharge points and adjacent ocean areas.

The consequences of these observed and measured phenomena to the health of the ecosystem or productivity of commercial species, or human health have been difficult to evaluate or predict. The regulation of water quality in California is entrusted by law to two governmental agencies, the California State Water Resources Control Board and the U.S. Environmental Protection Agency. For these water regulatory agencies to utilize the pollution effects measurements to evaluate the overall impacts of municipal, industrial and other pollutant sources, the measurements must be clearly related to detrimental impacts on marine ecosystems.

The regulation of waste discharge to California ocean waters has been established by the "Ocean Plan" (CSWRCB 1978). The protection of beneficial uses of ocean water, such as commercial fisheries, recreation and public health, from adverse effects of waste discharge is the major concern or focus of this document. In adopting this document, the Board has suggested several measures to evaluate the influence that a particular discharge might have on marine orgnisms (Table 1).

It was assumed that, from an economic perspective, the state could not afford to provide data and monitoring on all ecosystem measurements of the effects or influence of pollutant discharge. Certain techniques or methods have been judged to be more sensitive and can provide better "early warning" systems than other techniques to determine sublethal effects of environmental or pollution stress (Capuzzo 1981). It was our opinion, shared by a number of other scientists (Goldberg et al. 1978; Butler 1973; Phillips 1980), that potentially bioaccumulative trace pollutants (trace elements, xenobiotic halogenated organic compounds, petroleum hydrocarbons and radionuclides) were critically important problems to fisheries and wildlife resources and human resources. For this reason, a State Mussel Watch was established in 1977 to survey and monitor trace toxic pollutants in marine and estuarine waters of California.

Table 1. Water quality measures in California Ocean Plan

1.	Bacteriological Standards	- General body contact; shellfish harvesting.
2.	Physical Characteristics	- Floating particulates and grease and oil not allowed; undesirable discoloration of surface not allowed; altered natural transmittance of light now allowed; deposition of inert solids not allowed.
3.	Chemical Characteristics	- Dissolved oxygen within 10% natural; pH not more than 0.2 units of natural condition; dissolved sulfide in water/sediments not allowed to be significantly increased; trace elements/hydrocarbons not allowed to levels that degrade[1] marine life; organic materials in sediments not allowed to levels that degrade marine life; nutrients that cause objectionable growth not allowed.
4.	Biological Charateristics	- Marine communities not allowed to be degraded; natural taste, odor and color of marine resources used for human consumption not to be altered.
5.	Radioactivity	- Not to exceed specified limits in California Administrative Code.

[1]Definition of "degrade" shall be determined by analysis of the effects of waste discharge on species diversity, population density, contamination, growth anomalies, debility, or supplanting of normal species by undesirable plant and animal species.

SCOPE FOR GROWTH

The Concept

Normally, in a healthy organism, there exists a dynamic equilibrium between the amount of energy required for the organism to maintain its life including growth and reproduction, and the amount of energy it obtains from the environment. If the energy costs for body maintenance are greater than the energy obtained, the short term effect is a reduction in energy reserves, and, in the long run, death results.

When a change in the environment results in a change in the physiology of an individual organism and reduces the excess of reserved energy stores, stress occurs. Bayne (1975) defined stress as a measurable alteration of a physiological (or behavioral, or biochemical, or cytological) steady state which is induced by an environmental change and which renders the individual (or the population, or the community) more vulnerable to further environmental change. Here the definition implies a major impact of stress in that it reduces an organism's capacity to cope with additional environmental changes, thus causing the organism to be less "healthy." In the extreme case, stress can reduce an organism's chances of survival.

Using stress as an index to the physiological condition of the individual, this study compares the Scope for Growth (SFG) index between animals living in clean and polluted environments. SFG defines the theoretical amount of energy available to an organism for growth and reproduction, in excess of energy required for maintenance (Warren and Davis 1967). It is expressed as the difference between the energy value of all the food an animal consumes and the energy value of all uses and losses of food other than growth, under a particular set of environmental conditions (Warren 1971). It is based on a balanced energy equation of Winberg (1960):

$$C = P + R + U + F$$

where

- C is the total energy of food consumed, in a given time period
- P is the energy utilized per unit time for somatic growth and gamete production
- R is the energy used per unit time in respiration
- U is the energy value per unit time of excretia
- F is the energy value per unit time of feces.

The SFG equation determines the value of P:

$$P = (C - F) - (R + U),$$

Let (C - F) = A = net amount of energy absorbed from food, so that

$$P = A - (R + U).$$

The terms of this equation are expressed in units of energy per unit of time (joules hour^{-1}) because several different factors and processes must be accounted for by a common unit of measure.

Methodology

The hypothesis tested in the Biological Effects Assessment (BEA) Study is that elevated levels of pollutants in mussel tissues affect the SFG index. Three observations led to the development of this hypothesis. First, mussels living in waters with higher concentration of pollutants tend to accumulate trace toxic substances (xenobiotic substances, trace elements, trace petroleum hydrocarbons) in their tissues at a higher rate than animals living in waters with lower concentrations of these substances. Second, mussels exposed to greater concentrations of pollutants show increased body burdens. These two observations are the results of analysis of the Marine Monitoring and Surveillance Program data described earlier. The third observation is that mussels with elevated levels of pollutants in their tissues have lower SFG values than mussels living in nonpolluted waters (Widdows et al. 1981). This latter observation is that reduced SFG and elevated tissue burdens are negatively correlated. Further cause-effect experiments are planned to assess whether or not pollutants in the tissues are responsible for the reduced SFG. The study is designed to investigate in the laboratory the relationship between tissue burdens of identified toxic substances (trace elements and pesticides) and the SFG.

Many have questioned the influences of laboratory conditions upon the outcome of the experiment or test. The mussels are collected from field populations and are selected on the basis of size. The collection sites are selected on the basis of body burdens of accumulated pollutants. The animals are collected by "clean" techniques to assure lack of chemical contamination during collection. They are transported to the laboratory within 6 hours, and immersed in seawater. Potential sources of "stress" include temperature, salinity, food, handling, light regime and particulate concentrations. In the laboratory, the animals were tested in uniform salinity (33 ppt), fed 6000 cells/mL Tetraselmis suecica (maintenance ratio) and exposed to light 10 hours per day. Subsequent experiments have been conducted to evaluate the salinity stress question.

RESULTS OF CALIFORNIA BIOLOGICAL EFFECTS ASSESSMENT STUDY

The results of the physiological stress testing indicate that the SFG is an excellent indicator of pollutant stress and is sensitive enough to detect pollution gradients. Six collection sites for resident Mytilus edulis in San Francisco and Tomales Bay were selected along an increasing "pollutant" gradient (Figure 4). The increasing "pollutant" gradient was previously shown by State Mussel Watch monitoring data (Martin et al. 1980; Stephenson et al. 1980) for San Francisco Bay.

Table 2 lists for both stations the SFG values (P), the four physiological factors (respiration rate, ammonia excretion, assimilation efficiency, and feeding rate) used in calculating P, and as well as a second "index" for stress, the O:N ratios.

The San Francisco Bay sites listed in Table 2 are arranged in order from north to south; that order also represents increasing ambient pollution levels. Bradford and Luoma (1980) reported that municipal discharges of chromium, copper, mercury, zinc and silver were at least four times greater in the south bay than in the north bay. The body burden data for Mytilus californianus transplanted along this gradient are in agreement. Tables 3 and 4 show that declining SFG values are correlated with increasing pollution levels. Other environmental factors, such as salinity and food availability, could be correlated with declining SFG. Further evaluation of those factors and their influence on SFG is in progress.

An important finding in the first year of physiological stress testing is the identification of specific toxic constituents in mussel tissues that are positively correlated with decreased SFG. Table 3 gives the concentration of trace elements found to accumulate in transplanted mussels and the SFG values for the six San Francisco Bay sites. Of particular interest is the degree of correlation between the concentration of a particular element in the tissue and the SFG values when values along the pollution gradient are compared. Regression coefficients, listed in Table 3, identify a high correlation ($r \geq -0.9$, 4 df.) between increasing tissue burdens and decreasing SFG for silver, chromium, copper and mecury, and to a slightly smaller degree ($r \geq -0.85$, 4 df.) for aluminum and zinc.

Figure 5 shows the collection sites of resident M. californianus along the coast of California. Table 5 lists SFG, physiological parameters, O:N ratio and collection sites for resident M. californianus. Along the coast of California, there is not a "gradient" or clinal trend of increasing pollutants (Martin et al. 1980; Stephenson et al. 1980; Risebrough et al. 1980). Two stations in central and northern California (Bodega Head and Granite Canyon) had lower body burdens for most trace toxic materials (trace elements, petroleum hydrocarbons) than five other stations (Table 5).

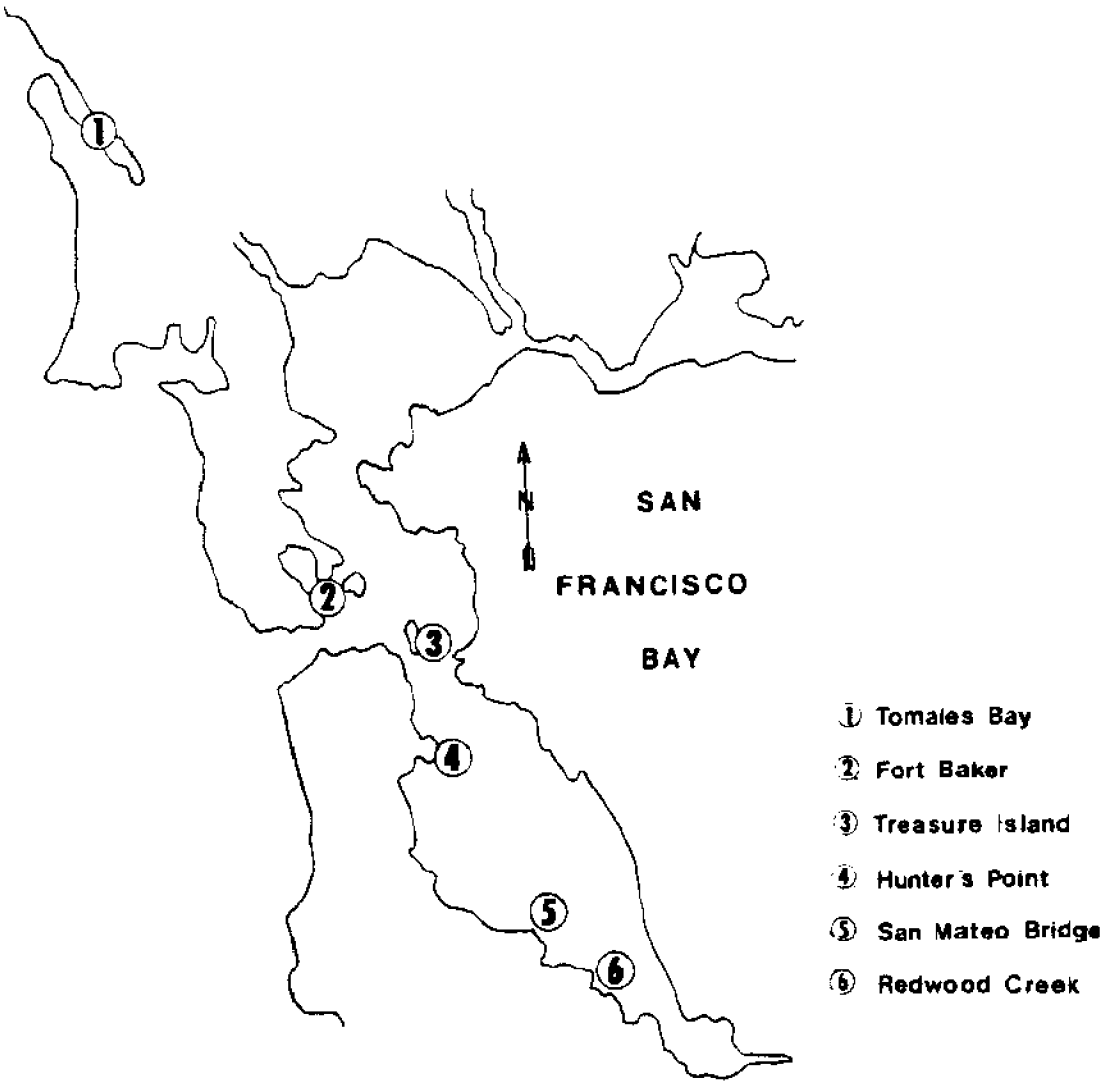

Figure 4. Sites for resident mussel collections (Mytilus edulis) in Tomales and San Francisco bays.

Table 2. Physiological stress index or scope for growth (P) for bay mussels (Mytilus edulis) resident in San Francisco and Tomales bays, calculated for a standard animal (1.0 g dry tissue weight).[1]

Station	N	Clearance Rate ($L\ h^{-1}$)	Oxygen Consumption ($ml\ h^{-1}$)	Ammonia ($\mu g\ h^{-1}$)	Assimilation Efficiency (%)	O:N Ratio	P ($J\ h^{-1}$)
Reference							
Tomales Bay	10	7.6 ± 1.1	0.6 ± .03	5.6 ± 0.7	45.9	148.6	43.8 ± 6.6
Chemically Influenced							
San Francisco Bay							
Fort Baker	10	6.0 ± 1.1	0.6 ± .06	19.1 ± 2.7	48.2	45.4	31.8 ± 9.0
Treasure Island	10	5.6 ± 0.8	0.4 ± .03	36.6 ± 7.0	50.4	15.7	26.3 ± 5.0
Hunters Point	10	3.3 ± 0.6	0.5 ± 0.1	27.8 ± 5.0	59.3	25.3	21.5 ± 5.2
San Mateo Bridge	10	5.1 ± 1.1	0.4 ± .05	11.6 ± 1.6	46.4	46.5	15.9 ± 4.5
Linear regression $(r)^2$		0.86	-0.36	-0.08	-0.23	0.39	
Level of significance		0.05	NS[3]	NS	NS	NS	

[1] Values are exressed as $\bar{x} \pm$ S.E.$_{\bar{x}}$, where S.E.$_{\bar{x}} = \frac{\text{S.D.}}{\sqrt{n-1}}$

[2] Linear regressions compare P with individual factors.

[3] NS—not significant.

Table 3. Trace element concentrations of transplanted mussel (Mytilus californianus) in San Francisco Bay. Element values are means of three replicates.

Station	Physiological Stress Index J h^{-1}	Trace Element Concentration ($\mu g\ g^{-1}$ dry wt.)[1]										
		Ag	Al	As	Cd	Cr	Cu	Hg	Mn	Pb	Se	Zn
Reference												
Tomales Bay	43.8	0.12	375	5.25	4.4	1.5	6.4	0.17	21.2	0.8	1.98	62
Chemically Influenced												
San Francisco Bay												
Fort Baker	31.8	1.08	350	6.26	10.6	1.7	12.1	0.25	15.6	3.7	2.15	116
Treasure Island	26.3	1.40	641	6.18	9.4	2.6	10.5	0.28	29.4	2.3	2.57	137
Hunters Point	21.2	1.13	689	7.57	12.1	2.8	12.2	0.39	23.1	2.8	3.79	160
San Mateo Bridge	15.9	1.86	660	7.69	18.9	3.0	13.9	0.44	26.3	2.6	2.92	198
Redwood Creek	3.8	4.35	750	6.66	12.6	3.6	15.6	0.56	28.9	2.8	3.11	162
Linear regression (r)		-0.91	-0.86	-.68	-.73	-.97	-.93	-.99	-.62	-.50	-.70	-.85
Level of significance		.05	.05	NS[2]	NS	.01	.01	.01	NS	NS	NS	.05

[1] From Stephenson et al. 1982.
[2] NS--not significant.

Table. 4. Higher molecular weight hydrocarbon concentrations in transplanted mussels (Mytilus californianus) from San Francisco Bay.

Station	Physiological stress index ($J\ h^{-1}$)	Hydrocarbon concentration (ng g^{-1} dry wt.)[1]		
		Total Chlordane	Dieldrin	PCB 1254
Reference				
Tomales Bay	43.8	4	4.7	27
Chemically Influenced				
San Francisco Bay				
Fort Baker	31.8	39	20	510
Treasure Island	26.3	67	24	1500
Hunters Point	21.2	51	27	1800
San Mateo Bridge	15.9	83	50	1300
Redwood Creek	3.8	78	44	1200
Linear regression (r)		-0.89	-0.91	-0.67
Level of significance		0.05	0.05	NS[2]

[1]Hydrocarbon data from Martin et al. 1982a
[2]NS--not significant

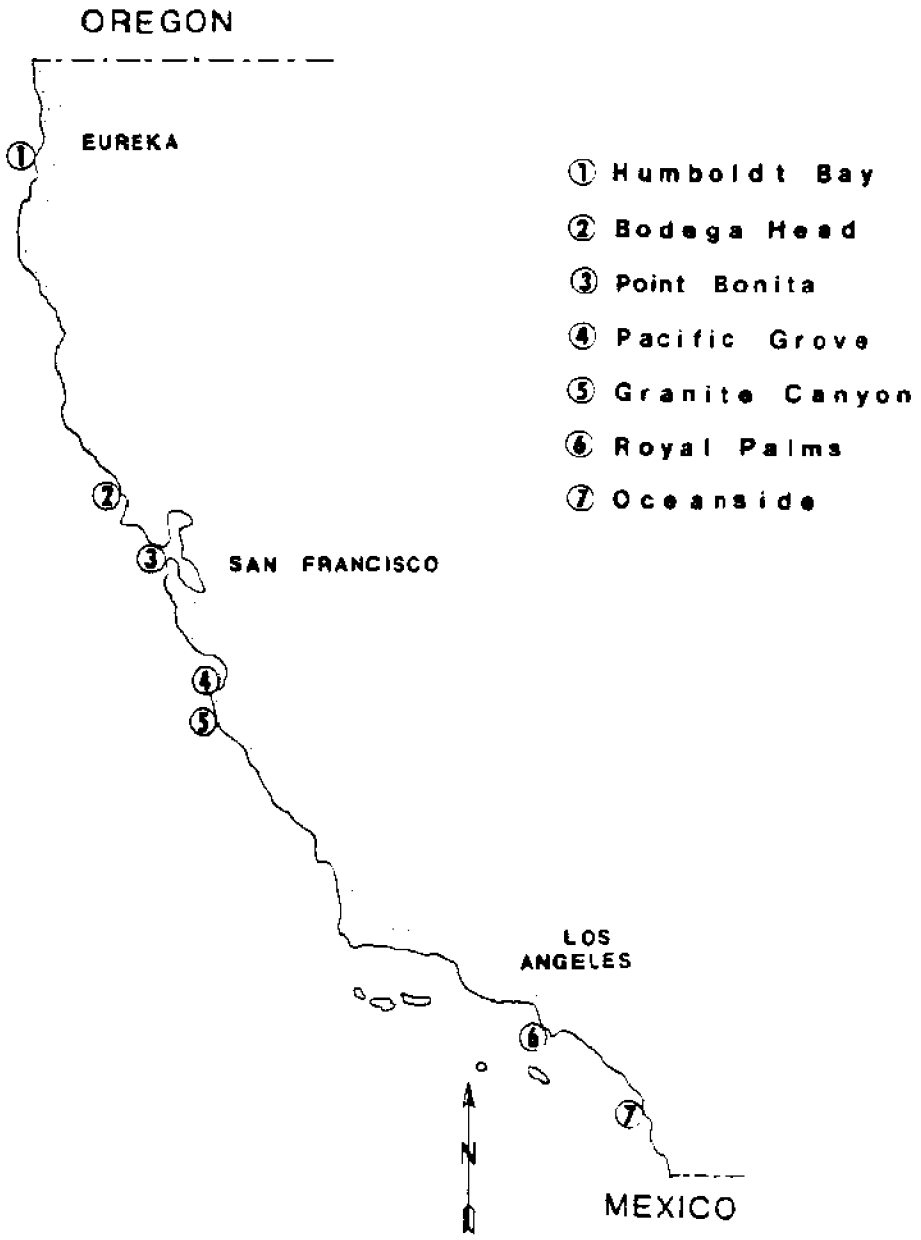

Figure 5. Sites for resident mussel collections (Mytilus californianus) along the California coast.

Table 5. Physiological stress index or scope for growth (P) for California mussel (Mytilus californianus) residents from the California coast, calculated for a standard animal (1.0 g dry tissue weight).[1]

Station	N	Clearance Rate ($L\ h^{-1}$)	Oxygen Consumption ($ml\ h^{-1}$)	Ammonia ($\mu g\ h^{-1}$)	Assimilation Efficiency (%)	O:N Ratio	P ($J\ h^{-1}$)
Reference							
Bodega Head	10	4.4±0.6	0.47±0.05	10.0±1.3	61.84	65.52	28.7±5.4
Granite Canyon	10	4.2±0.5	0.23±0.01	13.6±1.4	52.68	24.14	29.9±2.3
Chemically Influenced							
Oceanside	9	6.0±1.0	0.38±0.04	13.8±1.0	41.11	33.59	24.4±4.7
Humboldt Bay	10	3.6±0.06	0.63±0.06	27.8±1.33	57.04	28.23	14.1±5.4
Pacific Grove	10	5.2±1.0	0.37±0.03	13.8±1.8	39.52	35.30	23.9±5.8
Royal Palms	10	2.4±0.8	0.50±0.20	26.3±1.7	53.63	21.92	6.4±3.8
San Diego Bay-Shelter Island[2]	8	0.5±0.1	0.19±0.03	12.1±1.5	68.15	17.51	0.4±1.4
Linear regression (r)[3]		0.85	-0.02	-0.44	-0.49	0.64	
Level of significance		0.01	NS[4]	NS	NS	NS	

[1]Values are expressed as mean ± 1 S.E. of mean, where S.E. $_{\bar{x}} = \frac{S.D.}{\sqrt{n-1}}$
[2]Transplant
[3]Linear regressions compare P with individual factors
[4]NS--not significant

Table 6 gives the SFG values and tissue burdens of trace elements for coastal mussels, M. californianus. Coastal mussels do not show a statistical correlation between increasing tissue burdens of trace elements and decreasing SFG values. Table 7 shows the correlation between organic pollutants and SFG values. Only petroleum hydrocarbons (unresolved complex mixture) show a significant correlation with the SFG ($r = -0.96$, 4 df.). PCBs exhibit a strong ($r = -0.81$, 4 df.), but not significant at the 0.05 level, correlation with SFG.

ASSUMPTIONS FOR SCOPE FOR GROWTH

In our examination of the potential external influences on SFG, several assumptions have been made about the field and laboratory environments of trace toxic pollutant accumulations in mussels. Although these assumptions may qualify the research conclusions of the studies, we believe that the overall findings are a documentation of the influence of pollutants on a representative marine organism. We have made these assumptions in relation to the experimental design of our study:

1. Energy availability in excess of maintenance requirements is a measure of the well-being or relative health of the organism.
2. SFG is a relative measure of or an index for the organism's condition, and is proportional to the true condition by a constant factor (laboratory vs. field SFG).
3. The field sites vary only with regard to ambient pollution concentration; all other environmental variables are identical between field sites.
4. SFG is related to the reproductive condition of the animal by a constant factor.
5. With respect to the physiological factors measured, the organism responds in the experimental apparatus in a pattern consistent with the field environment.
6. A decrease in energy reserves of an individual causes a negative impact to the mussel population by a variety of factors, such as fecundity and survival of larvae, and could eventually result in population decline.
7. Mussels are representative organisms of a particular trophic level. SFG has been used in few experiments with other than bivalve molluscs.
8. The populations of mussels are normally distributed with respect to the SFG parameters and measurements.

Table 6. Trace element concentrations of California mussels (Mytilus californianus) along the California coast. Element concentrations are means of three replicates.[1]

Station	Physiological Stress Index J h^{-1}	Trace Element Concentration ($\mu g\ g^{-1}$ dry wt.)										
		Ag	Al	As	Cd	Cr	Cu	Hg	Mn	Pb	Se	Zn
Reference												
Bodega Head	28.7	.08	207	5.4	9.1	1.5	4.7	0.16	9.5	.77	2.3	83
Granite Canyon[2]	29.9	1.00	23	NA[3]	7.7	1.1	6.1	0.15	3.1	2.4	NA	156
Chemically Influenced												
Humboldt Bay	14.1	.13	506	7.0	5.5	2.0	9.1	0.17	17.0	1.9	1.4	109
Pacific Grove	23.9	.23	45	NA	11.0	ND	3.6	0.13	3.4	5.0	NA	65
Royal Palms	6.4	63.7	360	8.3	5.9	8.1	24.7	0.75	13.2	9.3	6.5	238
Oceanside	24.4	.12	494	8.7	9.9	1.3	16.0	0.34	19.8	3.1	2.8	214
San Diego Bay-Shelter Island	0.4	.22	333	8.9	4.9	1.1	9.4	0.22	14.3	3.0	2.3	167
Linear regression (r)		-0.45	-.26	-.65	-.58	-.44	-.49	-.42	-.39	-.15	-.31	-.40
Level of significance		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1]Data from Stephenson et al. 1982
[2]Data from Stephenson et al. 1979
[3]Element not analyzed

Table 7. Higher molecular weight halogenated hydrocarbon and petroleum concentrations in California mussels (Mytilus californianus) along the California coast.[1]

Station	Physiological Stress Index ($J\ h^{-1}$)	Hydrocarbon Concentrations ($ng\ g^{-1}$) DDE	PCB 1254	Total Chlordane	aBHC	Dieldrin	Petroleum ($\mu g\ g^{-1}$) Total UCM	Total Resolved
Reference								
Bodega Head	28.7	25	52	20	13	14	4	5
Chemically Influenced								
Humboldt Bay	14.1	5	34	4	5	ND[3]	39	11
Pacific Grove	23.9	58	55	26	11	5	21	4
Royal Palms	6.4	1300	390	46	5	4	92	7
Oceanside	24.4	390	220	73	6	22	20	2
San Diego Bay - Shelter Island	0.4	290	1000	75	7	20	ND	ND
Linear regression (r)		-.49	-.81	-.38	.66	.01	-.96	-.67
Level of significance		NS[4]	NS	NS	NS	NS	0.05	NS

[1]Data from Martin et al. 1982a
[2]Risebrough et al. 1980
[3]Below limit of detection
[4]NS--not significant

Obviously, one (#3) of these assumptions requires appropriate field conditions; another (#4) is dependent upon the biological condition of the organism. The assumption that field conditions vary only with pollution concentration could be violated (e.g., salinity could fluctuate); however, the experimental design tries to minimize these differences by sampling when environmental parameters are least variable and by attempting to duplicate field conditions in the laboratory. The assumption that the physiological responses of the mussels are identical between lab and field has not yet been evaluated. As long as the effect of the laboratory on the physiological parameters measured is constant for all individuals, the SFG index can be used to compare the relative amount of stress. The SFG measured then is proportional to the SFG in the field by a constant factor.

RELATIONSHIP OF SCOPE FOR GROWTH TO BIOLOGICALLY IMPORTANT RESPONSES

Evidence that the SFG is an overall integration of the energetic condition of the organism has been corroborated by other studies (Bayne et al. 1979, 1981; Widdows et al. 1981). Phelps et al. (1981) analyzed several different physiological and biochemical stress measurements of mussels (M. edulis) transplanted to three sites of varying pollution in Narragansett Bay, R.I. Phelps et al. (1981) found that trends observed in respiration rate and enzyme activity in excised gill and posterior adductor muscle correlated with trends in SFG measurements. Monthly sampling during summer indicated that mussels living in polluted sites (trace elements, petroleum, PCB) showed a trend of increased oxygen consumption rates, lowered gill glycolytic activity, and increased posterior adductor muscle lactate dehydrogenase activity, compared with mussels from less polluted stations. Elevated lactate dehydrogenase activity, statistically greater from mussels in polluted areas, indicates that energy reserves in the posterior adductor muscle are being or have been mobilized to support weakening gill metabolism. Pollutant stress causes a drastic change in glycogen metabolism, as glycogen stores in the adductor muscles are normally saved for gamete formation. This finding led Phelps et al. (1981) to conclude that, in polluted areas, energy stores normally reserved for gamete formation are used for physiological compensation as the result of pollution stress.

In another corroborating study Bayne et al. (1979) found that mussels in which they had induced low SFG values produced fewer eggs (55% decrease in egg productin between mussels with SFG 46 $J\ d^{-1}$ and -218 $J\ d^{-1}$ as well as an 84% decrease in the energy content of eggs). Bayne et al. (1979) attributed this to resorption of eggs due to release of lysosomal enzymes in areas of developing

oocytes. Also, the oocytes that did develop were reduced in size. Gametes apparently had decreased vitality and decreased chance of development to the larval state. Bayne et al. (1979) concluded that this deterioration of the fitness of the population was a result of environmental stress on the adult population.

Bayne et al. (1979) also found significant correlation between the labilization period of hexosaminidase from digestive gland cells of Mytilus and negative values of SFG. This suggests that at very negative values of SFG cellular damage would result from the release of free hydrolases into the cytoplasm, although lysosomal destabilization may not be significant at low positive SFG values.

Individual cytological/biochemical alterations are sensitive to environmental changes and provide supportive evidence that the SFG is a measurement of a natural response to stress (Bayne et al. 1979). These individual cytological/biochemical alterations do not demonstrate, in themselves, a decline in health of the affected organisms. The SFG measurement is the observable integrated response of all the cytological and biochemical changes induced by pollution, and provides a measure of the effect of pollutants on individuals. As a measure of productivity of an individual, SFG therefore has relevance in predicting the ecological effects of pollutants on the population (Bayne et al. 1979).

Field Validation

An ideal field validation of the hypothesis that high pollutant tissue burdens result in a decreased SFG would be to eliminate the sources of pollution to an area and observe an increase in the SFG values of mussels. Because a method and the equipment for adequately measuring the tested variables (oxygen consumption, ammonia excretion, clearance rate and assimilation efficiency) with organisms in situ have yet to be developed, field measurements of the variables do not appear feasible at this time.

Field validation requires a corroboration between field and laboratory derived data. In our study of California marine waters, the chemicals and/or elements correlated with decreased SFG have been identified. These include copper, chromium, silver, aluminum, mercury, zinc, chlordane, dieldrin and PCBs for M. edulis, and petroleum hydrocarbons for M. californianus. Laboratory dose responses are being tested to determine the effect that a chemical has on SFG.

After testing the responses of a specific chemical on SFG, it is important to test the effect of removing or reducing the concentration of that chemical in an analog test. If the analog test is positive, (i.e., if SFG increases) a study site is proposed for a field validation. Let us assume that chromium is identified as a contributor to increased Mytilus stress in San Francisco Bay. The mass balance of chromium discharge to that particular site in San Francisco Bay would need to be determined. Risebrough et al. (1978)

reported that chromium is discharged to San Francisco Bay at a rate of 67 metric tons yr^{-1} by municipal wastewater outfalls and 26 metric tons yr^{-1} by petroleum refineries. Surface runoff contributed 1.2 metric tons per year. By using a no-effect concentration determined in the dose response test as an estimate of the "safe" ambient level, maximum discharge limits of chromium can be set for all dischargers.

After the establishment of water quality requirements necessary to protect marine organisms from adverse effects of waste discharge, the feasibility of controlling major discharges, such as publicly owned treatment works, can be determined. In this example, a program of pretreatment or source control may be required to advance safe ambient levels. After chromium discharges have been reduced, the SFG values of mussels living in that area will be retested to determine their response. An on-site laboratory (onboard ship or a portable laboratory) might reduce the variability of conducting the experiments in a more remote laboratory.

It is important to quantify and relate other biologically important "health" indices, such as gonad condition or general body condition (e.g., shell volume vs. dry weight), with SFG. Major population changes (in diversity or numbers) are also important factors to be considered in field validation experiments.

SFG AS A SIGNIFICANT MEASUREMENT OF ECOSYSTEM PRODUCTIVITY

There is a direct relationship between the amount of energy available after basic metabolic needs are accounted for and growth (somatic and gamete formation) of the individual. Bayne et al. (1979) reported that rates of growth predicted from SFG data agree with observed rates of growth. Martin et al. (1982b) showed significant correlation between body condition index and SFG. Criteria that measure the effects of pollution on the productivity of the individual give an indication of the likely consequences of environmental change to a population. One aspect of the ecological fitness of a population is fecundity. A decline in number and viability of gametes could be a direct cause of deterioration in the ecological fitness of the population. The goal of an SFG measurement is to calculate energy available for growth. Although it may not reflect the exact energies available to field animals, it is directly related to the actual value and can be used in a relative manner to predict actual production (somatic and gametic formation) in populations (Bayne et al. 1979).

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REFERENCES

Bayne, B.L. 1975. Aspects of physiological condition in Mytilus edulis (L) with special reference to the effects of oxygen tension and salinity. In: H. Baines (ed.), Proceedings of the 9th European Marine Biology Symposium, Aberdeen, Scotland, pp. 213-238. Aberdeen Univ. Press, Aberdeen, Scotland.

Bayne, B.L., M.N. Moore, J. Widdows, D.R. Livingstone and P. Salkeld. 1979. Measurements of the responses of individuals to environmental stress and pollution: Studies with bivalve molluscs. Philos. Trans. R. Soc. Lond. B. 286: 563-581.

Bayne, B.L., K.R, Clarke and M.N. Moore. 1981. Some practical considerations in the measurement of pollution effects on bivalve molluscs, and some possible ecological consequences. Aquat. Toxicol. 1(3,4): 159-174.

Bradford, W.L. and S.N. Luoma. 1980. Some perspectives on heavy metal concentrations in shellfish and sediment in San Francisco Bay, California. In: R.A. Baker (ed.), Contaminants and Sediments, Vol. 1, pp. 501-532. Ann Arbor Science Publ., Ann Arbor, Mich.

Butler, P.A. 1973. Residues in fish, wildlife and estuaries: Organochlorine residues in estuarine mollusks, 1965-72 National Pesticide Program. Pestic. Monit. J. 6(4): 238-362.

Capuzzo, J.M. 1981. Predicting pollution effects in the marine environment. Oceanus 24(1): 25-33.

CSWRCB (California State Water Resources Control Board). 1978. Water Quality Control Plan for Ocean Waters of California. Sacramento, Calif.

De Lappe, B.W., W.R. Sistek, E.F. Letterman, M. Firestone-Gillis and R.W. Risebrough. 1980. Predischarge studies, San Francisco South-West Ocean Outfall Project: The distribution of higher-molecular weight hydrocarbons in the coastal environment. Rep. to CH2M Hill, San Francisco by Bodega Marine Lab., Univ. of California, Bodega, Calif.

Fowler, S.W., M. Heyraud and J. LaRosa. 1978. Factors affecting methyl and inorganic mercury dynamics in mussels and shrimp. Mar. Biol. 46: 267-76.

Goldberg, E.D. 1981. The oceans as waste space: The argument. Oceanus 24(1): 2-9.

Goldberg, E.D., V.T. Bowen, J.W. Farrington, G. Harvey, J.H. Martin, P.L. Parker, R.W. Risebrough, W. Robertson IV, E. Schneider and E. Gamble. 1978. The Mussel Watch. Environ. Conserv. 5: 101-125.

Martin, M., D. Crane, T. Lew and W. Seto. 1980. California Mussel Watch 1979-1980--Synthetic organic compounds in mussels, Mytilus californianus and M. edulis, along the California coast and selected harbors and bays. Water Quality Monitoring Rep. 80-8, California State Water Resources Control Board, Sacramento, Calif.

Martin, M., D. Crane, T. Lew, and W. Seto. 1982a. California Mussel Watch: 1980-1981 Part III: Synthetic organic compounds in mussels, from California's coast, bays and estuaries. Water Quality Monitoring Rep. 81-11 TS, California State Water Resources Control Borad, Sacramento, Calif.

Martin, M., G. Ichikawa, J. Goetzl, and M. de los Reyes. 1982b. An evaluation of physiological stress in mussels related to toxic substance-tissue accumulation in California marine waters. Special Projects Rep. 82-2 sp, California State Water resources Control Board, Sacramento, Calif.

Mearns, A.J. 1981. Ecological effects of ocean sewage outfalls: Observations and lessons. Oceanus 24(1): 45-54.

Phelps, D.K., W. Galloway, F.P. Thurberg, E. Gould and M.A. Dawson. 1981. Comparison of several physiological monitoring techniques as applied to the blue mussel, Mytilus edulis, along a gradient of pollutant stress in Narragansett Bay, Rhode Island. In: F.J. Vernberg, A. Calabrese, F.P. Thurberg and W.B. Vernberg (eds.), Biological Monitoring of Marine Pollutants, pp. 335-355. Academic Press, New York.

Phillips, D.J.H. 1976. The common mussel Mytilis edulis as an indicator of trace metals in Scandinavian waters. 1. Zinc and cadmium. Mar. Biol. 43: 283-91.

Phillips, D.J.H. 1980. Quantitative Aquatic Biological Indicators--Their Use to Monitor Trace Metal and Organochlorine Pollution. Applied Science Publ. Ltd., London. 488 pp.

Risebrough, R.W., J.W. Chapman, R.K. Okazaki and T.T. Schmidt. 1978. Toxicants in San Francisco Bay and Estuary. Special Rep., Assoc. Bay Area Government, Berkeley, Calif.

Risebrough, R.W., B.W. de Lappe, E.F. Letterman, J.L. Lane, M. Firestone-Gillis, A.M. Springer and W. Walker, II. 1980. California Mussel Watch 1977-1978. Vol. III. Organic pollutants in mussels, Mytilus californianus and M. edulis, along the California coast. Water Quality Monitoring Rep. 79-22, California State Water Resources Control Board, Sacramento, Calif.

Stephenson, M.D., M. Martin, S.E. Lange, A.R. Flegal and J.H. Martin. 1979. California State Mussel Watch 1977-1978. Vol. II. Trace metal concentrations in the California mussel, Mytilus californianus. Water Quality Monitoring Rep. 79-22, California State Water Resources Control Board, Sacramento, Calif.

Stephenson, M.D., S.L. Coale, M. Martin, and J.H. Martin. 1980. California Mussel Watch 1979-1980--Trace metal concentrations in the California mussel, Mytilus californianus, and the bay mussel, M. edulis, along the California coast and selected harbors and bays. Part 1. Water Quality Monitoring Rep. 80-8, California State Water Resources Control Board, Sacramento, Calif.

Stephenson, M.D, S.L. Coale, M. Martin, D. Smith, E. Armbrust, E. Faurot, B. Allen, L. Cutter, G. Ichikawa, J. Goetzl, J.H. Martin. 1982. California Mussel Watch: 1980-1981 Part II: Trace metal concentrations in the California mussel, Mytilus californianus, from California's coast, bays and estuaries. Water Quality Monitoring Rep. 81-11TS, California State Water Resources Control Board, Sacramento, Calif.

Warren, C.E. 1971. Biology and Water Pollution Control. W.B. Saunders, Philadelphia, Pa.

Warren, C.E. and G.E. Davis. 1967. Laboratory studies on the feeding, bioenergetics, and growth of fishes. In: S.D. Gerking (ed.), The Biological Basis of Freshwater Fish Production, pp. 175-214. Blackwell Scientific Publ., Oxford, England.

Widdows, J., D.K. Phelps and W. Galloway. 1981. Measurement of physiological condition of mussels transplanted along a pollution gradient in Narragansett Bay. Mar. Environ. Res. 4: 181-194.

Winberg, G.G. 1960. Rate of metabolism and food requirements of fishes. Translation Ser. 194, Canada Fisheries Research Board, Ottawa, Ontario.

Mussel Madness: Use and Misuse of Biological Monitors of Marine Pollution

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INTRODUCTION

A sentinel network to monitor the distribution, and sometimes the effects, of marine contaminants has been proposed in a number of different forms. Of course, many surveys of suspended and dissolved contaminants in the water column have been made (e.g., see Chester and Stoner (1974) for one of the rare surveys covering several oceans). There have also been numerous surveys of distribution of contaminants in marine sediments, and Simpson and Lockwood (1981) even proposed that a national system of sediment measurements be adopted in the United States. For some trace metal contaminants in Hong Kong waters, Phillips and Yim (1981) considered sediments to be a better indicator than the mussel Septifer bilocularis, because the organism exhibits regulation of some metals. Butler et al. (1978) proposed that a national network of sediment gas chromatograms be established, archived, and analyzed for long-term trends as well as sudden appearances of anthropogenic compounds such as Kepone. Lytle and Lytle (1977) examined sediment hydrocarbons as environmental indicators in the northeast Gulf of Mexico.

A number of living organisms have been proposed or used as indicators of contaminant distribution. The most popular of these is the mussel, especially Mytilus edulis (Goldberg et al. 1978), but many substitute bivalves have also been suggested. Eide and Jensen (1979) demonstrated the feasibility of using two diatom species (Thalassiosira pseudonana and Phaeodactylum tricornutum) enclosed in situ in dialysis bags to monitor heavy metal pollution. A number of authors (e.g., Preston et al. 1972; Bryan and Hummerstone 1973a; Preston 1973; Pentreath 1976; Hansen 1980; de Kock

and Kuiper 1981) described the use of seaweeds to monitor heavy metal and radionuclide contamination. Papadopoulou and Kanias (1977) outlined the use of tunicates as marine pollution indicators, and Phillips (1980) suggested that Ciona intestinalis was sufficiently common and widely distributed to deserve further study as an indicator organism. Popham and D'Auria (1982) proposed using the polychaete Eudistylia vancouveri as a sentinel organism for monitoring a suite of trace metals, especially vanadium and titanium, which are not concentrated by mussels. On the other hand, Bryan and Hummerstone (1973b) found that the polychaete Nereis diversicolor can regulate zinc, and is thus a poor indicator of that metal, while it is an excellent indicator of cadmium. Albrecht et al. (1981) chose the dermistid shrimp Heterocarpus ensifer as an indicator organism to monitor cadmium, copper, and zinc contaminants associated with dredge spoil mounds. Ireland (1973, 1974), Navrot et al. (1974), Walker et al. (1975), Goldberg (1976), Barbaro et al. (1978), De Kock and Kuiper (1981) and Barber and Trefry (1981) all discussed the possibility of employing barnacles (mostly of the genus Balanus) to monitor for heavy metals. Barber and Trefry (1981) pointed out the ubiquity of barnacles in harbors and marinas, even when mussels and other bivalves are absent, and examined the former as monitoring organisms. The United Kingdom Atomic Energy Authority has used a variety of organisms, including macroalgae, limpets, oysters, crabs and fish, to monitor discharges from the Winfrith, Dorset, reactor development facility (Flew 1977). Johnels et al. (1967) noted the several advantages of the pike (Esox lucius) as an efficient sentinel for mercury, and Dix et al. (1976) suggested using the sand flathead (Platycephalus bassensis) as a sentinel for this element. Portmann (1970, 1972) reported the use of several common fish species in a surveillance program for PCBs, organochlorine pesticides and heavy metals in British waters. Weiss (1965) reviewed the use of many fish species as insecticide monitors. The National Academy of Sciences (NAS 1980) discussed the development of an "artificial mussel" using polyurethane foam, and Eide and Jensen (1979) actually followed the strategy by deploying a chelating resin column in the field. Without citing further instances, I simply observe that sediments and a host of organisms have been proposed as sentinels to detect the presence of a variety of contaminants in marine waters.

ADVANTAGES AND DISADVANTAGES OF VARIOUS SENTINELS

Each of the above sentinels has unique advantages, and each has attracted its advocates and lobbyists. Phillips (1977a, 1978a, 1980) reviewed many of the physiological characteristics that render different plants and animals fit to monitor one or more contaminants or one or more environments. Certain bivalves have received far

more attention than other potential sentinels. As a result, we have become familiar with their many useful properties, such as their wide distribution, abundance, size, ease of collection, long life span, euryhalinity, general stress tolerance and high bioconcentration of a number of chemicals. A considerable literature exists on the uptake and elimination of contaminants by bivalves (Lee et al. 1972; Stegeman and Teal 1973; DiSalvo et al. 1975; Fossato 1975; Fossato and Canzonier 1976; Boehm and Quinn 1977; Fowler et al. 1978). Although there is still disagreement over the dynamics of uptake and release, and both known and unknown factors appear to control those processes, it is a mark of the attention given to bivalves that simple bioconcentration models have been formulated (e.g., Majori and Petronio 1973; Burns and Smith 1981).

On the other hand, each proposed sentinel organism has its share of disadvantages. Water column surveys suffer from many inadequacies such as extremely high natural variability, low contaminant levels (necessitating preconcentration of large volumes of water), and analytical contamination problems. Sediments enjoy the advantage of nearly ubiquitous distribution, but sediment contaminant levels do not necessarily indicate bioavailability (Malins et al. 1983 inter alia). Phillips (1977a, 1978a, 1980) made a penetrating review of the many important natural factors (species, weight, age, sex, lipid content, season, reproductive state, shoreline position, salinity, temperature, interactions, etc.) that strongly interfere with the relationship between contaminant levels in macroalgae, bivalves, and teleosts, and contaminant levels in the ambient environment. Other literature affirms that interfering parameters are as multifarious as Phillips described, and include such factors as chemical speciation (Fowler et al. 1978), chelation (George and Coombs 1977), feeding rate (Janssen and Scholz 1979), sediment type (Jackim et al. 1977), pre-exposure history (Roesijadi et al. 1982) and so on and on.

Besides these confounding factors, different sentinel organisms suffer from a variety of strategic inadequacies. Algae (micro- and macro-) are insensitive to particulate-bound contaminants. Albeit with some qualification, deposit-feeding infauna do not accumulate a significant fraction of contaminants from the overlying water column, while filter-feeding organisms remain relatively unresponsive to sediment contaminants. Tidal and subtidal species are exposed to a very small proportion of an estuary's volume, and are nearly useless as monitors of the continental shelves and beyond. No animal sentinel exhibits a direct response to eutrophication, a growing problem in most estuaries and a severe problem in some. Indeed, there is no single sentinel organism, or even group of organisms, that is free of either strategic or technical difficulties. Consequently, the adequacy of any organism to monitor contaminants must be qualified, usually extensively.

For example, Table 1 summarizes heavy metal contaminants and circumstances for which mussels have been regarded as sub-optimum indicators. Davies and Pirie (1980), paraphrasing Phillips (1977c, 1978b), pointed out that it would be more correct to envisage the mussel as an indicator of metal availability to the mussels themselves, rather than an indicator of the total metal concentration in the seawater. It is therefore no wonder that various "mussel watch" programs have discovered nothing more than the trivial result that mussel contaminant burdens loosely parallel patterns of industrialization and urbanization along adjacent seaboards (Risebrough et al. 1979; Murray and Law 1980; Farrington et al. 1982a, b). It is equally unsurprising that mussel monitoring has so far failed to uncover contaminant "hot spots" that were not known or suspected before (Goldberg et al. 1978; Goldberg 1979; Farrington et al. 1982). These lackluster conclusions are not a special indictment of mussel monitoring; it is likely that any other monitoring organism, applied in so narrow a context, would perform equally poorly.

BEYOND SENTINELS

The environmental manager is thus in a quandary, presented with a nearly endless litany of imperfect sentinel organisms whose individual virtues are touted by one expert or another. How does one discover the "right" sentinel?

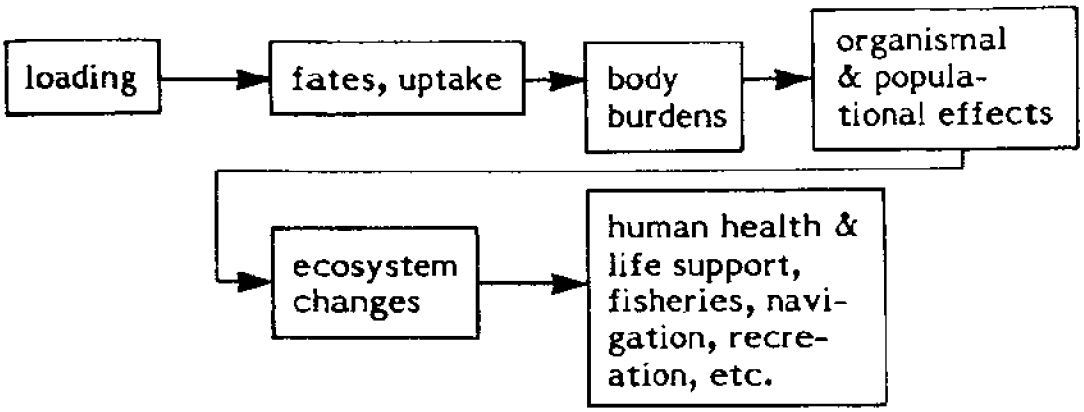
To answer this question, the environmental manager must first clearly define the purpose for gathering sentinel data. Sentinels are posted to warn of danger. The environmental manager must do what he has nearly always avoided: clearly designate the entity he is protecting from danger. Is it fishery resources? Recreational resources? Human health? Balanced ecosystems? Endangered species?

Next, the environmental manager must select an effective sentinel to guard that entity from danger. A canary is no better a sentinel in an army camp than a soldier is in a coal mine. Similarly, mussels (as an example) are poor sentinels for environmental contamination by copper (Phillips 1976a; Davenport 1977), manganese (Phillips 1978b), and titanium and vanadium (Popham and D'Auria 1982). Because those contaminants and conditions interfere with the normal patterns of valve closure in mussels, sentinels free of that problem, perhaps macroalgae, should be considered as monitors for those contaminants. Environmental managers usually must protect a number of interests in an array of environmental regimes. It is unrealistic and naive to expect a single taxon of organisms simultaneously to serve as sentinel for all interests.

Table 1. Heavy metals and circumstances for which mussels have been regarded as sub-optimum indicators.

Contaminants	Circumstances	Reference
Mn	Mytilus edulis from low-salinity Scandinavian waters	Phillips (1978b)
Mn	Mytilus edulis from Yaquina Bay, Oregon, USA	Latouche and Mix (1982)
Zn	Mytilus edulis in waters experiencing large, rapid salinity changes	Phillips (1977b)
Zn	Trichomya hirsuta from tropical, nearshore Australian waters	Klumpp and Burden-Jones (1982)
Cu	Mytilus edulis from Port Phillip Bay, Australia	Phillips (1976 a, b)
Cu	Mytilus edulis from Menai Strait, U.K.	Davenport (1977)
Cu, Zn	Septifer bilocularis from Hong Kong	Phillips and Yim (1981)
Cu, Zn, Ag, Ni	Mytilus edulis from Cape Henlopen, New Jersey and Wachapreaque, Virginia, U.S.A.	Goldberg et al. (1978)
Hg	Mytilus edulis from the Firth of Forth, Scotland	Davies and Pirie (1978)
Hg	Mytilus californianus from southern California, U.S.A.	Eganhouse and Young (1978)
As	Mytilus galloprovincialis from Monaco	Unlu and Fowler (1979)
Ti, V	Mytilus edulis from Vancouver, B.C., Canada	Popham and D'Auria (1982)

But in truth the sentinel concept is too simplistic and restrictive. A much larger perspective is demanded, for one must ultimately understand the very complex relationship between the contaminant's loading, the sentinel's response, and the final entity being protected:



The rationale and urgent need for this holistic perspective is fully developed elsewhere (White and Lockwood 1982); it is not an original thesis. The report of the 1978 International "Mussel Watch" Workshop (NAS 1980) states:

> Implicit in what is proposed is a conceptual model of the physical, biological, and social systems affected. It is impossible to construct any monitoring system in the absence of such a concept, and its development must be a primary goal of the program. (Emphasis mine.)

Yet not another word on this topic appears in that or any other report. A mussel monitoring system actually tested in the United States lacked this conceptual framework considered so essential (Goldberg et al. 1978).

A consolation is that systems employing other monitoring organisms are conceptually still more primitive than "Mussel Watch", not even having recognized the need for a holistic model. Furthermore, some mussel monitoring programs have tenderly begun to examine the relationship between body burdens and effects. This is at least a slight advance out of the middle box in the above model (strict body burden monitoring). The report on the International "Mussel Watch" Workshop (NAS 1980) did make a plea for research on the relationship between tissue residues, physiological stress indices, and effects on growth, reproduction and survival. The New Jersey Marine Sciences Consortium measures not only mercury, cadmium and lead in mussels deployed in the New York Bight, but also survival rate, shell length and weight (Koepp et al. 1981). The U.S. Environmental Protection Agency's Mussel Watch monitors

histopathological effects, along with body burdens (Goldberg et al. 1978). The California Mussel Watch program is perhaps the most advanced, measuring scope for growth in mussels (Martin and Severeid 1984).

However, this approach is backwards. We must reconsider the direction that has been taken to develop monitoring systems of any kind. We should not first select a particular monitoring system or organism, then attempt to squeeze it into the relationship between contaminant loading and environmental entities we wish to protect. Rather, we should first develop a model of that latter relationship, then monitor only those components and those organisms whose values are required by terms in the model. It may well be that tissue levels of contaminants in certain species are necessary data to compute the effects of a contaminant on environmental entities of concern; it may be that such data are irrelevant to the computation. Let us construct the model first.

REFERENCES

Albrecht, W.N., C.A. Woodhouse and J.N. Miller. 1981. Nearshore dredge-spoil dumping and cadmium, copper and zinc levels in a dermestid shrimp. Bull. Environ. Contamin. Toxicol. 26: 219-223.

Barbaro, A., A. Franceson, B. Polo and M. Bilio. 1978. Balanus amphitrite (Cirripedia: Thoracica)--A potential indicator of fluoride, copper, lead, chromium, and mercury in North Adriatic lagoons. Mar. Biol. 46: 247-257.

Barber, S. and J.H. Trefry. 1981. Balanus eburneus: A sensitive indicator of copper and zinc pollution in the coastal zone. Bull. Environ. Contamin. Toxicol. 27: 654-659.

Boehm, P.D. and J.G. Quinn. 1977. The persistence of chronically accumulated hydrocarbons in the hard shell clam Mercenaria mercenaria. Mar. Biol. 44: 227-233.

Bryan, G.W. and L.G. Hummerstone. 1973a. Brown seaweed as an indicator of heavy metals in estuaries in South-West England. J. Mar. Biol. Assoc. (U.K.) 53: 705-720.

Bryan, G.W. and L.G. Hummerstone. 1973b. Adaptation of the polychaete Nereis diversicolor to estuarine sediments containing high concentrations of zinc and cadmium. J. Mar. Biol. Assoc. U.K. 53: 839-857.

Burns, K.A. and J.L. Smith. 1981. Biological monitoring of ambient water quality: The case for using bivalves as sentinel organisms for monitoring petroleum pollution in coastal waters. Estuar. Coast. Shelf Sci. 13: 433-443.

Butler, P.A., R. Huggett, K. Macek, R. Reinert and R. Risebrough. 1978. Synthetic organics. In: E.D. Goldberg (ed.), Proceedings of a Workshop on Scientific Problems Relating to Ocean Pollution, Estes Park, Colo., July 10-14, 1978. NOAA Environmental Research Laboratories, Boulder, Colo.

Chester, R. and J.H. Stoner. 1974. The distribution of zinc, nickel, manganese, cadmium, copper and iron in some surface waters from the world ocean. Mar. Chem. 2: 17-32.

Davenport, J. 1977. A study of the effects of copper applied continuously and discontinuously to specimens of Mytilus edulis (L.) exposed to steady and fluctuating salinity levels. J. Mar. Biol. Assoc. U.K. 57: 63-74.

Davies, I.M. and J.M. Pirie. 1978. The mussel Mytilus edulis as a bio-assay organism for mercury in seawater. Mar. Pollut. Bull. 9: 128-132.

Davies, I.M. and J.M. Pirie. 1980. Evaluation of a "mussel watch" project for heavy metals in Scottish coastal waters. Mar. Biol. 57: 87-93.

de Kock, W.C. and J. Kuiper. 1981. Possibilities for marine pollution research at the ecosystem level. Chemosphere 10: 575-603.

DiSalvo, L.H., H.E. Guard and L. Hunter. 1975. Tissue hydrocarbon burden of mussels as potential monitor of environmental hydrocarbon insult. Environ. Sci. Tech. 9: 247-251.

Dix, T.G., A. Martin, G.M. Ayling, K.C. Wilson and D.P. Ratkowsky. 1976. Sand flathead (Platycephalus bassensis) as an indicator species for mercury pollution in Tasmanian waters. Mar. Poll. Bull. 6: 142-144.

Eganhouse, R.P. and D.R. Young. 1978. In situ uptake of mercury by the intertidal mussel, Mytilus californianus. Mar. Pollut. Bull. 9: 214-217.

Eide, I. and A. Jensen. 1979. Application of in situ cage cultures of phytoplankton for monitoring heavy metal pollution in two Norwegian fjords. J. Exp Mar. Biol. Ecol. 37: 271-286.

Farrington, J.W., R.W. Risebrough, L. Parker, A.C. Davis, B. de Lappe, J.K. Winters, D. Boatwright and M.M. Frew. 1982a. Hydrocarbons, Polychlorinated biphenyls, and DDE in mussels and oysters from the U.S. coast, 1976-1978--The Mussel Watch. Tech. Rep. WHOI-82-42, Woods Hole Oceanographic Institution, Woods Hole, Mass.

Farrington, J.W., E.D. Goldberg, R.W. Risebrough, J.H. Martin and V.T. Bowen. 1982b. U.S. "Mussel Watch" 1976-1978: An overview of the trace metal, DDE, PCB, hydrocarbon, and artificial radionuclide data. Environ. Sci. Tech.

Flew, E.M. 1977. Radioactive waste disposal by UKAEA establishments in 1976 and associated environmental monitoring results Rep. AERE-R8779, United Kingdom Atomic Energy Authority, Harwell, U.K.

Fossato, V.U. 1975. Elimination of hydrocarbons by mussels Mar. Poll. Bull. 6: 7-10.

Fossato, V.U. and W.J. Canzonier. 1976. Hydrocarbon uptake and loss by the mussel Mytilus edulis. Mar. Biol. 36: 243-250.

Fowler, S.W., M. Heyraud and J. LaRosa. 1978. Factors affecting methyl and inorganic mercury dynamics in mussels and shrimp. Mar. Biol. 46: 267-276.

George, S.G. and T.L. Coombs. 1977. The effects of chelating agents on the uptake and accumulation of cadmium by Mytilus edulis. Mar. Biol. 39: 261-268.

Goldberg, E.D. 1976. Toward a global monitoring program for transuranics and other marine pollutants. In: M.W. Miller and J.N. Stannard (eds.), Environmental Toxicity of Aquatic Radionuclides: Models and Mechanisms. Ann Arbor Science Publ., Ann Arbor, Mich.

Goldberg, E.D. 1979. The surveillance of coastal marine waters with bivalves--The Mussel Watch. In: J. Albaignes (ed.), Proceedings of the International Congress on Analytical Techniques in Environmental Chemistry. Pergamon Press, Oxford.

Goldberg, E.D., V.T. Bowen, J.W. Farrington, G. Harvey, J.H. Martin, P.L. Parker, R.W. Risebrough, W. Robertson, E. Schneider and E. Gamble. 1978. The Mussel Watch. Environ. Conserv. 5: 101-125.

Hansen, M.M. 1980. A critical evaluation of the use of chemical analysis of organisms to monitor seawater chemistry. Marine Environmental Quality Committee Report No. CM 1980/E: 53, International Council for the Exploration of the Sea, Copenhagen, Denmark.

Ireland, M.P. 1973. Result of fluvial zinc pollution on the zinc content of littoral and sub-littoral organisms in Cardigan Bay, Wales. Environ. Pollut. 4: 27-35.

Ireland, M.P. 1974. Variations in the zinc, copper, manganese, and lead content of Balanus balanoides in Cardigan Bay, Wales. Environ. Pollut. 7: 65-75.

Jackim, E., G. Morrison and R. Steele. 1977. Effects of environmental factors on radiocadmium uptake by four species of marine bivalves. Mar. Biol. 40: 303-308.

Janssen, H.H. and N. Scholz. 1979. Uptake and cellular distribution of cadmium in Mytilus edulis. Mar. Biol. 55: 133-141.

Johnels, A.G., T. Westermark, W. Berg, P.I. Persson and B. Sjostrand. 1967. Pike (Esox lusius L.) and some other aquatic organisms in Sweden as indicators of mercury contamination in the environment. Oikos 18: 323-333.

Klumpp, D.W. and C. Burden-Jones. 1982. Investigations of the potential of bivalve molluscs as indicators of heavy metal levels in tropical marine waters. Aust. J. Mar. Freshwater Res. 33: 285-300.

Koepp, S., E.D. Santoro, R. Zimmer and J.E. Nadeau. 1981. Selected metal concentrations in Mytilus edulis transplanted in and around a dredged material disposal site in the New York Bight. Marine Environmental Quality Committee Report No. CM 1981/E:18, International Council for the Exploration of the Sea, Copenhagen, Denmark.

Latouche, Y.D. and M.C. Mix. 1982. The effects of depuration, size and sex on trace metal levels in bay mussels. Mar Pollut. Bull. 13: 27-29.

Lee, R.F., R. Sauerheber and A.A. Benson. 1972. Petroleum hydrocarbons: Uptake and discharge by the marine mussel Mytilus edulis. Science 177: 344-346.

Lytle, J.S., and T.F. Lytle. 1977. Sediment hydrocarbons as environmental indicators in the northeast Gulf of Mexico. In: D.A. Wolfe (ed.), Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms. Pergamon Press, New York.

Majori, L. and F. Petronio. 1973. Marine Pollution by metals and their accumulation by biological indicators (accumulation factor). Rev. Int. Oceanogr. Med. 31-32: 55-90.

Malins, D.C., H.O. Hodgins, U. Varanasi, W.D. MacLeod, Jr., B.B. McCain and S.L. Chan. 1983. Chemical measurements of organic pollutants and effects criteria. (this volume)

Martin, M., and R. Severeid. 1984. Mussel Watch monitoring for the assessment of trace toxic constituents in California marine waters. (This volume.)

NAS (National Academy of Sciences). 1980. Report of the International "Mussel Watch" Workshop, Barcelona Spain, December 4-7, 1978. National Academy of Sciences, Washington, D.C.

Navrot, J., A.J. Amiel and J. Kronfeld. 1974. Patella vulgata: A biological monitor of coastal metal pollution--A preliminary study. Environ. Pollut. 7: 303-308.

Papadopoulou, C. and G.D. Kanias. 1977. Tunicate species as marine pollution indicators. Mar. Pollut. Bull. 8: 229-231.

Pentreath, R.J. 1976. The monitoring of radionuclides. In: Biological Accumulators, pp. 9-26. Food and Agriculture Organization of the United Nations, Rome.

Phillips, D.J.H. 1976a. The common mussel Mytilus edulis as an indicator of pollution by zinc, cadmium, lead, and copper. I. Effects of environmental variables on uptake of metals. Mar. Biol. 38: 59-69.

Phillips, D.J.H. 1976b. The common mussel Mytilus edulis as an indicator of pollution by zinc, cadmium, lead and copper. II. Relationship of metals in the mussels to those discharged by industry. Mar. Biol. 38: 71-80.

Phillips, D.J.H. 1977a. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments--A review. Environ. Poll. 13: 281-317.

Phillips, D.J.H. 1977b.- Effects of salinity on the net uptake of zinc by the common mussel Mytilus edulis. Mar. Biol. 41: 79-88.

Phillips, D.J.H. 1977c. The common mussel Mytilus edulis as an indicator of trace metals in Scandinavian waters. I. Zinc. and cadmium. Mar. Biol. 43: 283-292.

Phillips, D.J.H. 1978a. Use of biological indicator organisms to quantitate organochlorine pollutants in aquatic environments--A review. Environ. Pollut. 16: 167-229.

Phillips, D.J.H. 1978b. The common mussel Mytilus edulis as an indicator of trace metals in Scandinavian waters. II. Lead, iron, and manganese. Mar. Biol. 46: 147-156.

Phillips, D.J.H. 1980. Quantitative Aquatic Biological Indicators. Applied Science Publ., London.

Phillips, D.J.H. and W.W.S. Yim. 1981. A comparative evaluation of oysters, mussels and sediments as indicators of trace metals in Hong Kong water. Mar. Ecol. Prog. Ser. 6: 285-293.

Popham, J.D. and J.M. D'Auria. 1982. A new sentinel organism for vanadium and titanium. Mar. Pollut. Bull. 13: 25-27.

Portmann, J.E. 1970. Monitoring of organo-chlorine residues in fish from around England and Wales, with special reference to polychlorinated biphenyls (PCBs). Report No. CM 1970/E:9, Internationa Council for the Exploration of the Sea, Copenhagen, Denmark.

Portmann, J.E. 1972. The levels of certain metals in fish from coastal waters around England and Wales. Aquaculture 1: 91-96.

Preston, A. 1973. Heavy metals in British waters. Nature 242: 95-97.

Preston, A., D.F. Jefferies, J.W.R. Dutton, B.R. Harvey and A.K. Steele. 1972. British Isles coastal waters: The concentrations of selected heavy metals in sea water, suspended matter and biological indicators—A survey. Environ. Pollut. 3: 69-82.

Risebrough, R.W., B.W. de Lappe, W. Walker, II, A.M. Springer, M. Firestone-Gillis, J. Lane, W. Sistek, E.F. Letterman, J.C. Shropshire, R. Vick and A.S. Newton. 1979. Patterns of hydrocarbon contamination in California coastal waters. In: J. AlBaiges (ed.), Proceedings of the International Congress of Analytical Techniques in Environmental Chemistry: 33-40. Pergamon Press, Oxford.

Roesijadi, G., A.S. Drum, J.M. Thomas and G.W. Fellingham. 1982. Enhanced mercury tolerance in marine mussels and relationship to low molecular weight, mercury-binding proteins. Mar. Pollut. Bull. 13: 250-253.

Simpson, D.C. and M. Lockwood. 1981. Pollution monitoring: Its role in marine environmental management. In: Conference Record, OCEANS 81, Boston, Mass., Sept. 16-18, 1981, pp. 715-719. Pub. No. 81CH1685-7, Institute of Electrical and Electronic Engineers, New York.

Stegeman, J.J. and J.M. Teal. 1973. Accumulation, release, and retention of petroleum hydrocarbons by the oyster Crassostrea virginia. Mar. Biol. 22: 37-44.

Unlu, M.Y. and S.W. Fowler. 1979. Factors affecting the flux of arsenic through the mussel Mytilus galloprovincialis. Mar Biol. 51: 209-219.

Walker, G., P.S. Rainbow, P. Foster and D.J. Crisp. 1975. Barnacles: Possible indicators of zinc pollution? Mar. Biol. 30: 57-65.

Weiss, C.M. 1965. Use of fish to detect organic insecticides in water. J. Water Pollut. Control. Fed. 37: 647-658.

White, H.H. and M. Lockwood. 1982. A holistic approach to solving marine pollution problems: The basis for wise management. Presented at International Society of Ecotoxicology and Environmental Safety, Tokyo, Japan, September 6-7, 1982.

Factors Affecting Bioaccumulation of Organic Pollutants by Marine Animals

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INTRODUCTION

Accumulation of organic pollutants by marine animals depends on various pharmacokinetic processes including uptake, distribution in tissues, metabolism and elimination. This paper deals with the effects of these processes on bioaccumulation and, in turn, the effects on these processes of various biological, chemical and physical factors.

UPTAKE

The mechanism of entry of lipid-soluble pollutants into the body is simple non-mediated transport across epithelial layers (Kotyk 1973), the sites of uptake being the integument, respiratory surfaces (gills) and gut. Marine animals can take up organic pollutants by each of these routes (Harris et al. 1977; Lee et al. 1976; Neff et al. 1976; McClain et al. 1978).

The form of the compound (i.e., dissolved, sorbed to particles or contained in food) and the site of entry significantly influence the bioavailability of the pollutant compound. As particles deliver pollutants to membrane vesicles, the rate-limiting step is the desorption from the particle (Lackowicz et al. 1980; Bevan et al. 1981). Such processes are certain to be important in the absorption of pollutants through membranes of marine animals.

The degree of correlation between accumulation of lipophilic foreign compounds by fish and octanol-water partition coefficients (Veith et al. 1979) supports the idea that a major mechanism of entry in fish is partitioning into and uptake from water through the gills (Hunn and Allen 1974).

Gill tissues of bivalves have a micellar layer on their surfaces which adsorbs hydrophobic compounds (Pasteels 1968). Since many bivalves filter large volumes of water while feeding, pollutant concentrations in gill tissues can be several orders of magnitude greater than concentrations in the water. Clams, oysters and mussels differ in their rates of hydrocarbon uptake, possibly because of differences in filtering rates and amounts of body lipids (Clark and Finely 1974, Neff et al. 1976). Oysters with high lipid content have been found to take up more fuel oil (314 $\mu g\ g^{-1}$) from the water than low lipid oysters (161 $\mu g\ g^{-1}$) (Stegeman and Teal 1973).

Various studies have indicated that the nature of the compound determines the importance of pollutant uptake by way of the gut. In fish the uptake of hexadecane from contaminated food differed markedly from that of benzo(a)pyrene (Whittle et al. 1977). In copepods (Calanus helgolandicus) dietary uptake of naphthalene was more important than uptake from the water (Harris et al. 1977, Corner et al. 1976). In blue crabs (Callinectes sapidus) hydrocarbons in the food were only slowly taken up and, in fact, most were voided in the feces (Lee et al. 1976). Uptake of pollutants through the gills occurs in fish (Lee et al. 1972a) but uptake from the food is often low (Whittle et al. 1977). Benthic fish can take up petroleum hydrocarbon from the sediment (Varanasi and Gmur 1981), probably by desorption from the sediment. Polychlorinated biphenyls were taken up from sediment by fiddler crab (Uca minax) and pink shrimp (Penaeus duorarum) (Nimmo et al. 1971).

In grass shrimp Palaemontes pugio exposed to pentachlorophenol, increased mortalities occurred during the early post-ecdysal period because of a large increase in the uptake of pentachlorophenol immediately after molting (Rao et al. 1979). Thus, uptake rates of pollutants can also vary depending on the life history stage of an animal. Temperature and salinity can affect uptake. Greater uptake of polynuclear aromatic hydrocarbons occurred in clams at reduced temperatures, but changes in salinity had little or no effect (Fucik and Neff 1977). The elimination rate was not affected by temperature or salinity.

DISTRIBUTION IN TISSUES

The distribution of pollutants within vertebrates is accomplished largely by circulation of pollutants associated with components of the blood, that is, serum proteins and lipoproteins. Association of chlorinated hydrocarbons with serum lipoproteins in fish has been described (Plack et al. 1979). The nature of the compound and its association with serum components may influence deposition in various tissues. Circulation associated with serum constituents is also likely to be important in many invertebrates.

Although, in general, the levels of pollutants in exposed fish are greatest in the liver, petroleum hydrocarbons reach high levels in neural tissues of fish after exposure to petroleum hydrocarbons (Neff et al. 1976; Collier et al. 1980). This seems to be consistent with the high lipid content and vascularization of neural tissues. Roubal et al. (1977) found that higher molecular weight aromatics were retained more than lower molecular weight aromatics in the brain of coho salmon (O. kisutch), a pattern also seen in other tissues such as the hepatopancreas and digestive gland. Studies with shrimp showed that DDT was rapidly taken up from the water and concentrated in the hepatopancreas (Nimmo et al. 1971).

METABOLISM—IN VITRO

An important process in the evolution of marine animals was the development of detoxification systems that allow them to metabolize foreign organic compounds. Many foreign compounds, often referred to as xenobiotics, are lipophilic and are converted by reduction, oxidation, hydrolysis or conjugation to more water soluble metabolites, which facilitates their elimination from the animal. Cytochrome P-450 mediated mixed-function oxygenase systems (MFO) oxidizes foreign compounds by hydroxylation, O-dealkylation, N-dealkylation or epoxidation reactions. The polar metabolites formed can be conjugated with sugars, sulfate or peptides and disposed of in the urine and feces.

The characteristics, functions and presence of P-450 systems in aquatic, principally marine, species have been reviewed in detail (Bend and James 1978, Stegeman 1981; Lee 1981). The liver is the primary site of pollutant metabolism in fish; the hepatopancreas serves this function in invertebrates. MFO activity has been found in fish whenever sought. There have been fewer studies to determine the presence or absence of MFO systems in marine invertebrates. To date 18 marine invertebrate species, belonging to four phyla (Anelida, Arthropoda, Echinodermata and Mollusca) are known to contain MFO activity in their hepatopancreas, digestive gland or other tissues (Lee 1981).

The levels of P-450 in a given organ and rates of monoxygenase reactions catalyzed can change with many environmental and physiological variables. This may be due in large part to changes in the complement or set of isozymes of P-450, each of which may preferentially transform a limited set of substrates. The rate of pollutant metabolism and elimination can be influenced by a variety of environmental and physiological factors that might affect the catalytic function of the P-450 system, alter the pharmacokinetics of parent or product, or affect the response of an animal to inducers of P-450. One of the most important factors is the induction of cytochrome P-450 by environmental pollutants such as aro-

matic hydrocarbons. Induction in numerous marine and freshwater fish has been described (e.g., Payne and Penrose 1975; Bend et al. 1977) including induction in embryonic and larval forms (Binder and Stegeman 1980). Induction has also been noted in polychaetes and crabs after exposure to xenobiotics (Lee 1981; Lee et al. 1982). Induction can result in increased rates of pollutant metabolism in liver and some extrahepatic tissues of fish and some invertebrates.

There is evidence that induction occurs in fish after exposure to petroleum in the environment (Payne 1976; Kurelec et al. 1977; Stegeman 1978). There is also increasing evidence of widespread induction of P-450 in fish by chemicals of unknown origin in the environment (Bend 1980; Stegeman et al. 1981). The causes of such induction have not been established, although there have been correlations with polycyclic aromatic hydrocarbons in the sediment. In the polychaete Capitella capitata exposed to crude oil, the third generation had much higher mixed function oxygenase activity than the first or second generations (Lee 1981). Grassle and Grassle (1976, 1977) showed that on the basis of electrophoretic patterns, C. capitata is a complex of at least six sibling species. Thus exposure to oil may result in selection for species or strains that are resistant to oil because of high mixed function oxygenase activity.

METABOLISM—IN VIVO

While one significant route of excretion of metabolites in fish is the gall bladder (Lee et al. 1972b; Melancon and Lech 1978; Collier et al. 1978; Solbakken et al. 1980) the liver is the major site of metabolism of xenobiotics. Consequently, it has been suggested that analysis of bile might serve to monitor exposure of fish to various foreign chemicals (Statham et al. 1976). Most of the metabolites appearing in bile are conjugated derivatives of oxidized metabolics of the pollutant. Metabolites eliminated by other routes, either gill, urine or skin, have also been shown for fish and invertebrates.

Many marine animals have enzyme systems to metabolize various xenobiotics, including polycyclic aromatic hydrocarbons and monohalogenated compounds. However, some of the xenobiotics found in the ocean, including highly chlorinated hydrocarbons (e.g., mirex, polychlorinated biphenyls and hexachlorobenzene) are highly resistant to biotransformation. Thus, polychlorinated compounds would be expected to accumulate and persist in the tissues of marine animals. Seven days after injection of mirex into the flounder Pseudopleuronectes americanus, no metabolism or excretion of the compound was detected, whereas 2% of injected DDT had been excreted as polar metabolites (Pritchard et al. 1973). A review of DDT uptake by fish reveals that different species store and metabolize DDT at very different rates. The dogfish Squalus acanthias

showed little metabolism of DDT in 17 days; storage occurred in the fatty livers (Dvorchik 1971; Dvorchik and Maren 1972). In contrast, DDT taken up from the water by the eel Anguilla rostrata was rapidly metabolized (18% in 6 hours); most DDT was stored in the blood, gill and liver (Guarino et al. 1971). Thus, in analytical studies of organochlorine pesticides in fish, it is important to consider species differences when selecting fish to examine.

Injection of ^{14}C-pentachlorobiphenyl into the lobster Homarus americanus resulted in the hepatopancreas having most of the activity in the form of unchanged pentachlorobiphenyl (Bend et al. 1973). The feces began to show hydroxylated derivatives after 2 weeks, and after 8 weeks the hepatopancreas had lost 60% of its activity. The half life of the compound was 45 and 4 days in the hepatopancreas and muscle tissues, respectively.

Aromatic hydrocarbons are metabolized by most groups of animals, but transformation rates differ greatly between groups, being extremely slow in bivalves and relatively fast in fish. The final equilibrium concentration within tissues depends on the ability of the animal to metabolize the hydrocarbon and physical-chemical properties of the compounds which affects the animals rate of uptake and elimination. In many cases transformation rates may balance uptake rates with low apparent bioaccumulation.

Several studies have disclosed an effect of temperature on disposition of naphthalene in vivo. In coho salmon (Onchorhynchus ksutch) and starry flounder (Platichthys stellatus) there was a pronounced increase in the retention of naphthalene and its metabolites in tissues of animals at lowered temperatures (Collier et al. 1978; Varanasi et al. 1981). Moreover, lowered temperature also shifts the pattern of naphthalene metabolism in starry flounder, resulting in a substantially greater proportion of glucuronides and 1,2-dihydrodiol in liver at the lower temperature a week after exposure (Varanasi et al. 1981). Further evidence also indicates that seasonal and sex-linked factors can influence the metabolism of foreign compounds and responses to inducers (Stegeman and Chevion 1980; Forlin 1980). Moreover, the many different types of xenobiotics present in the environment can influence the metabolism, disposition and effects on each other. Gruger et al. (1981) found that the pattern of metabolism of 2,3-dimethylnaphthalene in a starry flounder was substantially altered by the presence of either naphthalene or cresol in the animal. Such results have implication for any extrapolation of bioaccumulation of compounds in the environment based on studies of biotransformation of single compounds.

ELIMINATION

Generally, for studies of elimination animals are allowed to accumulate the pollutant for some period of time and are then transferred to "clean" seawater to allow depuration or elimination of the compound. Elimination rates can be fast or slow depending to some extent on the hydrophobic properties of the compound, that is, the octanol-water partition coefficient. The half-lives of accumulated compounds are influenced by numerous factors and can be as long as hours, weeks, months or even years. Elimination can often be rapid in an early phase but slow during a second phase. Thus, depuration studies with filtering bivalves containing petroleum showed an initial rapid discharge that resulted in short half-lives for accumulated petroleum; however, a small amount was retained for a long period after the initial rapid discharge (Clark and Finley, 1975; Lee et al. 1972a; Stegeman and Teal 1973). Mussels from the oil-polluted Lagoon of Venice which initially contained 250 $\mu g\ g^{-1}$ retained 30 $\mu g\ g^{-1}$ of petroleum hydrocarbons after 56 days of depuration (Fossato 1975). Similarly, the elimination of accumulated photochlordane from bluegill fish was rapid in the first three weeks and slower in a second phase (Sudershan and Khan 1980). Elimination occurs by several routes. One often suggested for fish is direct partitioning through the gills into water. Thomas and Rice (1981) present evidence that substantial proportions of naphthalene and toluene could be excreted directly through the gills. In fish and invertebrates, stored xenobitics may be mobilized and subsequently transported to and eventually shed in lipid-rich eggs. Such a pathway has been demonstrated for polychlorinated biphenyls in fish (Guiney et al. 1979). Biliary, renal and epidermal routes may be used for disposition of xenobiotics. For example, substantial amounts of napthalene were seen in bile of fish exposed to the compound (Roubal et al. 1977; Varanasi et al. 1979).

In copepods, depuration of naphthalene taken up from the food was significantly slower than naphthalene taken up from the water (Corner et al. 1976). In benthic crustaceans, polychaetes and sipunculids, exposure to petroleum or individual hydrocarbons followed by transfer to clean sea water resulted in the accumulated hydrocarbons being released within 2 to 10 days (Rossi 1977; Anderson et al. 1977; Lyes 1979; Rossi and Anderson 1977; Cox et al. 1975; Lee et al. 1976; Neff et al. 1976). In one experiment when the polychaete Neanthes arenaceodenta was exposed to fuel oil and then transferred to clean water (Rossi and Anderson 1977) males gradually discharged naphthalenes to undetectable levels whereas gravid females retained most of the accumulated naphthalenes. After egg release there was a dramatic decrease in naphthalenes of the females due to release of naphthalenes that had accumulated in the eggs.

When mussels and clams collected from heavily oiled areas are transferred to clean water, the hydrocarbon depuration rate can be slow, the hydrocarbons having half-lives of up to several weeks (DiSalvo et al. 1975; Vandermeulen et al. 1977). It has been suggested that in bivalves from chronically polluted areas the hydrocarbons enter stable compartments from which release is very slow. Slow depuration by bivalves from oil polluted waters may occur if the animals are in a weakened physiological state. Another factor affecting bioaccumulation and release of pollutants by bivalves is the peaks in lipid reserves prior to spawning. Pollutants accumulated in the lipid-rich gametes will be discharged during gamete release. Thus, the seasonal reproductive cycle is an important factor in pollutant accumulation and discharge. Maxima for benzo(a)pyrene and perylene concentrations in Mytilus edulis from the Lagoon of Venice, Italy, occurred in January and minima in May (Fossato et al. 1979). Spawning took place from March to April.

PHARMACODYNAMICS OF UPTAKE AND ELIMINATION

Metcalf (1977) has discussed the pharmacodynamics of uptake and elimination of xenobiotics by aquatic animals. Uptake is generally a first-order process so that the rate of uptake from water is a function of the concentration of xenobiotic times the length of exposure. Both uptake and elimination occur simultaneously so the concentration, c_o, measured in an animal can be represented by the equation

$$c_o = c_e k_a / k_c V \, (1 - e^{-k_c t})$$

where k_c is the rate constant for clearance of the xenobiotic due to metabolism and elimination; k_a is the uptake or absorption constant which is determined by the lipid/water partition coefficient of the compound; c_e is the concentration of the xenobiotic in the water and V is the animal mass or volume.

If the rate of uptake is constant and the rate of clearance is exponential, the concentration, c_o, will increase until a steady state is reached where elimination is equal to uptake. As t becomes infinite, $1-e^{-k_c t}$ becomes unity, and at the steady state

$$c_o = c_e k_a / k_c V.$$

Goldstein (1969) refers to this as the plateau principle. For compounds such as polychlorinated biphenyls where lipid/water partition coefficients are very high and degradation/elimination are slow, bioconcentration is a linear function with age. The term k_c for a particular compound can be influenced by such factors as temperature, salinity and reproductive status.

SUMMARY AND RECOMMENDATIONS

1. The extent of bioaccumulation of a particular organic pollutant by marine animals is a reflection of the relative importance of uptake, metabolism and elimination of that compound. Because they filter large volumes of water and have limited ability to metabolize many organic pollutants, the filtering bivalves (e.g., oysters and mussels) have been used to monitor marine waters for toxic organic chemicals. Knowing the consequences of elevated pollutant concentrations in bivalves (e.g., scope for growth) may be useful in that concentrations of a pollutant can be related to a biological effect.

2. One of the limitations in using marine animals for monitoring studies is the fact that pollutants can be stored in lipid-rich eggs. Thus, if animals are analyzed just before and after spawning, the concentrations of the pollutants in an animal can be quite different. It may prove useful to analyze eggs for pollutants where pollutant concentrations could be related to effects on reproduction or on juvenile growth rates.

3. The extent of bioaccumulation by a marine animal is related to the hydrophobic properties of the compound, that is, the octanol-water partition coefficient. Compounds with very low water solubility (e.g., mirex, benzo(a)pyrene) are concentrated in animal tissues several orders of magnitude over their concentration in the water. Analysis of such compounds in animal tissues has proved useful for a number of monitoring studies. Compounds with high water solubility (e.g., phenols) are accumulated in animals only to a very limited extent. Elimination is very rapid, so that monitoring for this class of compounds can be carried out by analysis of the water.

4. Polychaetes, crustaceans and fish, because of their ability to metabolize a variety of organic pollutants, are less useful in bioaccumulation type monitoring studies. However, the response of their cytochrome P-450 dependent mixed-function oxygenase system, which can metabolize organic pollutants, has useful applications. Fish and some invertebrates show increased mixed-function oxygenase activity and cytochrome P-450 content as a result of exposure to such pollutants as petroleum compounds or polychlorinated biphenyls. In addition, different classes of pollutants result in the production of different cytochromes P-450. To demonstrate the usefulness of these responses for environmental pollution studies, more work is needed, for example, early warning programs.

REFERENCES

Anderson, J.W., L.J. Moore, J.W. Blaylock, D.L. Woodruff and S.L. Kiessen. 1977. Bioavailability of sediment-sorbed naphthalenes to the sipunculid worm, Phascoloscoma agassizii. In: D.A. Wolfe (ed.), Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms, pp. 276-285. Pergamon Press, New York.

Bend, J.R. 1980. Induction of drug-metabolizing enzymes by polycyclic aromatic hydrocarbons: Mechanism and some implications in environmental health research. In: Environmental Chemicals, Enzymic Mechanisms and Human Disease, pp. 83-99. Ciba Foundation Symposium No. 76. Excerpta Medica, Amsterdam, Netherlands.

Bend, J.R. and M.O. James. 1978. Xenobiotic metabolism in marine and freshwater species. In: D.C. Malins and J.R. Sargent (eds.), Biochemical and Biophysical Perspectives in Marine Biology, Vol. 4, pp. 128-188. Academic Press, New York.

Bend, J.R., S.G. Bend, A.M. Guarino, D.P. Rall and J.R. Fouts. 1973. Distribution of ^{14}C-2, 4, 5, 2', 5'-pentachlorobiphenyl in the lobster Homarus americanus at various times after a single injection into the pericardial sinus. Bull. Mt. Desert Isl. Biol. Lab. 13: 1-4.

Bend, J.R., M.O. James and P.M. Dansette. 1977. In vitro metabolism of xenobiotics in some marine animals. Ann. N.Y. Acad. Sci. 298: 505-521.

Bevan, D.R., S.C. Riemer and J.R. Lakowicz. 1981. Effects of particulate matter on rates of membrane uptake of polynuclear aromatic hydrocarbons. J. Toxicol. Environ. Health 8: 241-250.

Binder, R.L. and J.J. Stegeman. 1980. Induction of aryl hydrocarbon hydroxylase activity in embryos of an estuarine fish. Biochem. Pharmacol. 29: 949-951.

Clark, R.C. and J.S. Finley. 1974. Acute effects of outboard motor effluent on two marine shellfish. Environ. Sci. Technol. 8: 1009-1014.

Clark, R.C. and J.S. Finley. 1975. Uptake and loss of petroleum hydrocarbons by mussels, Mytilus edulis, in laboratory experiments. Fish. Bull. 73: 508-515.

Collier, T.K., L.C. Thomas and D.C. Malins. 1978. Influence of environmental temperature on disposition of dietary naphthalene in coho salmon (Oncorhynchus kisutch): Isolation and identification of individual metabolites. Comp. Biochem. Physiol. 61C: 23-28.

Collier, T.K., M.K. Krahn and D.C. Malins. 1980. The disposition of naphthalene and its metabolites in the brain of rainbow trout (Salmo gairdneri). Environ. Res. 23: 35-41.

Corner, E.D.S., R.P. Harris, C.C. Kilvington and S.C.M. O'Hara. 1976. Petroleum compounds in the marine food web: Short-term experiments on the fate of naphthalene in Calanus. J. Mar. Biol. Assoc. U.K. 56: 121-133.

Cox, B.A., J.W. Anderson and J.C. Parker. 1975. An experimental oil spill: The distribution of aromatic hydrocarbons in the water, sediment, and animal tissues within a shrimp pond. In: Proceedings 1975 Conference on Prevention and Control of Oil Pollution, Mar. 25-27, 1975, San Francisco, Calif., pp. 607-612. American Petroleum Inst., Washington, D.C.

DiSalvo, L.H., H.E. Guard and L. Hunter. 1975. Tissue hydrocarbon burden of mussels as potential monitor of environmental hydrocarbon insult. Environ. Sci. Tech. 9: 247-257.

Dvorchik, B.H. 1971. Binding, fate and toxicity studies of ^{14}C-2,2-bis (p-chlorophenyl)-1, 1-trichloroethane (p, p'-DDT) in the dogfish Squalus acanthias. Bull. Mt. Desert Island Biolog. Lab. 11: 7-10.

Dvorchik, B.H. and T.H. Maren. 1972. The fate of p,p'-DDT (2, 2-bis (p-chlorophenyl)-1, 1, 1-trichloroethane) in the dogfish, Squalus acanthias. Comp. Biochem. Physiol. 42A: 205-210.

Forlin, L. 1980. Effects of Clophen A50, 3-methylcholanthrene, pregnenolone 16a-carbonitrile and phenobarbital on the hepatic microsomal cytochrome P-450 dependent monooxygenase system in rainbow trout, Salmo gairdneri, of different age and sex. Toxicol. Appl. Pharmacol. 54: 420-430.

Fossato, V.U. 1975. Elimination of hydrocarbons by mussels. Mar. Pollut. Bull. 6: 7-10.

Fossato, V.U., C. Nasci and F. Dolci. 1979. 3,4-Benzopyrene and perylene in mussels, Mytilus sp., from the Laguna Veneta, Northeast Italy. Mar. Environ. Res. 2: 47-53.

Fucik, K.W. and J.M. Neff. 1977. Effect of temperature and salinity of naphthalene uptake in the temperate clam, Rangia cuneata, and the boreal clam, Protothaca staminea. In: D.A. Wolfe (ed.), Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems, pp. 305-312. Pergamon Press, New York.

Goldstein, J.A. 1969. The structure-activity relationship of halogenated biphenyls as enzyme inducers. Ann. New York Acad. Sci. 320: 164-178.

Grassle, J.F. and J.P. Grassle. 1977. Temporal adaptations in sibling species of Capitella. In: B. Coull (ed.), Ecology of Marine Benthos, pp. 177-189. Univ. of South Carolina Press, Columbia, S.C.

Grassle, J.P. and J.F. Grassle. 1976. Sibling species in the marine pollution indicator Capitella (polychaete). Science 192: 567-569.

Gruger, E.H., Jr., J.V. Schnell, P.S. Fraser, D.W. Brown and D.C. Malins. 1981. Metabolism of 2,6-dimethylnaphthalene in starry flounder (Platichthys stellatus) exposed to naphthalene and p-cresol. Aquat. Toxicol. 1:37-48.

Guarino, A.M., R.H. Janicki and W.B. Kinter. 1971. Distribution and metabolism of ^{14}C-DDT in the eel (Anguilla rostrata) after six hours of uptake from ambient water. Bull. Mt. Desert Island Biol. Lab. 11: 23-24.

Guiney, P.D., M.J. Melancon, Jr., J.J. Lech and K.E. Peterson. 1979. Effects of egg and sperm maturation and spawning on the distribution and elimination of a polychlorinated biphenyl in rainbow trout (Salmo gairdneri). Toxicol. Appl. Pharmacol. 53: 521-529.

Harris, R.P., V. Berdugo, E.D.S. Corner, C.C. Kilvington and S.C.M. O'Hara. 1977. Factors affecting the retention of a petroleum hydrocarbon by marine planktonic copepods. In: D.A. Wolfe (ed.), Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms, pp. 286-304. Pergamon Press, New York.

Hunn, J.B. and J.L. Allen. 1974. Movement of drugs across the gills of fishes. Ann. Rev. Pharmacol. 14: 47-55.

Kotyk, A. 1973. Mechanisms of non-electrolyte transport. Biochim. Biophys. Acta 300: 183-210.

Kurelec, B., S. Britvic, M. Rijavec, W.E.G. Muller and R.K. Zahn. 1977. Benzo(a)pyrene monooxygenase induction in marine fish--Molecular response to oil pollution. Mar. Biol. 44: 211-216.

Lakowicz, J.R., D.R. Bevan and S.C. Riemer. 1980. Transport of a carcinogen, benzo(a)pyrene, from particulates to lipid bilayers: A model for the fate of particle-adsorbed polynuclear aromatic hydrocarbons which are retained in the lungs. Biochim. Biophys. Acta 629: 243-258.

Lee, R.F. 1981. Mixed function oxygenases (MFO) in marine invertebrates. Mar. Biol. Lett. 2: 87-105.

Lee, R.F., G. Sauerheber and A.A. Benson. 1972a. Petroleum hydrocarbons: uptake and discharge by the marine mussel, Science 177: 344-346.

Lee, R.F., R. Sauerheber and G.H. Dobbs. 1972b. Uptake, metabolism, and discharge of polycyclic aromatic hydrocarbons by marine fish. Mar. Biol. 17: 201-208.

Lee, R.F., C. Ryan and M.L. Neuhauser. 1976. Fate of petroleum hydrocarbons taken up from food and water by the blue crab, Callinectes sapidus. Mar. Biol. 37: 363-370.

Lee, R.F., S.C. Singer and D.S. Page. 1981. Responses of cytochrome P-450 systems in marine crabs and polychaetes to organic pollutants. Aquat. Toxicol. 1: 355-365.

Lee, R.F., J.W. Conner, D. Page, L.E. Ray and C.S. Giam. 1982. Cytochrome P-450 dependent mixed-function oxygenase systems in marsh crabs. In: W.B. Vernberg, A. Calabrese, F.P. Thurberg and F.J. Vernberg (eds.), Physiological Mechanisms of Marine Pollutant Toxicity, pp. 145-159. Academic Press, New York.

Lyes, M.C. 1979. Bioavailability of a hydrocarbon from water and sediment to the marine worm Arenicola marina. Mar. Biol. 55: 121-127.

McClain, B.B., H.O. Hodgins, W.D. Gronlund, J.W. Hawkes, D.W. Brown, M.S. Meyers, and J.H. Vandermeulen. 1978. Bioavailability of crude oil from experimentally oiled sediments to English sole (Parophrys vetulus), and pathological consequences. J. Fish. Res. Board Can. 35: 657-664.

Melancon, M.J. and J.J. Lech. 1978. Distribution and elimination of naphthalene and 2-methylnaphthalene in rainbow trout during short- and long-term exposures. Arch. Environ. Contam. Toxicol. 7: 207-220.

Metcalf, R.L. 1977. Biological fate and transformation of pollutants in water. In: I.H. Suffet (ed.), Fate of Pollutants in the Air and Water Environments, pp. 195-221. John Wiley and Sons, New York.

Neff, J.M., B.A. Cox, D. Dixit and J.W. Anderson. 1976. Accumulation and release of petroleum-derived aromatic hydrocarbons by four species of marine animals. Mar. Biol. 3: 279-289.

Nimmo, D.R., P.D. Wilson, R.R. Blackman and A.J. Wilson. 1971. Polychlorinated biphenyl absorbed from sediment by fiddler crabs and pink shrimp. Nature 231: 50-52.

Pasteels, J.J. 1968. Pinocytose et athrocytose par l'epithelium branchial de Mytilus edulis. Z. Zellforsch. 92: 239-259.

Payne, J.F. 1976. Field evaluation of benzo(a)pyrene hydroxylase induction as a monitor for marine pollution. Science 191: 945-946.

Payne, J.F. and W.R. Penrose. 1975. Induction of aryl hydrocarbon hydroxylase in fish by petroleum. Bull. Environ. Contam. Toxicol. 14: 112-116.

Plack, P.A., E.R. Skinner, A. Rogie and A.I. Mitchell. 1979. Distribution of DDT between lipoproteins of trout serum. Comp. Biochem. Physiol. 62C: 119-126.

Pritchard, J.D., A.M. Guarino and W.B. Kinter. 1973. Distribution metabolism and excretion of DDT and Mirex by a marine teleost, the winter flounder. Environ. Health Perspect. 2: 45-53.

Rao, K.R., F.R. Fox, P.J. Conklin, A.C. Cantelma and A.C. Brannon. 1979. Physiological and biochemical investigations of the toxicity of pentachlorophenol to crustaceans. In: W.B. Vernberg, F.P. Thurberg, A. Calabrese and F.J. Vernberg (eds.), Marine Pollution: Functional Responses, pp. 307-340. Academic Press, New York.

Rossi, S.S. 1977. Bioavailability of petroleum hydrocarbons from water, sediments and detritus to the marine annelid Neathes arenaceoentata. In: Proceedings 1977 Oil Spill Conference, Mar. 8-10, 1977, pp. 621-625. American Petroleum Inst., Washington, D.C.

Rossi, S.S. and J.W. Anderson. 1977. Accumulation and release of fuel-oil-derived diaromatic hydrocarbons by the polychaete Neanthes arenaceodentata. Mar. Biol. 39: 51-55.

Roubal, W.T., T.K. Collier and D.C. Malins. 1977. Accumulation and metabolism of carbon-14 labeled benzene, naphthalene, and anthracene by young coho salmon (Oncohynchus kisutch). Arch. Environ. Contam. Toxicol. 5: 513-529.

Solbakken, J.E., K.H. Palmork, T. Neppelberg and R.R. Scheline. 1980. Urinary and biliary metabolites of phenanthrene in the coalfish (Pollachius virens). Acta Pharmacol. Toxicol. 46: 127-132.

Statham, C.N., M.J. Melancon, Jr. and J.J. Lech. 1976. Bioconcentration of xenobiotics in trout bile: A proposed monitoring aid for some water-borne chemicals. Science 193: 680-681.

Stegeman, J.J. 1978. Influence of environmental contamination on cytochrome P-450 mixed-function oxygenases in fish: Implications for recovery in the Wild Harbor Marsh. J. Fish. Res. Board Can. 35: 668-674.

Stegeman, J.J. 1981. Polynuclear aromatic hydrocarbons and their metabolism in the marine environment. In: H. Gelboin and P.O. Ts'O (eds.), Polycyclic Hydrocarbons and Cancer, Vol. 3, pp. 1-60. Academic Press, New York.

Stegeman, J.J. and M. Chevion. 1980. Sex differences in cytochrome P-450 and mixed-function oxygenase activity in gonadally mature trout. Biochem. Pharmacol. 28: 553-559.

Stegeman, J.J. and J.M. Teal. 1973. Uptake, release and retention of petroleum hydrocarbons by the oyster, Crassostrea virginica. Mar. Biol. 22: 37-44.

Stegeman, J.J., A.V. Klotz, B.R. Woodin and A.M. Pajor. 1981. Induction of hepatic cytochrome P-450 in fish and the indication of environmental induction in scup (Stenotomus chrysops). Aquat. Toxicol. 1: 197-212.

Sudershan, P. and M.A.O. Khan. 1980. Metabolism of cis - {^{14} C} chlordane and cis - {^{14} C} photochlordane in bluegill fish. J. Agric. Food Chem. 28: 291-296.

Thomas, R.E. and S.D. Rice. 1981. Excretion of aromatic hydrocarbons and their metabolites by freshwater and saltwater Dolly Varden char. In: F.J. Vernberg, A. Calabrese, F.P. Thurberg and W.B. Vernberg (eds.), Biological Monitoring of Marine Pollutants, pp. 425-448. Academic Press, New York.

Vandermeulen, J.H., P.D. Keizer and W.R. Penrose. 1977. Persistence of non-alkane components of bunker C oil in beach sediments of Chedabucto Bay, and lack of their metabolism by molluscs. In: Proceedings 1977 Oil Spill Conference, Mar. 8-10, 1977, pp. 469-473. American Petroleum Inst., Washington, D.C.

Varanasi, U. and D.J. Gmur. 1981. Hydrocarbons and metabolites in English sole (Parophrys vetulus) exposed simultaneously to (^{3}H) benz(a) pyrene and (^{14}C) naphthalene in oil-contaminated sediment. Aquat. Toxicol. 1: 49-68.

Varanasi, U., D.J. Gmur and P.A. Treseler. 1979. Influence of time and mode of exposure on biotransformation of naphthalene by juvenile starry flounder (Platichthys stellatus) and rock sole (Lepidopsetta bilineata). Arch. Environ. Contam. Toxicol. 8: 673-692.

Varanasi, U., D.J. Gmur and W.L. Reichert. 1981. Effect of environmental temperature on naphthalene metabolism by juvenile starry flounder (Platichthys stellatus). Arch Environ. Contam. Toxicol. 10: 203-214.

Veith, D.G., D.L. Defoe and B.V. Bergstedt. 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish. Res. Board Can. 36: 1040-1047.

Whittle, K.J., J. Murray, P.R. Mackie, R. Hardy and J. Farmer. 1977. Fate of hydrocarbons in fish. Rapp. P-V. Reun. Cons. Int. Explor. Mer 171: 139-142.

Determining the Biological Significance of Contaminant Bioaccumulation

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INTRODUCTION

Bioaccumulation data are useful in that they provide direct information on the actual bioavailability of contaminants. This is particularly important as bioavailability and toxicity may be substantially altered by site-specific variations in water chemistry (e.g., salinity, pH, dissolved organics) or by the presence of other contaminants. This latter point is crucial in complex effluent situations where a myriad of trace metals and organics results in complex matrices which make modeling of uptake and toxicity of individual compounds exceedingly difficult. By measuring bioaccumulation of contaminants in organisms from the environment, it is possible to skip past the alterations described above and determine the contaminants' actual bioavailability. Laboratory simulations are also useful in predicting bioaccumulation for complex mixtures such as dredge spoils. The major problem currently facing these approaches, however, is determining the relevance of a specific tissue or body burden to the fitness of an organism or community. These correlations are made difficult because organisms have the ability to detoxify and acquire tolerance to a wide range of environmental contaminants.

In spite of these substantial obstacles, recent studies of detoxification systems in marine organisms may provide a basis for effectively estimating biological response to environmental perturbation on the cellular, organismal and population levels (Bayne et al. 1980). These studies suggest that the assimilative capacity of an

organism's detoxification system may be a sensitive and highly relevant index of "cellular fitness." In this approach, rather than total body burden, the partitioning of contaminants between sites of detoxification and sites of toxic action is determined. This will indicate if the contaminants are sequestered by molecules which function in detoxification such as metallothionein (for trace metals) or glutathione (for trace organic metabolites), or if they are interfering in general metabolism (Jenkins et al. 1982a; Brown et al. 1982). Only those contaminants at sites of toxic action present a direct problem to the organism. This approach also has the added advantage of being contaminant specific since it makes it possible to determine which contaminants are present at a site of toxic action and are thus no longer detoxified. This is particularly important when dealing with complex effluents for it not only makes it possible to determine the degree of impact, but it also provides information as to which contaminant is responsible.

In the following sections we examine the mechanisms by which trace metals and trace organics are detoxified and we discuss studies concerned with estimating biological significance of organic contaminant burdens. Finally, we review the current limitations of the approaches and suggest how they might be dealt with experimentally.

TRACE METAL DETOXIFICATION/TOXIFICATION

The detoxification of trace metals by marine organisms is due, in part, to the presence of intracellular metal-binding proteins which bear a strong resemblance to mammalian metallothioneins (Learch et al. 1982; Noel-Lambot et al. 1978; Overnell and Coombs 1979; Overnell and Trewhella 1979). Metallothioneins are characterized by their low molecular weight, high cystiene content, lack of aromatic amino acids and high affinity for metals including Ag, Au, Cd, Cu, Hg and Zn (Kägi and Nordberg 1979; Sabbioni and Marafante, 1975). Since their discovery in 1957, metallothionein-like proteins have been isolated from a wide variety of organisms including humans (Kissling and Kägi 1977), mice (Huang et al. 1977), fish (Marafante 1976), crabs (Learch et al. 1982; Olafson et al. 1979) and molds (Ammer et al. 1978), and they appear to be ubiquitous.

Metallothionein synthesis can be induced by low concentrations of Zn or Cu (Brady et al. 1979; Bremner and Davies 1975; Richards and Cousins 1975; and Webb 1972), making these proteins an effective nontoxic reservoir for these essential trace metals (Bremner 1979; Brown and Chatel 1978). Nonessential trace elements such as Cd, Hg and Ag are also strong inducers of metallothionein and are effectively sequestered by the newly synthesized metallothionein (Bouquegneau 1979; Glick et al. 1981; Hildebrand et al. 1979; Rugstadt and Norseth 1978).

Metallothioneins, functioning as sequestering agents can be seen in the livers of white croakers collected from a control site in the Southern California Bight (Figure 1). In these organisms, 97% of the total liver Cd was associated with the metallothionein pool. When Cu and Zn are included, over 55% of the total liver metals are effectively sequestered on metallothioneins (Jenkins et al. 1982a).

This sequestration of trace metals by metallothioneins appears to be effective over a wide range of tissue metal concentrations. For example, in sea urchins collected from a number of sites in the Southern California Bight (Jenkins et al. 1982b) the range of body burdens could be accounted for entirely by metals associated with metallothioneins (Figure 2). The high molecular weight enzyme-containing pool, which represents a major site of toxic action for excess metals, does not change significantly over the entire range of metal body burdens. The baseline level of metals in this pool represents Zn and Cu associated with metalloenzymes. These data suggest that the metallothionein system is very effective in sequestering nonessential trace metals and that a precise mechanism exists for maintaining constant levels of essential trace metals in the enzyme-containing pool. These data also provide insights into the difficulties associated with extrapolating from total tissue metal burdens to biological effects.

Many marine organisms are also capable of sequestering substantial concentrations of trace metals in membrane-limited vesicles or granules (Coombs and George 1978). These structures are particularly prominent in mollusks and much of the high metal burden observed in these organisms is attributable to metals in membrane-limited vesicles rather than the free cytoplasm (George and Piere 1979, 1980). The relationship between metallothionein and these membrane-limited vesicles is currently under study. Metallothioneins, however, are thought to occur in lysosomal membrane-bound vesicles (Porter 1974; George and Pirie 1979). Also, Viarengo, et al. (1981) have demonstrated that in mussels exposed to a 3-day Cu pulse, initial accumulation of metals on metallothionein is followed by a transient accumulation in lysosomal granules and clearance of metal from the tissue. Finally, George and Piere (1980) have demonstrated exocytosis and sloughing of Zn-containing granules from the kidneys of mussels. These data, though not conclusive, strongly favor a model in which excess metals are initially accumulated on metallothioneins and the metallothioneins are later sequestered in lysosomal granules for storage and/or excretion.

As can be seen from the above examples, marine organisms have the ability to detoxify trace metals through sequestration on proteins and/or in membrane-bound vesicles. This detoxification capacity, however, is finite, and under conditions of high metal uptake or severe stress the capacity to sequester these metals can be

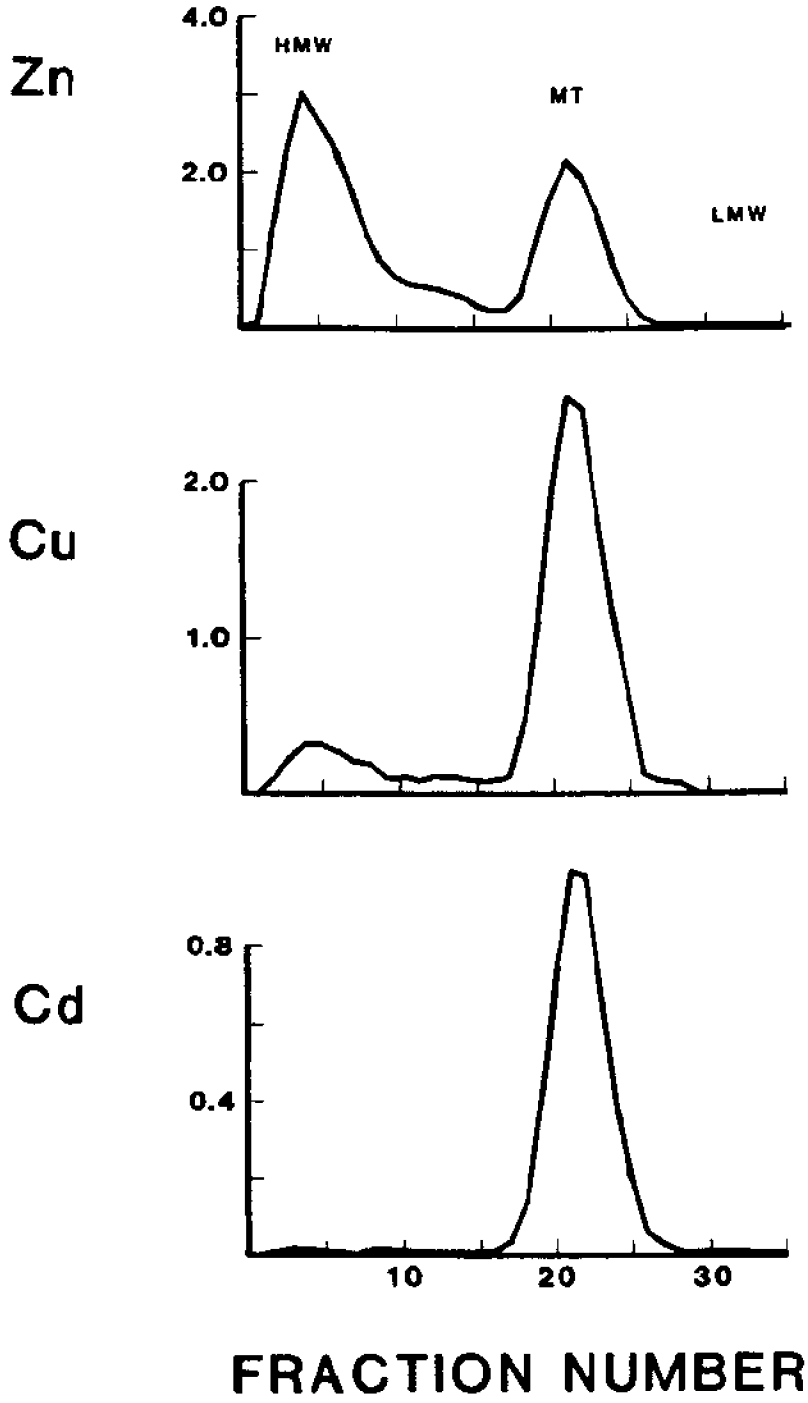

Figure 1. Sephadex G-75 elution profile of a composite (N=10) of livers from croakers collected from a control station (Point Dume) in the Southern California Bight. Zn and Cu were found to be associated with the high-molecular-weight, enzyme-containing pool (HMW) and the metallothionein-containing pool (MT) while Cd was associated predominantly with the MT pool. The low-molecular-weight pool (LMW) did not contain significant quantities of the three metals examined in this study. Metal concentrations are expressed as $\mu g\ ml^{-1}$ (from Jenkins et al. 1982a).

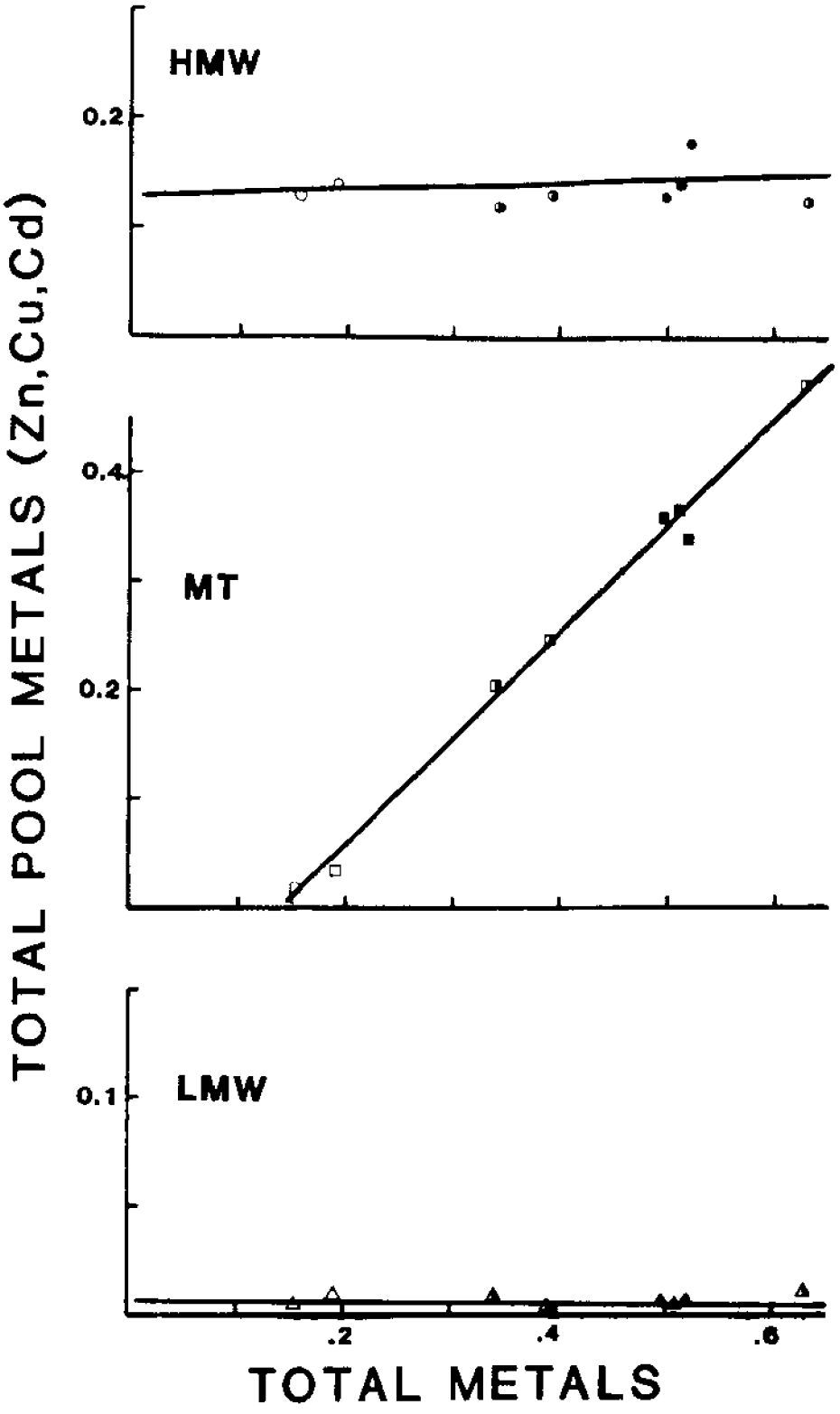

Figure 2. Relationship between total metal levels in tissue and cytosolic metal distribution. The relative metal distributions for the HMW, MT and LMW pools over a range of tissue metals in sea urchins collected from the Southern California Bight. Metal concentrations are expressed as $\mu g\ ml^{-1}$ (from Jenkins et al. 1982b).

exceeded. Under these conditions, excess metals would spill over and have direct impact on sites of toxic action, such as the enzyme-containing pool. This phenomenon was initially suggested by Winge et al. (1973) who found a strong correlation in mice between the onset of pathological effects due to cadmium exposure and the spillover of cadmium from metallothionein to the enzyme-containing pool. Likewise, Pruell and Engelhardt (1980) have shown a correlation between Cd spillover and reduced catalase activity in the cytosol of the killifish. Data consistent with these observations have also been reported for bivalves exposed to Cd or Cu (Engel and Fowler 1979; Roesijadi 1982).

In studies in our laboratories (Bay et al. 1983) we have found that in polychaete worms exposed to acute levels of Cd, there is good correlation between the concentration at which spillover occurs and the onset of histopathological damage (Figure 3). Likewise, in scorpionfish exposed to an acute range of cadmium concentrations (Brown et. al. 1983) the degree of histopathological damage in the gills was proportional to the degree of cadmium loading in the enzyme-containing pool (Figure 4). In these same studies, however, the livers of fish exposed to Cd concentrations representing 85% of the acute LC50 level showed a fortyfold increase in Cd when compared with control organisms, and all of this cadmium was effectively sequestered on metallothionein. As might be expected, the liver tissues in the exposed organisms were found to have no obvious histopathological effects. These studies support the importance of spillover in the onset of acute metal toxicity. They also underline the importance of understanding detoxification mechanisms at the tissue level so that the appropriate tissues can be utilized when carrying out bioaccumulation or detoxification studies.

Observations such as those described above have led to the suggestion that the toxicological impact of trace metals may be evaluated most effectively by examining their relative distribution between sites of detoxification and sites of toxic action such as the enzyme-containing pool (Bayne et al. 1980; Brown et al. 1977; Engel and Fowler 1979). An added benefit of this is that it would automatically take into account metal interactions as well as other stresses that could reduce the ability of the organism to synthesize metallothionein. These characteristics along with the contaminant-specific nature of the test make it particularly attractive for evaluating the metal status of organisms obtained directly from the environment (Brown et al. 1977).

TRACE ORGANIC DETOXIFICATION/TOXIFICATION

While several studies have been concerned with detoxification of trace metals in aquatic organisms exposed in their natural habi-

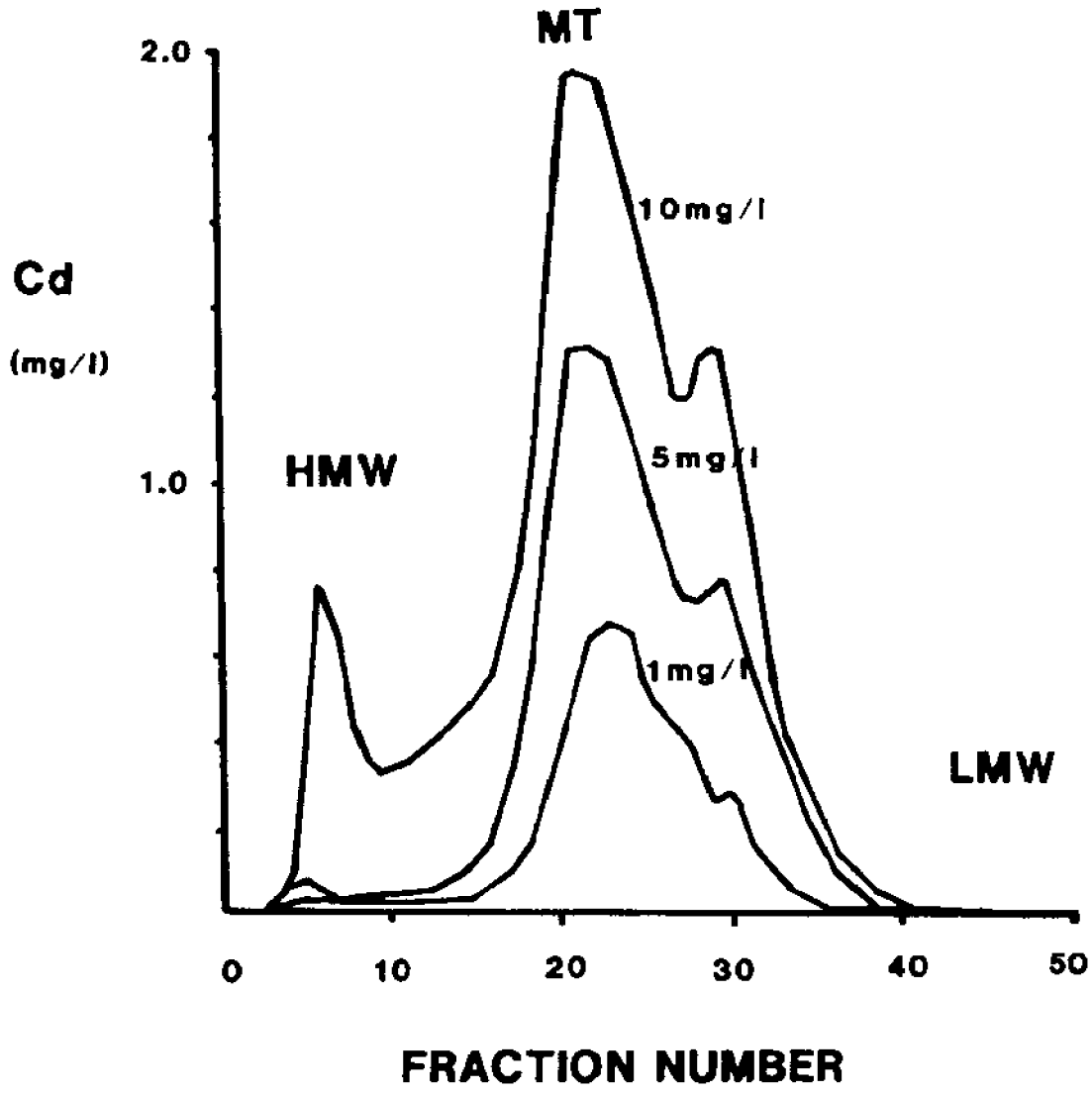

Figure 3. Sephadex G-75 profile of cytosolic Cd in Neanthes exposed to various Cd concentrations (as indicated) for 96 hours. Metal pools are labeled as in Figure 1, and concentrations are expressed as mg L^{-1}. Following exposure animals were examined histologically and the gut rated for sloughing and necrosis where 0 = normal, 1 = low, 2 = moderate and 3 = high levels of pathology. The mean ratings for four animals at each concentration were 0 mg L^{-1} = 0, 1 mg L^{-1} = 0, 5 mg L^{-1} = 0.25 and 10 mg L^{-1} = 1.75 (from Bay et al. 1982).

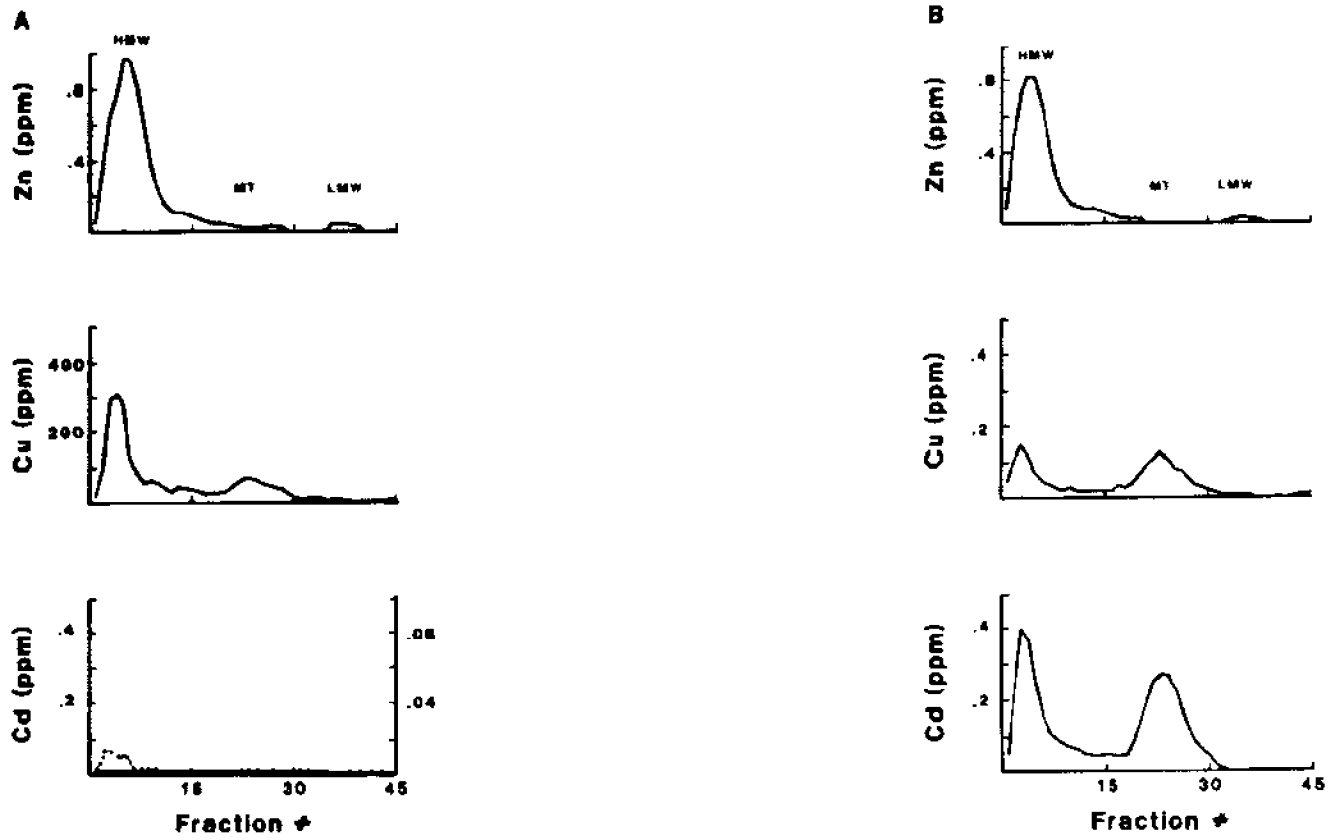

Figure 4. Cytosolic metal distributions in gills of (a) control and (b) scorpionfish exposed to Cd. Exposed fish were maintained at 50 ppm Cd (85% of the LC50) for 4 days. Metal pools are labeled as in Figure 1, and concentrations are expressed as µg/ml. Following exposure these tissues were examined histologically and rated according to the degree of observed hyperplasia where 0 = none, 1 = low, 2 = moderate and 3 = high. The mean ratings for three fish at the various exposures were 0 mg L^{-1} = 0, 25 mg L^{-1} = 0 and 50 mg L^{-1} = 2.3 (from Brown et al. 1982).

tats (Brown et al. 1977; Jenkins et al. 1982a), the ability of these organisms to detoxify trace organics has not been thoroughly investigated. Studies involving exposure of fish to pesticides indicate that fish can acquire tolerance to these by means of preexposure and suggest that fish have the ability to increase their capacity to detoxify these substances (Vinson et al. 1963; Ferguson et al. 1964). More recent studies suggest that aquatic organisms like mammals do indeed have the ability to detoxify trace organics (Lee et al. 1981; Stegeman et al. 1981).

Although similar in concept, detoxification of trace organics is more complex than trace metal detoxification, owing in part to the number and variety of trace organics which must be studied. It is also more complex mechanistically as the initial organic parent compounds are metabolized through several steps into a variety of metabolites with varying solubility, reactivity and toxicity. Thus, in studying trace organics, we must deal not only with the parent compounds, but also with a myriad of metabolites which complicate bioaccumulation studies considerably.

In the remainder of this section we review the partitioning and metabolism of parent organics and the fate of the resulting metabolites. We also review recent studies which may provide a basis for determining the toxic impact of given body burdens of parent trace organics and their metabolites.

When parent trace organics enter organisms they are, because of their hydrophobic nature, initially partitioned into lipid pools (Allen et al. 1974, 1976) and are thus distributed among tissues according to lipid availability (Brown et al. 1982 [Table 1]). If

Table 1. Amounts of DDT + DDE + DDD in livers and muscle of white croakers from Dana Point and Palos Verdes. Data are presented in terms of wet weight and lipid weight. Composites of N = 10.

	Tissue Wet Weight (g)		mg of DDT + DDE + DDD per kg of Tissue Wet Weight		mg of DDT + DDE + DDD per kg of Lipid	
	DP	PV	DP	PV	DP	PV
LIVER	2.98	4.47	0.55	35.7	9.2	238
MUSCLE	142	147	0.05	8.1	6.3	369

DP = Dana Point (Control Station)
PV = Palos Verdes (Outfall Station)

peripheral lipid pools, however, are overloaded with trace organics, then these parent compounds can accumulate in lipids of nervous tissues, producing neurotoxic effects (Quraishi 1977).

Trace organics also accumulate in liver tissue where they stimulate a proliferation of endoplasmic reticulum and an increased synthesis and accumulation of lipids (Allen et al. 1974, 1975, 1976). Concomitantly, there is an increase in serum lipids which may serve to transport trace organics from peripheral tissues to the liver. Trace organics entering the liver are partitioned into the lipid vacuoles associated with the endoplasmic reticulum. The enzymes which metabolize the trace organics are located in the membranes of the endoplasmic reticulum, and their synthesis is also induced by the presence of excess trace organics (Hart and Fouts 1963).

The metabolism of trace organics results in their conversion from a hydrophobic to a more hydrophilic form (Hodgson 1974). For chlorinated hydrocarbons, this metabolism initially involves the removal of chlorines (Figure 5). Next, the mixed function oxygenases catalyze the addition of an oxygen to form a highly reactive epoxide intermediate. This bioactivated metabolic intermediate can attach to macromolecules including proteins, DNA and RNA with resultant toxic effects, or alternatively, can be further metabolized to less reactive hydrophilic metabolites. These latter deactivation reactions include spontaneous conversion to phenols, enzymatic catalyzation of dehydrodiols by epoxide hydratases, or spontaneous or enzymatic conversion by glutathione-s-epoxide transferases to glutathione conjugates (Jerina and Daley 1974). It has been suggested that whether toxic effects will occur or not (i.e., whether epoxide intermediates will react with cellular macromolecules) depends upon the ratio of the rate of the bioactivation processes to the rate of the deactivation processes (Brodie et al. 1971; Jerina and Daley 1974). If the rate of the deactivation processes is less than that of the bioactivation processes, then levels of toxic epoxide intermediates will increase, causing toxic effects.

Extrapolations from acute laboratory toxicity tests for trace organics, to chronic environmental exposure are difficult because the modes of both detoxification and toxification are different for acute and chronic exposures (McKinney 1981). Acute effects of trace organics result from the overloading of lipid pools with unmetabolized parent trace organics and their subsequent accumulation in lipid-containing membranes of nerve tissue. However, chronic effects of trace organics occur when the rate of deactivation processes exceeds the rate of bioactivation processes, and reactive metabolic intermediates accumulate and attach to macromolecules.

A number of marine organisms have been shown to possess enzymes necessary for both the bioactivation and deactivation of trace organics. These include the presence of mixed function

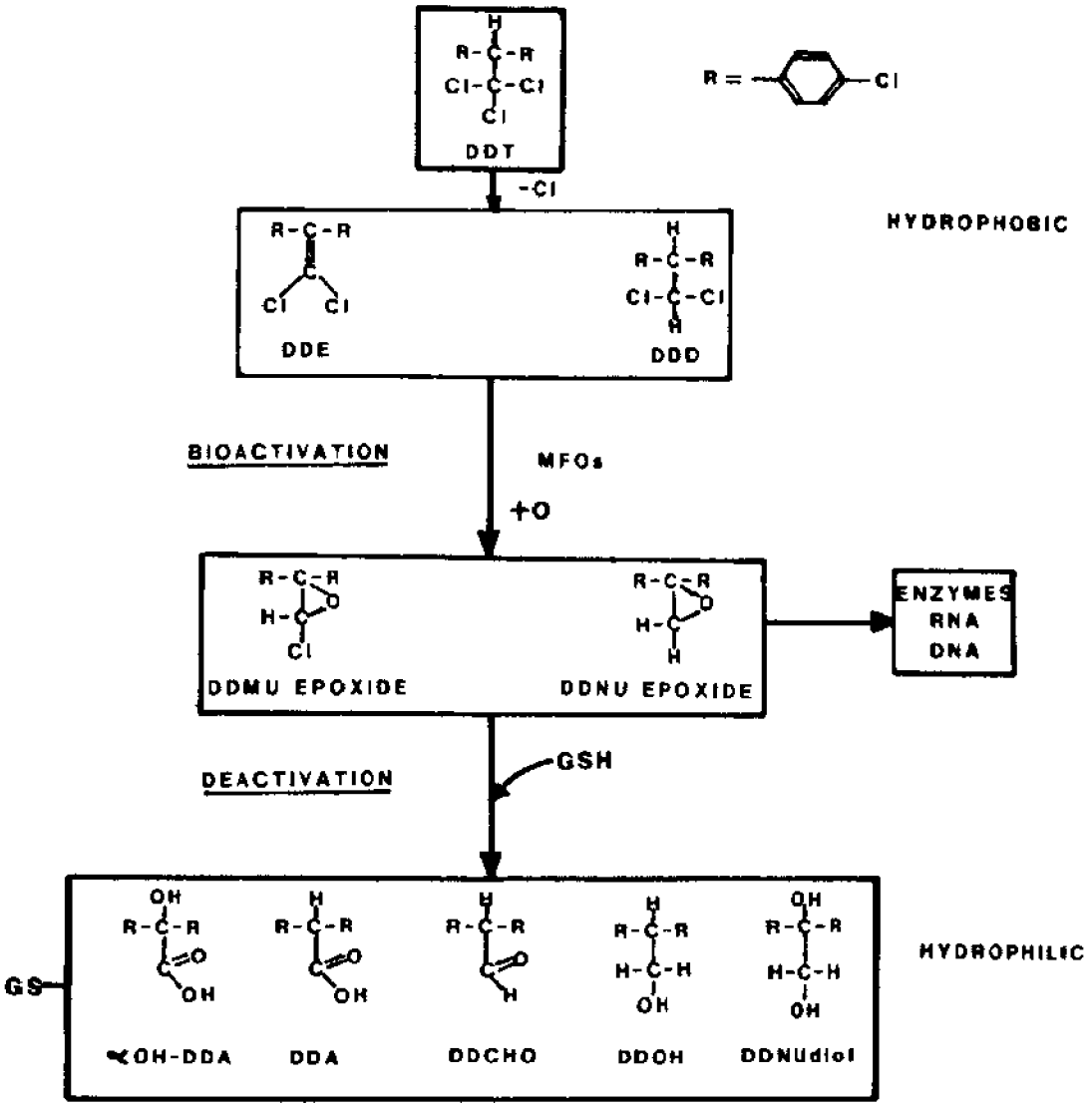

Figure 5. Outline of the steps in the metabolism of DDT. MFOs are mixed function oxygenases (from Brown et al. 1982).

oxidase activity in mussels (Moore 1979), polychaetes, crabs (Lee et al. 1981) and fish (Stegeman et al. 1981), and epoxide hydratase and glutathione-s-transferase activity in mollusks, crustacea, elasmobranchs and teleosts (James et al. 1979). In addition, a number of investigators have demonstrated the presence of trace organic metabolites in marine organisms including crustacea (Lee 1976; Lee et al. 1976) and fish (Lee et al. 1972; Malins et al. 1979).

Unfortunately, neither the demonstration of these enzyme activities nor the presence of metabolites gives any indication as to whether sensitive cellular sites are being impacted. Recent studies, however, have been carried out to determine the partitioning of trace organic metabolites between a site of detoxification and sites of toxic action (Brown et al. 1982). This work was carried out in parallel with metal detoxification studies, and like these studies, makes use of Sephadex G-75 molecular weight separations (see Figure 1). In these studies, most of the metabolites (>95%) present in the low-molecular-weight (LMW) pool were present as conjugates since they were not extractable without a heat-catalyzed base hydrolysis of this pool (Gold et al. 1981). This suggests that the metabolites present in the LMW pool are not free to impact macromolecules. Furthermore, recent studies indicate that almost all cytosolic glutathione is present in the LMW pool (Figure 6). It has previously been demonstrated that glutathione is a key substance in the conjugation of trace organic metabolites and that trace organics do not bind to macromolecules unless glutathione reserves are severely depleted or overloaded (Reid and Krishna 1973). This further implicates the LMW glutathione (GSH)-containing pool as a major site of detoxification of trace organic metabolites.

Whereas the LMW, GSH-containing pool appears to function as a site of detoxification of trace organic metabolites, the HMW, enzyme-containing pool and the medium-molecular-weight, metallothionein (MT)-containing pool may represent major sites of toxic action for metabolites. When Sephadex G-75 gel chromatography profiles were examined for croakers from a control site, it was found that all measured trace organic metabolites occurred in the LMW, GSH-containing pool (Figure 7). When similar profiles were examined for croakers from a highly contaminated site it was found that higher levels of metabolites occurred in the GSH-containing pool and that additional metabolites appeared to have accumulated in the HMW and MT pools. Enzyme activities were reduced in croaker livers in which metabolites had accumulated in the HMW pool. In these same organisms, the trace metal content MT-containing pool was decreased, suggesting that metabolites were interfering with metallothionein interactions (Jenkins et al. 1982). The higher levels of metabolites in the MT-containing pool may be a consequence of the fact that MT, like GSH, has a high ratio of cystiene residues.

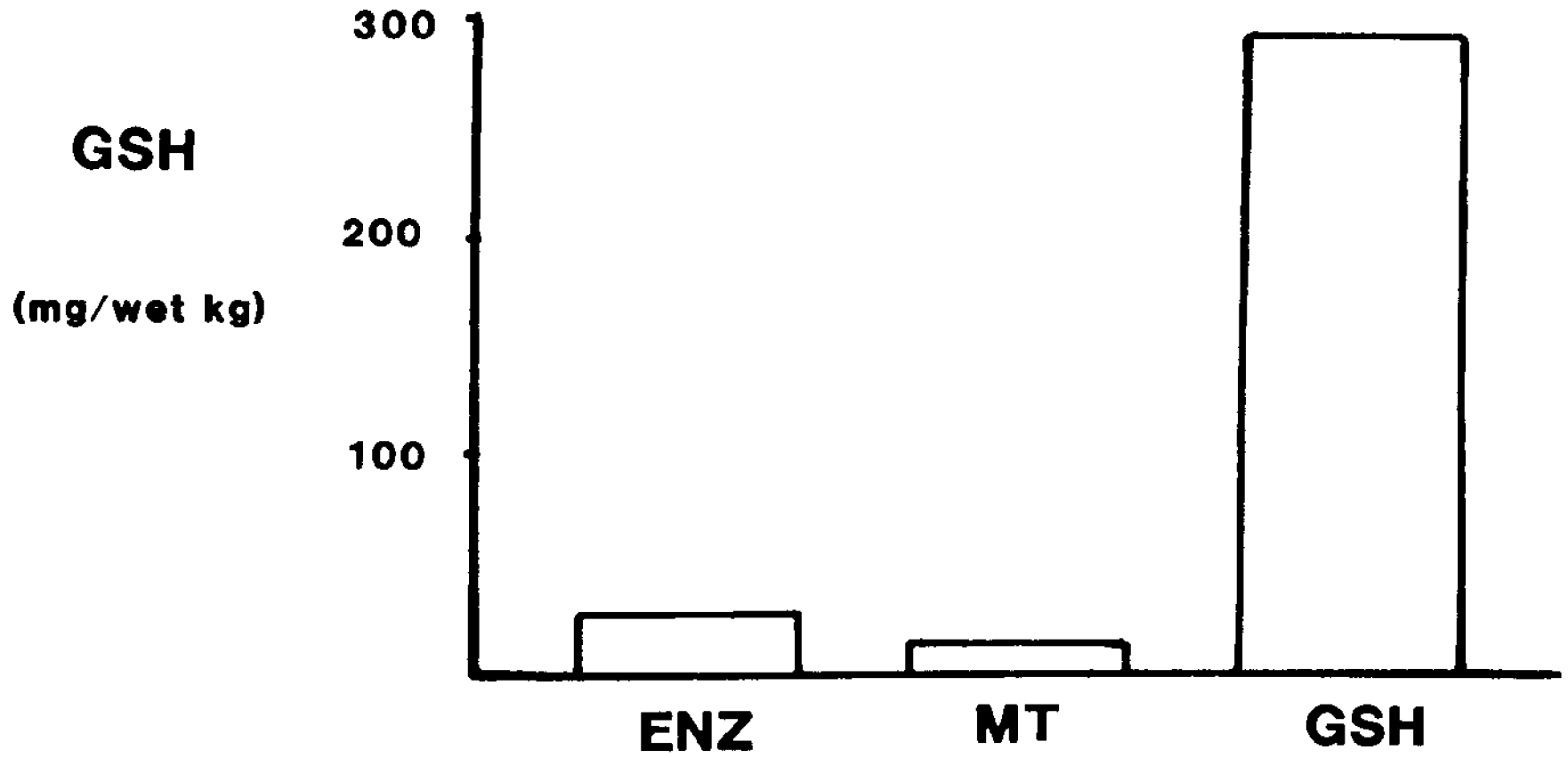

Figure 6. Distribution of glutathione (GSH) in the high-molecular-weight, enzyme-containing pool (ENZ), metallothionein-containing pool (MT) and the low-molecular-weight, glutathione-containing pool (GSH) in scorpionfish liver.

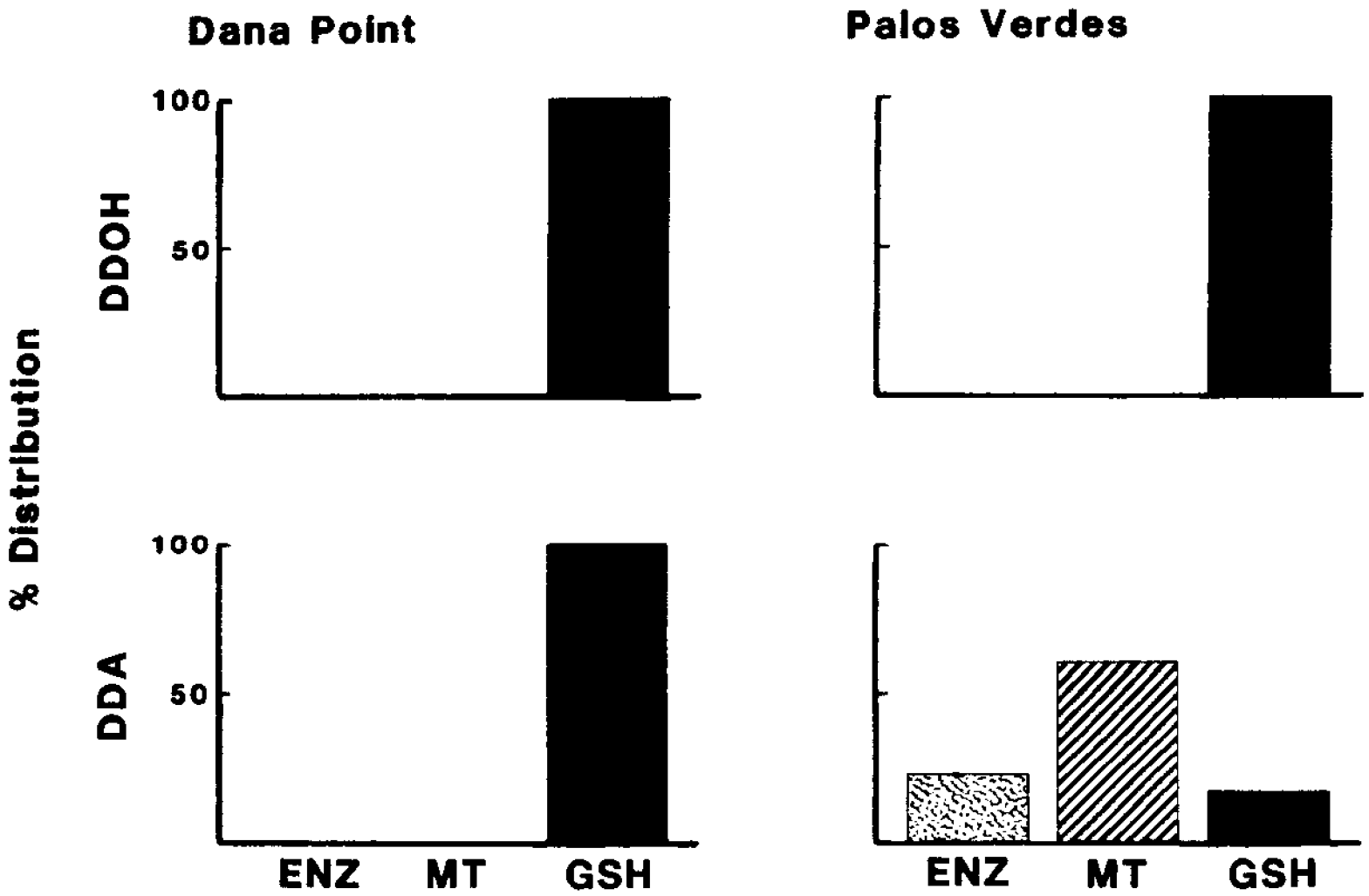

Figure 7. Percentage distribution of the DDT metabolites, DDA and DDOH, in the ENZ, MT and GSH pools in Dana Point (control) and Palos Verdes (contaminated) scorpionfish liver.

The above observations suggest that excess trace organic metabolites may impact metal detoxification by binding directly to metallothioneins. Along these lines, recent studies have demonstrated that the enzymes responsible for detoxification of metabolites are particularly sensitive to metal poisoning (Hjelle et al. 1978). Studies such as these serve to stress the importance of contaminant interactions and accentuate the limitations of single-contaminant bioassays for dealing with complex effluents. They also reinforce the difficulties in interpreting body burden data at a more superficial level.

CURRENT LIMITATIONS

In the approaches discussed above, spillover is often presented as an end point, the tacit assumption being that metals and organics will have no impact on an organism prior to spillover. On the surface, this makes good sense, as those contaminants which are effectively sequestered are not available to have direct toxic impact on the organism. This approach, however, ignores the potential indirect cost of detoxification to an organism. This cost may represent the energy required for the synthesis of detoxification proteins, competition for amino acids or the physical cost of accumulating large quantities of proteins and/or membrane-bound vesicles within a cell. Ultimately, however, the cost of detoxification must be related not only to basic energetics but to factors such as growth and reproduction if we are to be able to extrapolate from this cellular and molecular data to biological impact at the level of the population and community.

Another question which must be dealt with in studies of this type relates to the variability of detoxification limits seasonally, within populations, between populations and between species. These variations may be genetic or may be a consequence of other stresses such as temperature or salinity changes, molting or reproduction which organisms must deal with. As the organism's ability to adapt physiologically to these additive stresses is finite, it is important to determine the degree to which these factors impact detoxification/toxification.

Studies are currently under way in a number of laboratories which may make it possible to calibrate the loading or overloading of the metal detoxification system with a number of other indicators of stress at a variety of levels of organization (e.g., from tissue and organism level to population and community levels). The variability of these systems is also being studied, including the effects of additional stresses. If these studies prove fruitful, it may indeed soon be possible to determine the relevance of tissue trace metal levels and/or trace organic levels to biological impact at the organism, population and community levels.

REFERENCES

Allen, J.R., L.A Carstens and D.A. Barsotti. 1974. Residual effects of short-term, low-level exposure of nonhuman primates to polychlorinated biphenyls. Toxicol. Appl. Pharmacol. 30: 440-451.

Allen, J.R., L.A. Carstens, L.J. Abrahanson and R.J. Marlar. 1975. Responses of rats and nonhuman primates to 2,5,2',5'-tetrachlorobiphenyl. Environ. Res. 9: 265-273.

Allen, J.R., L.A. Carstens and L.J. Abrahanson. 1976. Responses of rats exposed to polychlorinated biphenyls for fifty-two weeks. I. Comparison of tissue levels of PCB and biological changes. Arch. Environ. Contam. Toxicol. 4: 404-419.

Allison, A.C. 1969. Lysomes and cancer. In: J.T. Dingle and H.B. Fell, (eds.), Lysomes in Biology and Pathology, pp. 178-204 North Holland-American Elsevier, Amsterdam, Netherlands.

Ammer, D., R. Budry and K. Learch. 1978. Structure and function of a copper-metallothionein from Neurospora crassa. Experientia 35: 905.

Bay, S.M., K.D. Jenkins, P.S. Oshida and D.A. Brown. 1983. Cadmium detoxification/toxification and histopathology in polychaete worms Neanthes arenaceodentata exposed to acute levels of Cd.

Bayne, B.L., D.A. Brown, F.L. Harrison and P.P. Yevich. 1980. Mussel health. In: E. Goldberg, (ed.), International Mussel Watch, Report on a Workshop at Barcelona, Spain, December, 1978, pp. 196-235. National Academy of Sciences, Washington, D.C.

Bouquegneau, J.M. 1979. Evidence for protective effects of metallothioneins against inorganic injuries to fish. Bull. Environ. Contam. Toxicol. 23: 218-219.

Brady, F.O., M. Panemangalore, F.A. Day, A.M. Fiskin and G. Peterson. 1979. In vivo and ex vivo induction of rat liver metallothionein. In: J.H.R. Kägi and M. Nordberg (eds.), Metallothionein: Proceedings of the First International Meetings on Metallothionein and Other Low Molecular Weight Metal-Binding Proteins, pp. 262-271. Brikhäuser Verlag, Boston, Mass.

Bremner, I. 1979. Factors influencing the occurrence of copper-thioneins in tissues. In: J.H.R. Kägi and M. Nordberg (eds.), Metallothionein: Proceedings of the First International Meetings on Metallothionein and Other Low Molecular Weight Metal-Binding Proteins, pp. 273-280. Brikhäuser Verlag, Boston, Mass.

Bremner, I. and N.T. Davies. 1975. The induction of metallothionein in rat liver by zinc injection and restriction of food intake. Biochem. J. 149: 733-738.

Brown, D.A. and K.W. Chatel. 1978. Interactions between cadmium and zinc in cytoplasm of duck liver and kidney. Chem.-Biol. Interactions 32: 271-279.

Brown, D.A., C.A. Bawden, K.W. Chatel and T.R. Parsons. 1977. The wildlife community of Iona Island Jetty, Vancouver, B.C., and heavy-metal pollution effects. Environ. Conserv. 4: 213-216.

Brown, D.A., R. Gossett and K.D. Jenkins. 1982. Contaminants in white croakers (Genyonemus lineatus) from the Southern California Bight. II. Trace organic detoxification/toxification. In: F.J. Vernberg, A. Calabrese, F.P. Thurberg and W.B. Vernberg (eds.), Physiological Mechanisms of Marine Pollutant Toxicity, pp. 197-214. Academic Press, New York.

Coombs, T.L. and S.C. George. 1978. Mechanisms of immobilization and detoxification of metals in marine organisms. In: D.S. McLusky and A.J. Berry (eds.), Physiology and Behavior of Marine Organisms, pp. 179-187. Pergamon Press, Oxford.

Engle, D.W. and B.A. Fowler. 1979. Factors influencing cadmium accumulation and its toxicity to marine organisms. Environ. Health Perspec. 28: 81-88.

Ferguson, D.E., D.D. Culley, W.D. Cotton and R.P. Dodds. 1964. Resistance to chlorinated hydrocarbon insecticides in three species of freshwater fish. Bioscience 14: 43-44.

George, S.P. and B.J.S. Pirie. 1979. The occurrence of cadmium in subcellular particles in the kidney of marine mussel, Mytilus edulis, exposed to cadmium. Biochem. Biophys. Acta. 380: 234-244.

George, S.P. and B.J.S. Pirie. 1980. Metabolism of zinc in the mussel, Mytilus edulis (L), a combined ultrastructural and biochemical study. J. Mar. Biol. Assoc. (U.K.) 60: 575-590.

Glick, G., K.S. McCarthy and K.S. McCarthy. 1981. The role of metallothionein synthesis in cadmium- and zinc-resistant CHO-KLM cells. Exp. Cell Res. 132: 23-30.

Gold, B., T. Leuschen, G. Brunk and R. Gingell. 1981. Metabolism of a DDT metabolite via a chloroepoxide. Chem.-Biol. Interactions 35: 159-178.

Hart, L.G. and J.R. Fouts. 1963. Effects of acute and chronic DDT administration on hepatic microsomal drug metabolism in the rat. Proc. Soc. Exp. Biol. Med. 114: 388-392.

Hildebrand, C.E, R.A Tobey, E.W. Campbell and M.D. Enger. 1979. A cadmium resistant strain of the Chinese hamster (CHO) cell with increased metallothionein induction capacity. Exp. Cell Res. 124: 237-246.

Hjelle, J.J., M. Kemal and A. Pokiis. 1978. Effects of acute inorganic lead administration on in vivo drug metabolism and cytochrome P-450 in the rat. Toxicol. Lett. 2: 361-364.

Hodgson, E. 1974. Comparative studies of cytochrome P-450 and its interaction with pesticides. In: M.Q. Khan and J.P. Bederka, Jr. (eds.), Survival in Toxic Environments, pp. 213-254. Academic Press, New York.

Huang, I.R., A. Yoshida, H. Tsunoo and H. Nakajima. 1977. Mouse liver metallothioneins. J. Biol. Chem. 252(22): 8217-8221.

James, M.O., E.R. Bowen, P.M. Dansette and J.R. Bend. 1979. Epoxide hydrase and glutathione -S- transferase activities with selected alkene and arene oxides in several marine species. Chem.-Biol. Interactions 25: 321-344.

Jenkins, K.D., D.A. Brown, G.P. Hershelman and W.C. Meyer. 1982a. Contaminants in white croakers, Genyonemus lineatus (Ayres, 1855), from the Southern California Bight. I. Trace metal detoxification/toxification. In: F.J. Vernberg, A. Calabrese, F.P. Thurberg and W.B. Vernberg (eds.), Physiological Mechanisms of Marine Pollutant Toxicity, pp. 177-196. Academic Press, New York.

Jenkins, K.D., D.A. Brown, P.S. Oshida and E.M. Perkins. (1982b). Cytosolic metal distribution as an indicator of toxicity in sea urchins from the Southern California Bight. Mar. Pollut. Bull.

Jerina, D.M., and J.W. Daley. 1974. Arene oxides: A new aspect of drug metabolism. Science 185: 573-582.

Kägi, J.H.R. and M. Nordberg (eds.) 1979. Metallothionein: Proceedings of the First International Meetings on Metallothionein and Other Low Molecular Weight Metal-Binding Proteins. Brikhäuser Verlag, Boston, Mass.

Kissling, N.M. and J.H.R. Kägi. 1977. Primary structure of human hepatic metallothionein. FEBS Lett. 82: 247-250.

Learch, K., D. Ammer and R.W. Olafson. 1982. Crab metallothionein: Primary structure of metallothioneins 1 and 2. J. Biol. Chem. 257: 2420-2426.

Lee, R.F. 1975. Fate of petroleum hydrocarbons in marine zooplankton. In: Proceedings 1975 Conference on Prevention and Control of Oil Pollution, Mar. 25-27, San Francisco, Calif., pp. 549-553. American Petroleum Inst., Washington, D.C.

Lee, R.F., R. Sauerheber and G.H. Dobbs. 1972. Uptake, metabolism and discharge of polycyclic aromatic hydrocarbons by marine fish. Mar. Biol. 17: 201-208.

Lee, R.F., C. Ryan and L. Neuhauser. 1976. Fate of petroleum hydrocarbons taken up from food and water by the blue crab, Callinectes sapidus. Mar. Biol. 37: 363-370.

Lee, R.F., S.C. Singer and D.S. Page. 1981. Response of cytochrome P-450 systems in marine crab and polychaetes to organic pollutants. Aquat. Toxicol. 1: 355-365.

Malins, D.C., T.K. Collier and H.R. Sandborn. 1979. Deposition and metabolism of aromatic hydrocarbons in marine organisms. In: M.O.Q. Khan, J.J. Lech and J.J. Menn (eds.), Pesticide and Xenobiotic Metabolism in Aquatic Organisms, pp. 251 - 275. American Chemical Society, Washington, D.C.

Marafante, E. 1976. Binding of mercury and zinc to cadmium-binding protein in liver and kidney of goldfish (Carassius auratus L.) Experientia 32: 149-150.

McKinney, J.D. 1981. Environmental health chemistry--definitions and interrelationships: A case in point. In: J.D. McKinney (ed.), Environmental Health Chemistry, pp. 3-14. Ann Arbor Science Publ., Ann Arbor, Mich.

Moore, M.M. 1979. Cellular responses to polycyclic aromatic hydrocarbons and phenobarbitol in Mytilus edulis. Mar. Environ. Res.

Noel-Lambot, F., C. Gerday and A. Disteche. 1978. Distribution of Cd, Zn and Cu in liver and gills of the eel Anguilla anguilla with special reference to metallothioneins. Comp. Biochem. Physiol. 61C: 177-187.

Olafson, R.W., R.G. Sim and A. Kearns. 1979. Physiological and chemical characterization of invertebrate metallotionein-like proteins. In: J.H.R. Kägi and M. Nordberg (eds.), Metallothionein: Proceedings of the First International Meetings on Metallothionein and Other Low Molecular Weight Metal-Binding Proteins, pp. 177-204. Brikhäuser Verlag, Boston, Mass.

Overnell, K. and T.L. Coombs. 1979. Purification and properties of plaice metallothionein, a cadmium-binding protein from the liver of the plaice (Pleuconectes platessa). Biochem. J. 183: 277-283.

Overnell, J. and E. Trewhella. 1979. Evidence for the natural occurrence of (cadmium, copper) metallothionein in the crab Cancer pagurus. Comp. Biochem. Physiol. 64C: 69-76.

Porter, H. 1974. The particulate half-cystine-rich copper protein of new-born liver: Relationship to metallothionein and subcellular localization in non-mitochondrial particles possibly representing heavy lysosomes. Biochem. Biophys. Res. Commun. 56: 661-668.

Pruell, R.J. and F.R. Engelhardt. 1980. Liver cadmium uptake, catalase inhibition and cadmium thionein production in the killifish (Fundulus heteroclitus) induced by experimental cadmium exposure. Mar. Environ. Res. 3: 101-111.

Quraishi, M.S. 1977. Biochemical Insect Control. John Wiley and Sons, New York.

Reid, M.D. and G. Krishna. 1973. Centrolobular hepatic necrosis related to covalent binding of metabolites of halogenated aromatic hydrocarbons. Exper. Mol. Pathol. 18: 80-99.

Richards, M.P. and R.J. Cousins. 1975. Influence of parenteral zinc and actinomycin D on tissue zinc uptake and synthesis of a zinc-binding protein. Bioinorg. Chem. 4: 215-224.

Roesijadi, G. 1982. Uptake and incorporation of mercury into mercury-binding proteins of gills of Mytilus edulis as a function of time. Mar. Biol. 66: 151-157.

Rugstadt, H.E. and T. Norseth. 1978. Cadmium resistance and content of cadmium-binding protein in two enzyme-deficient mutants of mouse fibro blasts (L-cells). Biochem. Pharmacol. 27: 647-650.

Sabbioni, J.E. and E. Marafante. 1975. Heavy metals in rat liver cadmium binding protein. Envir. Physiol. Biochem. 5: 132-141.

Stegeman, J.J. and R.L. Binder. 1979. High benzocal pyrine hydroxylase activity in the marine teleost fish Stenotomus versicolor. Biochem. Pharmacol. 28: 1686-1690.

Stegeman, J.J., A.V. Kotz, B.R. Woodin and A.M. Pajor. 1981. Induction of hepatic cytochrome P-450 in fish and the indication of environmental induction in a scup (Stenotomus chrysops). Aquat. Toxicol. 1: 197-212.

Viarengo, A., G. Zanicchi, M.N. Moore and M. Orunesu. 1981. Accumulation and detoxification of copper by the mussel Mytilus galloprovincialis Lam: A study of the subcellular distribution in the digestive gland cells. Aquat. Toxicol. 1: 147-157.

Vinson, S.B., C.E. Boyd and D.E. Furgeson. 1963. Resistance to DDT in the mosquito fish Gambusia affinis. Science 139: 217-218.

Webb, W. 1972. Protection by zinc against cadmium toxicity. Biochem. Pharmacol. 21: 2767-2771.

Winge, D., J. Krasno and A.V. Colucci. 1973. Cadmium accumulation in the rat liver: Correlation between bound metal and pathology. In: W.G. Hoefkstra, J.W. Suttie, H.E. Gantner and W. Mertez (eds.), Trace Element Metabolism in Animals, Vol. 2., pp. 500-501. University Park Press, Baltimore, Md.

Chapter 5. Chemical Measurements and Effects Criteria

Introduction

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Those who have been involved with chemical measurements in the marine environment have seen numerous changes over the past decade or so. Advances in instrumentation now allow scientists to look at substances which previously went undetected. In addition, many of the substances now entering the marine environment had not even been developed several decades ago. We are just now starting to determine as many compounds as possible in our analyses rather than limiting ourselves to those on some list which have been deemed important. Much progress is being made.

It is not likely, however, that the chemist of twenty to fifty years ago would be totally lost in our modern laboratories. This is because many of the analyses performed then are still required and are accomplished in similar ways today. I sometimes question if some of these are really necessary or just required by outdated regulations. There is no reason to believe that the needs or demands for certain types of chemical analyses should remain constant nor should we feel that everything we have done in the past was useless. Rather we should be in a position of reevaluating and perhaps modifying our contributions to environmental protection of the marine environment as needs and advances dictate. The papers in this session are two attempts at this.

Meaningful Chemical Measurements in the Marine Environment—Transition Metals

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The following comments present a critical, but not negative, point of view that seeks identification of improved approaches to "marine pollution effects" studies. The current literature has many examples of the disquiet that the authors experience in reviewing or participating in recent studies. As pointed out by Dayton (1982), in reviewing the proceedings of a symposium: The Shore Environment, "Environmental protection programs are increasingly criticized by ecologists, regulatory and management agencies, and private business as being of questionable quality and value. Because regulatory agencies and many ecologists are uncomfortable with the highly probabilistic nature of ecology, there is a tendency, often a legal necessity, for impact studies to be very detailed and specific and to collect reams of data that have no underlying logic and defy generalization or test. This prevents the growth of coastal ecology as a science." It seems interesting and paradoxical that the collection of a large amount of data prevents the growth of a particular science, but it seems to be true.

Further on in Dayton's review, he expresses what may be the cause of the paradox: "Though the editors have been successful in presenting integrated holistic studies and many of the papers are current and innovative, explicit recognition of the importance of attempting to falsify hypotheses as a scientific method is generally lacking. Assuredly, emphasis on rigorous testing can be overdone when the hypotheses are trivial. Nevertheless, I would have hoped

to see more evidence of the use of the experimental methods and an explicit recognition of the scientific futility of testing general hypotheses with computer analyses of data collected without concern for mechanistic questions. . . . It is to be hoped that increasing sophistication of regulatory agencies and decreasing funds will soon result in a merger of mechanistic science and environmental monitoring." This example illustrates the point that data collection without clear hypothesis formulation is really the cause of the "prevention of the growth of coastal ecology as a science."

A feeling of dissatisfaction is expressed also by Gross (1981) in a review of Industrialized Embayments and Their Environmental Problems: A Case Study at Swansea Bay. He remarks that, "the volume will be a useful reference to researchers interested in coastal ocean areas and their alteration by human activities. Its weaknesses are the familiar failure to demonstrate the relevance of the science presented and the failure to explain the science to nonscientists. It is worth noting that none of the papers in the volume refers to comparable U.S. environmental studies such as the Corps of Engineers' Dredged Material Research Program, or NOAA's New York Bight Project. So far as I know, the neglect is mutual: Environmental studies of the 1970's were apparently done in nearly scientific isolation. Perhaps this accounts for the slight progress made in environmental studies during the past ten years." It seems a reasonable guess that the lack of "relevance of science presented" can be ascribed to the absence of clearly stated hypotheses to guide the work.

We cite one more example of unavoidable critical appraisals. The NAS-NRC committee to evaluate outer continental shelf (OCS) environmental studies came to this conclusion: "The program does not now effectively contribute to leasing decisions or to the accrual of sound scientific information adequate for OCS management, both offshore and onshore. While the Bureau of Land Management (BLM), which administers the program, does not define it as a research program as such, a scientifically sound activity is nevertheless required. Our concern for the scientific content of the program, as distinguished from its utility to BLM, is that we could find very little evidence of explicit hypotheses or statements of scientific purpose for which the data were intended. Thus gathering of the data prescribed through formal bid instructions often leads to descriptive data for unknown purpose but does not necessarily lead to invalid scientific information. We do not wish to imply that we judge all work of this BLM program unscientific. However, the general lack of a scientific construct and specific hypotheses combined with uncertain relevance for departmental decisions, greatly erode the potential value of the program. Therefore, we urge BLM to execute a problem analysis to identify the information required to develop a program design suitable to obtain this information. Without a second scientific design focused on the rel-

evant issues, the present program will continue to produce inconclusive descriptions."

Current examples of distress in "marine pollution effects" studies could also be found in the drafts of "synthesis reports" for the NOAA New York Bight program and the EPA Chesapeake Bay Toxic Substances Program. The point is that chemical measurements have formed a substantial part of the data collection, and identification of improved "meaningful" measurements can be based only on recognition of current deficiencies. Our perception is that the dissatisfaction with many recent marine pollution effects studies results from failure to apply the scientific method to these problems. Table 1 is a simplistic outline of application of the scientific method to the study of estuarine and coastal processes. Perhaps the perplexity concerning the quality of the current literature comes from a failure to recognize that most work does not progress very far along the sequence in Table 1.

To illustrate this point, Boehm (1982) has given permission for quotation of a figure from a manuscript that describes measurements of some organic compounds in the New York Bight region. The data suggested to Dr. Boehm that several processes (resuspension and transport) need to be considered in the system that was sampled (Figure 1). The point that we would like to make is that Boehm's excellent work went only as far as Step 3 in Table 1. A limited amount of new data permitted him to formulate some speculative conclusions. The reader is frustrated in that there is no indication that the work is to be carried forward in terms of testing the significance of these hypothesized processes to produce a scientifically "meaningful" study.

Table 1. Scientific method for studying estuarine and coastal processes.

1. Exploratory Data Collection
2. Summary Description - Qualitative or Semiquantitative
3. Formulation of Process Hypotheses (Modelling Equations)
4. Data Collection for Hypotheses Evaluations (Model Quantitative Evaluation)
5. Scientific, Quantitative Tentative Conclusions
6. Use and Testing of Conclusions
A. Evaluation of Forecasts
B. Verification of Management Decisions

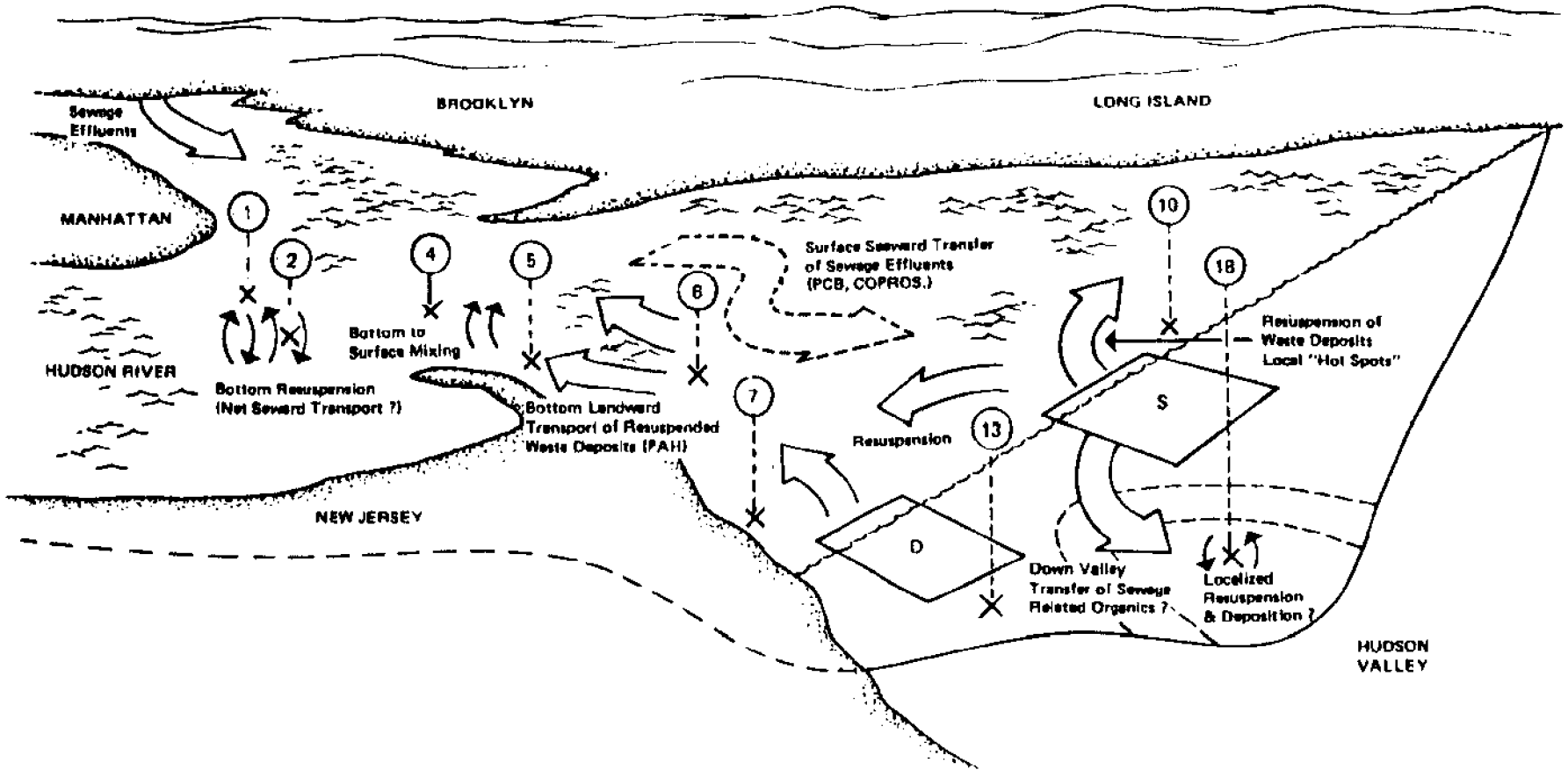

Figure 1. Organic pollutant resuspension and transport systems as suggested by data. Numbers refer to stations; arrows represent surface transport; arrows represent bottom or middle water transport (Boehm 1982).

We think that Dayton's hopes for increasing sophistication of regulatory agencies may lie primarily in the direction of recognition that many "research" programs have been stopped short of the scientific phases in Table 1 or have been continued for many years as monitoring or "baseline" data collection efforts without recognition that the scientific method has not been invoked.

The final inference that we would like to draw from the above briefly described examples is that the scientific method can be cost effective. Mindless monitoring will never produce understanding of environmental processes that will be useful in formulating public policy and regulations. Assessment of probable environment effects (impacts) should become realistic and meaningful as understanding, rather than larger data files, becomes the specified goal of environmental studies.

LIMITATIONS OF CURRENT TRACE METAL DATA

For inorganic materials, marine "pollution effects" often imply a focus on toxic substances such as some of the transition metals (Cu, Zn, Cd, Hg, Pb, Ni, Cr, etc.). In contrast to the synthetic organic materials that are entirely anthropogenic these metals are naturally present and "pollution effects" need to be discerned against the natural background occurrences.

Unanticipated Temporal and Spatial Viability

Many of the data for the transition metals cannot be interpreted in terms of the processes that control metal abundance in estuarine and coastal waters because the designs of the sampling programs did not adequately anticipate the temporal and spatial variability that is present. Variability can be considered as composed of two parts.

1. Components considered in the equations that are the quantitative hypotheses (models). These may have annual, seasonal, daily, tidal and diurnal time scales and spatial scales of meters to kilometers. The sampling design should be derivative from the hypotheses and, in absence of clear statements of hypotheses, the data interpretation is frequently based on an ad hoc scheme developed posteriorly.

2. Random variability (noise) that comes from the lack of homogeneity in the system being sampled. While it is trivially obvious that the sampling must be carried out with sufficient replication to provide "representative" data, much of the current information is woefully deficient in this regard, as will be illustrated in the following examples. However, particular note should be taken that regular variability not considered in the sampling design then contributes to the noise; for example, processes that are operative on daily,

tidal or diurnal time scales would contribute variability if monthly sampling is used to look at seasonal cycles and samples are collected at different times of the day or stages of the tide.

Some data from the recent literature illustrate these features. For the toxic metals, anthropogenic damage (pollution) may occur primarily in the estuarine and coastal environments. The processes that cause variability in metal concentrations are diagramed in Figure 2. Attention should be directed to the obvious feature (which is neglected to a remarkable extent in much current work) that the estuarine and coastal environments are places where upland drainage mixes with water from the open oceans. A major cause of temporal and spatial variations in metal concentrations for these environments may be the processes that take place outside the study area; that is, the drainage basin chemical dynamics may be reflected in the estuarine or coastal area. The major variations in the source water can be ascribed frequently to seasonal variations in discharge rates, particularly with respect to suspended solids and associated metals, as shown in Figure 3, for samples from the mouth of the Susquehanna River as it enters the Chesapeake Bay.

Further perspective on the challenge to understanding the dynamics of anthropogenic metal damage is displayed in Figure 4. It is important to remember that these temporal variations at the head of Chesapeake Bay will be expressed as spatial variations in the estuary as these waters continue to move seaward. Downstream "pollution" would need to be discerned against this substantial variability. Zinc is predominantly associated with settleable solids, but does not correlate very well with the variations in iron concentrations. Week-to-week variations of twofold or more were found, with a pulse of soluble zinc in January. Copper and nickel were roughly equally distributed between the solid phase and filter-passing or "soluble" phase. The winter pulse of nickel might be a vegetative input, but the possibility of inputs from the burning of fossil fuels needs to be studied using rainwater samples from the watershed. However, the lack of large metropolitan areas on the Susquehanna watershed may rule out fossil fuels as a quantitatively important source. The lack of an increase in the copper and nickel concentrations during February, March and May, comparable with the increase shown by iron, manganese, and zinc suggests that the solids carrying the copper and nickel have a different source and character.

The challenge to understanding is reflected further by the data plotted in Figure 5, which are the same values as those shown in Figure 4 except that the observed metal contents of the samples are expressed as weight concentrations in either the settleable or filterable solids. The most striking feature is the seasonal varia-

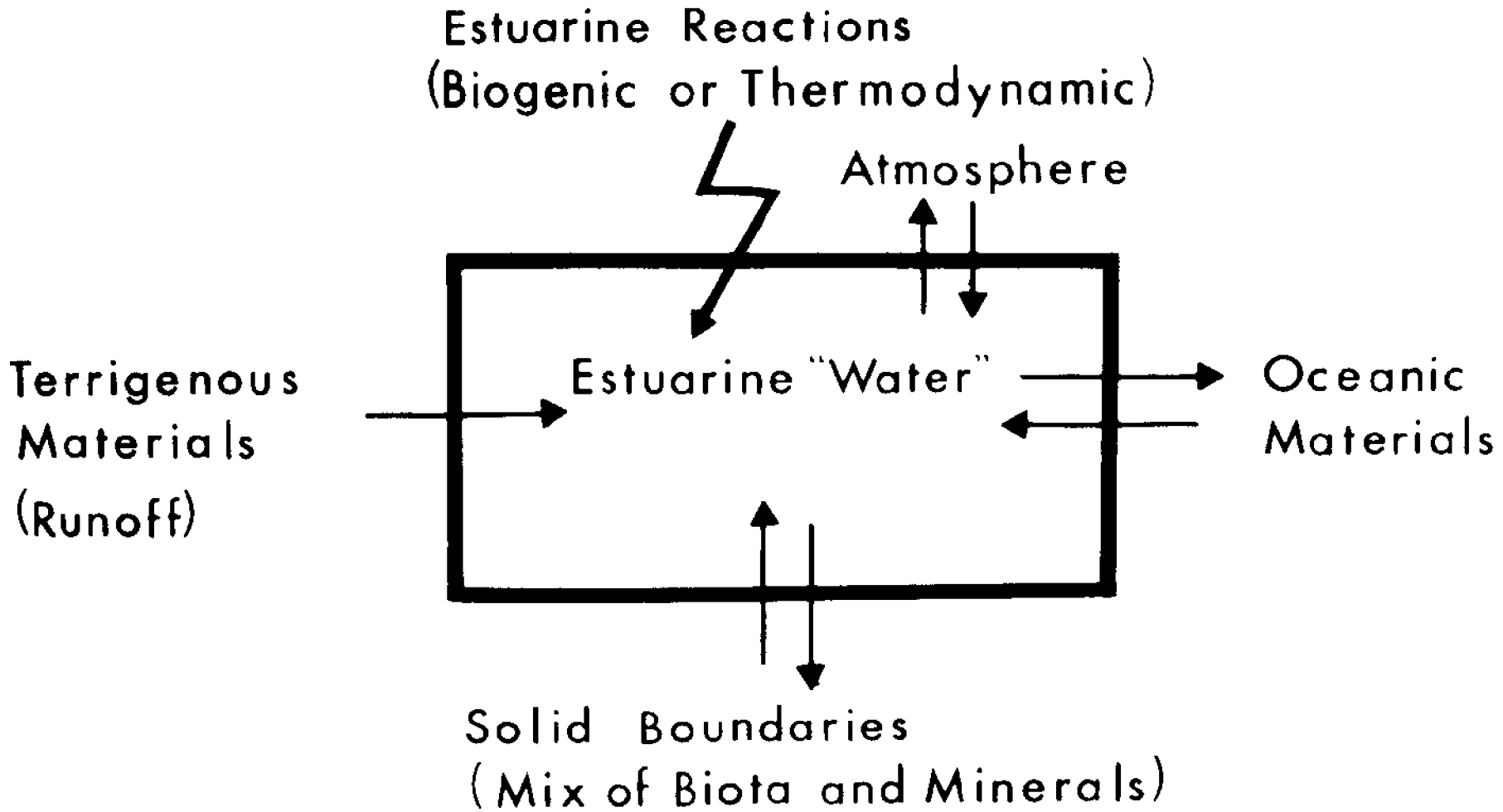

Figure 2. Schematic representation of the processes that affect the composition of estuaries (Carpenter et al. 1975).

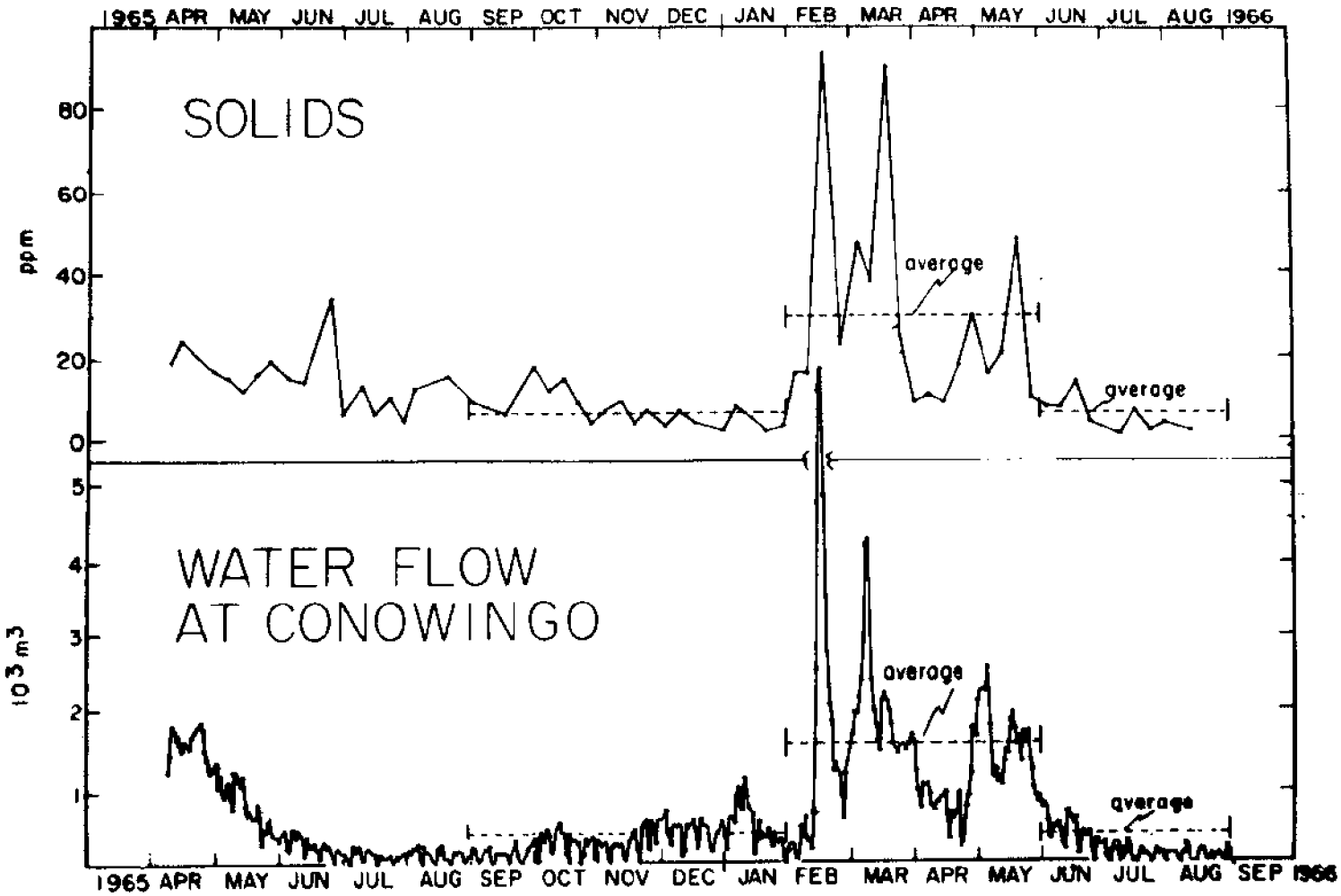

Figure 3. Flow rates of the Susquehanna River and the concentrations of suspended solids in the samples (Carpenter et al. 1975).

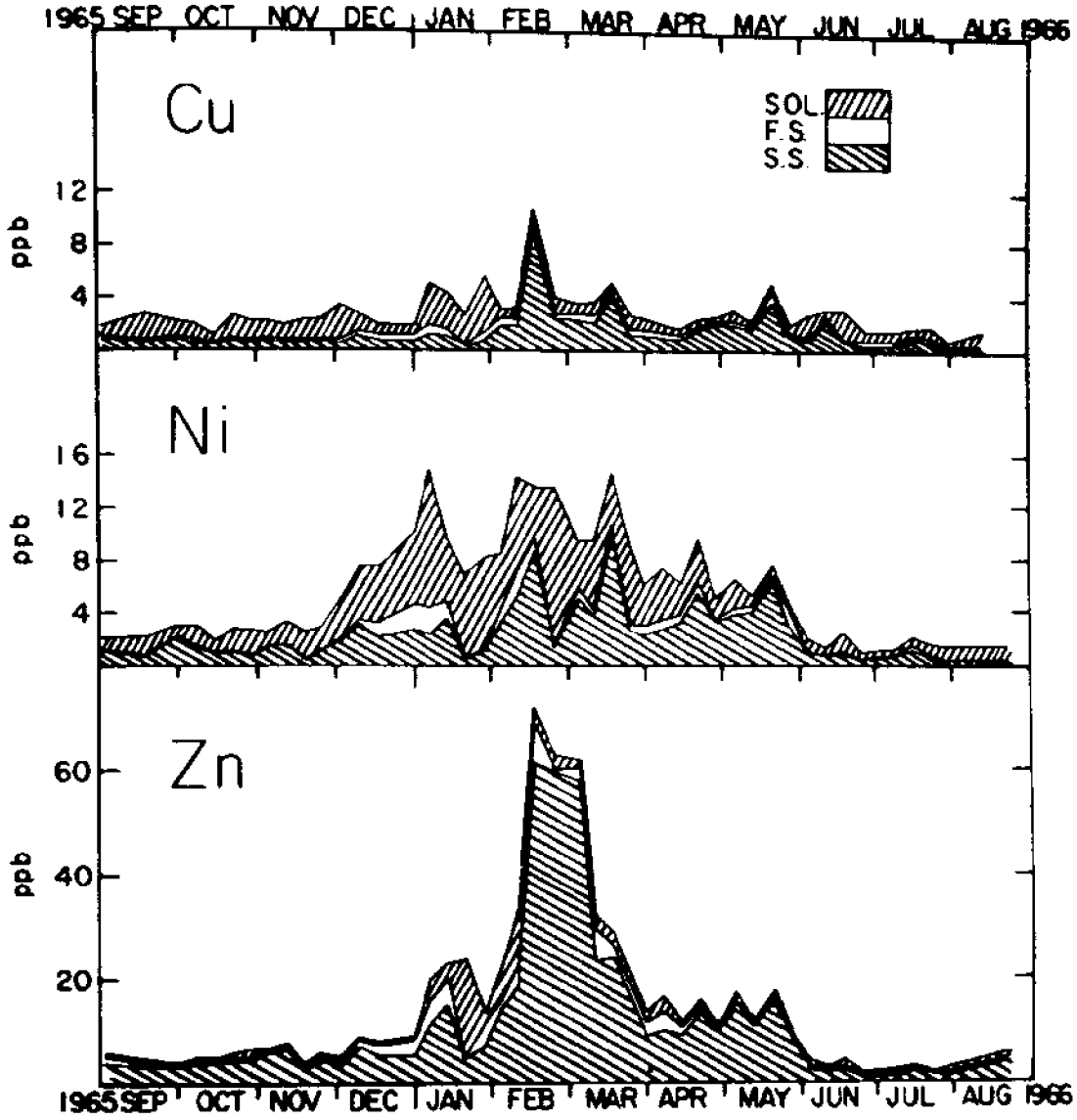

Figure 4. Copper, nickel and zinc concentrations in the soluble, filtered-solids and settled-solids fractions of the samples (Carpenter et al. 1975).

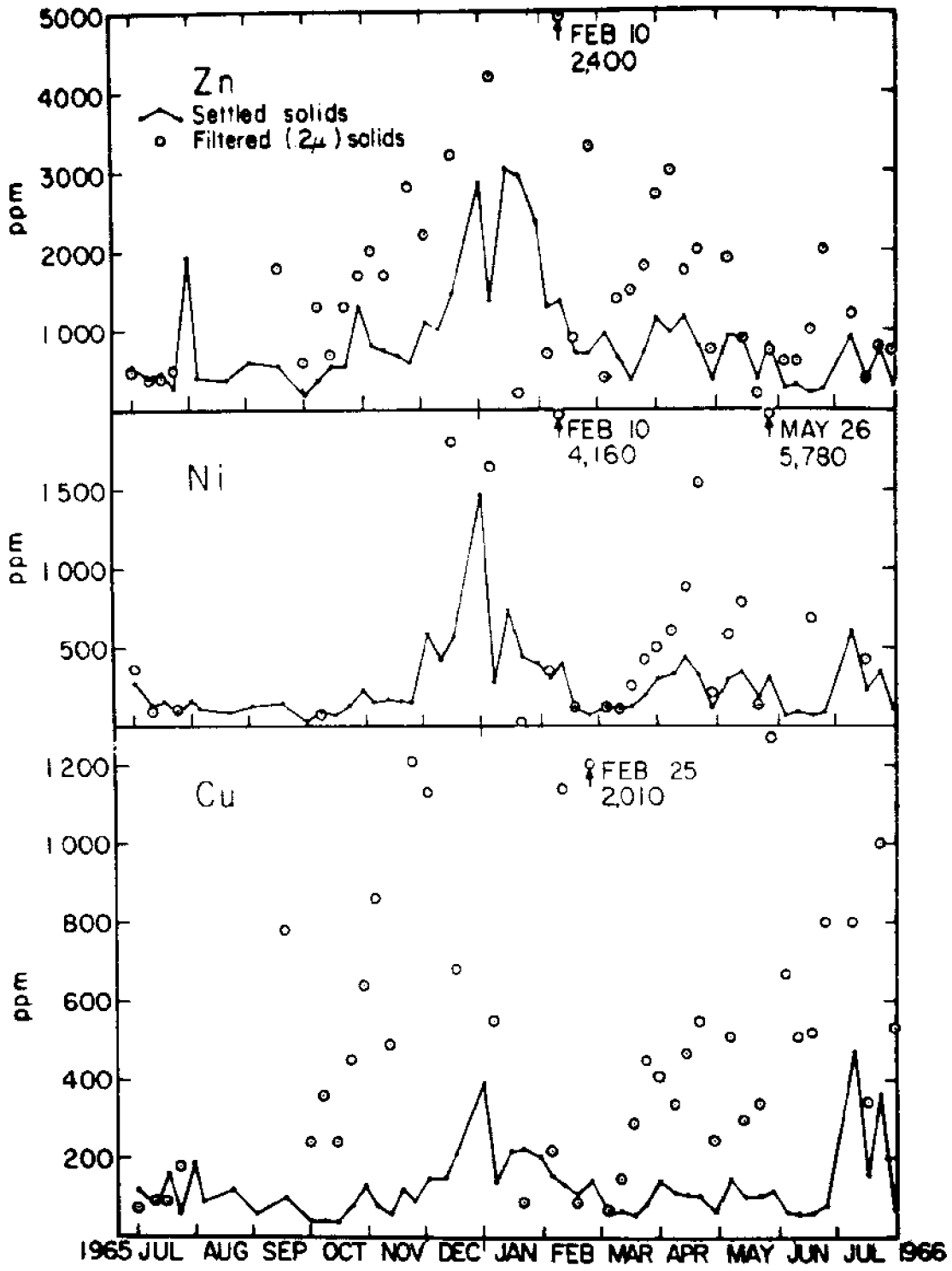

Figure 5. Zinc, nickel and copper concentrations in the separated solids (Carpenter et al. 1975).

tion in the composition of the solids, metal-rich materials being more abundant during December and January. The non-settleable or filterable materials (either small size or low density) probably were high in organic and aluminosilicate content; they frequently had much higher concentrations of metals, but were extremely variable.

The variable metal content of the solids as a function of particle size or density is present also in estuarine sediments. Huggett (1981) reported the data shown in Figure 6 that displays an intimidating large variation in the copper content of Chesapeake Bay sediments. Detection or realistic perception of estuarine damage due to anthropogenic copper in the face of this observed variability requires greater skill and thoughtfulness than has been present in much recent work.

That the metal content of suspended material in estuaries like Chesapeake Bay is highly variable has been documented recently by Nichols et al. (1982). Many observations (5576) were made and Table 2 summarizes the data. The expectable variability due to the processes diagramed in Figure 1 is clearly present but the absence of process hypotheses in the program design impairs interpretation of this large data set in terms of the relative significance of the various processes and resolution of anthropogenic contributions to this system.

One source of variability that was unusually well documented by Nichols et al. (1982) was the temporal variation in suspended solids and associated metals. As shown in Figure 7, variations greater than twofold were found over a tidal cycle. This source of variability was mitigated by scheduling observations close to slack water ($\pm$ 1 hour) and does not contribute to the data summarized in Table 2. However, from the point of view of aquatic toxicology and evaluation of possible anthropogenic damages, these time variations in the concentrations of suspended solids and associated metals need to be considered in terms of possible responses by organisms. Bioassay data in the literature have been focused on time invariant exposure to various toxicants and one cannot help but wonder whether, in the "real-world" environments such as Chesapeake Bay, the organisms respond to the mean metal concentrations or to the complex temporal variations.

Seasonal variability in both natural and anthropogenic metal concentrations derives largely from variations in the values of upland runoff. Consider an anthropogenic input to the river or near the head of an estuary and the resulting concentration distribution as shown in Figure 8, which might be a plot of a single survey. Measurement of chlorinity or salinity makes it possible to compute the effects of dilution with sea water, and such plots have been used to estimate the effects of processes other than dilution. However, simple interpretation is not possible; for example, the difference labeled with a question mark in Figure 8 might be due to

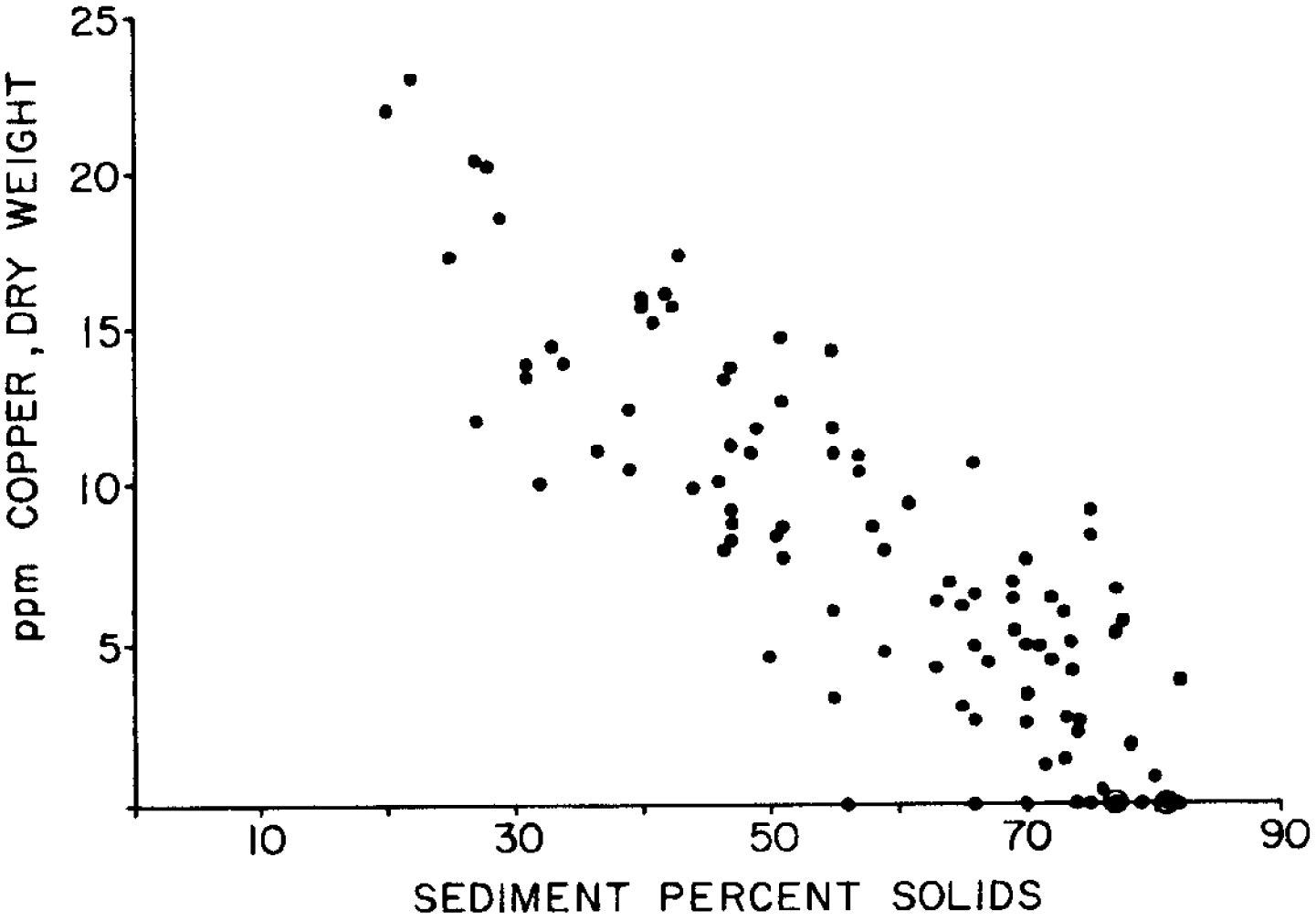

Figure 6. Copper concentrations in the top 2 cm of bottom sediments from Chesapeake Bay plotted with the respective percent solids (Huggett 1981).

Table 2. Summary of mean metal concentrations and range of Bay-wide values in suspended material, expressed as weight per weight and weight per volume*.

$\mu g\ g^{-1}$			$\mu g\ L^{-1}$		
Metal	Mean	Range	Metal	Mean	Range
As	13.00	0.55-100.00	As	0.32	0.006-5.00
Cd	14.16	0.12-790.00	Cd	0.14	0.003-3.80
Cu	127.96	9.90-570.00	Cu	1.84	0.068-17.00
Fe(%)	3.11	0.29-17.00	Fe	0.88	0.01-12.00
Pb	160.30	21.00-730.00	Pb	2.27	0.10-15.00
Mn	2.88	0.08-46.00	Mn	65.13	0.48-1000.00
Hg	3.89	0.05-59.00	Hg	0.035	0.01-0.47
Ni	95.80	4.80-770.00	Ni	2.00	0.03-34.00
Sn	17.97	0.25-290.00	Sn	0.20	0.01-4.80
Zn	0.75	0.10-7.10	Zn	11.02	0.55-94.00

* Nichols et al. (1982)

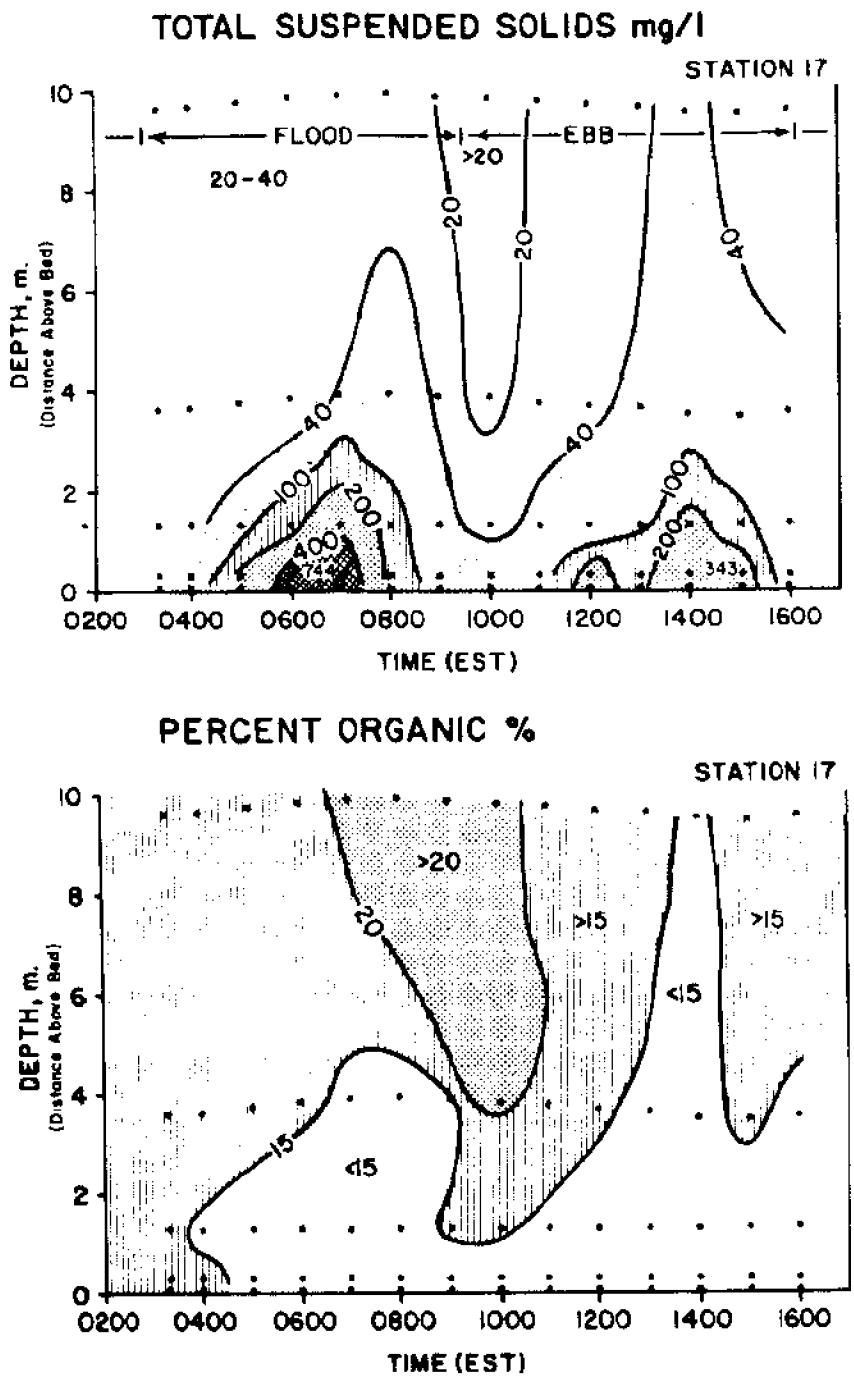

Figure 7. Temporal variations of total suspended solids and percent organic content with depth (Nichols et al. 1982).

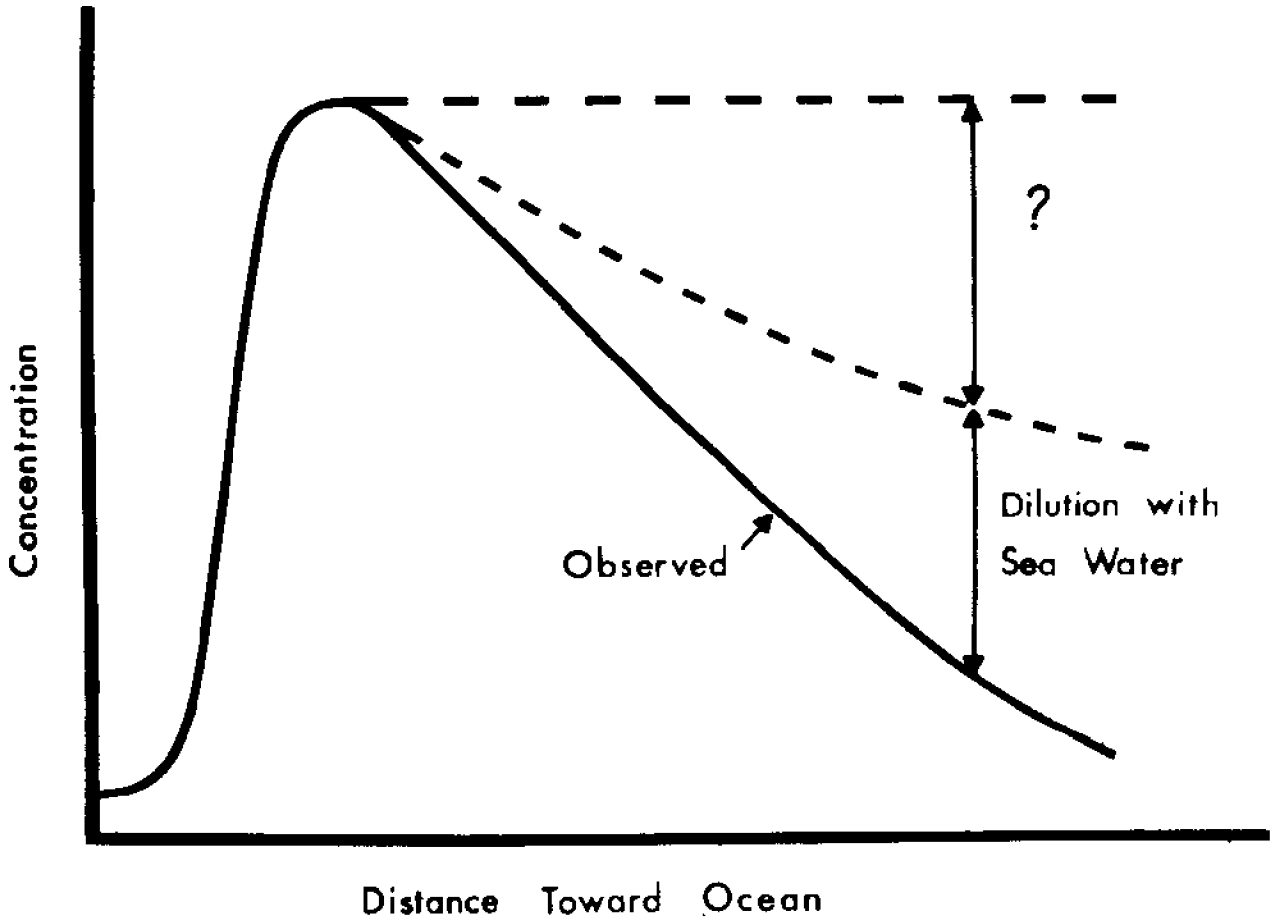

Figure 8. Example of the longitudinal concentration variation from a point source input (contamination), solid line. Small-dash curve represents estimate of concentration that would have been found if no dilution had been present. Difference between small-dash curve and large-dash curve requires identification of additional process from several possibilities.

(a) transformation of the constituent into a form not determined in the analytical procedure, (b) loss to the sediments, (c) input of the constituent having varied with time, or (d) the flow rate of the river having varied with time. The last possibility always exists, and the curve in Figure 8 may simply indicate that the water in the more seaward part of the estuary passed the point of contaminant input at a high rate of flow and never had the concentration that was being produced at the source point at the time of the survey. Evaluation of this effect requires adequate time-varying "models" (equations) for estuarine flow and mixing and a comprehensive set of data for variations as a function of both position and time. Because of these features, our understanding of the fate of contaminants has not progressed very far, and progress awaits simultaneous developments in theory and observation.

Natural variabilities in trace metals are present in marine and estuarine organisms as well as in bottom and suspended sediments. An example of this phenomenon in organisms which supposedly integrate varying inputs of substances over time and thus smooth out temporal variations is presented utilizing copper and zinc in oysters, Crassostrea virginica (Huggett et al. 1973). In this study replicate oganisms were collected from the same bed at the same time and were analyzed. The resulting data showed that the metal concentrations in individuals, which were presumed to be the same age, often differed as much as 100% and occasionally 300%. In addition, it was shown in this and further studies that even in the absence of pollution sources, oysters in fresher waters of estuarine systems contain more metals than those from more saline waters (Huggett et al. 1975).

Obviously if natural variations such as these are not incorporated into the sampling design, then valid conclusions relative to the impact of human activity will be difficult if not impossible.

Total Metal Content as Subject of Data

Most data are for total metal content. Although the total concentrations of various metals in suspended solids and sediments are of interest in simple geochemical studies, such data are of limited use in assessing anthropogenic damage. The recent, extensive study of Chesapeake Bay sediments by Helz et al. (1981) provides a good example of geochemical studies of estuarine sediments. This group carried out 8000 individual analyses of 12 metals and came to the conclusion that "a number of processes may influence the observed vertical profiles (of sediment metal concentrations). No single process, such as anthropogenic contamination, provides an adequate explanation for all the data." This work provides a good example of the labor required to provide a description of the metal distributions in Chesapeake Bay sediments and indicates that in the absence of hypothesis formulations, resolution of the magnitudes of the various processes awaits further scientific studies based on this work.

However, knowledge of the abundance of the metals in the sediments does not provide a basis for evaluating anthropogenic damage. Helz et al. used refluxing in a 90-10 mixture of nitric and hydrochloric acids to solubilize the metals from the sediment samples. This harsh technique had the virtue of giving reproducible results but probably solubilized much metal that would not be available to benthic organisms. Data obtained by such means do not appear to be applicable to understanding the toxicity or lack of toxicity of metals in estuarine sediments.

This point is demonstrated very clearly in the recent work of Rubinstein et al. (1982) of the EPA Gulf Breeze laboratory. Sandworms, hard clams and grass shrimps were exposed for 100 days to sediments from New York harbor that had been contaminated with PCBs, mercury and cadmium. Some transfer of PCBs to the animals was found but no transfer of mercury or cadmium was observed. As they state, "Results from this study support the contention that sediment concentration alone does not reflect bioavailability and that bioassays and field monitoring remain the most direct method for estimating bioaccumulation potential of sediment bound contaminants at this time." It seems clear that new, appropriate chemical techniques for characterization of the metal content of sediment solids are needed for "meaningful measurements of marine pollution".

Another example of the importance of bioavailability in assessing impacts from anthropogenic inputs was demonstrated in a study by Haven and Morales-Alamos (1979). In this work Kepone-contaminated sediments were collected from the James River in Virginia and presented to oysters as a suspension under controlled laboratory conditions. Analysis of the oyster feces and pseudofeces showed that the Kepone levels averaged 3.5 times higher in the feces than in the pseudofeces. The explanation is that oysters selectively differentiate the particles that they filter. Those that they pass through their gut are voided as feces and the remainder exit as pseudofeces. Since Kepone is associated with the organic fraction of the sediments (Huggett et al. 1980) and since the oysters obtain their energy requirement from it and hence "eat" this fraction, the feces are relatively enriched with the pesticide. Obviously, in the case of Kepone and oysters, more relevant information would be obtained if the organic fraction of sediment were analyzed rather than the total. Even though this example concerns an organic compound, it appears likely that such differentiations are important for metals as well.

Dependence of Toxicity on Undetermined Chemical Speciation

The attitude of administrators and regulators that inexpensive, simple testing techniques for "marine pollution" are what is needed is not surprising. However, the truth stands squarely in the way of simplistic "quick-fixes" and biogeochemical quackery. Even for the

simple "dissolved" toxic metal content of estuarine and coastal waters, the toxicity depends on the chemical form of the metal. Regulatory strategy that seeks reasonable protection of marine life and public health by shying away from both overprotection and underprotection will have to be based on the translation of scientific knowledge into policy, rather than vice versa. The intricacy that must be faced may be illustrated by drawing on the recent literature concerning the aquatic toxicity of copper. The purpose is not to review the literature on copper, but rather to cite some work that shows the nature of the "tiger that we have by the tail".

At the present time, many states have adopted water quality standards for copper; for example, the State of Florida 1979 standard was promulgated at 0.015 mg L^{-1} (15 ppb) for marine waters from the older general value of 0.5 mg L^{-1} (500 ppb). The guidance provided by the NAS/NAE (ESB 1972) states "on the basis of data available at this time, it is suggested that concentrations of copper equal to or exceeding 0.05 mg L^{-1} (50 ppb) constitute a hazard in the marine environment and levels less than 0.01 mg L^{-1} (10 ppb) present minimal risk of deleterious effects." Presumably that guidance was considered in setting the revised Florida water quality standard for copper.

The NAS/NAE report drew primarily on bioassay work with larger marine animals. Subsequent research (Sunda and Gillespie 1979; Sunda and Ferguson 1982) has shown that smaller organisms, particularly bacteria and phytoplankton, are remarkably sensitive to ionic copper, responding (with growth reduction) at levels of approximately 0.0005 ppb free cupric ion. In seawater, inorganic complexes (with hydroxide and carbonate anions) form with a ratio of inorganically complexed copper to free copper of roughly 60 to 1. If this inorganic complexing were the only detoxification present, the water quality criterion would need to be roughly 0.03 ppb to protect bacteria and phytoplankton. Since the natural copper concentrations in estuarine and coastal waters range from 1 to 0.1 ppb, copper toxicity would be widespread were it not for detoxification by complexing with organic materials. As Sunda and coworkers (ESB 1972; Sunda and Gillespie 1979) have shown, the organic complexing of copper is extensive and nearly all the copper (96-99%) in their samples was organically bound.

It seems obvious that site-specific knowledge is necessary to establish water quality standards for copper. Bioassay techniques would be useful except that they are extremely tedious and require great skill and care by the observer. Chemical techniques may be an attractive alternative but, in view of the extremely low concentration of copper that causes biological responses, the chemical techniques will not be as simple or inexpensive as regulatory personnel might hope. However, such costs may be minuscule compared with the economic burdens of overprotecting or underprotecting our aquatic environments.

One candidate technique for observing the capacity of natural waters to bind or detoxify copper is anodic stripping voltametry (ASV). Discussions in the recent literature have raised questions as to whether there are undiagnosed artifacts in the use of ASV. Some recent unpublished work by M. J. Spencer in her doctoral research at the University of Miami will be briefly outlined to show that the ASV technique, in which the copper complexing capacity of samples is determined by titration with copper, provides data that, if properly interpreted, are a good measurement of copper complexing capacity (Figure 9).

The first point brought out by Spencer's research is that the copper-binding compounds can be isolated from seawater by ultrafiltration (500 daltons nominal pore size). Having found a way to concentrate the compounds, she could determine the complexing capacity by an independent method based on an equilibrium binding gel filtration technique, obtaining results such as those shown in Figure 10. The integral quantity of copper shown in this chromatogram corresponds to 38 nM (2.4 ppb) complexing capacity in the original sample, and the titrimetric value for this sample was 35 nM. This agreement between the two measurements that involve entirely different techniques seems to be strong evidence that her ASV procedure and data interpretation are sound.

A second point in Spencer's work is that pseudopolarograms (like those shown in Figure 11), constructed from stripping peak versus plating potential data, showed that the copper-organic complex was electroactive--albeit to a lesser extent than would be possible for the inorganic copper complexes or no-titration. This previously unidentified property of the compounds has led to erroneous interpretations of the ASV titration data by a number of authors. Spencer derived equations for the calculation of the conditional stability constant based on an electroactive copper-organic complex mode. To test the soundness of this methodology, she titrated irradiated seawater samples containing known additions of EDTA with a standardized copper solution. The average conditional stability constant determined from these titration data was 2.3×10^{8}, which agrees quite well with the value of 1.9×10^{8} calculated from the literature for the various competing reactions with the calcium, magnesium, carbonate and hydroxide in seawater.

Using this new knowledge, Spencer determined an average conditional stability constant of 1.0×10^{9} for a number of samples from southeastern Florida coastal waters. This value is an order of magnitude higher than that calculated using the methodology of previous investigators in which the complexes were assumed to be nonelectroactive. When this average value is used together with typical values for the total copper and complexing capacity in southeastern Florida waters, 98.6% of the copper is predicted to be in the organic form and only 0.02% in the free copper ion or toxic form.

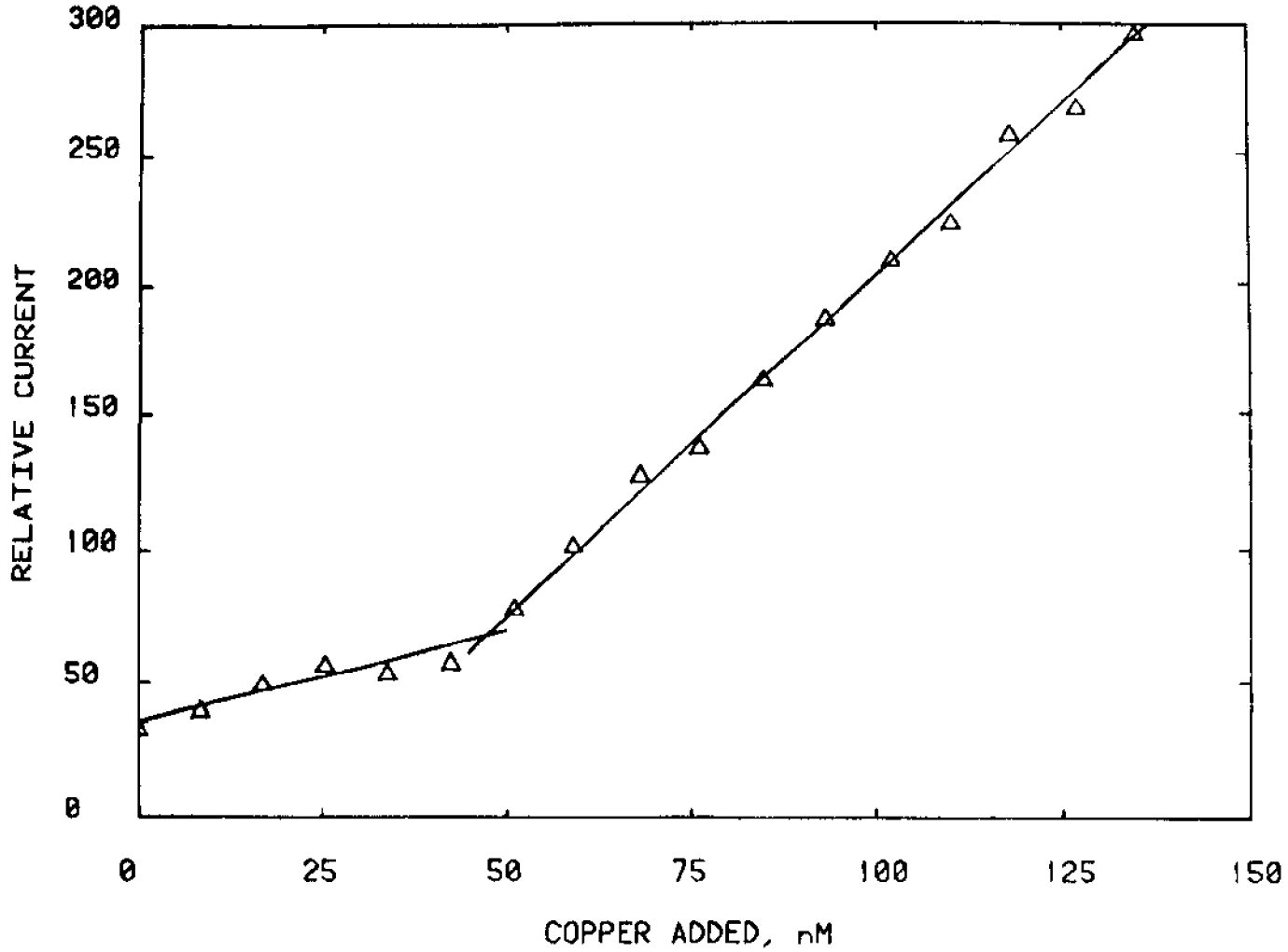

Figure 9. Titration of a Biscayne Bay sample with a copper solution. ASV was used to follow the course of the titration.

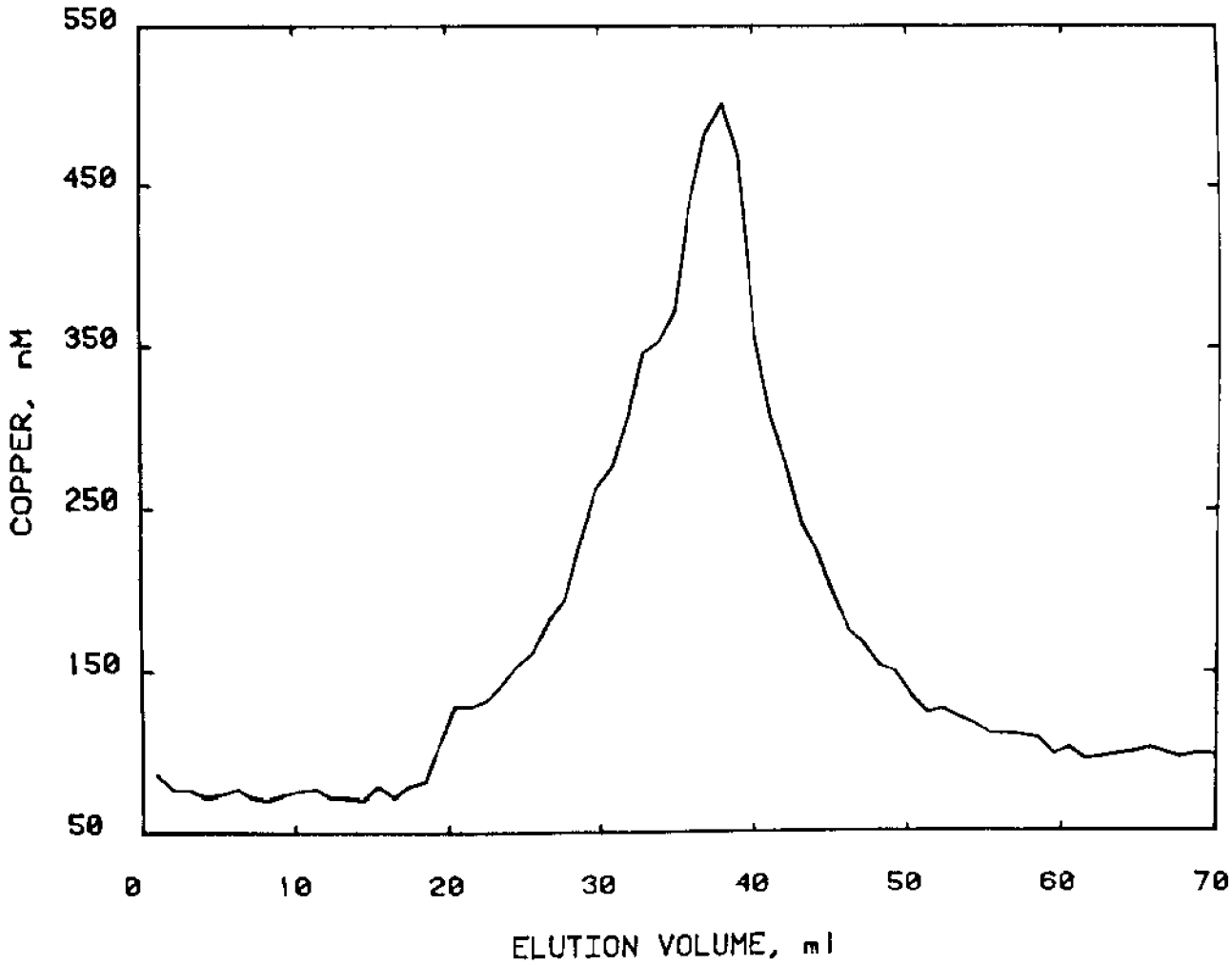

Figure 10. Chromatogram using equilibrium binding gel filtration of isolated copper complexing compounds to confirm titrimetric measurement of complexing capacity.

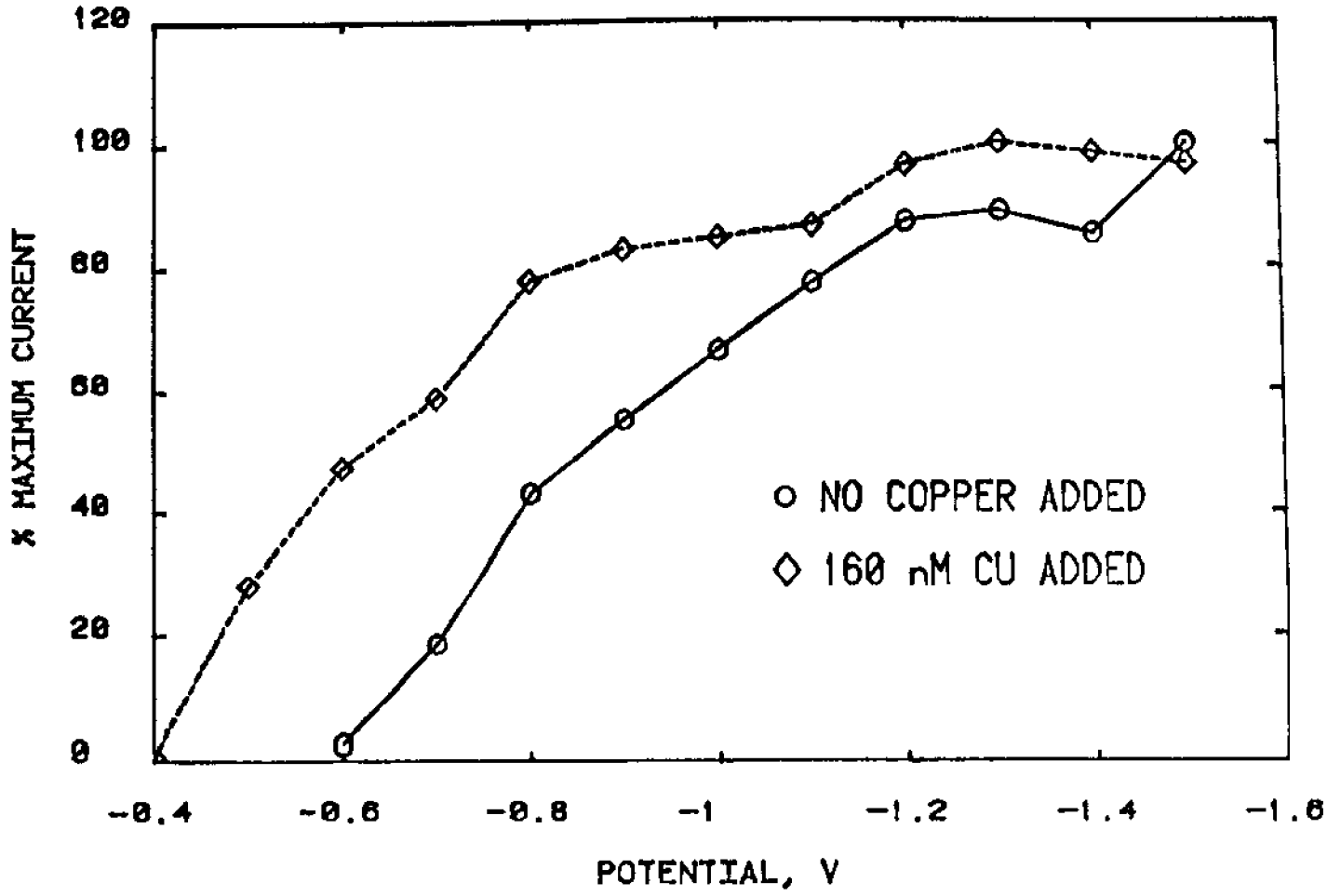

Figure 11. Pseudopolarograms of original sample showing one wave corresponding to reduction of organically complexed copper, and of copper spiked sample showing overlapping waves from inorganic and organic reductions.

It seems probable that the ASV titration technique with copper can yield observations having the same information content as that obtained with the tedious and tricky bioassay techniques.

Recent research clearly shows that aquatic organisms do not respond to the "total" toxic metal concentration but that the metal speciation controls the observed responses. "Meaningful" chemical measurements must reveal the speciation. The penalty of cost and complexity with the appropriate techniques should be more than offset in the utility of the resulting data.

CONCLUSION

This brief survey has been intended to support the following views. Many of the data (measurements) that might have some bearing on "marine pollution effects" from transition metals do not appear to be meaningful. This deficiency exists because the scientific method has been inadequately applied and the nature of the problems have not been well understood. In the positive sense it appears that, currently, sufficient descriptive data exist and new techniques are being developed for chemical measurements that will permit work with increased "meaning" or understanding in the future. "More of the same" doesn't look useful, but the groundwork for improvement has been laid. Research that seeks quantitative understanding of estuarine and coastal biogeochemical processes, including anthropogenic damage, must be carried out before straightforward monitoring or proctoring will be useful.

REFERENCES

Boehm, P.D. 1982. Investigations of estuarine/continental shelf and benthic/water column coupling of organic pollutant-bearing water column particulates in the New York Bight region. Can. J. Fish. Aquat. Sci. (in press).

Carpenter, J.H., W.L. Bradford and V. Grant. 1975. Processes affecting the composition of estuarine waters. In: L.E. Cronin (ed.), Estuarine Research, Vol. 1, pp. 188-214. Academic Press, New York.

Dayton, P.K. 1982. Book Review of "The Shore Environment: Proceedings of a Symposium." Science 215: 393-394.

ESB (Environmental Studies Board). Water Quality Criteria. 1972. National Academy of Sciences, National Academy of Engineers, Washington, D.C.

Gross, M.G. 1981. Book Review of "Industrialized Embayments and Their Environmental Problems: A Case Study of Swansea Bay." Am. Sci. 69: 330.

Haven, D.S. and R. Morales-Alamo. 1979. Uptake of Kepone from oysters and other bivalve molluscs. Final Report to U.S. Environmental Protection Agency (Grant No. R804993-01-0).

Helz, G.R., S.A. Sinex, G.H. Setlock and A.Y. Cantillo. 1981. Chesapeake Bay Sediment Trace Elements. Univ. of Maryland, College Park, Md.

Huggett, R.J. 1981. The importance of natural variabilities in the total analytical scheme. Biomed. Mass Spectrom. 8: 416-418.

Huggett, R.J., M.E. Bender and H.D. Slone. 1973. Utilizing metal concentration relationships in the eastern oyster (Crassostrea virginica) to detect heavy metal pollution. Water Res. 7: 451.

Huggett, R.J., F.A. Cross and M.E. Bender. 1975. Distribution of copper and zinc in oysters and sediments from three coastal-plain estuaries. In: F.G. Howell, J.B. Gantry and M.H. Smith (eds.), Proceedings of a Symposium on Mineral Cycling in Southeastern Ecosystems, Augusta, Ga., pp. 224-238, ERDA Symposium Ser. (CONF-740513, National Technical Information Service, Springfield, Va.)

Huggett, R.J., M.M. Nichols and M.E. Bender. 1980. Kepone contamination in the James River Estuary. In: R.A. Baker (ed.), Contaminants and Sediments, Vol. pp. 33-52. Ann Arbor Science Publ., Ann Arbor, Mich.

NRC (National Research Council). 1977. OCS Oil and Gas: An Assessment of the Department of the Interior Environmental Studies Program. National Academy of Sciences, Washington, D.C.

Nichols, M., R. Harris, G. Thompson and B. Nelson. 1982. Significance of suspended trace metals and fluid mud in Chesapeake Bay. Final Report to U.S. Environmental Protection Agency, (Grant No. R806002-01-1), EPA, Annapolis, Md.

Rubinstein, N.I., E. Loves and N. Gregory. 1982. Accumulation of PCB's, mercury and cadmium by Nereis virens, Mercenaria mercenaria and Palaemonetes pugio from contaminated harbor sediments. (Submitted).

Sunda, W.G. and R.L. Ferguson. 1982. Sensitivity of natural bacterial communities to additions of copper and the cupric ion activity: A bioassay of copper complexation on seawater. In: C.S. Wong (ed.), Trace Metals in Seawater, Proceedings of the NATO Advanced Research Institute Symposium in Erite, Sicily.

Sunda, W.G. and P.A. Gillespie. 1979. The response of a marine bacterium to cupric ion and its use to estimate cupric ion activity in seawater. J. Mar. Res. 37: 761-777.

Chemical Measurements of Organic Pollutants and Effects Criteria

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INTRODUCTION

There is a tendency among those of us working on marine pollution problems to foster and perpetuate certain unreal expectations. Principal among these is the myth that definitive answers are available, or that answers could soon be forthcoming, to any selected questions of pollutant impacts on marine environments. The simple truth is that we can provide few definitive answers. Moreover, the problems are so complex, the unknowns so many, and the research efforts so fragmented and poorly focused, that, if we proceed as we have been proceeding, it seems inescapable that we will contribute only marginal amounts of substantive information in the future on pollutant fates and effects.

Despite this rather disturbing perspective, it is our intent to focus our discussion on the linking of chemical measurements of organic pollutants with effects. The approach will be first to review the state of the art of analysis of organic chemicals in the environment and methods of determining effects of organic xenobiotics on marine biota. Next will be a more in-depth treatment of problems, attempting to link the analytical data with observed effects. We will draw on our recent research—including a recently completed survey of pollutants and abnormalities in biota of Puget Sound (Malins et al. 1980, 1982)—for examples of real world observations.

Finally, ideas will be presented about approaches for more closely relating analytically determined organic chemicals to effects, and for making progress in solving the extremely complex problems of marine pollutant impacts.

STATE OF THE ART AND LIMITATIONS OF CHEMICAL ANALYSIS OF ORGANIC SUBSTANCES

There are an estimated 63,000 chemicals in common use (Maugh 1978). Many of these chemicals eventually find their way into the marine environment. Clearly any attempt to analyze for even one-tenth of them presents a task of Herculean dimensions. Hence, it is necessary to focus on the more hazardous and bioavailable chemicals, while at the same time avoiding an over-simplified approach that fails to protect the environment.

To detect and quantify chemical contaminants in seawater, sediments and organisms, trace amounts of pollutants often have to be analyzed against a background of complex mixtures of hundreds of "natural" compounds. This is indeed a formidable task. In fact, the linking of chemical and biological parameters is profoundly limited because not all chemicals in sediment, biota or the water column can be identified or quantified at present.

In a recent study (Malins et al. 1980, 1982), more than 500 aromatic hydrocarbons (AHs) were revealed in a chromatogram of sediment from Puget Sound's Commencement Bay. Moreover, scores of halogenated compounds were shown to be present, but the identities of most of them could not be confirmed because reference mass spectra were not available. It is a regrettable fact that we have only a few hundred standards for positively identifying the thousands of compounds now detected in such gas chromatography (GC) profiles of chemicals.

We might ask, "How effectively have we addressed the identification and quantitation of foreign chemicals in the marine environment?" Overall, significant progress has been made in the past 5-6 years. In large measure it was due to advances in glass capillary GC and high performance liquid chromatography (HPLC). Figure 1 shows glass capillary GC profiles of unsaturated hydrocarbons extracted from the hepatopancreas of crab (Cancer gracilis) (Malins et al. 1980). The upper tracing shows the olefinic and aromatic hydrocarbons isolated by silica gel chromatography.

By rechromatographing this fraction on Sephadex gel and obtaining the simpler mixture shown in the lower tracing, it is possible to determine individual AHs characteristic of both fossil fuel and combustion products (Ramos and Prohaska 1981). Intralaboratory precision in these types of determinations generally has about 20-50% coefficient of variation, which is to be expected. However, interlaboratory agreement is usually less.

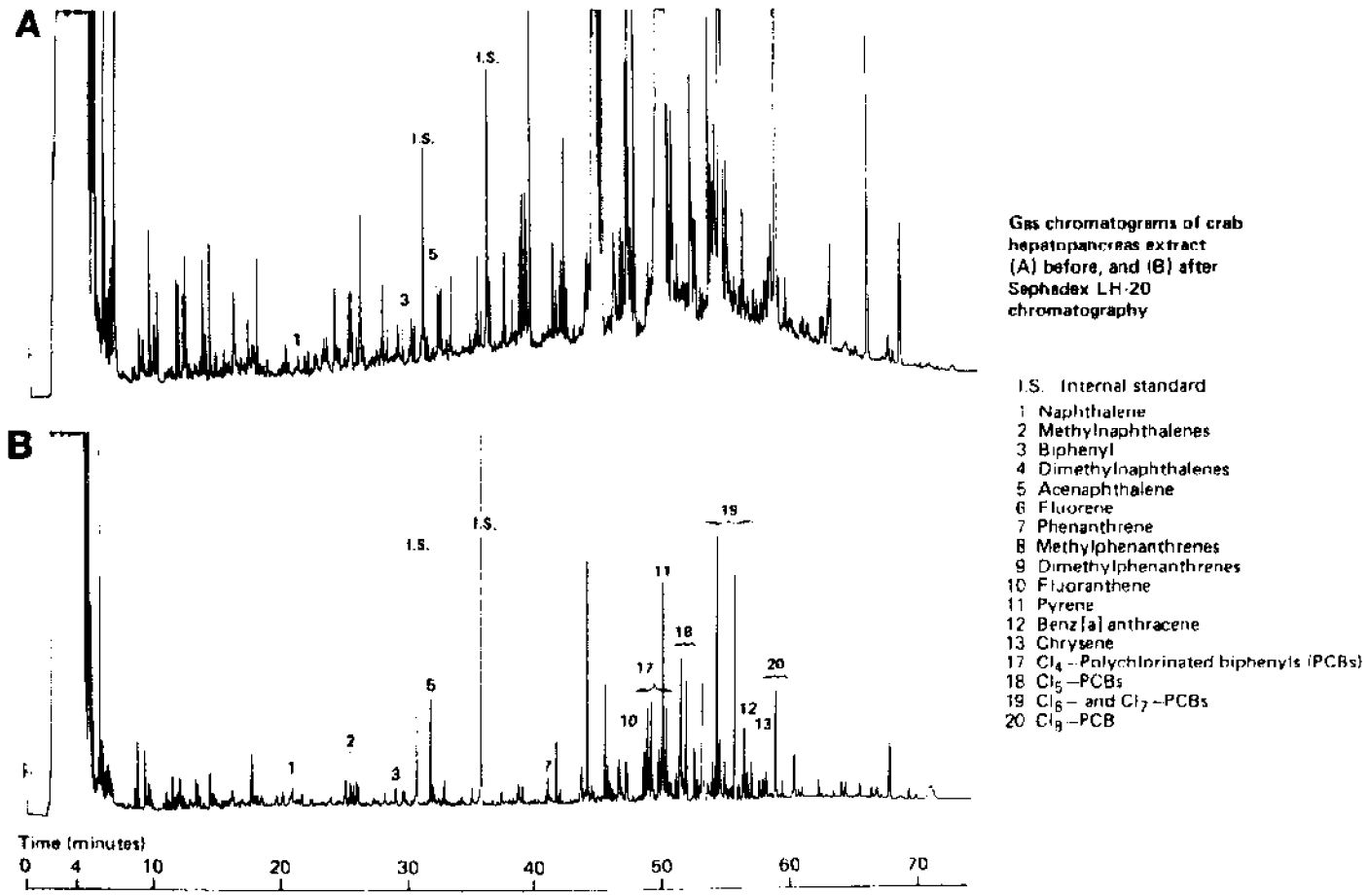


Figure 1. High resolution capillary gas chromatogram of unsaturated hydrocarbons extracted from crab (Cancer gracilis) hepatopancreas. Upper tracing (A) shows pattern after isolation by silica gel chromatography; lower tracing (B) was obtained after further chromatography on Sephadex LH-20.

Analytical methods have also improved recently for another class of marine pollutants, the chlorinated hydrocarbons. Among these ubiquitous compounds are the familiar chlorinated pesticides, such as dieldrin and DDT; PCBs; and chlorinated benzenes, butadienes and cyclopentadienes. Progress in resolving and quantifying chlorinated compounds is illustrated in Figure 2 which shows a typical GC profile of PCBs (Aroclor 1254) prior to glass capillary GC. Figure 3 demonstrates, however, how more detailed information on PCBs can now be obtained by glass capillary GC using an electron capture detector (Malins et al. 1982). Yet despite this improved resolution, environmental mixtures of chlorinated hydrocarbons may be far more complex than these data would indicate. For instance, in Figure 3 it is apparent why we should not limit our perspective to the PCBs alone. Had the analysis been restricted to PCBs, complex suites of other chlorinated hydrocarbons would have been completely overlooked, despite the fact that they may be just as toxic, or more so. In reflection, we sometimes tend to use PCBs as an overall indicator of pollution when in actuality they provide a very myopic perspective of impacted estuarine and coastal marine environments.

Comparable analytical procedures for other classes of organic compounds, such as chloro-and nitro-phenols and phthalate plasticizers, are being actively developed and the results show considerable promise (Giam et al. 1975; Sorensen 1978; Coutts et al. 1980). Research efforts such as these are essential for delineating the nature and extent of chemical pollution in marine environments.

There are other pollutants of considerable concern to both the analytical chemist and the toxicologist. These compounds arise from either chemical (e.g., photochemical oxidation) or biochemical (metabolite formation) transformations in the marine environment (Lee 1977; Bend and James 1978; Malins and Hodgins 1981). For example, when AHs, such as benzo(A)pyrene and benz(A)anthracene, are oxidized either photochemically or enzymatically, the parent compounds seem to "disappear"—that is, from our "analytical window"--and new compounds (oxidized products) are formed, some of which may be teratogenic, mutagenic and/or carcinogenic or otherwise toxic.

The fact is that most chemical and biological transformation products cannot be detected in marine samples, even with our most sophisticated analytical techniques (Malins 1980; Malins and Hodgins 1981). To illustrate the point, let us again refer to the Puget Sound research (Malins et al. 1980, 1982). Concentrations of AHs from virtually all biota samples (worms, crabs, fish) were found to be lower than the concentrations of AHs in sediment from areas in which the animals were captured. Only a small number of AHs were detected in biota, whereas as many as 500 AHs, as indicated earlier, were detected in the sediment. Because we were unable to analyze for the transformation products, it was not possible

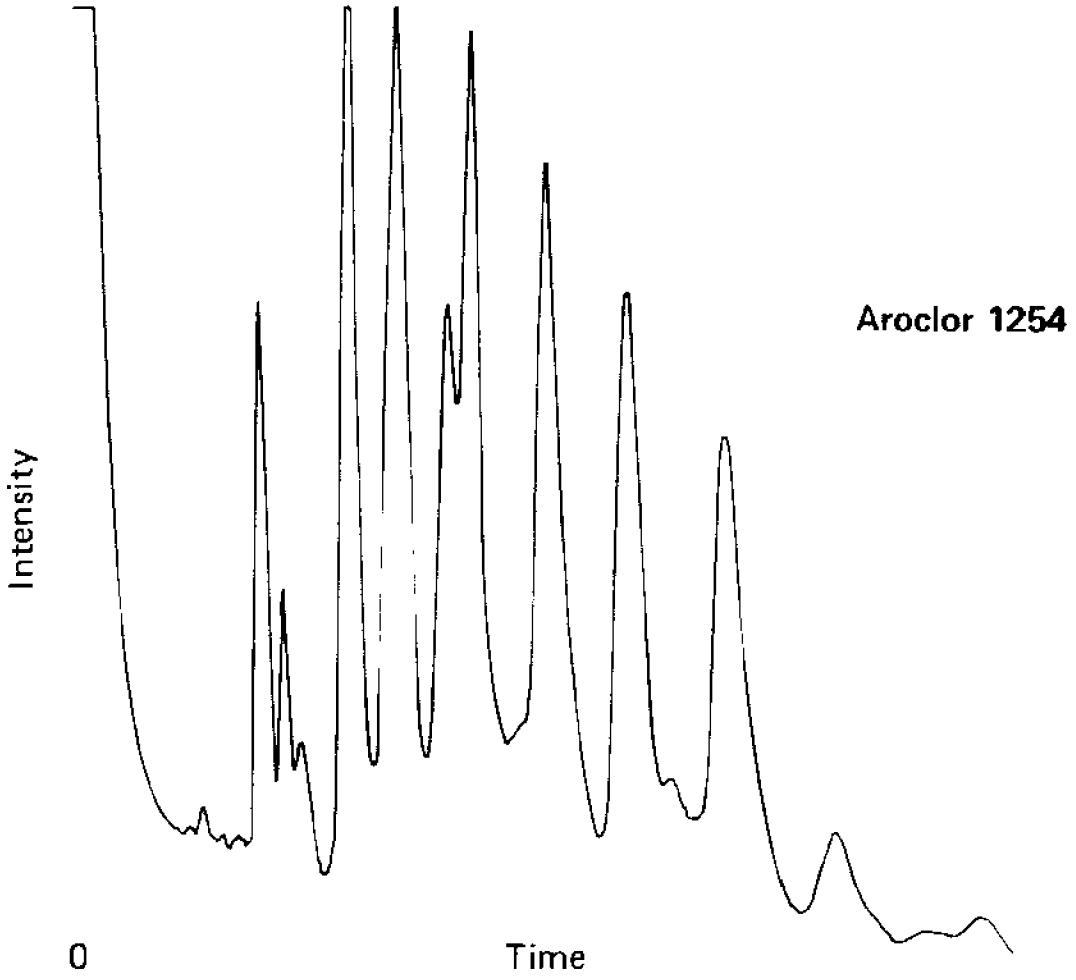


Figure 2. Gas chromatogram of typical PCBs (Aroclor 1254) by conventional packed column technique.

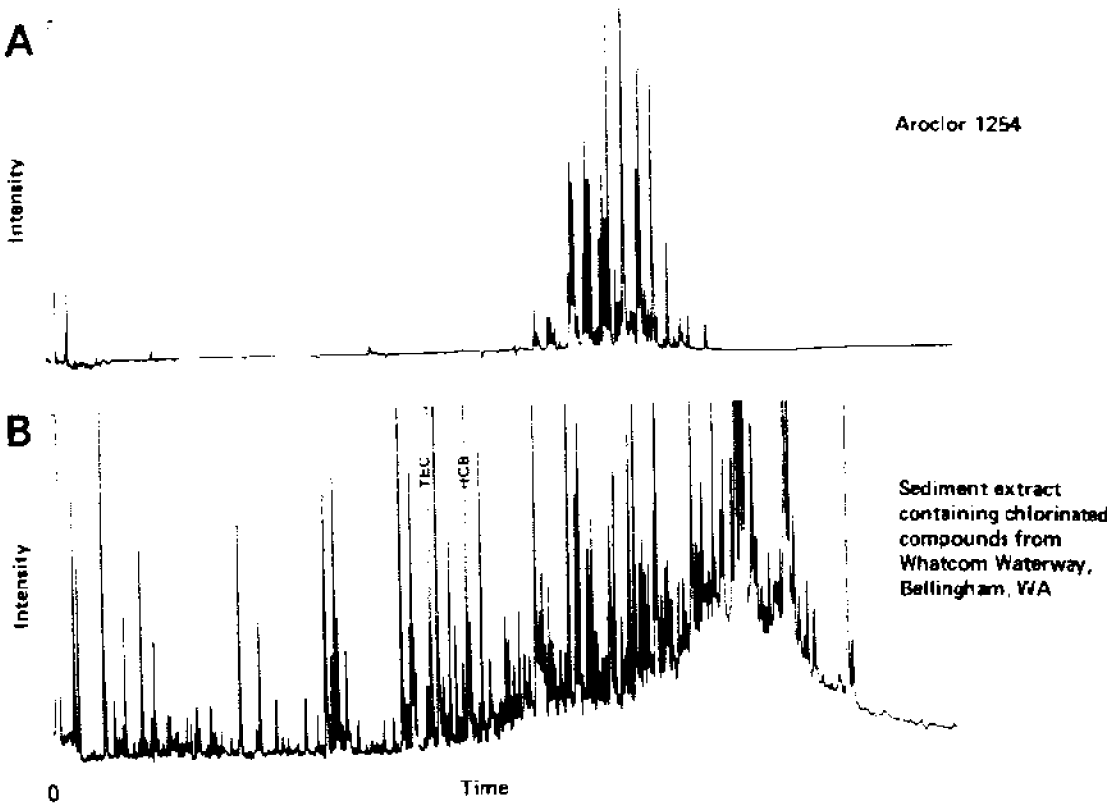


Figure 3. High resolution capillary gas chromatograms of (A) typical PCBs (Aroclor 1254) and (B) PCBs plus other chlorinated hydrocarbons extracted from sediment from Bellingham Bay, Wash.

to ascertain directly whether biota took up the AHs or whether the AHs were extensively converted. In essence, therefore, the absence of AHs in biological samples cannot be construed to mean that the compounds were not taken up.

It should be recognized that the potential toxicity of the oxidized compounds has only recently been appreciated, and the analytical methodology for them has not progressed as far as that for the other pollutants, that is, the parent compounds. In fact, it can be argued that present methodology is actually totally inadequate because we have little capability for routinely analyzing for these types of compounds.

During the last 2-3 years, HPLC techniques have allowed for modest advances in the analysis of highly complex mixtures of oxidized products (Krahn et al. 1980, 1981, 1982; Krahn and Malins 1982). These techniques have been particularly valuable because many of the oxidized products are simply not amenable to analysis by GC because of their low volatility and/or lability. Notwithstanding, we still have not succeeded in finding ways of applying present HPLC techniques to the routine analysis of field samples. An urgent need exists for identifying and quantifying both the chemical and biological transformation products if we are to understand links between pollution and effects in exposed organisms and links between pollution and the health of the consumer of fish and shellfish.

EFFECTS STUDIES: STATE OF THE ART

Three general approaches are available for studying the effects of pollutants on marine biota:

1. Effects can be inferred from field observations.
2. Controlled laboratory experiments can be conducted in which all but one or a few variables are controlled.
3. Semicontrolled experiments can be conducted in situ in the field in pens or other enclosures, or in model ecosystems under "natural" conditions.

Each approach, unfortunately, has limitations (Malins and Hodgins 1981). Definitive cause-and-effect relationships are usually impossible to establish by field studies alone, and there are still many undefined variables even in semicontrolled systems, so that cause-and-effect relationships are still difficult to establish. These relationships can be established in controlled laboratory studies, but events observed in the laboratory may not occur in the same way, or to the same degree, as they do under the complex conditions of natural marine environments.

Effects studies on marine species have ranged from examinations of acute toxic effects in controlled laboratory studies (Hansen et al. 1974; Swartz et al. 1979) and in field situations after

spills (Hyland and Schneider 1976) or other polluting incidents, to examinations of longer term effects on species and ecosystems using a spectrum of scientific disciplines (Haensly et al. 1982; Mearns and Sherwood 1977; Smith et al. 1979; Malins et al. 1980).

It is important to recognize that in effects studies the major concern is not really whether pollutants are present in the marine environment, but whether they are available to organisms and whether they are transported up the food chain to higher marine forms and humans. For example, many pollutants are found in sediments, but of course they are not all taken up by marine life to the same extent. PCBs in sediment tend to accumulate in tissues of bottom-dwelling fish to a greater degree than do AHs. This difference may be explained, in part, by the fact that the AHs are rapidly oxidized in fish, as previously indicated, whereas most PCBs are transformed relatively slowly. With this in mind, it is obvious that unless we know the bioavailability and biodisposition of chemical pollutants, we are severely hampered in deducing the causes of biological effects.

The bioavailability of a pollutant will depend on a host of factors such as the structure of the compound, its ability to bind to particulate matter, the nature of the particles to which the chemical is bound, the anatomy and physiology of the organism and the length of exposure. It is clearly not possible in every instance to know the actual contribution of these individual factors; however, analyses of chemicals in the environment of an organism, together with analyses of chemicals in tissues, provide useful information on bioavailability. Knowledge of this type is essential in linking pollution to biological change.

Elucidating the metabolic fate of pollutants is also a very important step in understanding pollutant-induced biological change. For example, the metabolism of hydrocarbons is a process whereby animals convert these lipophilic compounds to derivatives that are generally water soluble and readily excreted. While such detoxification processes clearly are beneficial, there may be negative consequences the organism must also face. For example, certain metabolites bind with DNA (Varanasi and Gmur 1980; Varanasi et al. 1981), a process that may lead to tumor formation.

Laboratory studies with radiotracers allow us to obtain data on uptake, metabolism and retention of chemical pollutants. While radiotracer studies have the advantage of providing in-depth information on the structures of individual oxidized products, their limitations are numerous. For example, they can be conducted only in the laboratory, and translation of the data to the environment is obviously fraught with many difficulties. For technical reasons, such studies can frequently be readily carried out with only two individual compounds (e.g., using carbon 14 and tritium labels independently), whereas organisms are exposed to a host of chemicals in the marine environment.

Another important question arises: "How do the bioavailability and biotransformation of one chemical influence the fates of other chemicals that have accumulated in exposed organisms?" In this regard, we are now beginning to realize that synergism and antagonism can bring about significant changes in the biochemistry of individual xenobiotics in exposed marine life (Malins and Collier 1981). For example, in one study (Gruger et al. 1981) the pattern of metabolism of 2,6-dimethylnaphthalene in starry flounder (Platichthys stellatus) was substantially altered by the administration of either naphthalene or p-cresol. Such results have implications for the toxicity of mixed chemicals in the marine environment, and also for extrapolations based on studies of the biotransformations of single compounds.

All of us who study the marine environment are well aware of the variability that exists in natural ecosystems over time and space. In more naive times--curiously, only a few years ago--we talked knowingly about needing "baseline" information. That is, we needed to go out and sample "important" areas and in a few years we would have all the information necessary to make statistically verifiable quantitative evaluations of pollutant impacts, when and if they occurred.

It didn't take us more than 5 or 10 years and some millions of dollars worth of effort, however, to decide that there was indeed great difficulty in differentiating the signal from the noise in all but a few instances, such as when the immediate environment was overwhelmed with a single, non-degradable, readily quantifiable xenobiotic. " Baseline" then became a tarnished concept, and we talked of "reconnaissances" instead.

In the Pacific Northwest and Alaska, we are particularly familiar with salmon runs and the marked variability that occurs in them from year to year. Many of the factors contributing to freshwater and ocean mortality are known--and we won't belabor this issue--but some contributing factors remain ill defined, and we are frequently unable to measure even known influences with precision. Consequently, predictions of numbers of fish in a given year, of runs such as those of Bristol Bay, Alaska, sockeye salmon (Oncorhynchus nerka), may miss by 100% or more; and this is the present status of the predictive science even after 30 years of intensive study. This variability in salmon runs is shown in Figure 4 which depicts the estimated number of sockeye salmon entering the Columbia River in the years 1938-70 (Anonymous 1971).

So the point is this: it has been stated (with considerable validity) that it takes at least an order of magnitude of change in marine fish populations, as the result of a pollutant impact, before the effect can be differentiated from natural variability. That is, in most instances it is very difficult to detect changes induced by xenobiotics in natural ecosystems because of the large amount of natural variability. This inherent problem plagues us now and will

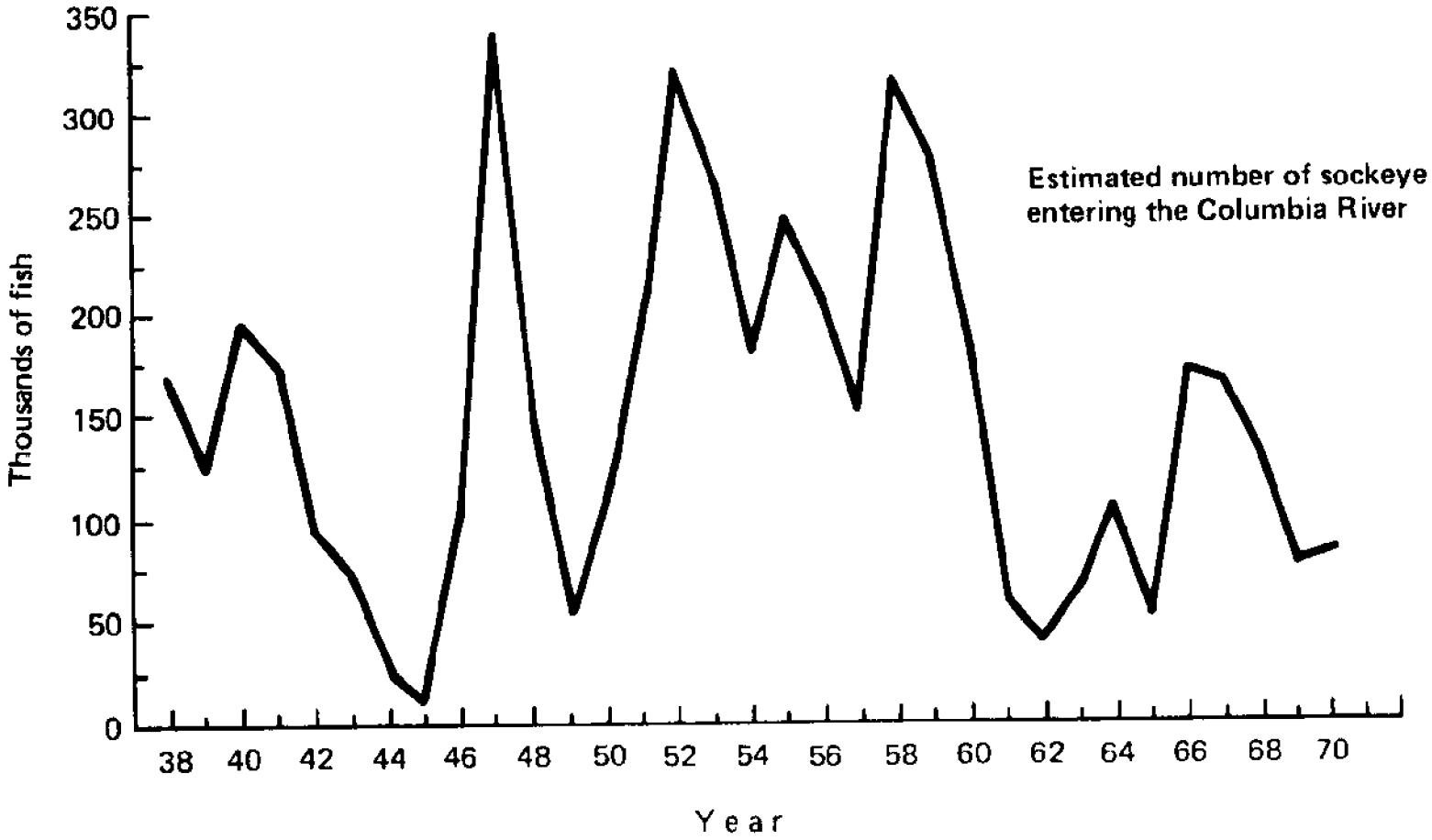


Figure 4. Estimated number of sockeye salmon entering the Columbia River 1938-70 (modified from Anonymous, 1971).

probably continue to do so. Quite frankly, we have tremendous difficulties in handling it.

Much rhetoric has been uttered and penned about "field verifying" laboratory results and performing "environmentally realistic" tests. However, accomplishing these is not a simple matter, nor even feasible in many instances.

As stated earlier, in order to ascertain that an effect is related to a specific pollutant, controlled laboratory exposures must usually be conducted. Over the years, for practical reasons, emphasis has been placed on studies of short-term effects, including acute toxicity or LD 50 tests. Yet it is becoming increasingly clear that at best these tell only part of the story.

Unfortunately, long-term studies of effects on marine species are still almost unexplored terrain. Such studies are difficult, costly and time consuming. Those who demand quick responses and those who live on year-to-year funding naturally tend to close their minds to long-term studies. In the current climate of one-year-at-a-time research projects, followed by all too many changes of direction, such studies appear likely to remain few in number. When then will we really come to grips with the issue of long-term effects?

Finally, even if we do find, for example, that 90% of the larvae of a species are severely impacted by a realistic pollutant exposure, what does that mean, precisely, in terms of ecosystem impact? All of us involved in marine pollutant research have faced the wolves on that one, and rightly so. Again, in most instances, we simply do not know. We don't know because such findings essentially reflect altered mortality rates of a certain developmental stage of a single species in the overall dynamics of ecosystems.

So to sum the points we have just made:

1. There are sufficient shortcomings in pollutant-effects tests that they are frequently relevant only to the individuals and ad hoc exposure conditions of the specific experiments.
2. Even if the effects information obtained is generic, it is usually not applied to marine ecosystem analysis in such a way that meaningful evaluations and predictions of natural environmental impacts are possible.

APPROACHES FOR BETTER CORRELATING ANALYTICAL CHEMICAL DATA WITH EFFECTS

Thus far in our discussion we have generally presented a jaundiced view of state of the art capabilities for establishing cause-and-effects relations and for quantifying, or even detecting, pollutant impacts on marine ecosystems. However, it is our consensus that such a perspective is justified and that we must face the pol-

lutant problems in marine environments realistically if significant progress is to be made in understanding them.

In Figure 5 we present a protocol for relating xenobiotics in the marine environment to biological effects. This represents synthesis more than innovation, but because we believe the general concepts are those that must be applied, we will briefly review some of these methods we are using and give examples of some of the data obtained. We will particularly highlight some of the results from our recent Puget Sound studies (Malins et al. 1980, 1982) and some of the attempts to relate sediment-associated xenobiotics to effects.

In the Puget Sound study, beginning in 1978 and continuing through the spring of 1981, sediments and selected bottom-dwelling fish and invertebrates were collected at regular intervals from urban embayments and from nonurban areas in Puget Sound and adjacent waters.

The locations of sampling stations are displayed in Figure 6; the major urban-associated sampling areas were Seattle's Elliott Bay, Tacoma's Commencement Bay, Olympia's Budd Inlet, and Bremerton's Sinclair Inlet. The nonurban areas were Case Inlet in south Puget Sound, Port Madison in central Puget Sound, Port Susan in north Puget Sound and Discovery Bay just outside the entrance to Puget Sound.

Sediment and tissue samples were analyzed for a large number of organic and inorganic chemicals, including AHs, PCBs, chlorinated pesticides, other chlorinated organic compounds and metals. In most cases, the same animals from which tissues were taken for chemical analyses were also examined for grossly visible and microscopic abnormalities. In addition, the community characteristics (i.e., abundance and species diversity) of the sediment-associated invertebrates and fish were investigated.

The taxonomic data for the benthic invertebrates were used to calculate several indices of community composition. The indices included the Infaunal Trophic Index (ITI) (a measure of feeding method), diversity measures such as taxon richness (the number of taxa in a sample) and the Shannon-Weaver diversity index. These indices are thought to be indicative of the effects of environmental stress on benthic marine communities.

Statistical methods were used to evaluate possible relationships between the prevalence of fish with liver lesions, the ecological community indices, and the chemical composition of the sediment in the areas from which the fish or invertebrates were captured. Two methods, principal components analysis and cluster analysis, were used to analyze sediment chemistry data.

Examples of relations between chemical "clusters" and biological parameters are as follows:

English sole (Prarophrys vetulus) and rock sole (Lepidopsetta bilineata) with three types of hepatic lesions--neoplasia, "preneo-

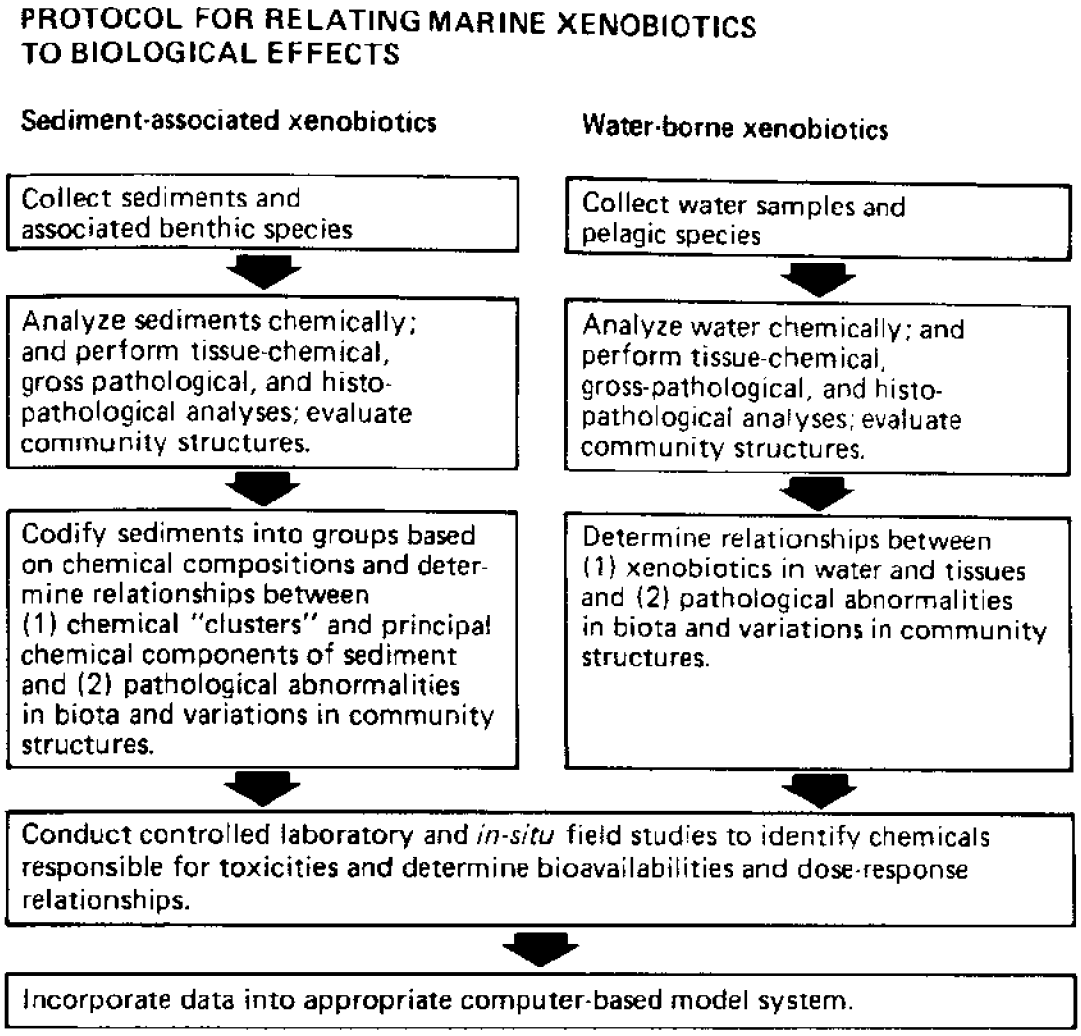


Figure 5. Protocol for relating marine xenobiotics to biological effects.

Map of Puget Sound showing sampling areas.

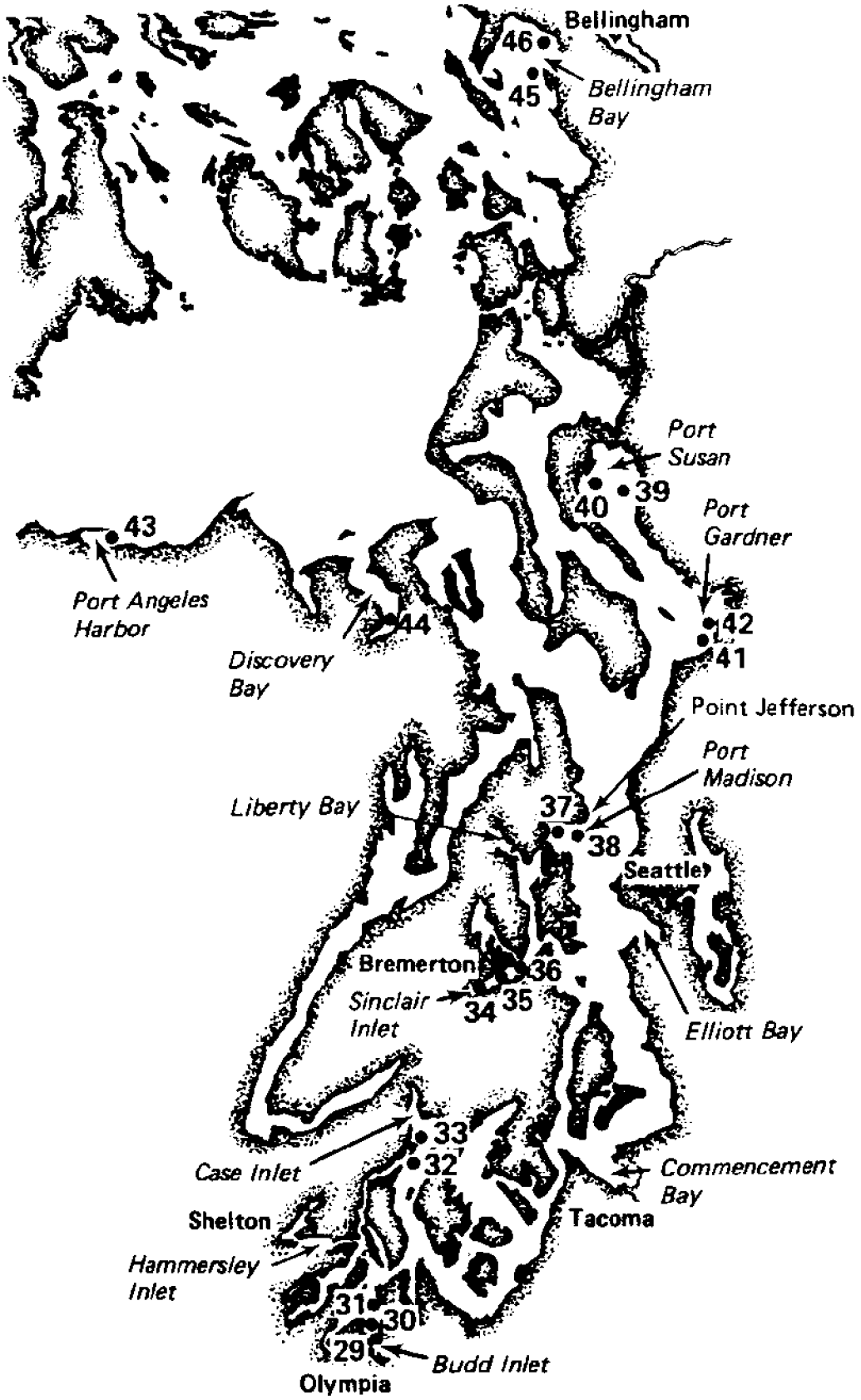


Figure 6. Locations of sampling areas in Puget Sound.

plasia" and specific degeneration/necrosis--were significantly ($p \leq 0.05$) more prevalent at Cluster Group Stations 4 (the Duwamish Waterway) and/or 7 (Sitcum and Hylebos Waterways) (Figure 7). Cluster Group 4 and 7 stations had relatively high concentrations of metals and intermediate levels of AHs. Sole with these lesions were either not found at Cluster Group 1 stations (the least contaminated group of stations) or the prevalence was significantly lower ($p \leq 0.05$) than the mean prevalence value for all the clusters.

The mean values for taxon richness of the benthic communities obtained during this study were calculated for the sampling stations in each of the Cluster Groups. Cluster Group stations 3, 4 and 7 had significantly ($p \leq 0.001$) lower values than did the other Cluster Groups. The mean taxon richness value was statistically higher for Cluster Group 1 than for any of the other Cluster Groups ($p \leq 0.05$).

A different statistical method, Spearman's rank correlation, was used to evaluate the relationships between infaunal benthic invertebrate community indices and the relative concentrations of chemicals in sediment. Taxon richness was strongly correlated (negatively) with the first, second and third principal components (high levels of AHs, toxic metals and PCBs) of the sediment chemistry data.

Spearman's rank correlation was also used to examine the relationships between the prevalence of fish with certain types of liver lesions and the chemical composition of the sediments at sampling stations from which they were captured. Positive correlations obtained agreed in general, as would be predicted, with the results obtained with the cluster group analysis; that is, these diseases were more prevalent at stations where concentrations of sediment-associated AHs and toxic metals were high.

Therefore, in relation to the protocol discussed (Figure 5), we have progressed to the point of needing controlled laboratory and *in situ* field studies to identify chemicals particularly associated with sediment toxicity, and to determine their bioavailabilities to and effects on selected phylogenetically diverse species. Laboratory studies would involve the use of model systems and fractions of chemicals obtained by selectively extracting sediments and suspended particles with solvents of differing polarity. The fractions would be studied for their biological effects and, in addition, an attempt would be made to rank extracted chemicals according to their bioavailability.

Information from these types of studies, together with chemical analyses of each of the fractions, would bring us a step closer to understanding whether certain identifiable and bioavailable chemical(s) are causing biological effects.

As state-of-the-art toxicity data are obtained, they must be integrated with relevant site-specific data on ecosystem dynamics.

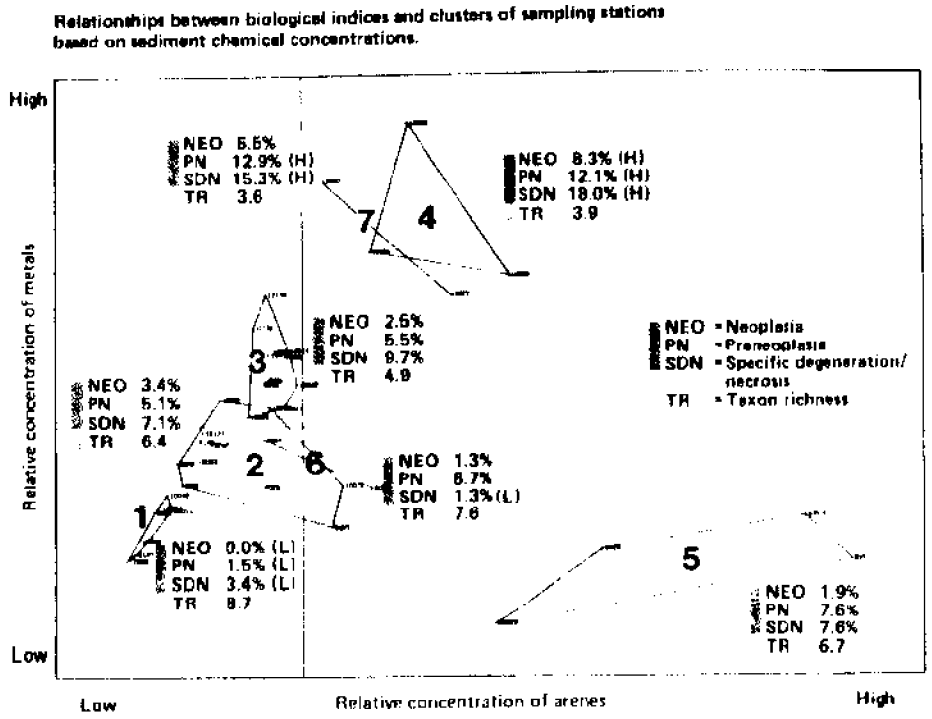


Figure 7. Relationships between a benthic community index (taxon richness), the prevalence of hepatic lesions in English sole and clusters of sampling stations, based on sediment chemical concentrations. Some prevalence values were significantly higher (H) or lower (L) ($p \leq 0.05$). The clusters are identified by boldfaced numbers.

This usually leads to incorporating such data into a model. As a case in point, an example of a commercially developed computer-based model system is shown in Figure 8. This system of computer models is designed to simulate the evolution and fate of an oil spill, and the impact of the spill on commercially fished species. It should be reemphasized that our contributions, in the final analysis, to such a model system would be analytical chemical data for the oil spill fates model and effects data for the mortality estimate in the fishery population model. The environmental impact would be derived from the several models in the system and the stronger the data base, the better the final impact estimates.

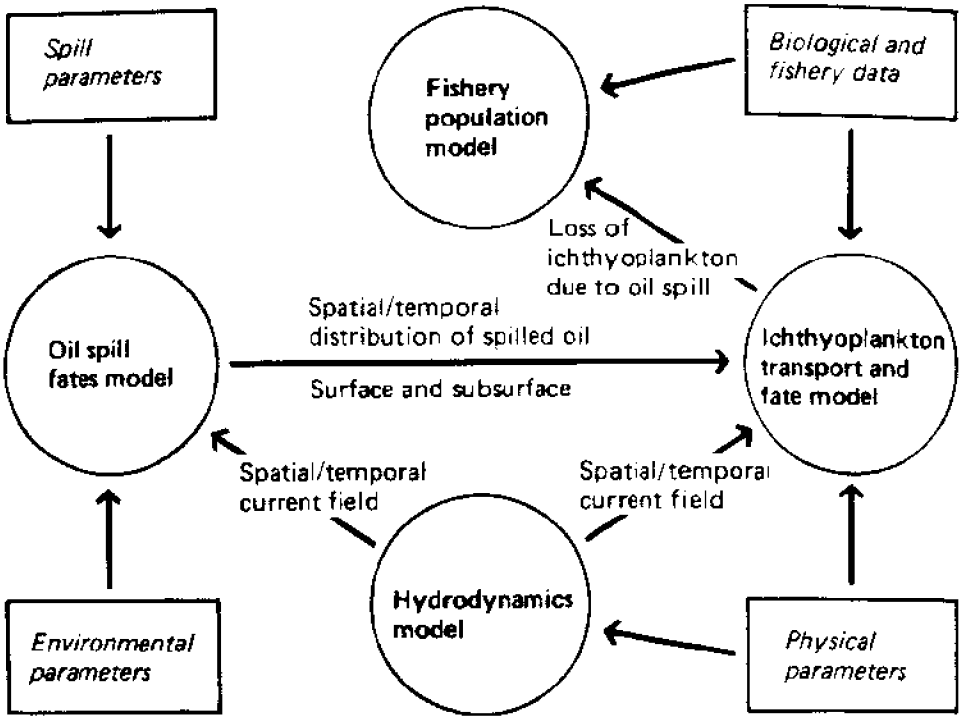
CODA AND PROSPECTUS

In an attempt to put the problems of marine pollution into a proper perspective, we propose that certain analogies and comparisons can be made between the problems that we face and those faced by researchers working in the cancer field. In both situations the complexity is mind-boggling and many of the important parameters probably haven't even been identified, much less addressed. A "war" was declared on cancer about a decade ago. The money, thought and effort that have gone into cancer research as part of this intensified effort make commitments to the aquatic pollution field seem miniscule indeed. And yet, the complexities and difficulties of pollutant fates and effects in marine ecosystems, we submit, are greater than those faced by workers in cancer research.

With the admittedly immense problems of human cancer, including determining the nature of this disease and developing improved procedures for its detection and treatment, how well has the intensified effort paid off? While human cancer has not been eliminated or converted into a negligible problem, substantial and steady progress has been made. In a recent newspaper article (The Seattle Times, March 8, 1982) Dr. Vincent DeVita, Director of the National Cancer Institute , is quoted as predicting that 5 years from now scientists will be shaking their heads in wonder at the new and unexpected things they've learned about cancer. He states, "Cancer is the most curable of the chronic diseases. In the 1950s, we estimated that about 30 percent of cancers were curable. In 1977, the estimate was 41 percent. This year, our best estimate is that 50 percent of serious cancers are curable. This is evidence of steady progress."

Important lessons should be drawn for future marine pollution research activities from the cancer research effort. First, marine pollution is going to have to be identified as an important enough national problem to require a substantially greater commitment of research funds and scientific expertise than now exists if substan-

Relationships among system components of an oil spill-fisheries impact model.



From Applied Science Associates, Inc., Wakefield, RI

Figure 8. Model of the impact of an oil spill on fisheries (Applied Science Associates, Inc., Wakefield, Rhode Island).

tive progress is to be made. Second, unreal expectations for quick solutions should be summarily abandoned. Finally, the research must be broadly based and better than it has been.

In summary, we have argued that serious problems exist in the determination of pollutants in marine environments and in elucidating their effects on ecosystems. Some of our colleagues appear to see little hope for resolving these complex issues. Consider, for example, the views of Professor O. Kinne, a highly respected biologist, of Biologische Anstalt, Helgoland (Kinne 1980):

> "Most important for man's long-term survival, ecosystem management remains problematic. Aims, ends and principles are difficult to define. We are just in the process of attempting to build the necessary scientific framework, and the political difficulties seem, at present, unsurmountable. Man's egotism and interest conflicts allow only unequivocal evidence of critical damage to be transformed into effective political action. But such evidence is likely, at least for the present time, to remain unobtainable prior to severe damage.
>
> The often-heard argument 'We cannot act unless ecologists present hard facts on which our action can be based' is unrealistic. Ecologists may in fact never be in the position to provide the kind of solid data presently asked for by politicians. Ecosystem dynamics may turn out to be too complex and too unpredictable to bring sufficient light. . .for reliable forecasting of impact consequences at the ecosystem level."

Our perspective is clearly more optimistic, but it is based on the hope that we will indeed make the dedicated, appropriately funded, long-term commitments that are necessary for progress. Failure to do this may well mean that Professor Kinne's dire prediction will actually come to pass.

REFERENCES

Anonymous. 1971. Columbia River fish runs and commercial fisheries, 1938-70. Joint Investigational Rep., Vol. 1, No. 1, p. 37. Fish Commission of Oregon and Washington Department of Fisheries.

Bend, J.R. and M.O. James. 1978. Xenobiotic metabolism in marine and freshwater species. In: D.C. Malins and J.R. Sargent (eds.), Biochemical and Biophysical Perspectives in Marine Biology, Vol. 4, pp. 126-188. Academic Press, New York.

Coutts, R.T., E.E. Hargesheimer and F.M. Pasutto. 1980. Application of a direct aqueous acetylation technique to the gas chromatographic quantitation of nitrophenols and 1-naphthol in environmental water samples. Fac. Pharm. Pharm. Sci., Univ. Alberta (Edmont.), B, 34: 397-400.

Giam, C.S., H.S. Chan and G.S. Neff. 1975. Sensitive method for determination of phthalate ester plasticizers in open-ocean biota samples. Anal. Chem. 47: 2225.

Gruger, E.H. Jr., J.V. Schnell, P.S. Fraser, D.W. Brown and D.C. Malins. 1981. Metabolism of 2,6-dimethylnaphthalene in starry flounder (Platichthys stellatus) exposed to naphthalene and p-cresol. Aquat. Toxicol. 1: 37-48.

Haensly, W.E., J.M. Neff, J.R. Sharp, A.C. Morris, M.F. Bedgood and P.D. Boehm. 1982. Histopathology of Pleuronectes platessa from Aber Wrac'h and Aber Benoit, Brittany, France: Long term effects of the Amoco Cadiz crude oil spill. J. Fish Dis. 5: 365-391.

Hansen, D.J., P.R. Parrish and J. Forester. 1974. Aroclor 1016: Toxicity to and uptake by estuarine animals. Environ. Res. 7: 363-373.

Hyland, J.L. and E.D. Schneider. 1976. Petroleum hydrocarbons and their effects on marine organisms, populations, communities and ecosystems. In: Sources, Effects and Sinks of Hydrocarbons in the Aquatic Environment, pp. 464-506. American Inst. of Biological Sciences, Washington, D.C.

Kinne, O. 1980. 14th European Marine Biology Symposium "Protection of life in the sea": Summary of symposium papers and conclusions. Helgolander Meeresunters. 33: 732-761.

Krahn, M. M., D. W. Brown, T. K. Collier, A. J . Friedman, R. G. Jenkins and D. C. Malins. 1980. Rapid analysis of naphthalene and its metabolites in bioilogical systems: Determination by high performance liquid chromatography/fluorescence detection and by plasma desorption/chemical ionization mass spectrometry. J. Biochem. Biophys. Meth. 2: 233-246.

Krahn, M. M., J. V. Schnell, M. Y. Uyeda and W. D. MacLeod, Jr. Determination of mixtures of benzo(a)pyrene, 2,6-dimethylnaphthalene and their metabolites by high-performance liquid chromatography with fluorescence detection. Anal. Biochem. 113:27-33.

Krahn, M.M., T.K. Collier, and D.C. Malins. 1982. Aromatic hydrocarbon metabolites in fish: Automated extraction and high-performance liquid chromatographic separation into conjugate and non-conjugate fractions. J. Chromatog. 236: 441-452.

Krahn, M.M. and D.C. Malins. 1982. Gas chromatographic-mass spectrometric determination of aromatic hydrocarbon metabolites from livers of fish exposed to fuel oil. J. Chromatog. 248: 99-107.

Lee, R.F. 1977. Fate of petroleum components in estuarine waters of the southeastern United States. In: Proceedings 1977 Oil Spill Conference, Mar. 8-10, 1977, pp. 611-616. American Petroleum Inst., Washington, D.C.

Malins, D.C. 1980. Pollution of the marine environment: A NOAA interdisciplinary team in Seattle searches for answers. Environ. Sci. Technol. 14: 32-37.

Malins, D.C. and T.K. Collier. 1981. Xenobiotic interactions in aquatic organisms: Effects on biological systems. Aquat. Toxicol. 1: 257-268.

Malins, D.C. and H.O. Hodgins. 1981. Petroleum and marine fishes: A review of uptake, disposition, and effects. Environ. Sci. Technol. 15: 1272-1280.

Malins, D.C., B.B. McCain, D.W. Brown, A.K. Sparks and H.O. Hodgins. 1980. Chemical contaminants and biological abnormalities in central and southern Puget Sound. Tech. Memo. OMPA-2, NOAA Office of Marine Pollution Assessment, Boulder, Colo.

Malins, D.C., B.B. McCain, D.W. Brown, A.K. Sparks, H.O. Hodgins and S-L. Chan. 1982. Chemical contaminants and abnormalities in fish and invertebrates from Puget Sound. Tech. Memo. OMPA-19, NOAA Office of Marine Pollution Assessment, Boulder, Colo.

Maugh, T.H., II. 1978. Chemicals: How many are there? Science 199: 162.

Mearns, A.J. and M.J. Sherwood. 1977. Distribution of neoplasms and other diseases in marine fishes relative to the discharge of waste water. Ann. N.Y. Acad. Sci. 298: 210-224.

Ramos, L.S. and P.G. Prohaska. 1981. Sephadex LH-20 chromatography of extracts of marine sediment and biological samples for the isolation of polynuclear aromatic hydrocarbons. J. Chromatog. 211: 284-289.

Smith, C.E., T.H. Peck, R.J. Klauda and J.B. McLaren. 1979. Hepatomas in Atlantic tomcod Microgadus tomcod (Walbaum) collected in the Hudson River estuary in New York. J. Fish Dis. 2: 313-319.

Sorensen, O. 1978. Trace analysis of chlorophenols in drinking water. Vom Wasser 51: 259.

Swartz, R.C., W.A. DeBen and F.A. Cole. 1979. A bioassay for the toxicity of sediment to marine macrobenthos. J. Water Pollut. Control Fed. 51:944-950.

Varanasi, U. and D.J. Gmur. 1980. Metabolic activation and covalent binding of benzo(a)pyrene to deoxyribonucleic acid catalyzed by liver enzymes of marine fish. Biochem. Pharmacol. 29: 753-761.

Varanasi, U., J.E. Stein and T.H. Hom. 1981. Covalent binding of benzo(a)pyrene to DNA in fish liver. Biochem. Biophys. Res. Commun. 103:780-787.

Chapter 6. Anomalies in Field Specimens

Introduction

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Effects of marine pollution are difficult, at best, to establish. The use of indicator microorganisms and pathobiological effects on marine animals appears to offer good promise in measuring biological response. In particular, bacteria can provide a rapid response, or "early warning system." In this chapter the role of bacteria in marine ecosystems, both as a component of the communities of oceanic waters and as the agents of mineralization, is evaluated.

Recent studies of the microbiology of the surface waters of deep ocean dump sites have been examined (Peele et al. 1981; Singleton et al. 1982; Grimes et al. 1983), and a clear indication of stability of species composition of bacterial communities has been obtained. Destabilization of these communities, upon introduction of allochthonous substances, has been established. Thus, the relatively "conservative" property of microbial community structure and the ability, with the aid of more recently developed methods, to assess species composition, provide a new approach for estimating rapid response of microorganisms to pollution. Bacteria possess a very short generation time and are readily induced to respond upon introduction of a new substrate or toxic substance. In this section Singleton et al. describe the approach taken for developing an index and evaluate the limitations of the approach, as well as the advantages. Since bacterial community studies provide a very quickly and reliably measured index, it appears that this approach should be explored further, particularly for direct identification by specific stains and for immunological detection using a modification of the epifluorescent direct viable enumeration method.

The role of bacteria in polluted marine ecosystems is discussed by Azam et al., who describe in situ measurements of growth rate, a powerful method for establishing bacterial biomass and microbial turnover time and response to specific pollutants such as heavy metals, petroleum compounds, or other materials entering the oceans accidentally or deliberately. The power of this approach is elaborated. Other methods, besides nucleotide determinations,

have been suggested by microbiologists to estimate microbial response to pollutants, but the measurements of growth rate, discussed in this section, offer a particularly helpful quantitative estimate not requiring in every instance the culturing of microbial populations, a clear advantage in field work.

Ecosystem stresses offer useful insight into the physiological effects of pollutants. Metabolic responses to stress are discussed by Patton and his colleagues, who offer changes in adenylate charge, glycogen and lipidmeasures of total system effects. Measurements of energy charge have provided useful insight into the health of an ecosystem, as well as of individual animal species. Because application of the measurement to ecology is relatively new, refinement in methodology is in progress. Nevertheless, useful results have been obtained with the crude estimates now possible. This approach provides a very good physiological index of stress and response to stress, mainly in elucidating the ability of a total system, or whole animal, to adjust to a changing environment.

Ultimately, the response of interest is that of the whole animal. One approach to measuring response of marine animals is to examine effects on larvae, possibly the most sensitive component of animals in a marine ecosystem. Epifanio describes the use of decapod crustacean larvae in assessing effects of pollutants, calling upon the extensive experimental data gathered in his laboratory. This approach to measuring the effects of pollutants offers reproducible and informative insights to animal response at the developmental stages. Toxicity tests are conducted in the laboratory, but care must be exercised in extrapolating results to the field, caveats for which are discussed by the author.

Tissue anomalies, including tumors, symptoms of overt disease, and morbidity in wild fish populations, are the basis of an intriguing approach to detect specific pollutant chemicals in the environment. However, Patton and Couch provide a realistic assessment of the validity of this method for establishing effects of pollution. Variability of natural populations, and the need for a very careful experimental design for statistical analysis, are two of the major factors that have rendered questionable some of the results published to date. If this approach is to be used for assessing the effects of pollution on natural populations, the caveats described by the authors clearly must be taken into account. However, the significant advantages of examining field specimens, in an appropriate experimental context, are very attractive, especially in the description of physiological, morphological and biochemical manifestations by the animal.

In every attempt to relate a significant biological response to pollution by a specific chemical or mix of chemicals, it is obvious that there is no single cause and effect relationship. The environment, indeed, is a complex mosaic of environmental, physical and biological parameters, no single one of which can be the ultimate

single measure or index of health or disease. However, each approach provides a facet or a phase in a "multiphasic system," and the multidimensional view can be provided when several approaches are taken simultaneously. Indeed, the caveat in every instance is that statistical rigor and precise and accurate measurements are fundamental requirements, without which no measure has value.

The microbiological approaches offer significant promise because of the rapid response of microorganisms to stress. Whole system measurements, described by Azam et al. and Hodson and Vetter, are also exciting because of their potential use as a "thermometer" of stress. Finally, the whole animal response, the effects indeed most closely approximating those of human health, can ultimately be determined only by direct measurement of the well-being of the whole animal. Laboratory approaches and field assessments should be simultaneous, to provide useful information from both.

Sudden revelations and instant successes have not been discovered in any case cited above, where methodology has been developed to implicate or explain biological effects of pollution. However, there is no need for despair or alarm. The procedures applied are, indeed, being reevaluated continuously, as well as examined from aspects of seeking improvement, extension and refinement. As newer methods and improved "old" methods become available, our knowledge of the marine ecosystem and its components will expand. Our probing and questioning should not cease nor should we be inhibited because the methods we use are not perfect. Understanding the limitations of what we do, as we carry out our experiments, will prevent us from being dogmatic and narrow-minded. Progress is, indeed, being made and the challenge ahead is stimulating.

REFERENCES

Grimes, D.J., F.L. Singleton and R. R. Colwell. 1984. Allogenic succession of marine bacterial communities in response to pharmaceutical waste. J. Appl. Bacteriol. (In press.)

Peele, E.R., F.L Singleton, J.W. Deming, B. Cavari and R.R. Colwell. 1981. Effects of pharmaceutical wastes on microbial populations in surface waters at the Puerto Rico dump site in the Atlantic Ocean. Appl. Environ. Microbiol. 41: 873-879.

Singleton, F.L., D. J. Grimes, H.S. Xu and R.R. Colwell. 1984. Microbiological Studies of Ocean Dumping. In: D.R. Kester, W.V. Burt, J.M. Capuzzo, P.K. Park, B.H. Ketchum and I.W. Duedall (eds.), Deep-Sea Waste Disposal. John Wiley & Sons, New York.

Role of Bacteria in Polluted Marine Ecosystems

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INTRODUCTION

Until quite recently, studies of the ecology of marine bacteria, and hence the pollution ecology of marine bacteria, were severely limited in scope for lack of suitable methodology. Bacteria were enumerated by plate counts which can yield gross underestimates. Bacterial activity was inferred from the rates of uptake of added radiolabeled organic nutrients (Wright and Hobbie 1965; Azam and Holm-Hansen 1973). Until recently, it was not feasible to measure in situ growth rates of bacteria, or the rates of predation on bacteria, measurements necessary for quantifying the flux of energy and material (and associated pollutants) through bacterioplankton to organisms at higher trophic levels.

A significant change has taken place during the past five years in our view of the role of bacteria in pelagic marine ecosystems. The use of new techniques has led to the following observations. (1) Bacterial abundance in coastal waters is on the order of 10^6 ml^{-1} (Hobbie et al. 1977; Fuhrman et al. 1980); bacterial biomass is about 10% of the total plankton biomass in seawater (comparable with the zooplankton biomass); (2) bacteria grow rapidly, with 0.5–3 d doubling times (Fuhrman and Azam 1980, 1982; Hagström et al. 1979); thus they are significant as secondary producers; (3) bacteria are avidly preyed upon by a variety of plankton organisms, thereby providing an important source of food for higher trophic levels (Hollibaugh et al. 1980; King et al. 1980; Wright et al. 1982; Fenchel 1982).

Figure 1 depicts the material and energy flow in a marine food web, and suggests plausible routes of pollutant transfer. It emphasizes the "bacterial route" as a major path for energy and material (and possible pollutant) transfer (Pomeroy 1979; Hagström et al. 1979; Fuhrman and Azam 1980, 1982; Williams 1981; Azam et al. 1982). This pathway contrasts with the generally accepted notion of a grazing foodchain (e.g., Steele 1974), in which essentially all primary production is grazed by herbivores. It now appears that both pathways are important.

Bacteria account for about 80% of all "bio-surface" in seawater. From first principles it seems likely that bacteria would dominate the biotic absorption and adsorption of dissolved pollutants. The appearance of soluble pollutants in animals (salps, bivalves, fish) may be due in part to pollutant transfer along the "bacterial route".

The discovery of high bacterioplankton secondary productivity has stimulated research on the trophic fate of bacteria. From recent studies using natural populations of marine bacteria (rather than larger cultured bacteria) it is apparent (Figure 2) that bacteriovory is widespread among a variety of marine organisms, ranging in body size from a few micrometers (colorless flagellates) to many centimeters (bivalves, salps). Wright et al. (1982) found that the mussel Geukensia demissa efficiently filtered bacteria from the water of a salt marsh estuary, and they argued that predation on bacteria was an important nutritional adaptation in this mollusc. Hollibaugh et al. (1980) found that another mussel, Mytilus edulis, fed on bacteria; they also found that the larvacean Oikopleura dioica and a ciliate, Helicostomella subulata, efficiently fed on bacterioplankton. King et al. (1980) found that O. dioica could obtain its daily body equivalent of carbon from bacterioplankton; however, most of the predation pressure on bacterioplankton was judged to be by colorless flagellates. O. dioica can be eaten directly by juvenile fish. Much of the bacterial biomass follows a high mineralization pathway (bacteria → μ-flagellates → ciliates → copepods → fish), but a certain fraction would follow shorter, more

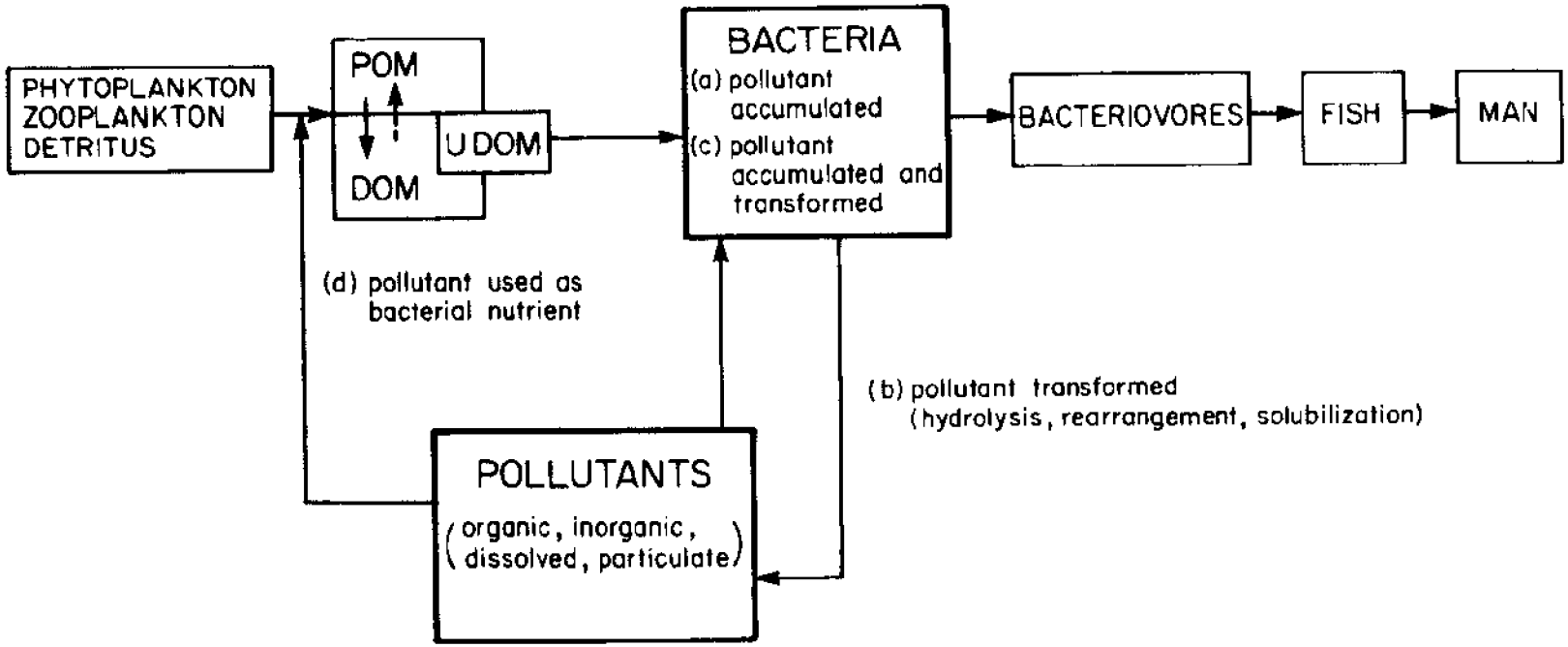

Figure 1. Flow of matter and energy through a marine food web, with emphasis on the "bacterial route". Four categories of bacteria-pollutant interactions are also illustrated. DOM = dissolved organic matter; UDOM = utilizable dissolved organic matter; POM = particulate organic matter.

efficient food chains (e.g., bacteria→larvaceans→fish, or bacteria→bivalve molluscs; Figure 2). These observations have implications for the foodchain transfer of pollutants accumulated by bacteria.

HYPOTHESES

On the basis of the foregoing we can formulate the following hypotheses regarding the roles of bacteria in the pollution ecology of marine ecosystems. These hypotheses may serve as a framework for developing bacterial indices of pollution effects.

Hypothesis 1: Some Bacterial Species Always Survive Ecosystem Perturbations

The physiological diversity of chemoorganoheterotrophs in nature is axiomatic. In a perturbed ecosystem, barring persistent biocidal conditions, some species of bacteria will always survive and grow rapidly to an abundance compatible with the new organic nutrient regime. This was observed, for example, in the Controlled Ecosystem Pollution Experiment (CEPEX) mesocosm experiment (Azam et al. 1977). The addition of 1 $\mu g\ L^{-1}$ $HgCl_2$ inhibited bacterial metabolic activity by more than 99%, but the activity recovered to the control level within 4 days (Figure 3). The succeeding populations could tolerate much higher Hg concentrations (Figure 4).

Whether the pollutant-resistant bacteria arise through selection or by adapting to the pollutant is often difficult to determine. Their dominance, however, is a valuable indicator of the persistence of a pollutant, a bioassay of sorts. It is apparent that a pollutant need not be directly detrimental to certain species to exert a selection pressure; it could do so by selectively stimulating the growth of other species. Given the short generation time of bacterioplankton, such selection could occur quickly. Oil pollution, for example, may cause the rapid dominance of oil utilizers (Atlas 1981). Similarly, the bacteria respond rapidly to removal of the pollutant from the environment. Given an assemblage turnover time of 1 or 2 days, bacteria have a "short memory". Thus the bacterioplankton response would sensitively reflect the pollutant stress on the environment (Atlas 1981).

Hypothesis 2: Bacterial Accumulation and Transformation of Dissolved Pollutants is a Function of the Bacterial Surface Area and Cell Metabolism

Most marine bacteria are very small (0.2-0.6 μm; Fuhrman 1981) and hence have very large surface/volume ratios. Per unit biomass, therefore, marine bacteria have a very high capacity for adsorption and intracellular accumulation of dissolved pollutants

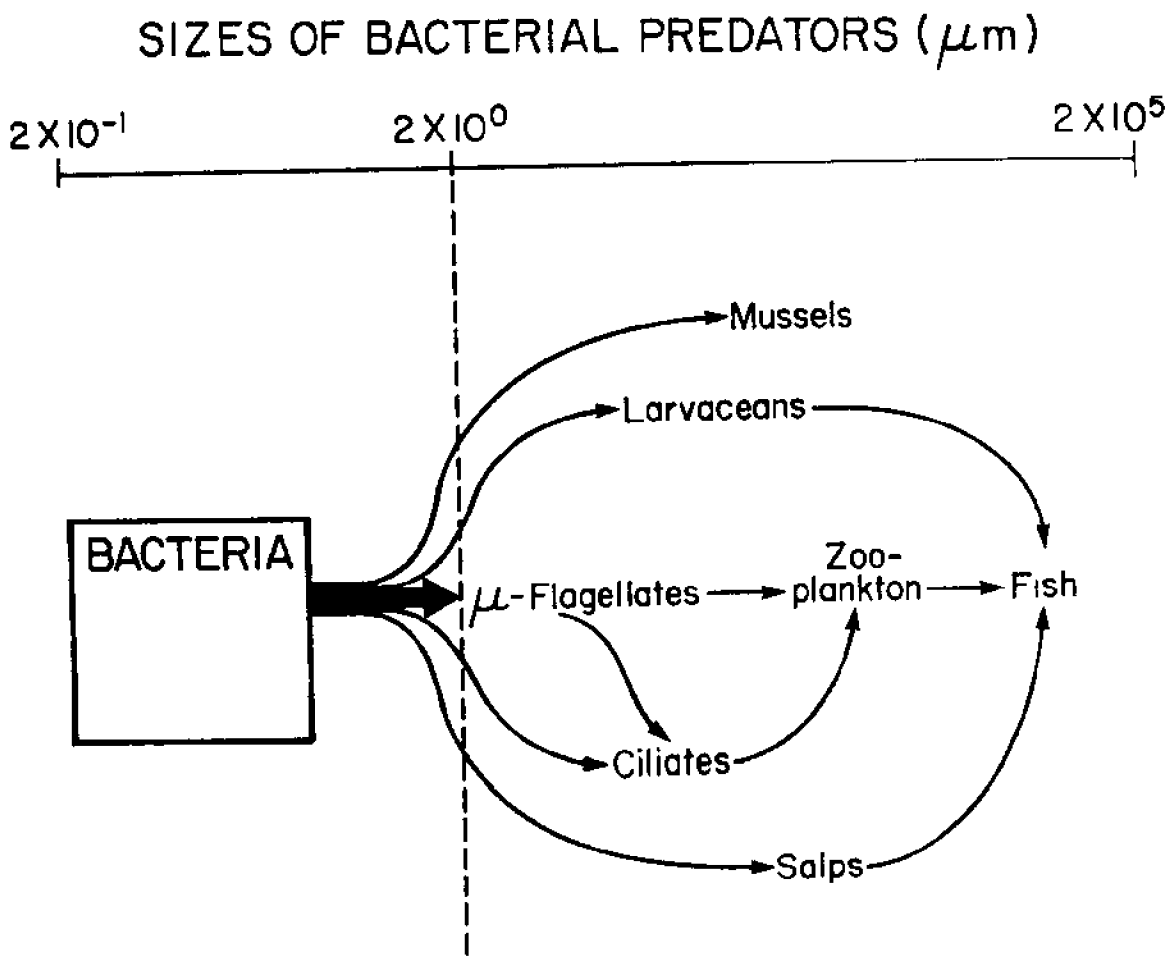

Figure 2. Pathways of the flow of bacterial secondary production through bacteriovores and higher trophic levels. Approximate body size (μm) is shown by the scale at the top.

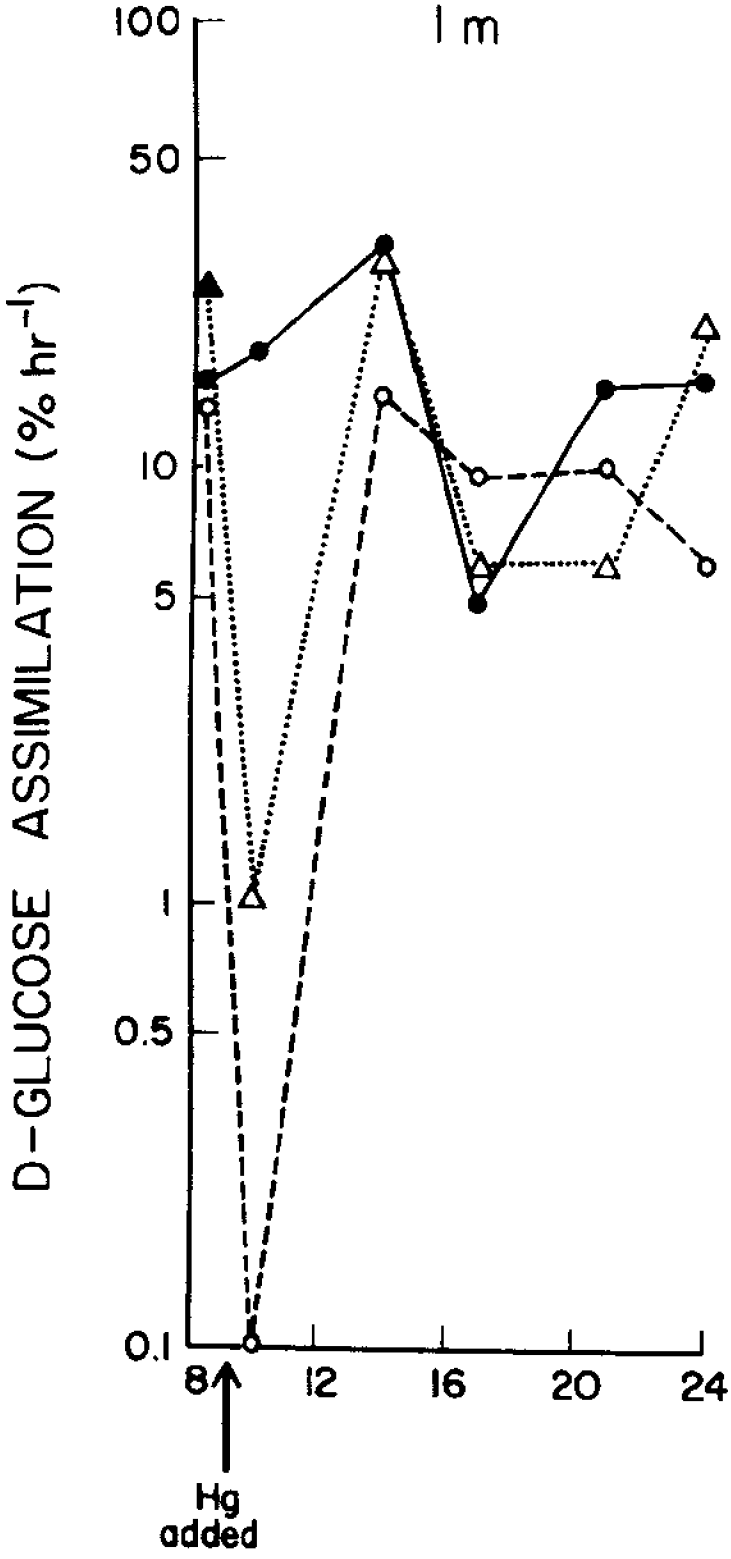

Figure 3: D-Glucose assimilation, expressed as % [6^3 H] D-Glucose assimilated h^{-1}. Open triangles = controlled experimental ecosystem (CEE) 1 (1 µg L^{-1} Hg added); closed circles = CEE 2 (control, no Hg added); open circles = CEE 5 (5 µg L^{-1} Hg added). (Reprinted from Azam et al. 1977, p. 320, by courtesy of Marcel Dekker, Inc.)

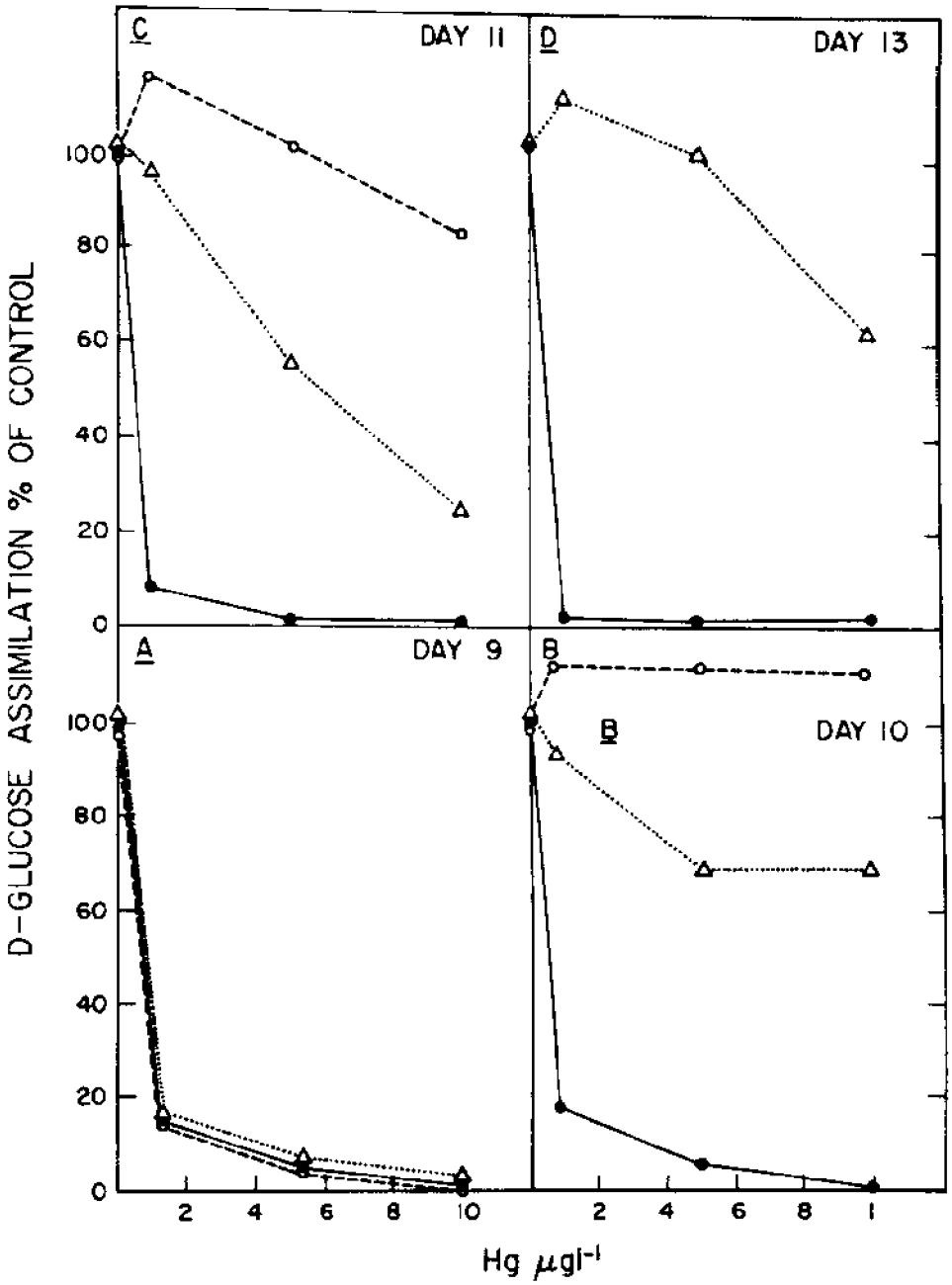

Figure 4. Inhibition of D-glucose assimilation by further addition of mercury to CEE 1, 2 and 5 populations ("tolerance development"). D-Glucose assimilation in the sub-sample receiving no further mercury addition is expressed as 100% ("control") for each CEE; the values for sub-samples receiving further additions of mercury are expressed as % of the "control" for each CEE. Symbols are same as in Figure 3. Tolerance of the three populations was tested just before mercury addition to CEEs (Day 9), and three times thereafter as indicated in Figure 4B, C and D. Tolerance of CEE 5 population was not measured on Day 13. All samples were from 10 m depth. (Reprinted from Azam et al. 1977, p. 321, by courtesy of Marcel Dekker, Inc.)

such as heavy metals, some hydrocarbons, polychlorinated biphenyls (PCBs), etc. Also, marine bacteria account for about one-half of the total respiration of all plankton (Williams 1981); it is thus probable that, per unit biomass, bacteria are highly active in pollutant transformation. In the CEPEX mesocosm experiment (Azam et al. unpublished), addition of 1 $\mu g\ L^{-1}$ $HgCl_2$ led to the appearance of 0.2 $\mu g\ L^{-1}$ Hg in the bacterial size fraction (0.2-0.6 μm). Probably some Hg in this size fraction was associated with detritus, some adsorbed on the surface of bacteria, and some present within the cells. If we assume that 10% of the Hg in the 0.2-0.6 μm size fraction was associated with bacterial cells, there would be 1 mg Hg (g bacterial cell carbon)$^{-1}$; bacteria carbon was about 20 $\mu g\ L^{-1}$. If only 1% of the Hg found in the bacterial size fraction were in bacterial metabolic pools, the intracellular concentration would be about 50 μM, a ten-thousand fold greater concentration than that added to the seawater. (This calculation assumes a carbon content of 10 fg bacterium^{-1} and a bacterial cell volume of 0.1 μm^3; Fuhrman and Azam 1980). If our assumptions are correct (or conservative) then this rough calculation indicates that (Hg-resistant) bacteria could accumulate Hg to very high intracellular concentrations. An experimental system for distinguishing abiotic adsorption from bacterial uptake is needed before we can quantify pollutant accumulation by bacteria. One such system is proposed below.

Hypothesis 3: Pollutant Transfer to Bacteriovores Via Bacterioplankton is a Function of the Bacterial Secondary Production

Bacterial abundance in coastal surface waters varies within remarkably narrow limits, generally from 1×10^6 to 2×10^6 ml^{-1} (Fuhrman et al. 1980), despite the rapid growth of bacteria. The mechanisms responsible for this population homeostasis are not fully understood. However, the relative constancy of the bacterial population allows one to assume that bacterial production (now a measurable parameter) can be equated with bacterial mortality, including mortality due to predation. Assuming that predation is the main cause of bacterial mortality, the rate of pollutant transfer via bacterioplankton is directly proportional to the bacterial secondary production.

PROPOSED BACTERIAL INDEX OF POLLUTION

It would be naive to think that simple and universal indices of pollution can be formulated at the present time. Owing to the diverse nature of marine pollutants it would appear impossible to develop uniform indices of transformation, accumulation, and trophic transfer of pollutants. While recognizing the complexity of the problem, we suggest that some useful insights into bacteria-pollu-

tant interactions might be obtained from measurements of bacterial abundance and production, based on the above hypotheses. However, it is essential, in addition, to experimentally determine the nature and the extent of bacterial accumulation or transformation of the pollutant.

For the purpose of this discussion, the bacteria-pollutant interaction might be divided into four categories: (1) pollutant accumulated, for example, some heavy metals; (2) pollutant transformed, for example, extracellular methylation of Hg (Summers and Silver 1978); (3) the combination of (1) and (2); (4) pollutant used as a bacterial nutrient (Figure 1), for example, petroleum-hydrocarbons (Atlas 1981). The extent of pollutant accumulation and transformation will be a function of metabolic activity. A convenient experimental system is, therefore, needed to determine bacteria-specific activity (activity per bacterial cell) with respect to a pollutant (see below). For instance, if we could determine PCB accumulation in mol PCBs bacterium^{-1} h^{-1} under experimental conditions approaching those in nature, this information could then be combined with bacterial abundance and growth rates, and used to calculate the flux of PCBs to higher trophic levels via bacteria.

Isolates grown in rich media are unsuitable as an experimental system to simulate what occurs in the field because the properties of the cells and of the growth medium may differ greatly from those in nature. These factors may, for instance, influence the measurement of the concentrative capacity of the cells. On the other hand, direct measurements with natural samples are difficult to interpret because bacteria coexist with other organisms and with detritus, as in the Hg experiment cited above. In fact, it is not now possible to distinguish between uptake of bacteria and that of other organisms and detritus.

The contribution of nonbacterial components can be essentially eliminated by growing natural assemblages of bacteria in particle-free unenriched seawater. A 0.6 µm-filtrate (Nuclepore membrane) of seawater (2 to 10% of final volume) is inoculated into 0.22 µm sterile-filtered seawater and grown as a batch culture, "seawater cultures" (Ammerman and Azam 1982). Most marine bacteria are small when free-living and will pass through a 0.6 µm Nuclepore filter (Azam and Hodson 1977). Continuous cultures can also be maintained on sterile, unenriched seawater (Hagström et al. unpublished manuscript). In batch culture, bacteria demonstrate average doubling times of 8-10 h, reaching natural assemblage levels in 1-2 d. Continuous culture steady-states are maintained at doubling times of 6-39 h. Obviously, the small amount of detritus in the inoculum should not increase. In batch culture, after initial increase in cell size, a morphologically-diverse population of bacteria having a size range similar to the initial natural assemblage is obtained. Details of the physiology of growth of seawater cultures are described elsewhere; the relevant point here is that both the

properties of the medium and the properties of the bacterial assemblage are substantially similar to those in nature. Thus, seawater cultures in the laboratory can be useful for measuring pollutant accumulation and transformation by bacteria.

CONCLUSION

To determine the role of bacteria in pollutant transfer through the food web one needs to determine the following: (1) the type of pollutant and its concentration in the water; (2) bacterial abundance, best determined by the acridine orange direct count method (Hobbie et al. 1977); and (3) bacterial secondary production, measured either by thymidine incorporation (Fuhrman and Azam 1982) or by frequency of dividing cells (FDC) (Hagström et al. 1979). Details of the precision and reproducibility of the above methods are discussed by the authors cited. These methods can provide estimates that are correct within a factor of 2. An attractive feature of this approach of estimating pollutant transfer is that, if one uses FDC for production rate measurements, only preserved samples are needed. Of course, as stated above, experiments with seawater cultures to determine the bacterial specific activity are a necessary adjunct to these measurements.

We hasten to point out that the proposed index of pollutant transfer by bacteria is only a general approach, having the potential of being refined to provide a useful method for some pollutants. Considering the potential importance of bacterioplankton in pollutant transfer, and the present lack of methods for its verification, the index suggested here may be a useful general approach.

ACKNOWLEDGMENTS

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REFERENCES

Ammerman, J.W. and F. Azam. 1981. Dissolved cyclic adenosine monophosphate (cAMP) in the sea and uptake of cAMP by marine bacteria. Mar. Ecol. Prog. Ser. 5: 85–89.

Ammerman, J.W. and F. Azam. 1982. Uptake of cyclic AMP by natural populations of marine bacteria. Appl. Environ. Microbiol. 43: 869–876.

Atlas, R.M. 1981. Microbial degradation of petroleum hydrocarbons: An environmental perspective. Microbiol. Rev. 45: 180-209.

Azam, F., T. Fenchel, J.G. Field, J.S. Gray, L.-A. Meyer-Reil and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10: 257-263.

Azam, F. and R.E. Hodson. 1977. Size distribution and activity of marine microheterotrophs. Limnol. Oceanogr. 22: 492-501.

Azam, F. and R.E. Hodson. 1981. Multiphasic kinetics for D-glucose uptake by assemblages of natural marine bacteria. Mar. Ecol. Prog. Ser. 6: 213-222.

Azam F. and O. Holm-Hansen. 1973. Use of tritated substrates in the study of heterotrophy in seawater. Mar. Biol. 23: 191-196.

Azam F., R.F. Vaccaro, P.A. Gillespie, E.I. Moussalli and R.E. Hodson. 1977. Controlled ecosystem pollution experiment: Effect of mercury on enclosed water columns. II. Marine bacterioplankton. Mar. Sci. Commun. 3: 313-329, Marcel Dekker, Inc., N.Y.

Fenchel, T. 1982. Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. Mar. Ecol. Prog. Ser. 9: 35-42.

Fuhrman, J.A. and F. Azam. 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl. Environ. Microbiol. 39: 1085-1095.

Fuhrman, J.A. and F. Azam. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: Evaluation and field results. Mar. Biol. 66: 109-120.

Fuhrman, J.A., J.W. Ammerman and F. Azam. 1980. Bacterioplankton in the coastal euphotic zone: Distribution, activity, and possible relationships with phytoplankton. Mar. Biol. 60: 201-207.

Hagström, Å., U. Larsson, P. Horstedt and S. Normark. 1979. Frequency of dividing cells, a new approach to the determination of bacterial growth rates in aquatic environments. Appl. Environ. Microbiol. 37: 805-812.

Hobbie, J.E., R.J. Daley and S. Jasper. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33: 1225-1228.

Hollibaugh, J.T., J.A. Fuhrman and F. Azam. 1980. Radioactive labeling of natural assemblages of bacterioplankton for use in trophic studies. Limnol. Oceanogr. 25: 172-181.

King, K.R., J.T. Hollibaugh and F. Azam. 1980. Predator-prey interactions between the larvacean Oikopleura dioica and bacterioplankton in enclosed water columns. Mar. Biol. 56: 49-57.

Mopper, K. and P. Lindroth. 1982. Diel and depth variations in dissolved free amino acids and ammonium in the Baltic Sea determined by shipboard HPLC analysis. Limnol. Oceanogr. 27: 336-347.

Mopper, K., R. Dawson, G. Liebezeit and V. Ittekkot. 1980. The monosaccharide spectra of natural waters. Mar. Chem. 10: 55-66.

Pomeroy, L.R. 1979. Secondary production mechanisms of continental shelf communities. In: R.J. Livingston (ed.), Ecological Processes in Coastal and Marine Systems, pp. 162-186. Plenum Publ., New York.

Steele, J.H. 1974. The Structure of Marine Ecosystems. Harvard Univ. Press, Cambridge, Mass.

Summers, A.O. and S. Silver. 1978. Microbial transformation of metals. Ann. Rev. Microbiol. 32: 637-672.

Williams, P.J. leB. 1981. Incorporation of microheterotrophic processes into the classical paradigm of the planktonic food web. Proc., 15th European Symposium on Marine Biology, Kiel, F.R.G. Kieler Meeresforsch., Sonderh. 5: 1-18.

Wright, R.T. and J.E. Hobbie. 1965. The uptake of organic solutes in lake water. Limnol Oceanogr. 10: 22-28.

Wright, R.T., R.B. Coffin, C.P. Ersing and D. Pearson. 1982. Field and laboratory measurements of bivalve filtration of natural marine bacterioplankton. Limnol. Oceanogr. 27: 91-98.

Autochthony of Bacterial Communities in Ocean Surface Waters as an Indicator of Pollution

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INTRODUCTION

Although recognized for decomposition of organic materials and nutrient regeneration and cycling, the autochthonous (naturally occurring) bacterial community of aquatic systems also assists in maintenance of system homeostasis (Alexander 1971). The autochthonous bacterial community of aquatic systems may, in many instances, provide a means of detecting environmental perturbations (Bell et al. 1982; Erkenbrecher 1981; Guthrie et al. 1978; Sieburth 1968a; Bonde 1967). In the past, only certain physiological types of bacteria (i.e., coliforms) have been routinely employed as indicator organisms in the marine environment (Olson 1978; Sayler et al. 1975; Ogawa 1973; Rittenberg et al. 1958). Although coliforms are, in many instances, useful for monitoring fecal pollution, they are of little or no value in detecting the presence of other types of pollutants.

In the open ocean environment, the autochthonous bacterial community consists of numerous species (Sieburth 1979; Kaneko et al. 1979; Simidu et al. 1977, 1980; Baumann et al. 1972; Wood 1967; ZoBell and Upham 1944). The total number of organisms in this community is commonly between 10^5 and 10^6 per milliliter (Sieburth 1971, 1979; Kogure et al. 1979; Hagström et al. 1979; Watson et al. 1977; Jannasch 1958; Azam et al. this volume). Within this community certain physiological types of bacteria are present which, when placed onto a suitable culture medium, will develop visible colonies. These, the culturable bacteria, have been

the most widely studied and best characterized marine bacteria (Sieburth 1979; Kaneko et al. 1979; Austin et al. 1979; Orndorff and Colwell 1980).

The culturable bacteria of aquatic systems, especially freshwater systems, have been employed as an index of (1) abundance of the microbial community, (2) trophic level of the water source (Tanaka et al. 1977; Brasfield 1972), (3) potential heterotrophic activity of the microbial community (Gocke 1977; Robinson et al. 1973; Colwell and Walker 1977; Singleton and Guthrie 1977; Guthrie et al. 1974) and (4) recovery of an aquatic system from pollution (Cherry et al. 1977). Use of the culturable bacterial community as a means of monitoring for environmental perturbations has been criticized in the past. Such criticism has been based on the fact that the culturable bacteria constitute only a small fraction of the total bacterial community, often less than 0.1% (Bowden 1977; Hobbie et al. 1972; Jannasch and Jones 1959). Therefore, the large majority of the total community is not considered in the assay, since these organisms were not capable of growth under the conditions imposed by the investigator. Such criticism may be valid, if results obtained from studies of the culturable bacteria are extrapolated to describe the response of the non-culturable organisms also.

When a specific pollutant entering an aquatic system exerts a direct toxic effect on the indigenous biota, it is relatively simple to evaluate the impact of that pollutant. However, in the absence of direct, or acute, toxic effects, an evaluation of the impact of a given pollutant on the biota can be difficult. The potentially rapid growth rates and high metabolic activities of bacteria are characteristics of a bacterial community in an aquatic system which make it the first component of the biota to demonstrate a measurable response to a pollutant. The bacterial community can, therefore, be employed to evaluate the impact of pollutants on an aquatic system if one compares quantitative and qualitative parameters of the bacterial community in the presence and absence of pollution stress. It is in this way that we have employed the autochthonous bacterial community of surface waters of an open ocean waste disposal site to evaluate the impact of disposed wastes on the biota of the receiving waters.

Research Area

The Puerto Rico dumpsite is a site of approximately 500 km^2 located in the Puerto Rico Trench area of the Atlantic Ocean (Figure 1). Approximately 74 km north of Arecibo, Puerto Rico, the dumpsite received an estimated 3.6×10^8 L of pharmaceutical wastes annually when dumping was permitted from January 1972 until August 1981. The composition of the pharmaceutical waste varied depending upon production schedules of the various industries contributing to the total waste volume. In general, the waste

consisted of spent fermentation fluids and various organic solvents used in the processing of antibiotics; it contained approximately 4% (by weight) total organic carbon (T.P. O'Connor personal communication). The industrial wastes were collected and transported to a common holding facility, and, at 2 to 3 day intervals, were transferred to a barge and the barge towed to the dumpsite. The composited pharmaceutical wastes were released as the barge was towed in the dumpsite area.

Method

To accomplish our objective of evaluating the impact of pharmaceutical waste disposal on the biota of the receiving waters, several methods were employed initially. A detailed discussion of each method is given in Peele et al. (1981), Singleton et al. (1983) and Grimes et al. (1983). Methods evaluated for applicability in our study included acridine orange staining and epifluorescent microscopy (Hobbie et al. 1977; Daley and Hobbie 1975; Francisco et al. 1973) to determine the total number of bacterial cells by the acridine orange direct count (AODC). Numbers of culturable bacteria were determined by membrane filtration of water samples and cultivation of bacteria on marine agar 2216 (MA) (Difco Laboratories, Detroit, Mich.) and on a medium prepared without added salts, that is, plate count agar (PCA) (Difco), to enumerate those organisms not requiring the constituent salts of seawater for growth. Bacteria growing on either culture medium were purified and identified to genus following the identification schema of Bain and Shewan (1968), LeChevallier et al. (1980), Gibson et al. (1977), Furniss et al. (1978) and Gunn et al. (1981).

RESULTS

Initial Cruise Studies

Initial cruises to the dumpsite region, accomplished in May and October 1979, were designed to allow for comparison of microbiological parameters of the waste-impacted waters and waste-free waters (Figure 1). However, results of gas chromatographic analysis of water samples collected at the stations sampled all demonstrated the presence of pharmaceutical wastes, i.e., the wastes were not limited to the dumpsite proper (Schwab et al. 1981). This surprising result was obtained by measuring waste-specific organic compounds (i.e., toluene, dimethylaniline and benzene) and finding them present in the surface water samples. Since the pharmaceutical wastes were not limited to the dumpsite but were widely distributed throughout the region, a comparison of waste-impacted waters and waste-free waters was not possible. Therefore, in subsequent cruises, the cruise plan was altered to allow sampling of stations along transect lines extending from near the coast of

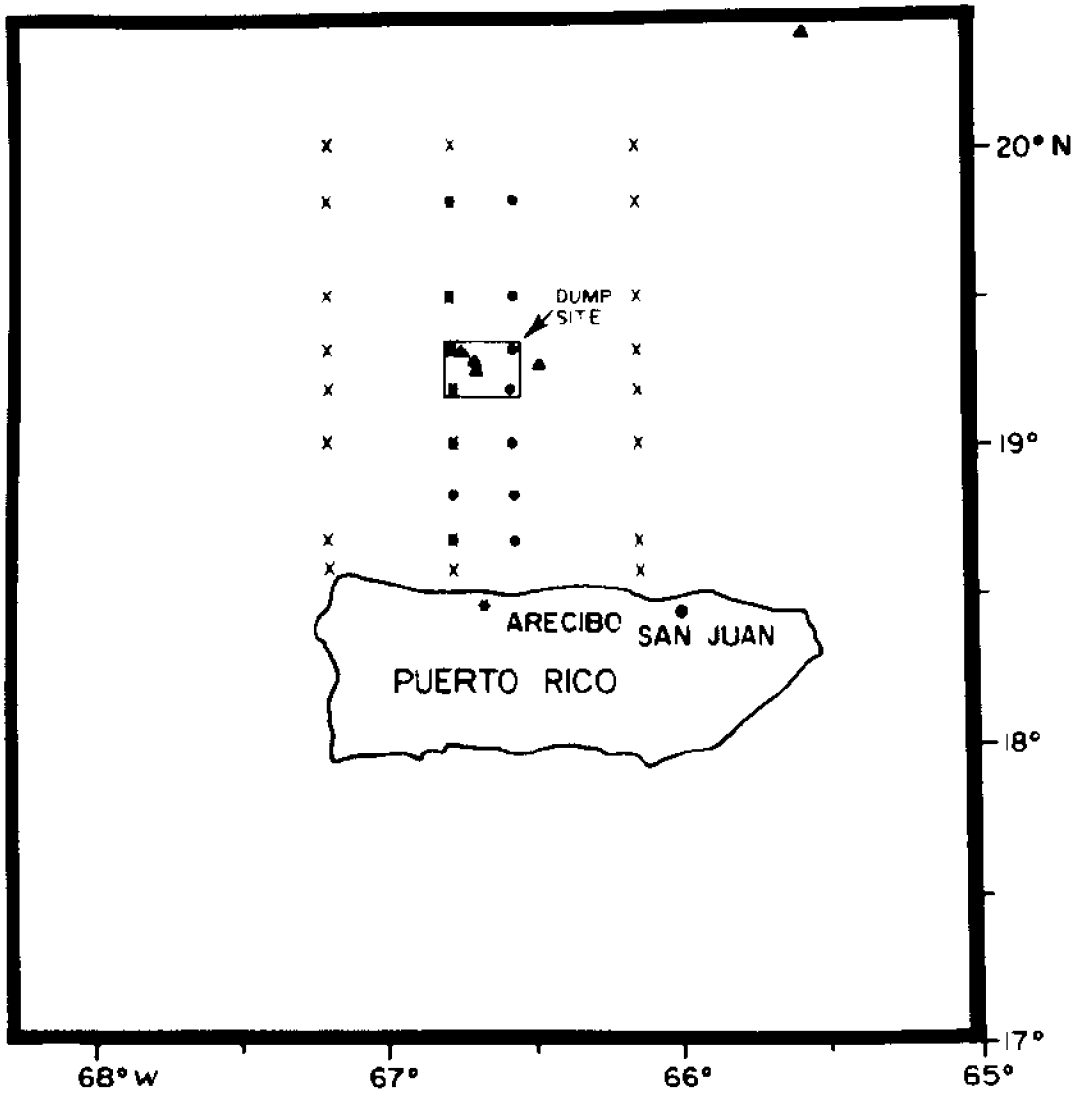

Figure 1. Dumpsite in Puerto Rico Trench area of Atlantic Ocean.

Puerto Rico, through the dumpsite, and into waters far beyond the dumpsite (Figure 1). Also, stations on transects to the east and west of the dumpsite were sampled to detect any large-scale impact on those bacterial communities that would be traced to waste disposal activities.

Transect Studies

Results obtained from the first transect study, accomplished in December 1979, demonstrated that the largest numbers of bacteria, determined by direct microscopic counting, were present in water samples collected at stations located along the western side of the dumpsite (Table 1). Differences in counts between stations along the two transects extending through the dumpsite were approximately 10% to 19%.

The culturable proportion of the bacterial community was also found to differ between the two transects (Table 1). Largest numbers of culturable bacteria were detected in samples collected from stations directly north of the dumpsite. The increased bacterial load in these waters was demonstrated by both direct and culturing enumeration methods. The larger numbers of bacteria in water samples collected north of the dumpsite were hypothesized to result from enrichment by the pharmaceutical wastes and currents in these waters carrying the waste material northeastward. Although the general pattern of bacterial counts detected in December 1979 (i.e., decreasing bacterial counts with increasing distance from land except within the dumpsite) was also detected during subsequent cruises (see Peele et al. 1981; Singleton et al. 1983; Grimes et al. 1983), the significant increase observed in bacterial counts for samples collected north of the dumpsite was not always detected. Such findings may be related to the variability in composition of the pharmaceutical wastes, an irregular schedule for dumping, and the current patterns in this region of the Atlantic Ocean.

For the increased numbers of culturable bacteria detected north of the dumpsite to be a result of pharmaceutical wastes in the dumpsite, the water movement would have to be in a northerly direction. However, the major current pattern of this region was reported to be the Antilles Current, which would move the waters in a westerly direction (Proni and Hansen 1981). Interestingly, some studies of the current patterns in the region, monitored by surface buoys, demonstrated water movement following a meandering course which creates large surface gyres. The gyres tend to recirculate waters through the dumpsite region, and allow for accumulation of material, including microorganisms, in this region (Duncan et al. 1982). It is doubtful, therefore, that wastes disposed in the dumpsite would move only in a westerly direction, as suggested by Proni and Hansen (1981).

Table 1. Location of stations sampled during December 1979 and results of bacterial enumeration employing the direct and plate count methods.

Station	Location		Total Number of Bacteria ml^{-1}*		
	Latitude N	Longitude W	AODC	MA	PCA
E1	18°40.0	66°37.0	2.5	3.5	11.9
E2	18°50.0	66°37.0	2.6	1.7	9.4
E3	19°00.0	66°37.0	3.2	4.1	2.3
E4	19°12.0	66°37.0	2.6	2.4	18.1
E5	19°18.0	66°37.0	2.3	4.4	-
E6	19°29.6	66°37.0	2.2	34.7	23.0
E7	19°50.2	66°37.0	2.2	12.2	13.9
W1	18°40.0	66°48.0	3.1	7.2	1.9
W2	18°50.0	66°48.0	3.0	5.3	1.9
W3	19°02.6	66°48.0	2.5	5.6	0.9
W4	19°12.0	66°48.0	3.1	5.0	0.6
W5	19°18.1	66°48.0	2.6	5.3	8.1
W6	19°30.0	66°48.0	2.6	180.0	48.0
W7	19°50.0	66°48.0	2.0	20.3	8.7

* AODC = Acridine orange direct count, $x10^5$
MA = Marine agar 2216
PCA = Plate count agar

Although there were quantitative differences in the bacterial community of surface waters of the dumpsite region, the culturable bacterial community was also examined for qualitative differences. Organisms growing on MA were purified and identified to genus. Results of identification of bacterial isolates yielded a community profile dominated by members of the genus Vibrio. Pseudomonas spp., commonly reported to dominate the culturable bacterial community of surface waters of the open ocean (Murchelano and Brown 1971; Sieburth 1971; Pfister and Burkholder 1965; ZoBell 1946), were conspicuously in the minority (Table 2). Results of taxonomic studies of the culturable bacterial community of surface waters in this region were consistent between cruises, demonstrating the presence of a relatively stable bacterial community response to the wastes (Table 2).

It has been accepted that the culturable bacterial community of open ocean waters is dominated by Pseudomonas spp. (Murchelano and Brown 1971; Sieburth 1971; Wood 1967; Pfister and Burkholder 1965; ZoBell 1946). However, a taxonomic analysis of bacterial

Table 2. Taxonomic distribution of bacterial strains isolated from marine agar 2216 for three cruises to the Puerto Rico Trench accomplished during 1979.

Genus	Percent Total Number of Strains		
	May	Oct	Dec
Vibrio	86	82	75
Pseudomonas	1	8	2
Photobacterium	3	0	1
Lucibacterium	0	4	0
Flavobacterium	1	5	4
Acinetobacter/Moraxella	4	1	2
Unknown gram-negative rods	0	0	1
Unidentified gram-positive (+) cocci	0	0	15
Total number of strains	227	99	140

isolates from waters within and surrounding the Puerto Rico dumpsite demonstrated that these communities were dominated by Vibrio spp., with Pseudomonas spp. comprising less than 10% of the total culturable community (Table 2). On the basis of these results, it was hypothesized that ocean disposal of pharmaceutical wastes resulted in a shift in bacterial species dominance in surface waters of this region (Peele et al. 1981; Singleton et al. 1983; Grimes et al. 1983).

Results of Confirmatory Cruises

To confirm the finding that pharmaceutical waste disposal activities resulted in a significant alteration in the species composition of the bacterial community of the receiving waters, additional cruises in this region were accomplished. However, the cruise plans were altered to allow sampling stations to be located along tracklines in waters far removed from the dumpsite and extending on into the dumpsite. These cruises, therefore, permitted comparison of bacteriological parameters of waters to the west, north

and south of the dumpsite.

The first trackline extended from Key West, Fla., to Puerto Rico and included repeated sampling along the transect through a western side of the dumpsite (Figure 2). Stations located in waters impacted by the Barceloneta Regional Treatment Plant outfall were also sampled.

As anticipated, the largest numbers of bacteria were detected in samples collected near the sewage outfall (Table 3). Also, significantly larger ($p \leq 0.05$) numbers of bacteria growing on PCA were detected at these stations. Water samples collected near land (i.e., Station 1) also contained large numbers of bacteria capable of growth on PCA. Such results demonstrate the influence of sewage discharges and surface runoff on the bacteriological quality of marine waters, with signficant contribution of allochthonous bacteria from these sources, further supported by the fact that, with increasing distance from land, the PCA culturable counts declined.

Numbers of bacteria growing on MA were observed to parallel closely those growing on PCA (Table 3). Largest numbers of bacteria grown on MA were detected near the sewage outfall and near land. Also, with increasing distance from land, culturable counts obtained on MA declined slightly.

Table 3. Locations of stations sampled during November 1980 and results of bacterial enumeration employing the direct plate count methods.

Station	Location		Total Number of Bacteria ml^{-1} *		
	Latitude N	Longitude W	AODC	MA	PCA
1	24°00.7	81°21.0	1.6	4.7	.19
2	20°31.0	72°39.9	1.3	4.9	.17
2A	19°10.0	68°30.3	1.1	4.8	.09
3C1	18°39.6	66°30.3	2.6	460.0	.34
3C2	18°30.9	66°33.7	3.7	12.0	.43
3C3	18°29.8	66°32.8	-	20.0	.88
4	19°00.2	67°19.2	1.9	11.0	.05
5	19°42.0	67°18.7	1.5	5.2	.02
6	20°01.7	67°19.0	1.7	4.7	.07

* AODC = Acridine orange direct count, $x10^5$
MA = Marine agar 2216
PCA = Plate count agar

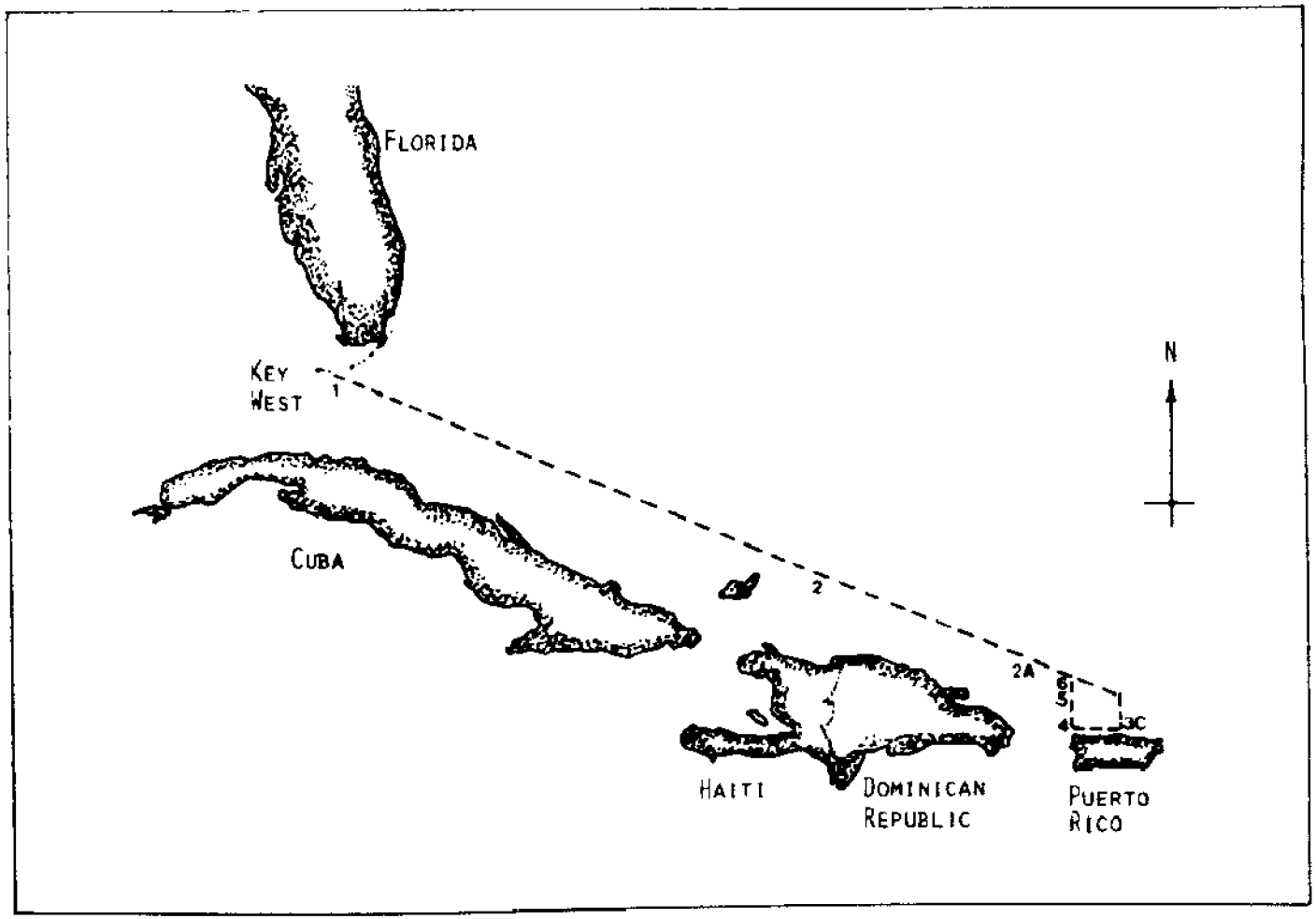

Figure 2. Transect of sampling area through western side of the dumpsite.

A cruise accomplished in June 1981 allowed sampling at stations located south of Puerto Rico, extending from Barbados to Puerto Rico through the dumpsite and northward to Bermuda (Figure 3). Water samples collected at the open ocean stations, E, F, I, J, K, L, M and N, yielded low culturable bacterial counts, averaging 5 ml^{-1} on MA and 0.1 ml^{-1} on PCA (Table 4). Larger numbers of bacteria were detected near land (Station D) and within the dumpsite (Stations G and H), but the largest numbers of culturable bacteria were detected in samples collected at the stations in the Caribbean Sea, A, B and C.

Total (AODC) bacterial counts were relatively stable along the tracklines of both cruises (Tables 2 and 4). However, an interesting relationship between total bacterial counts and culturable bacterial counts were detected. The proportion of the total community capable of growth on MA and PCA decreased with increasing distance

Table 4. Locations of stations sampled during June 1981 and results of bacterial enumeration employing the direct and plate count methods.

Station	Location		Total Number of Bacteria ml^{-1}*		
	Latitude N	Longitude W	AODC	MA	PCA
A	15°33.2	63:54.7	1.9	11.0	0.31
B	16°02.0	64°35.8	2.5	24.0	0.04
C	16°38.0	65°29.0	1.1	50.0	0.10
D	18°34.8	66°47.9	2.3	7.7	0.34
E	18°39.8	66°47.9	2.2	9.4	-
F	19°00.1	66°48.0	1.9	5.8	0.09
G	19°12.0	66°48.0	1.7	10.0	0.94
H	19°18.0	66°48.0	2.7	7.4	0.40
I	19:29.8	66°48.0	1.7	9.3	0.14
J	19°50.1	66°46.9	2.0	5.9	0.38
K	20°00.0	66°48.0	2.1	4.5	0.07
L	21°50.7	66°29.5	1.7	2.7	0.07
M	25°30.0	66°51.4	2.0	1.1	0.01
N	27°53.3	65°24.4	1.6	2.2	0.01

* AODC = Acridine orange direct count, $x10^5$
MA = Marine agar 2216
PCA = Plate count agar

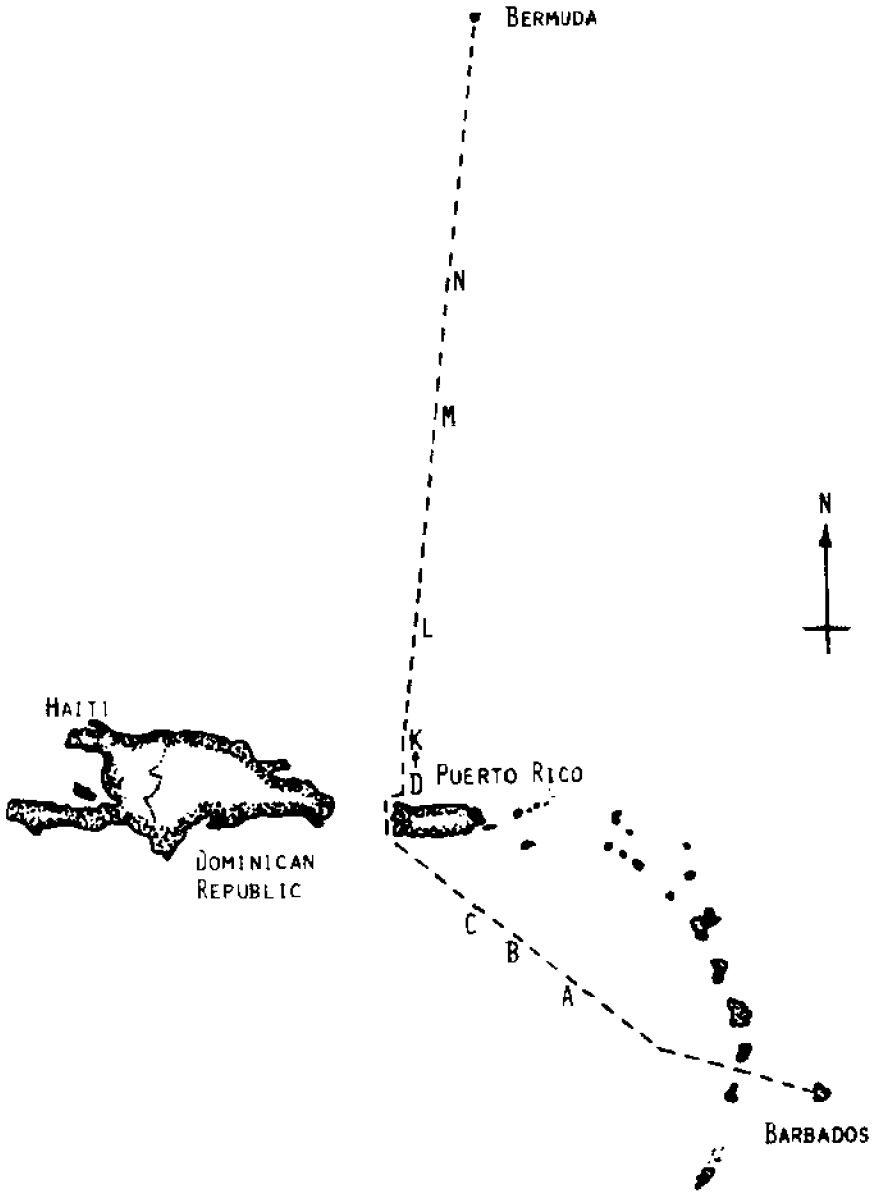

Figure 3. Sampling station from Barbados through dumpsite to Bermuda.

from land, but increased in areas impacted by sewage or pharmaceutical waste. For example, in June 1981 the percent of the total bacterial community culturable on MA decreased ten-fold from stations D to M, except within the dumpsite and at stations located near the dumpsite where a significant increase was noted. The percent of the total community capable of growth on PCA followed a similar pattern, decreasing by a factor of 30 as distance from land increased, and increasing more than ten-fold in the dumpsite.

The culturable bacterial community of waters sampled in November 1980 and June 1981 was, as previously detected, dominated by members of the genus Vibrio (Tables 5 and 6). Vibrio spp. accounted for 70% of the isolates recovered on MA during November 1980, and 57% in June 1981. The second most abundant species was Acinetobacter, averaging 13% in November 1980 and 32% in June 1981. Pseudomonads constituted only 3% of the culturable community. Interestingly, the genera Vibrio and Acinetobacter together constituted the majority of the bacteria recovered on MA from water samples collected at all stations sampled in June 1981, ranging from a low of 77% at Station M to 100% at Stations C, E and F. The mean percentage was relatively constant (88.4 ± 8.4%), so that an increase in the number of Vibrio spp. was accompanied by a decrease in Acinetobacter spp., and vice versa.

Table 5. Taxonomic distribution of marine agar 2216 isolates--November 1980.

Genus	Percent Total Number of Strains								
	Sta. 1	Sta. 2	Sta. 2A	Sta. 3C1	Sta. 3C2	Sta. 3C3	Sta. 4	Sta. 5	Sta 6
Vibrio	47	76	72	82	68	76	75	71	62
Acinetobacter	21	19	11	9	8	8	15	6	19
Pseudomonas	16	0	0	0	0	8	0	6	0
Flavobacterium	5	0	11	0	8	0	0	11	8
Staphylococcus	0	0	0	0	8	0	0	0	0
Arthrobacter	0	0	6	0	0	0	0	0	0
Enterobacter	0	0	0	0	8	0	0	0	0
Moraxella	0	0	0	0	0	0	0	0	4
Unidentified	11	5	0	9	0	8	10	6	8
Total number of strains	19	21	18	23	13	13	20	18	26

Table 6. Taxonomic distribution of marine agar 2216 isolates--June 1981--at Stations A-N.

	Percent Total Number of Strains Examined													
Genus	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Vibrio	21	18	100	18	95	86	45	27	81	65	52	50	59	70
Acinetobacter	67	64	0	68	5	14	40	60	14	21	44	29	18	0
Pseudomonas	0	9	0	0	0	0	0	13	5	7	0	14	0	0
Flavobacterium	0	0	0	4	0	0	0	0	0	0	0	0	6	20
Staphylococcus	8	0	0	5	0	0	10	0	0	0	0	0	0	0
Arthrobacter	0	0	0	5	0	0	0	0	0	0	0	7	0	0
Enteric	0	0	0	0	0	0	5	0	0	0	0	0	0	0
Moraxella	0	9	0	0	0	0	0	0	0	0	0	0	0	0
Micrococcus	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Unidentified	0	0	0	0	0	0	0	0	0	7	4	0	17	10
Total number of strains	24	11	29	22	22	7	20	15	21	14	25	14	17	10

Results of the November 1980 and June 1981 cruises supported the hypothesis of an alteration in the species composition of the culturable bacterial community of surface waters in the region of the Puerto Rico dumpsite. However, it must be noted that domination of the bacterial community by Vibrio spp. instead of Pseudomonas spp. was observed in other water samples collected from this region of the Atlantic Ocean.

Laboratory Studies

To evaluate the degree to which addition of pharmaceutical waste could affect the culturable bacterial community, laboratory experiments were carried out to determine the impact of pharmaceutical waste on the bacterial community of water samples, collected elsewhere than at, or near, the dumpsite, and expected to be free of pharmaceutical wastes. To approximate in situ conditions, as well as to ensure proper replication of experimental units, laboratory microecosystems (i.e., microcosms) were employed. Microcosms were prepared by addition of 50 ml seawater samples collected from the lower portion of the Chesapeake Bay to sterile, acid-cleaned Erlenmeyer flasks. All microcosms were then allowed to stabilize for 4 days prior to testing for effects of addition of filter sterilized pharmaceutical waste. Concentrations of pharmaceutical waste added to the microcosms were selected to approximate the initial dumpsite dilution after dispersal in surface waters of the open ocean, that is, ca. 10^{-3} to 10^{-4} (Csanady 1981).

Initial experiments consisted of addition of the pharmaceutical waste and sampling the culturable bacterial community during a 6-day test period. The culturable bacteria were found to increase in number after waste additions; the maximum population size was proportional to the concentration of added waste (Figure 4), demonstrating that some members of the bacterial community respond to components of the waste that serve as substrate for growth.

Since the culturable bacterial community was found to respond to pharmaceutical waste, with a significant increase in number, an additional series of microcosms was challenged with pharmaceutical waste to determine the impact on the bacterial community structure. Samples were taken 30 min after waste addition, assuming this was not sufficient time for growth of the bacteria present in the water samples. Hence, any immediate toxic effects could be detected. As shown in Figure 5, no detectable toxic effect of the pharmaceutical waste on the culturable bacterial community was observed. Also, the increase in cell count with time was linear, indicating that the pharmaceutical waste, or a component within it, was indeed being utilized as a substrate for growth.

Thus, the culturable bacterial community of Chesapeake Bay water microcosms responded to pharmaceutical waste additions by increasing in number. However, it was necessary to establish whether certain species were selected as a result of enrichment.

To evaluate the extent to which the pharmaceutical waste conferred a selective advantage upon specific members of the community, species-defined microcosms were prepared. A chemically defined artificial seawater medium and selected bacterial strains were used (Singleton et al. 1982). Predetermined numbers of nutrient-free cells were added to the sterile microcosms and each microcosm contained two bacterial strains, Vibrio PR-110, a strain typical of the Vibrio spp. isolated from the dumpsite, and either Vibrio PR-106, a strain typical of one of the other, less frequently occurring Vibrio strains isolated from water samples collected near the dumpsite, or Pseudomonas AO-66, isolated from the Atlantic Ocean near the Virginia coast. Approximately the same numbers of cells of each strain were added to the microcosms and, after a 48-h stabilization period, selected concentrations of filter-sterilized pharmaceutical waste were added to the microcosms. Following an additional 48-h stabilization period, pharmaceutical waste was again added to the microcosms. Samples for bacterial enumeration by spread plating were collected immediately after inoculation, before waste addition and after 48 h incubation.

In microcosms containing two Vibrio spp., the species composition of the community was not significantly affected except in the presence of the larger concentrations of pharmaceutical waste, in which case the Vibrio PR-110 increased in dominance by approximately 20% (Figure 6). However, in microcosms containing Vibrio PR-110 and Pseudomonas AO-66, the influence of pharmaceutical waste enrichment was dramatic (Figure 7). As the concentration of waste increased, the proportion of the community occupied by Vibrio PR-110 also increased, until, in the presence of 1000 µl pharmaceutical waste L^{-1}, after the first waste addition and incubation period, no Pseudomonas colonies were detected on the culture medium.

DISCUSSION

Significant changes in the bacterial community composition of surface waters of the Puerto Rico dumpsite, occurring as a result of ocean disposal of pharmaceutical wastes, were observed when water samples collected within the dumpsite were compared with samples collected north, south and west of the dumpsite. Also, in some of the studies, a series of transects were studied, extending from the Puerto Rico coast, through the dumpsite, and into waters well beyond the dumpsite. Although the total numbers of bacteria, determined by direct microscopic counting, were found to be relatively constant in waters of this region of the Atlantic Ocean, there were significant differences in the culturable bacteria both in numbers and in species composition (Tables 1, 3, 4). The species composition of the culturable bacterial community differed from

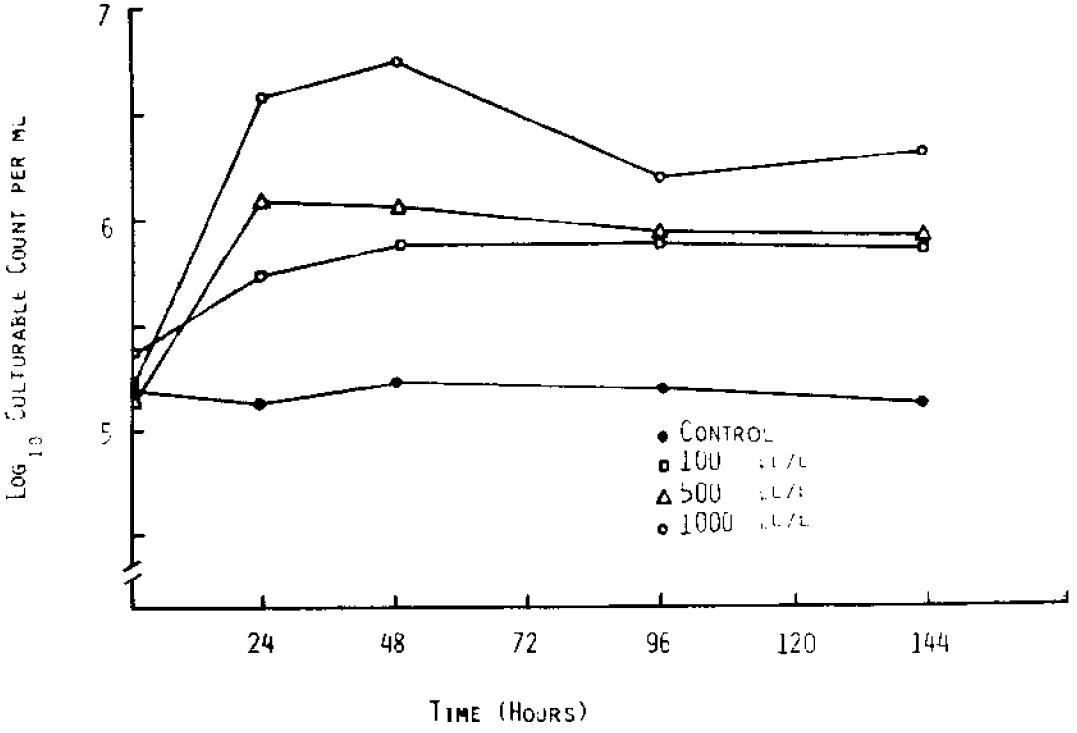

Figure 4. Long-term response of the culturable bacterial community in Chesapeake Bay water to composite pharmaceutical waste.

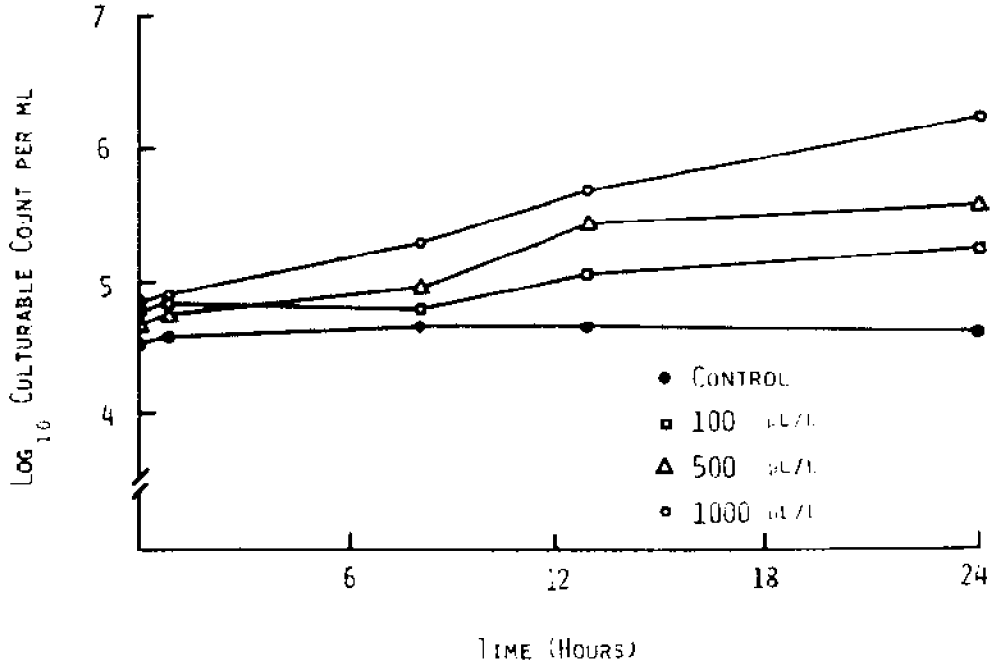

Figure 5. Short-term response of the cultural bacterial community in Chesapeake Bay water to composite pharmaceutical waste.

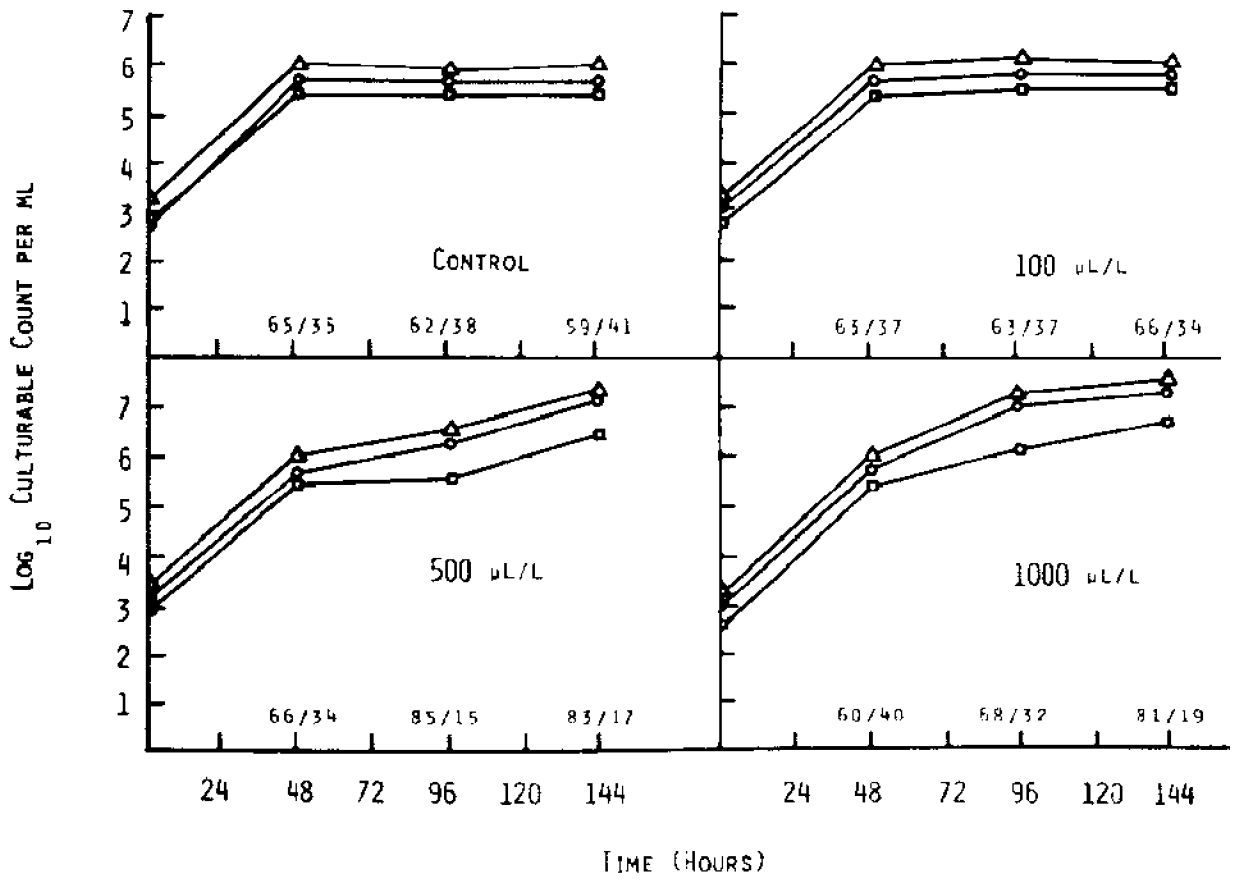

Figure 6. Effect of composite pharmaceutical waste on a mixed culture of Vibrio PR-110 (O) and Pseudomonas AO-66 (□). Numbers inset and located above the abscissa represent the percent of the total (△) community composed of PR-110 and AO-66, respectively. Waste was added immediately after the sample was taken at 48 h.

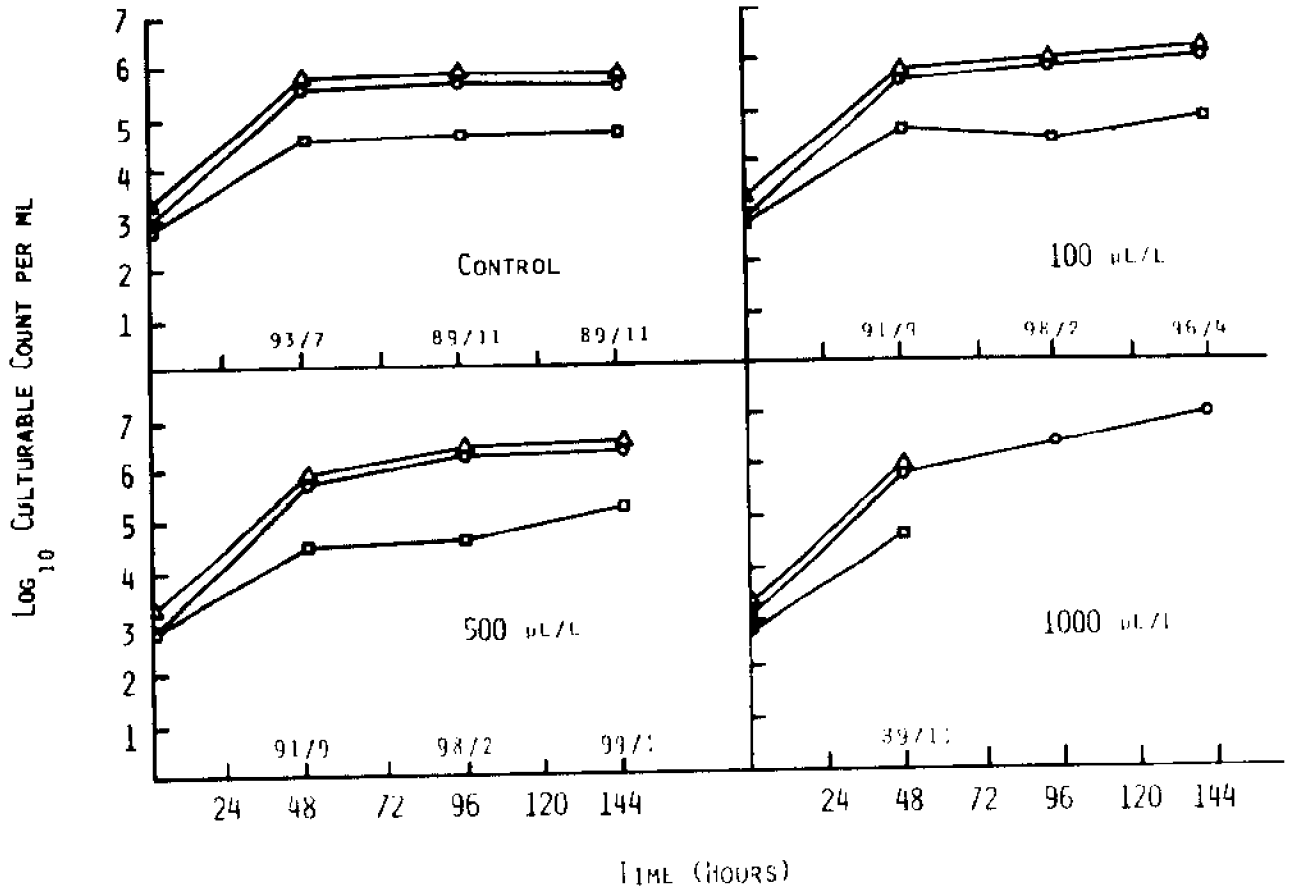

Figure 7. Effect of composite pharmaceutical waste of a mixed culture (△) composed of Vibrio PR-110 (○) and Pseudomonas AO-66 (□). Numbers inset and located above the abscissa represent the percent of the community composed of strains PR-110 and AO-66, respectively. Waste was added immediately after the sample was taken at 48 h.

that reportedly characteristic for waters of this region (Sieburth 1971; Pfister and Burkholder 1965). Notably, Vibrio spp. were found to dominate the culturable community, and not Pseudomonas spp. Also unexpected was the finding that, during the 1981 cruises, Acinetobacter spp. were second most abundant.

That Pseudomonas spp. represent the dominant culturable bacteria in surface waters of the open ocean in temperate and tropical regions is open to question, based on the studies reported here. In the past, occurrences of Pseudomonas spp. up to, and exceeding, 75% of the culturable bacterial community of open ocean waters have been reported. Sieburth (1971), for example, reported nearly 100% of isolates from samples collected from stations, approximating the June 1981 trackline, F through N (Figure 3) of this study, were Pseudomonas spp. Similarly, in a numerical taxonomy study of bacterial isolates from seawater samples collected near Puerto Rico, reported by Pfister and Burkholder (1965), approximately 66% of the isolates were identified as Pseudomonas spp.

One means of accounting for the apparent shift in dominance of the culturable bacterial community from Pseudomonas to Vibrio is that methods for identification and classification of bacteria currently employed differ from those used in the past. Certainly, vibrios and pseudomonads can now be more clearly differentiated. Therefore, it is possible that bacteria classified as Pseudomonas spp. a few decades ago would, today, be identified as Vibrio or Aeromonas spp. For example, several of the glucose-fermenting Pseudomonas spp. described by ZoBell and Upham (1944) and the glucose-fermenting, indole-producing bacteria reported by Pfister and Burkholder (1965) to be pseudomonads very likely would now be classified as vibrios.

However, is also possible that a shift has occurred in the dominance of species within the culturable bacterial community in waters of some regions of the temperate and tropical oceans. A dominance pattern as reported here has been observed in the East China Sea by Simidu et al. (1980), in Chesapeake Bay by Austin et al. (1979), and in the Gulf of Mexico (R.K. Sizemore, personal communication). An explanation for such a shift in dominance of the culturable bacterial community is suggested by studies of Sizemore and Colwell (1977) and Hada and Sizemore (1981). That is marine vibrios demonstrate a high degree of environmental adaptiveness, possibly because of the variety of cryptic plasmids carried by the majority of vibrios. However, pseudomonads are also known to possess a number of plasmid-mediated characteristics, including degradative and resistance traits. It is possible that, as a result of their environmental adaptiveness and ubiquitous distribution in the world oceans, that marine vibrios can more readily respond to ecological or selective pressures exerted upon them in the natural environment. Thus, the presence of certain components of pharmaceutical wastes could exert a selective pressure, enabling the vibri-

os to out-compete pseudomonads, in much the same manner as was observed in the microcosm studies reported here (Figures 4 and 5). Certain Vibrio spp. are known to metabolize short chain volatile fatty acids, alcohols and aromatic hydrocarbons (Baumann et al. 1971) similar to those compounds present in the pharmaceutical waste (Schwab et al. 1981). Furthermore, Acinetobacter spp., often the second most abundant group of bacteria in surface waters of this region of the Atlantic Ocean, are known to metabolize hydrocarbons (Juni 1978) of the type present in pharmaceutical waste. Therefore, hydrocarbons and related compounds may serve as substrates for the selective growth of Vibrio spp. and Acinetobacter spp. at the expense of other members of the bacterial community.

Plasmid-bearing Vibrio spp. have been isolated in significantly larger numbers from water samples collected in the oil fields of the Gulf of Mexico than from water samples collected in control areas (Hada and Sizemore 1981). In a study of the effect of petroleum on sediment bacterial communities in Chesapeake Bay, Walker et al. (1976) found that oil-polluted sediments contained Vibrio spp., Acinetobacter spp. and Pseudomonas spp., representing populations metabolically responsive to petroleum enrichment. Conversely, in non-oil-polluted sediments, the predominant culturable bacteria were Pseudomonas spp. and gram-positive coryneform bacteria.

The region of the Atlantic Ocean which we have studied serves as one of the world's busiest sea lanes (Gunkel and Gassmann 1980). Also, this region is subjected to a heavy burden of sea traffic involved with petroleum shipping, which has been reported to be the major source of hydrocarbons entering the marine environment (Gunkel and Gassman 1980). Therefore, the marine bacterial community of these waters are subjected to unknown quantities of petroleum-derived hydrocarbons, in addition to pharmaceutical wastes and other point pollution sources. Thus, a combination of these could affect the bacterial communities of vast expanses of ocean surface waters in this part of the world.

Another factor related to the observed change in the composition of the bacterial community is suggested from the findings of Sieburth (1968b), who observed in 1962-63 that both Flavobacterium and Pseudomonas spp. were dominant, that is, they constituted 50% of the isolates from Narragansett Bay water samples, during periods of diatom blooms. High diatom activity was found to suppress growth of Vibrio spp., which otherwise were dominant (i.e., up to 40%); the incidence of Flavobacterium spp. was inversely related to that of Vibrio spp. Reasons suggested for this relationship were diatom-bacterium interactions or bacterial antagonism. These results are supported by the recent studies of Shiba and Taga (1981) in which marine algae were found to suppress growth of vibrios. Likewise, Fukami et al. (1981) also observed that, during

phytoplankton decomposition, bacterial succession occurred, with Pseudomonas and Alcaligenes spp. becoming dominant during early stages of decomposition, followed by Acinetobacter and Moraxella spp. in later stages. Vibrio spp., often dominant in the inoculum were consistently absent during the decomposition process. Therefore, the natural interactions between bacteria and higher trophic level organisms can affect community structure. A change in one component of the biota, as a result of the input of allochthonous materials, can affect interactions between organisms and, thereby, influence the community structure.

In summary, the results of laboratory and field studies accomplished to date document a significant relationship between species composition of bacterial communities in surface waters and enrichment by pharmaceutical wastes. In laboratory microcosms, Vibrio spp. were, in general, superior competitors, and, as the concentration of added pharmaceutical waste increased, the proportion of the community consisting of Vibrio spp. also increased. Vibrio spp. were also found to dominate the culturable bacterial community of surface waters within and surrounding the dumpsite region. However, the effect on the bacterial community of surface waters of such a wide expanse of oceanic waters (i.e., from Barbados to Bermuda) is difficult to relate directly to pharmaceutical waste disposal alone. This is especially so because of the hydrocarbons that enter the vicinity of the study area from marine transportation. Petroleum hydrocarbon enrichment of the bacterial community similar to that observed from pharmaceutical wastes deserves consideration.

Quantitative and qualitative analyses of the autochthonous bacterial community of aquatic systems receiving allochthonous materials are, therefore, useful in evaluating effects of those materials on the microbiota. If, in fact, the species composition of the microbial flora is significantly altered to the extent that the community composition is significantly changed by influx of anthropogenic materials, as in the case of pharmaceutical wastes, the quantity of material a given water mass can assimilate must be carefully assessed if ocean disposal of wastes is to be a continuing, or permanent, means of solving waste disposal problems. It is, after all, the microbial populations upon which we depend, ultimately, for degradation and recycling of allochthonous materials deposited in the world's oceans.

REFERENCES

Alexander, M. 1971. Microbial Ecology. John Wiley and Sons, New York.

Austin, B., S. Garges, B. Conrad, E.E. Hardin, R.R. Colwell, U. Simidu and N. Taga. 1979. Comparative study of the aerobic, heterotrophic bacterial flora of Chesapeake Bay and Tokyo Bay. Appl. Environ. Microbiol. 37: 704-714.

Bain, N. and J.M. Shewan. 1968. Identification of Aeromonas, Vibrio and related organisms. In: B.M. Gibbs and D.A. Shapton (eds.), Identification Methods for Microbiologists, Part B., pp. 79-84. Academic Press, New York.

Baumann, L., P. Baumann, M. Mandel and R.D. Allen. 1972. Taxonomy of aerobic marine eubacteria. J. Bacteriol. 110:402-429.

Baumann, P., L. Baumann and M. Mandel. 1971. Taxonomy of marine bacteria: The genus Beneckea. J. Bacteriol. 107: 268-294.

Bell, C.R., M.A. Holder-Franklin and M. Franklin. 1982. Correlations between predominant heterotrophic bacteria and physicochemical water quality parameters in two Canadian Rivers. Appl. Environ. Microbiol. 43: 269-283.

Bonde, G.J. 1967. Pollution of a marine environment. J. Water Pollut. Control Fed. 39 (Part 2): R15-R63.

Bowden, W.B. 1977. Comparison of two direct-count techniques for enumerating aquatic bacteria. Appl. Environ. Microbiol. 33: 1229-1232.

Brasfield, H. 1972. Environmental factors correlated with the size of bacterial populations in a polluted stream. Appl. Microbiol. 24: 349-352.

Cherry, D.S., R.K. Guthrie, F.L. Singleton and R.S. Harvey. 1977. Recovery of aquatic bacterial populations in a stream after cessation of chemical pollution. Water Air Soil Pollut. 7: 95-101.

Colwell, R.R. and J.D. Walker. 1977. Ecological aspects of microbial degradation of petroleum in the marine environment. Crit. Rev. Microbiol. 5: 423-445.

Csanady, G.T. 1981. An analysis of dumpsite diffusion experiments. In: B.H. Ketchem, D.R. Kester and P.K. Park (eds.), Ocean Dumping of Industrial Wastes, pp. 109-129. Plenum Press, New York.

Daley, R.J. and J.E. Hobbie. 1975. Direct counts of aquatic bacteria by a modified epifluorescence technique. Limnol. Oceanogr. 20: 875-882.

Duncan, C.P., S.G. Schladow and W.G. Williams. 1982. Surface currents near the Greater and Lesser Antilles. Int. Hydrogr. Rev. 59: 67-78.

Erkenbrecher, C.W., Jr. 1981. Sediment bacterial indicators in an urban shellfishing subestuary of the lower Chesapeake Bay. Appl. Environ. Microbiol. 42: 484-492.

Francisco, D.E., R.A. Mah and A.C. Rabin. 1973. Acridine orange epifluorescence technique for counting bacteria in natural waters. Trans. Am. Microsc. Soc. 92: 416-421.

Fukami, K., U. Simidu and N. Taga. 1981. Fluctuation of the communities of heterotrophic bacteria during the decomposition process of phytoplankton. J. Exp. Mar. Biol. Ecol. 55: 171-184.

Furniss, A.L., J.V. Lee and T.J. Donovan. 1978. The vibrios. Public Health Laboratory Service Monograph Series, Her Majesty's Stationery Office, London, England.

Gibson, D.W., M.S. Hendrie, N.C. Houston and G. Hobbs. 1977. The identification of some gram-negative heterotrophic aquatic bacteria. In: F.A. Skinner and J.M. Shewan (eds.), Aquatic Microbiology, pp. 135-159. Academic Press, New York.

Gocke, K. 1977. Untersuchungen über die heterotrophe Aktivitat in der zentralen Ostee. Mar. Biol. 40: 87-94.

Grimes, D.J., F.L. Singleton and R. R. Colwell. 1984. Allogenic succession of marine bacterial communities in response to pharmaceutical waste. J. Appl. Bacteriol. (In press.)

Gunkel, W. and G. Gassmann. 1980. Oil, oil dispersants, and related substances in the marine environment. Helgol. Meeresunters. 33: 164-181.

Gunn, B.A., F.L. Singleton, E.R. Peele, R.R. Colwell, J.K. Keiser and C.O. Kapfer. 1981. A comparison of methods for identifying Staphylococcus and Micrococcus spp. J. Clin. Microbiol. 14: 195-200.

Guthrie, R.K., D.S. Cherry and R.N. Ferebee. 1974. A comparison of thermal loading effects on bacterial populations of polluted and nonpolluted aquatic systems. Water Res. 8: 143-148.

Guthrie, R.K., D.S. Cherry, F.L. Singleton and R.S. Harvey. 1978. The effect of coal ash basin effluent and thermal loading on bacterial populations in flowing streams. Environ. Pollut. 17: 297-302.

Hada, H.S. and R.K. Sizemore. 1981. Incidence of plasmids in marine Vibrio spp. isolated from an oil field in the northwestern Gulf of Mexico. Appl. Environ. Microbiol. 41: 199-202.

Hägstrom, Å., U. Larsson, P. Horstedt and S. Normark. 1979. Frequency of dividing cells, a new approach to the determination of bacterial growth rates in aquatic environments. Appl. Environ. Microbiol. 37: 805-812.

Hobbie, J.E., O. Holm-Hansen, T.T. Packard, L.R. Pomeroy, R.W. Sheldon, J.P. Thomas and W.J. Weibe. 1972. A study of the distribution and activity of microorganisms in ocean water. Limnol. Oceanogr. 17: 544-555.

Hobbie, J.E., R.J. Daley and S. Jasper. 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33: 1225-1228.

Jannasch, H.W. 1958. Studies on planktonic bacteria by means of a direct membrane filter method. J. Gen. Microbiol. 18: 609-620.

Jannasch, H.W. and G.E. Jones. 1959. Bacterial populations in sea water as determined by different methods of enumeration. Limnol. Oceanogr. 4: 128-139.

Juni, E. 1978. Genetics and physiology of Acinetobacter. Ann. Rev. Microbiol. 32: 349-371.

Kaneko, T., M.I. Krichevsky and R.M. Atlas. 1979. Numerical taxonomy of bacteria from the Beaufort Sea. J. Gen. Microbiol. 110: 111-125.

Kogure, K., U. Simidu and N. Taga. 1979. A tentative direct microscopic method for counting living marine bacteria. Can. J. Microbiol. 24: 415-420.

LeChevallier, M.W., R.J. Seidler and T.M. Evans. 1980. Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. Appl. Environ. Microbiol. 40: 922-930.

Murchelano, R.A. and C. Brown. 1971. Heterotrophic bacteria in Long Island Sound. Mar. Biol. 7: 1-6.

Ogawa, K. 1973. Distribution of coliform bacteria in the coastal water. J. Oceanogr. Soc. Jap. 29: 203-208.

Olson, B.H. 1978. Enhanced accuracy of coliform testing in seawater by a modification of the most-probable-number method. Appl. Environ. Microbiol. 36: 428-444.

Orndorff, S.A. and R.R. Colwell. 1980. Distribution and identification of luminous bacteria from the Sargasso Sea. Appl. Environ. Microbiol. 39: 983-987.

Peele, E.R., F.L. Singleton, J.W. Deming, B. Cavari and R.R. Colwell. 1981. Effects of pharmaceutical wastes on microbial populations in surface waters at the Puerto Rico dump site in the Atlantic Ocean. Appl. Environ. Microbiol. 41: 873-879.

Pfister, R.M. and P.R. Burkholder. 1965. Numerical taxonomy of some bacteria isolated from Antarctic and tropical seawaters. J. Bacteriol. 90: 863-872.

Proni, J.R. and D.V. Hansen. 1981. Dispersion of particulates in the ocean studied acoustically: The importance of gradient surfaces in the ocean. In: B.H. Ketchem, D.R. Kester and P.K. Park (eds.), Ocean Dumping of Industrial Wastes, pp. 161-173. Plenum Press, New York.

Rittenberg, S.C., T. Mittwer and D. Ivler. 1958. Coliform bacteria around three marine sewage outfalls. Limnol. Oceanogr. 3: 101-108.

Robinson, G.G.C., L.L. Hendzel and D.C. Gillespie. 1982. A relationship between heterotrophic utilization of organic acids and bacterial populations in West Blue Lake, Manitoba. Limnol. Oceanogr. 18: 264-269.

Sayler, G.S., J.D. Nelson, Jr., A Justice and R.R. Colwell. 1975. Distribution and significance of fecal indicator organisms in the Upper Chesapeake Bay. Appl. Microbiol. 30: 625-638.

Schwab, C.R., T.C. Sauer, Jr., G.F. Hubbard, H. Abdel-Reheim and J.M. Brooks. 1981. Effects of the extracellular products of Enteromorpha linza on its epiphytic bacterial flora. Bull. Jap. Soc. Sci. Fish. 47: 1193-1197.

Shiba and Taga. 1981. Effects of the extracellular products of Enteromorpha linza on its epiphytic bacterial flora. Bull. Jap. Soc. Sci. Fish. 47: 1193-1197.

Sieburth, J.M. 1968a. Observations on planktonic bacteria in Narragansett Bay, Rhode Island: Proceedings of the U.S.-Japan Seminar on Marine Microbiology. Bull. Misaki Mar. Biol. Inst. Kyoto Univ. 12: 49-64.

Sieburth, J.M. 1968b. The influence of algal antibiosis on the ecology of marine microorganisms. In: M.R. Droop and E.J.F. Wood (eds.), Advances in Microbiology of the Sea, Vol. 1, pp. 63-94. Academic Press, London.

Sieburth, J.M. 1971. Distribution and activity of oceanic bacteria. Deep-Sea Res. 18: 1111-1121.

Sieburth, J.M. 1979. Sea Microbes. Oxford Univ. Press, New York.

Simidu, U., E. Kaneko and N. Taga. 1977. Microbiological studies of Tokyo Bay, Microb. Ecol. 3: 173-191.

Simidu, U., N. Taga, R.R. Colwell and J.R. Schwarz. 1980. Heterotrophic bacterial flora of the seawater from the Nansei Shoto (Ryuko Retto) area. Bull. Jap. Soc. Sci. Fish. 46: 505-510.

Singleton, F.L. and R.K. Guthrie. 1977. Aquatic bacterial populations and heavy metals. I. Composition of aquatic bacterial populations in the presence of copper and mercury salts. Water Res. 11: 639-642.

Singleton, F.L., R.W. Attwell, M.S. Jangi and R.R. Colwell. 1982. Influence of salinity and organic nutrient concentration on survival and growth of Vibrio cholerae in aquatic microcosms. Appl. Environ. Microbiol. 43: 1080-1085.

Singleton, F.L., J.W. Deming, E.R. Peele, B. Cavari, B. Gunn and R.R. Colwell. 1983. Microbial communities in surface waters at the Puerto Rico dumpsite. In: I.W. Duedall, B.H. Ketchum, P.K. Park and D.R. Kester (eds.), Wastes in the Ocean, Vol. 1, pp. 201-218. Industrial and Sewage Wastes in the Ocean. Wiley-Interscience, New York.

Sizemore, R.K. and R.R. Colwell. 1977. Plasmids carried by antibiotic-resistant marine bacteria. Antimicrob. Agents Chemother. 12: 373-382.

Staley, J.T., L.G. Lehmicke, F.E. Palmer, R.W. Peet and R.C. Wissmar. 1982. Impact of Mount St. Helens eruption on bacteriology of lakes in the blast zone. Appl. Environ. Microbiol. 43: 664-670.

Tanaka, N., Y. Ueda, M. Onizawa and H. Kadota. 1977. Bacterial populations in water masses of different organic matter concentrations in Lake Biwa. Jap. J. Limnol. 38: 41-47.

Walker, J.D., R.R. Colwell and L. Petrakis. 1976. Biodegradation of petroleum by Chesapeake Bay sediment bacteria. Can. J. Microbiol. 22: 423-428.

Watson, S.W., T.J. Novitsky, H.L. Quinby and F.W. Valois. 1977. Determination of bacterial number and biomass in the marine environment. Appl. Environ. Microbiol. 33: 940-946.

Wood, E.J.F. 1967. Microbiology of Oceans and Estuaries. Elsevier Publ., Amsterdam, Netherlands.

ZoBell, C.E. and H.C. Upham. 1944. A list of marine bacteria including descriptions of sixty new species. Bull. Scripps Inst. Oceanogr. (Univ. Calif. Tech. Ser.) 5: 239-292.

ZoBell, C.E. 1946. Marine Microbiology. Chronica Botanica Co., Waltham, Mass.

Metabolic Indicators of Sublethal Stress: Changes in Adenine Nucleotides, Glycogen and Lipid

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INTRODUCTION

The possibility of using adenine nucleotides as indicators of pollution stress has been the subject of much interest and controversy over the past few years. In this paper we discuss the original rationale for using adenylate measurements, the assumptions and misconceptions that have often surrounded their use and a review of recent studies which have used adenylate measurements as indicators of stress. In the final section we discuss ways of incorporating adenylate measurements with measurements of energy storage compounds such as neutral lipid and glycogen to increase the sensitivity and information content of the basic adenylate measurements.

The basic premise of the metabolic approach for measuring stress effects is that pollution results in an increase in entropy, which results from either (1) diverting assimilated energy to nonproductive functions such as increased respiration, cell repair, cleaning and avoidance activity or (2) decreasing the efficiency of energy transfer reactions through toxic effects on enzyme systems, changes in membrane potentials or genetic damage. Either mechanism results in an increase in the dissipation of assimilated energy as heat and a concomitant decrease in growth and reproductive potential. The aim of using sublethal metabolic indicators of pollution stress is to measure pollutant effects before they are translated into increased mortality and reproductive failure. The central role of adenine nucleotides as the basis of energy transformation in the cells of all living organisms has made these compounds particularly attractive as possible indicators of pollutional stress

(Figure 1). Adenosine triphosphate (ATP) is regenerated aerobically through oxidative phosphorylation and anaerobically through glycolysis. During periods of rapid ATP consumption, for example, during muscle contraction, ATP is regenerated by direct rephosphorylation from a storage phosphogen, such as creatine phosphate or via the adenylate kinase mediated salvage pathway, whereby 2 moles of adenosine diphosphate (ADP) are converted to 1 mole of ATP, immediately available for use, and 1 mole of adenosine monophosphate (AMP) (Figure 1).

Adenine nucleotides are also important as regulators of metabolic processes. The concentrations of ATP, ADP and AMP can act as either positive or negative allosteric effectors of regulatory enzymes of intermediary metabolism, balancing anabolic and catabolic processes (Figure 2). For example, falling ATP levels stimulate glycolytic processes which regenerate ATP while high levels of ATP inhibit glycolysis and stimulate gluconeogenesis. This rapid counterbalancing or "cybernetic" control results in the relative proportion of the three adenylates remaining almost constant within the cell except under extreme conditions of energy consumption or metabolic inhibition. Atkinson and co-workers were among the first to appreciate the constancy of the ratios of the three adenylates. Furthermore, they found that when a variety of eukaryotic cell and bacterial cultures were exposed to nutrient limitation (stress), the adenylate ratios changed in similar and predictable ways in different organisms exposed to different levels of stress (Atkinson 1968; Chapman et al. 1971; Montague and Dawes 1974; Walker-Simmons and Atkinson 1977). Using these findings, Atkinson proposed a single unitless ratio, based on the mole fraction of adenylate in the charged form, ATP plus ADP (where ADP is 1/2 the value of ATP), to the total adenylate pool. This adenylate energy charge ratio (AEC) is as follows:

$$AEC = \frac{ATP + 1/2ADP}{ATP + ADP + AMP}$$

It can vary from 0.0 (all the adenylate as AMP) to 1.0 (all the adenylates as ATP) (Atkinson and Walton 1967; Atkinson 1968). Atkinson's group and others have amply demonstrated that the AEC exhibits cybernetic control and that the AEC remains remarkably constant at values between 0.87 to 0.95 in a wide variety of other organisms under normal growth conditions (see review by Atkinson 1977).

Although the AEC was originally proposed as a biochemical concept, many investigators and administrators were attracted to the concept as an indicator of stress since it was a simple unitless ratio which would allow comparison of stress effects in very different organisms in a common context (e.g., if an organism had an

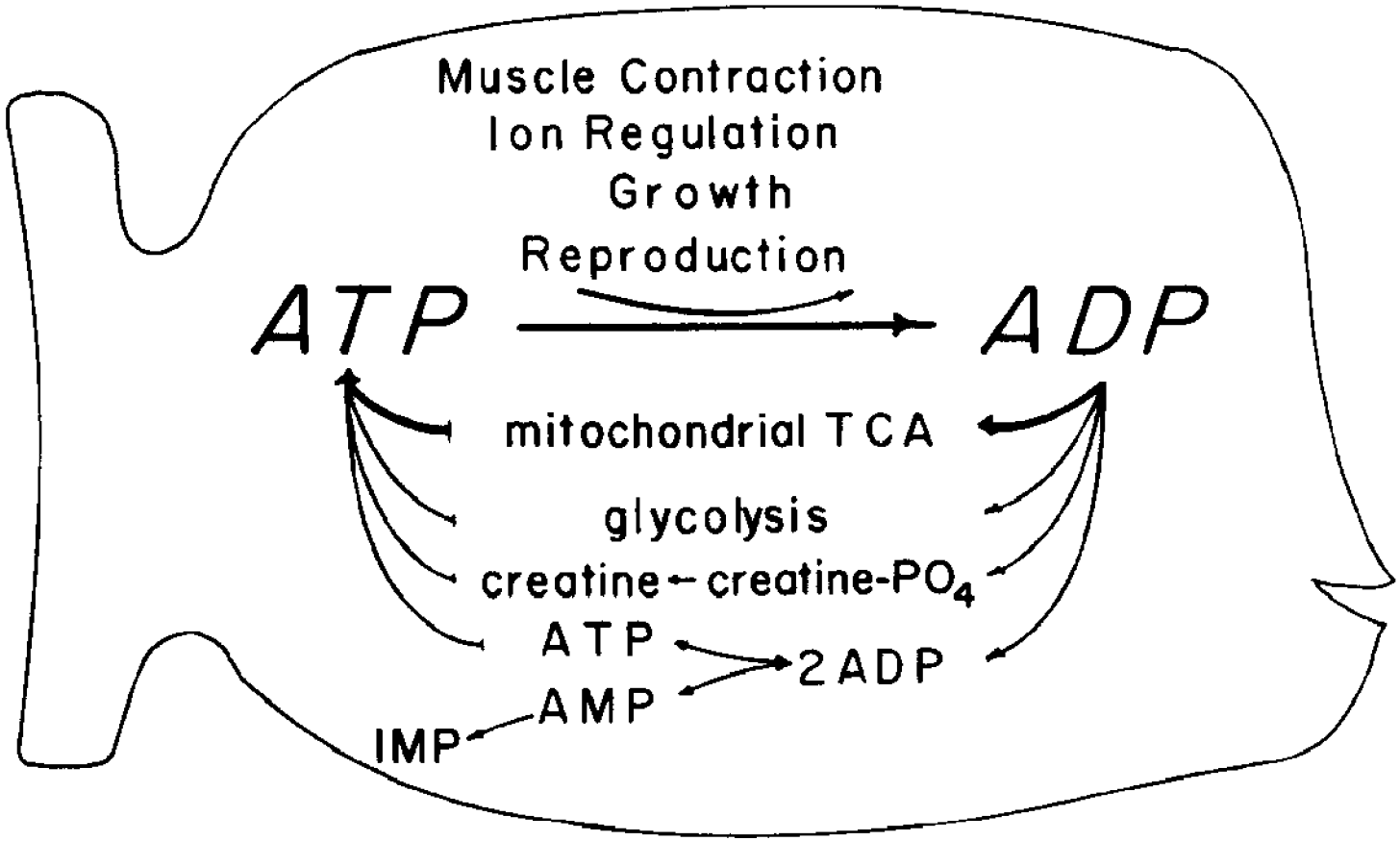

Figure 1. The adenine nucleotide system serves as the primary energy transducer of all living cells, linking anabolic and catabolic functions. Although oxidative phosphorylation and glycolysis are the primary means of ATP regeneration, phosphogens such as creatine--PO_4--and the adenylate kinase-mediated salvage pathway can regenerate limited amounts of ATP during periods of rapid depletion.

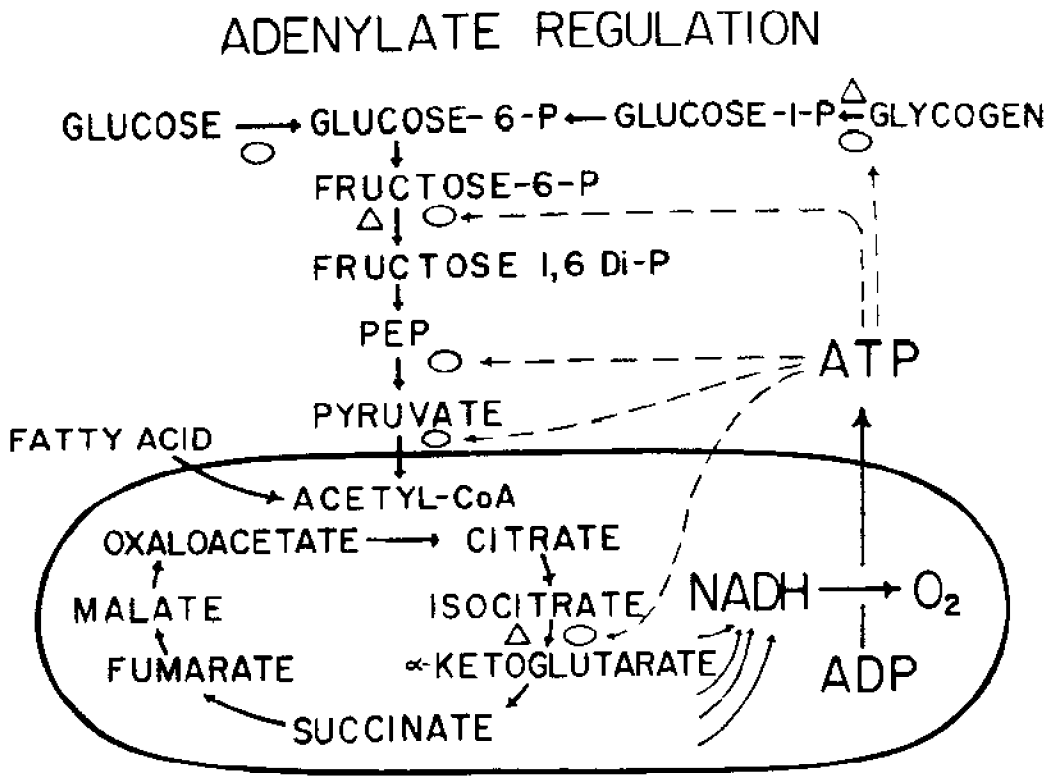

Figure 2. All three adenine nucleotides (ATP, ADP and AMP) act as allosteric modifiers of the enzymes that regulate the rates of ATP synthesized. High levels of ADP and/or AMP (Δ) positively stimulate glycogen mobilization, phosphofructokinase activation, tricarboxylic acid cycle activity and therefore the synthesis of ATP. High levels of ATP inhibit further ATP synthesis by inhibiting glycogen mobilization, phosphofructokinase activities and tricarboxylic acid cycle activity and positively stimulating gluconeogenic enzymes such as fructose 1,6 diphosphatase and glycogen synthetase. In this way, the ratios and concentrations of adenylates are kept tightly regulated and nearly constant under most conditions of normal metabolism.

AEC above 0.8 it was normal and below 0.8 it was stressed). However, as Atkinson himself has pointed out, large changes in the absolute concentrations of adenylates can occur and if the ratio among the three remains the same, the AEC will remain constant while the total adenylate pool size varies (Chapman et al. 1976; Atkinson 1977). A major objective of this paper is to differentiate clearly between measures of adenylate concentration and the AEC. We emphasize that the AEC is a secondary calculation which is only as good as the analytical measurement of adenylate concentration and that the AEC by its very nature is a conservative measure which will likely underestimate the effects of chronic low level stress. Changes in the absolute concentration of the three adenylates and the total adenylate pool are useful indicators of stress. However, the information content of the basic measurements can be vastly increased and the mechanism of toxic action can be suggested if the changes in adenylate concentration are considered in the light of changes in other energy compounds such as glycogen and neutral lipid reserves.

USE OF ADENINE NUCLEOTIDE CONCENTRATIONS AS INDICATORS OF STRESS IN ORGANISMS

Microbial

Adenylate measurements as part of basic investigations of cellular energy metabolism have been made for about 30 years. Early measurements are probably in error in terms of absolute concentration because of methodological problems and assumptions that will be discussed below. However, within a given study, values are probably internally consistent between experimental and control samples. Atkinson (1977) constructed a revealing model of the "evolution" of apparent AEC values as techniques improved during the past 30 years.

Since most of the original adenylate measurements were made on pure cultures of bacteria, it is not surprising that measurements of adenylates in natural marine microbial assemblages have been among the earliest and most intensively investigated applications of adenylate research to environmental science. An important early application was the use of particulate ATP concentrations in seawater as a measure of total microbial biomass (Holm-Hansen and Booth 1966) and marine sediments (Wiebe and Bancroft 1975; Hodson et al. 1976; Karl and LaRock 1976). Soon thereafter, ATP measurements were used to follow the effects on marine microbial biomass of stressors such as pulp mill effluent (Quammen et al. 1973), copper (Thomas et al. 1977) and mercury (Azam et al. 1977). For ATP to be a measure of biomass, its concentration per unit "living carbon" must be constant. However, under certain conditions this ratio was found not to be constant (e.g., Chapman et al.

1971); that is, the ATP per cell reflected not only the biomass, but also the average physiological state of the cells. These findings led to the use of alternative adenylate measurement parameters to assess the biomass and activity of microbial assemblages. Total adenylate concentration $(A_t; (ATP + ADP + AMP)]$ was proposed as a better measure of biomass than (ATP) (Karl 1980); energy charge was used as a measure of the activity of physiological state of natural microbial assemblages. Results of recent studies suggest that these more elaborate measurements may often not yield more or better information than ATP concentration alone. For instance, plots of ATP and A_t are often parallel, suggesting that the ratio of ATP to other adenylates and hence the energy charge may be held nearly constant at the expense of the size of the intracellular adenylate pool (Karl 1980). Thus, although it is often criticized, using ATP as an indicator of microbial biomass remains the most generally useful and certainly the simplest of the various methods available. Moreover, for most purposes, ATP measurements do track changes in total microbial biomass mediated by pollutional stress.

However, serious questions remain to be answered regarding interpretations of adenylate measurements on natural microbial assemblages and how adenylate concentrations change in response to pollutional stress. Some of the problems yet to be solved include (1) determining the extraction efficiencies of adenylates from seawater and sediments; (2) the relative quantitative significance of extracellular (dissolved) adenylate relative to cellular adenylates and, hence, to estimates of biomass or AEC; (3) the relative significance of bacterial, nonbacterial, microbial, and meiofaunal ATP to total ATP biomass in sediments. Karl (1980) has reviewed these and other potential problems and applications of adenylate measurements to marine microbial ecology. The reader is referred to Karl's report for further information. The remainder of the discussion will consider adenylate concentrations in marine fishes and invertebrates and the effects of stress on these concentrations.

Invertebrates

Studies of the effects of environmental stressors on the adenine nucleotide concentrations of invertebrates have been conducted almost entirely with molluscs and crustaceans. Earlier studies have examined natural environmental factors such as temperature and dissolved oxygen and only recently have investigators begun to measure the effect of anthropogenic stressors. Some of the first studies were those that used adenylate measurements to investigate anaerobic tolerance in mussels (Mytilus galloprovincialis) (Zs-Nagy and Ermini 1972; Zs-Nagy 1973). Since then Wijsman and Zwaan have used adenylate measurements to help determine the unusual metabolic pathways by which mussels are able to increase the efficiency of anaerobic ATP production (Wijsman 1976a, 1976b;

Zwaan and Wijsman 1976). Ivanovici (1980) measured the effects of salinity and temperature change on adenylate concentrations and AEC in the estuarine gastropod (Pyrazus ebinninus) under controlled laboratory conditions. AEC decreased 20% when the temperature increased from 20° to 29°C. Sensitivity to salinity was temperature dependent but, in general, the greater the decrease in salinity, starting from the acclimation salinity of 34 ppt, the greater the decrease in ATP concentration and AEC. In a similar field study, which included two other molluscs, Anadara trapezia and Saccostria commercialis, AEC responded in a qualitatively similar manner, decreasing significantly for all species when salinity was reduced from 34 ppt to 10 ppt. However, the amount of change in AEC was not proportional to the degree of sensitivity of the different species to salinity change (Rainer et al. 1979). In polychaetes, natural resistance to anaerobic conditions and habitat preference was strongly correlated with changes in AEC (Schottler 1979). Studies of the effects of anthropogenic stressors have only recently been done. Ivanovici (1979) conducted a field test of the effects of hydrocarbon pollution on the gastropod mollusc (Pyrazus ebinninus). She transplanted caged snails from an unpolluted estuary to either unpolluted sites in another estuary or polluted sites that were previously inhabited by the same species. She also performed a series of back transfers. Changes in adenylate concentrations were consistent with the degree of hydrocarbon pollutants at a site. However, as with most field studies, there were other gradients of salinity and dissolved oxygen which may have contributed to the results. Effects due to heavy metals have also been observed. Dickson et al. (1982) found that cadmium at a concentration of 10 ppb caused a significant drop in AEC after only 7 days in crayfish. This concentration resulted in mortality only after several months. Giesy et al. (in press) found significant decreases in adenylate concentrations and AEC in asian clams (Crobicula fluminea) when exposed to low levels of cadmium. Zaroogan et al. (1982) have probably conducted the most comprehensive evaluation of adenylate concentrations in predictors of metal stress. Exposure to nickel at 10 µg/kg seawater resulted in significant decreases in ATP in 10-week laboratory trials with the mussel (Mytilus edulis) (Table 1). Field populations exposed to polluted waters were different from control sites. There were seasonal changes in adenylates in both control and stressed populations. Similar results were found for the oyster (Crassostrea virginica), but in this species adenylate concentrations were also affected by their post spawning condition.

Fish

Much of the early interest in measuring adenylate concentrations in fish was the result of interest in basic muscle physiology and the effects of exercise. In general, high levels of muscular

Table 1. The response of adenine nucleotides in the muscle tissue of Mytilus edulis after treatment with ambient (control), 5 and 10 $\mu g\ Ni\ kg^{-1}$ seawater at ambient temperature and salinity for 10 weeks (from Zaroogian et al. 1982).

Treatment	Sample Size	$\mu mol\ g^{-1}$ wet weight		
		AEC	ATP	ATP + ADP + AMP
Control	6	0.85 0.01	2.82 0.12	3.89 0.20
5 $\mu g\ Ni\ kg^{-1}$	6	0.80 0.05	2.72 0.34	4.03 0.20
10 $\mu g\ Ni\ kg^{-1}$	6	0.75 0.04	1.84* 0.26	3.09* 0.25

*Significantly different from control ($p < .05$).

exercise result in a rapid decrease in creatine phosphate which acts as an energy buffer until the cell's rate of metabolism can be increased. As the work load increases, ATP concentration decreases as does total adenylate concentration. The decrease in total adenylate concentration is caused by the rapid removal of AMP, via 5'-AMP deaminase, to inosine monophosphate (IMP). An almost 1:1 stoichiometry between decreases in ATP and increases in IMP has been observed (Driedzic and Hochachka 1976). The activation of AMP deaminase is dependent upon falling cellular levels of guanosine triphosphate (GTP). A review by Driedzic and Hochachka (1978) summarizes much of the earlier work on the effects of exercise on fish muscle. In all cases, an increase in muscular exertion resulted in decreases in ATP and total adenylate concentration. AEC decreased significantly only at the point of exhaustion when maximum metabolic rates were unable to meet the work load. Reduced environmental oxygen concentrations are similar to muscular exertion in that there is an increased reliance on anaerobic respiration. The response of the adenylate system to decreased oxygen stress is, therefore, similar to exercise response. Van de Thillart et al. (1976) exposed goldfish (Carassius auratus) to 10 hours of complete anoxia. While ATP and creatine phosphate reserves decreased steadily throughout the 10 hour expo-

sure, AEC, after dropping from 1.0 to 0.8 in the first few minutes, remained constant at about 0.8. Vetter and Hodson (1982) compared the effects of environmental hypoxia on five marine fish species from three habitats that typically experience a range of natural diel oxygen regimes: pompano and silverside were from a well aerated surf zone, perch were from a tidal creek with brief periods of low oxygen during slack low tide, and killifish and juvenile mullet were from a eutrophic pool where aquatic macrophyte respiration caused nightly depletion of dissolved oxygen (Table 2). There was a strong positive relationship between resistance to low oxygen as reflected in adenylate change and the relative amount of oxygen stress occurring in the species' habitat of preference. Juvenile mullet, which typically inhabit higher oxygen waters, invade the eutrophic pools to escape predation. These fish showed no physiological resistance to low oxygen but survived nightly oxygen depletion by skimming the oxygen enriched surface layer. Decreases in ATP concentrations were again reflected in decreases in total adenylate concentrations as well as a change in AEC. Jorgensen and Mustafa (1980) compared differences in oxygen stress between different organs of the flounder (Platichthys flesus). They found that in all tissues except the heart, ATP as well as AEC decreased significantly. Liver, which had virtually no creatine phosphate reserves, was most severely affected by hypoxia, experiencing a 90% drop in ATP, a 58% drop in total adenylates and a large change in AEC (0.73–0.36). While studies determining the effects of anthropogenic stressors on adenylate concentrations have been few, many are currently being pursued. Christensen (1975) exposed brook trout (Salvelinus fontinalis) to methylmercury at a concentration of 1.03 μg/l for 38 days and found a significant decrease in ATP. MacFarlane (1981) measured the effects of decreased pH on different tissues of the Gulf killifish (Fundulus grandis) (Fig. 3). He found that the gill and brain tissues were most sensitive to pH changes in terms of decreases in ATP, total adenylate and AEC. In our laboratory we have recently found adenine nucleotides to be sensitive and useful predictors of the effects of dissolved DDT, DDT uptake from the diet, pulp mill effluent, and pulp mill effluent acting synergistically with low oxygen (unpublished data).

ADVANTAGES OF ADENINE NUCLEOTIDES AS STRESS INDICATORS

A clear advantage of adenylate concentration measurements as stress indicators is the fact that they can provide an instantaneous "snapshot" of the physiological state of field specimens. No incubations or prolonged handling of living material is required. However, what is required is a method for quickly killing and fixing the sample to inhibit post-collection changes in adenylate concentrations. Another important advantage of adenine nucleotide meas-

Table 2. Adenylate concentrations, μmol g^{-1} wet weight (± 1 SD), of five estuarine species exposed to hypoxic conditions (*) are significantly different from control groups at $p < 0.05$ (from Vetter and Hodson 1982).

Species	Experimental Condition	n	ATP	ADP	AMP	TOTAL	AEC
Florida pompano	control	6	0.40(0.77)	0.55(0.15)	0.11(0.13)	5.10(0.81)	0.93(0.02)
	hypoxic	6	1.46(0.55)*	0.47(0.11)	0.15(0.09)	2.08(0.59)*	0.79(0.08)*
Atlantic silverside	control	6	4.42(0.53)	0.66(0.11)	0.20(0.10)	5.24(0.52)	0.90(0.02)
	hypoxic	5	2.32(0.77)*	0.62(0.05)	0.28(0.05)	3.24(0.79)*	0.81(0.05)*
Silver perch	control	6	4.58(0.21)	0.64(0.11)	0.30(0.10)	5.52(0.28)	0.89(0.01)
	hypoxic	5	3.95(0.52)*	0.81(0.05)*	0.41(0.13)	5.16(0.50)	0.84(0.03)*
Killifish	control	6	4.64(1.75)	0.56(0.24)	0.10(0.14)	5.39(1.78)	0.93(0.03)
	hypoxic	6	5.43(0.77)	0.82(0.27)*	0.21(0.19)	6.45(0.94)	0.90(0.03)
Striped mullet	control	5	4.05(0.50)	0.45(0.13)	0.00(00)	4.50(0.53)	0.95(0.01)
	hypoxic	5	1.14(0.27)*	0.44(0.02)	0.14(0.08)	1.72(0.36)*	0.79(0.02)*
	control	6	4.21(0.82)	0.53(0.13)	0.12(0.09)	4.87(0.87)	0.92(0.03)
	access to surface	6	4.87(0.19)	0.42(0.16)	0.24(0.13)	5.53(0.28)	0.92(0.02)

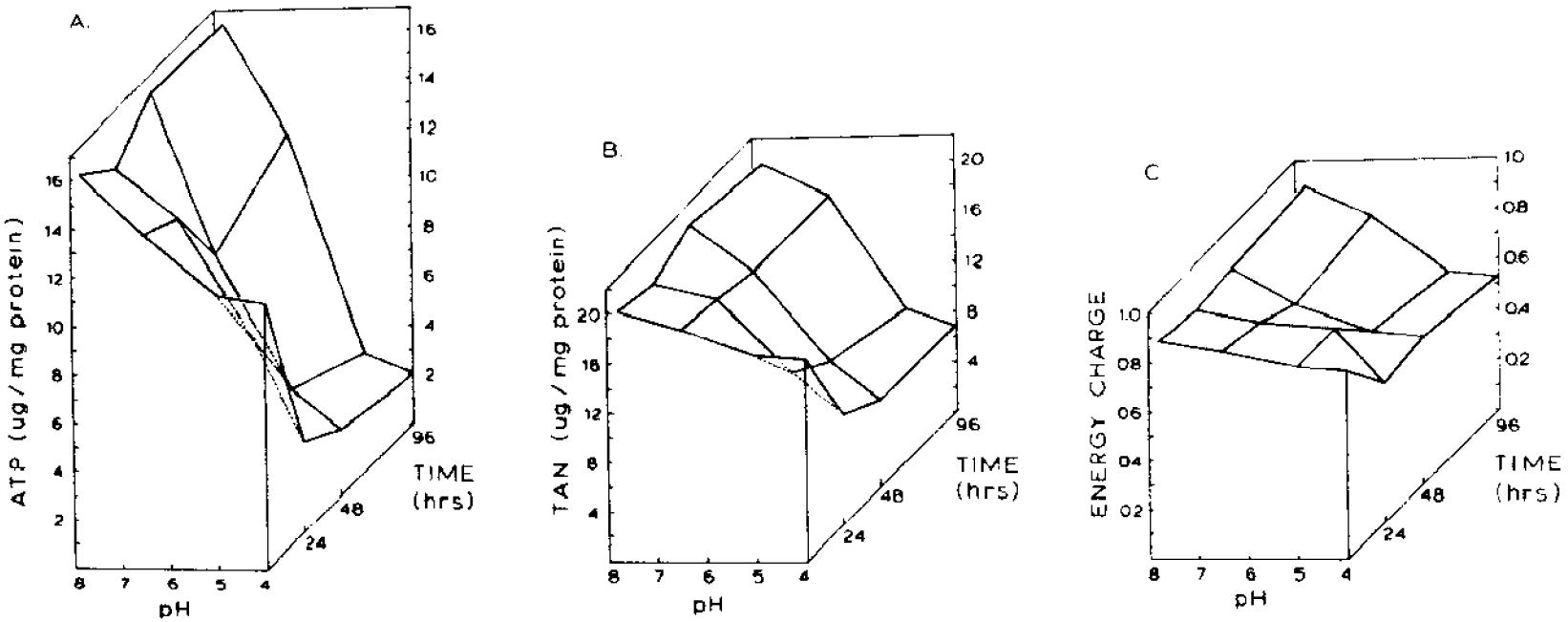

Figure 3. Effect of pH and time of exposure on (a) ATP concentration, (b) total adenine nucleotide concentration, and (c) adenylate energy charge in gill tissue of Fundulus grandis. Each intersection represents mean value of six fish, except 96 hr exposure at pH 6.5, 5.0 and 4.0 which are the mean of five fish (from MacFarlane 1981).

urements as stress indicators is the universal occurrence of these compounds and the central roles they play in the metabolism of all living cells. In addition, the adenine nucleotide concentrations in a variety of tissues and over a wide range of organisms are remarkably constant. For example, ATP concentration (μ mol g^{-1}) ranged from 3.91 to 6.59 in the white muscle of 11 different fish species (Vetter and Hodson 1982), 2.15 to 2.34 for tail muscle of three species of crayfish (Dickson and Franz 1980), 2.71 to 4.10 for a mollusc (Ivanovici 1980), 3.03 for red muscle in the rat (Edington et al. 1973) and 2.01 for rat brain (Ridge 1972). Since most invertebrate and vertebrate tissues are in the same range (1-10 μ mol g^{-1} fresh wt.), extraction and measurement techniques can be standardized. Another advantage is that the extreme sensitivity of the luciferin-luciferase assay allows accurate measurement of picomolar amounts of ATP, an important factor when the method is used for microbial samples from oligotrophic environments. AEC values for a wide range of organisms are also remarkably constant under "unstressed" conditions (Chapman et al. 1971; Atkinson 1977; Vetter and Hodson 1982). Although published values for AEC have slowly increased, as methods of measurement have improved, it now appears that virtually all organisms have a normal "unstressed" AEC value somewhere between 0.87 to 0.95. This constancy of "unstressed" AEC allows intercomparison of stress effects among a wide range of organisms and types of stressors.

An often overlooked advantage of the entire approach of measuring changes in energy metabolism as a response to stress is that the approach has a basis in "classical" respiratory physiology. For example, the "value" of a particular amount of glycogen, lipid or ATP can be expressed in comparable units such as calories per gram, joules per mole or moles of oxygen consumed, allowing data on the effects of anthropogenic pollutants to be compared with data in the literature on natural stress and adaptation (Selye 1952; Krogh 1959; Fry 1971; Precht 1973; Bayne 1976).

ASSUMPTIONS AND PROBLEMS INHERENT IN USING ADENINE NUCLEOTIDES AS STRESS INDICATORS

Investigators should not be deterred from using adenylate measurements as indicators of stress because of the problems cited here, but rather they should take note of the assumptions and analytical problems associated with the measurement of adenine nucleotide and, in particular, to emphasize the inherent insensitivity of the AEC measurement. Since virtually all metabolic energy flows through ATP as a link between catabolic and anabolic processes, a direct measure of energy flow through ATP would be an ideal measurement. However, current methods measure the static quantity, pool sizes, and attempt to imply something about flow.

In general the smaller the pool and the faster the flow, the less valid this is. The turnover time for ATP in metabolically active tissues can be less than 1 second (Lehninger 1977) and its concentration relative to energy storage compounds such as lipid, glycogen and the phosphogens (creatine phosphate and argenine phosphate) is, in general, exceedingly low. For example, the energy available in the standing pool of muscle ATP is sufficient to maintain muscle contraction for only 0.5 second (Lehninger 1977). As a further example, the caloric contribution of triglyceride, wax ester, glycogen and ATP during development of an estuarine fish egg is compared in Table 3. While the decrease in ATP was largest, on a percent basis its caloric contribution was negligible. However, many investigators still refer to ATP as an energy store and believe they are measuring the immediate energy reserves of the cell rather than the ability of the cell to generate energy.

Another potential problem when measuring adenylate concentrations without an adequate appreciation of turnover rates is the interpretation of "no effect" data. Figure 4 is a schematic representation of conditions under which ATP concentrations would be expected to change and those conditions which would not result in change. While investigators speak of the effects of stress, there are really two ways that a stressor may affect metabolism. The stessor may increase the demand for energy and/or it may inhibit the ability of the organism to produce energy. Since fish and other organisms can exhibit a 10-fold increase in metabolism between resting and active states yet maintain similar adenylate concentrations prior to exhaustion, there is obviously tremendous reserve capacity to regenerate ATP at higher than normal rates (Fry 1971; Driedzic and Hochachka 1978). An environmental stressor that increases metabolism without exceeding te ability of the cell to re-

Table 3. Net decreases in different energy reserves during an 18 h period between fertilization and hatching in red drum (Scianops ocellata) eggs. Values expressed on a wet wt. basis (from Vetter et al. 1983).

Compound	% Original Pool Consumed	Amount Consumed	Caloric Value	Total Caloric Contribution
Triglyceride	31%	2.13 mg g^{-1}	9 cal mg^{-1}	19.17
Wax Ester	33%	2.46 mg g^{-1}	9 cal mg^{-1}	22.14
Glycogen	63%	0.176 mg g^{-1}	4 cal mg^{-1}	0.704
ATP	75%	0.361 µmol g^{-1}	.007 cal µmol^{-1}	0.0025

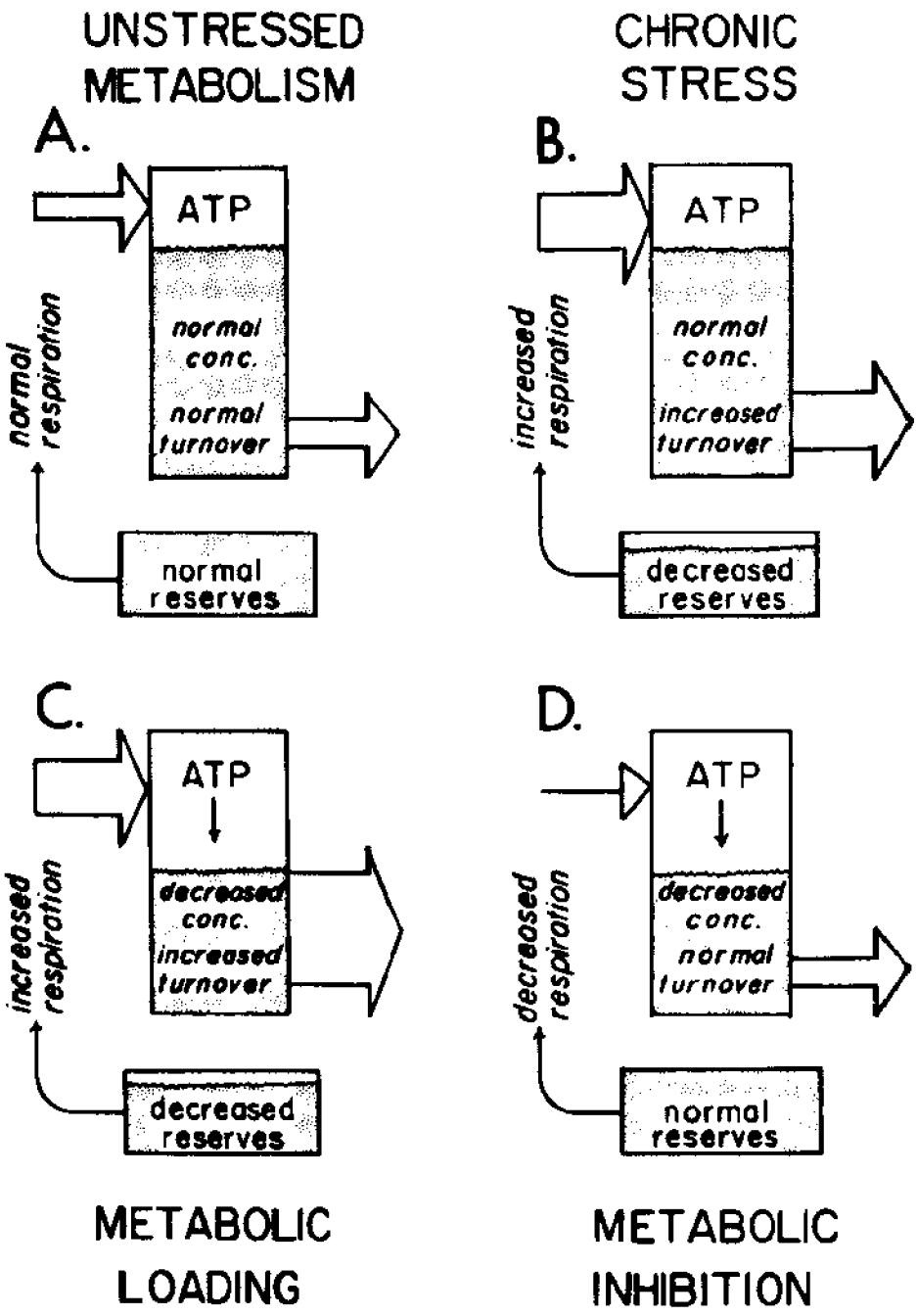

Figure 4. Different types of stress and the changes in ATP that would result from alterations in catabolic potential (input arrow) and the energy demands of mechanical, transport and biosynthetic work (output arrow). (a) control condition; (b) chronic low-level stress--the catabolic potential is not exceeded, no change in ATP concentration, no change in AEC; (c) acute metabolic loading--energy demand exceeds catabolic potential, decrease in ATP, possible decrease in AEC; (d) acute toxic inhibition catabolic potential decreased, decrease in ATP, effect on AEC will depend on site of lesion.

generate ATP will result in increased turnover rates without a visible change in ATP concentration (Figure 4b). Although adenylate concentrations may not change, energy reserves such as neutral lipid and glycogen will be rapidly depleted to maintain increased rates of ATP synthesis, diverting energy from growth and reproduction; "no effect" data should be interpreted cautiously and in this larger context. Changes in ATP concentration would be expected only under the conditions presented in Figures 4c and d, that is, when the environmental stressor results in a metabolic demand exceeding the ability of the organism to regenerate ATP (metabolic loading, Figure 4c) or if the stressor directly blocks the ability to resynthesize ATP (metabolic inhibition, Fig. 4d). The adenylate charge when considered alone is an even less sensitive indicator of chronic low level stress since it is a unitless ratio. If decreases in ATP result in a decrease in total adenylate concentration rather than a stoichiometric buildup in ADP and AMP, the AEC can remain constant even under the conditions presented in Figures 4c and d. A change in total adenylate concentration is precisely what does occur to a greater or lesser extent in almost all organisms studied to date. The thermodynamic reasons for this are simple. If ADP and AMP were allowed to accumulate, the energetic value of the remaining ATP on a per mole basis would be decreased (Vetter and Hodson 1982). In addition, the energy salvage pathway, 2ADP⟶ 1ATP + AMP is reversible and non-energy requiring so that AMP must be removed to "pull" the reaction in favor of ATP regeneration. AMP is generally deaminated to inosine monophosphate or dephosphorylated to adenosine depending on the organism. In experiments with tissue cultures, ATP decreased to 30% of the original concentration before AMP-deaminase was sufficiently inhibited to cause AEC to drop below 0.80 (Chapman et al. 1976). Large decreases in total adenylate prior to, or in conjunction with, decreases in AEC can be seen in Tables 1, 2, Figure 3 and in most published studies. Some molluscs appear to accumulate ADP and AMP to a greater extent than other invertebrates and vertebrates and thus respond to stress with decreased AEC prior to a change in total adenylates (Wijsman 1976; Rainer et al. 1979; Ivanovici 1980). The apparent insensitivity of AEC to very low level stress is not a criticism of the AEC as a biochemical concept. Atkinson has repeatedly called attention to the conservative and cybernetic nature of AEC (Atkinson 1977); however, his caution has not been sufficiently appreciated by many investigators.

When considering the application of adenylate measurements to field populations, important considerations are the sources and extent of variation in ATP concentrations due to natural causes such as seasonality, age and reproductive state. Some data on natural variation are available. Dickson and Giesy (1981) compared five species of crayfish from four different habitats, two from caves and the others from ponds, streams and artesian wells. When

ranked according to concentration, the species showed no significant differences in the adenylate concentrations or AEC of tail muscle. The highest and the lowest concentrations, which were significantly different, they felt represented the most and least active species. Vetter and Hodson (1982) compared 11 different species of estuarine fish which differed in their natural swimming activities. ATP content of the white muscle varied from 3.91-6.59 μmol g^{-1} wet wt. While the highest and lowest concentrations were significantly different ($p < .05$), there was no apparent relationship between activity and tissue adenylate concentrations. Ansell (1977) presented data from 23 bivalve species and found that ATP content varied from 0.26-1.26% of the dry wt. He concluded that respiration rate of the whole organism accounted for little of the observed variation but high maintained levels of ATP were associated with a species' ability to use energy rapidly for short periods.

Seasonal changes in adenylate concentrations, possibly associated with reproductive cycles, have also been reported for some organisms. Dickson and Giesy (1982a) reported that adenylate concentrations and AEC of crayfish tail muscle varied seasonally over the course of one year. The variation was not related to temperature, dissolved oxygen, pH, sex, breeding condition or limb regeneration. However, the seasonal peak appeared to coincide with the period of peak breeding activity. Skjoldal and Barkati (1982) found that ATP content and AEC varied seasonally in a European population of Mytilus edulis. The change in ATP was related to the nutritional and reproduction cycle. Zaroogan et al. (1982) also found seasonal differences in the ATP content and AEC of a North American population of Mytilus and the oyster (Crassostrea virginica). Seasonal changes in adenylate concentrations have also been reported for zooplankters (Bamstedt and Skjoldal 1976; Skjoldal and Bamstedt 1976, 1977) and microbial communities (Christian et al. 1975; Wiebe and Bancroft 1975).

The effect of nutritional state on adenylate concentration has been a subject of intensive investigation, particularly with regard to microorganisms. Much of the original work of Atkinson's group which led to refinement of the AEC concept was done with nutrient limited cultures (Chapman et al. 1971; Ball and Atkinson 1975; Walker-Simmons and Atkinson 1977). A complete summary of the types of microorganisms, the types of nutrient limitation and the types of culture conditions that have been studied has been presented by Karl (1980). As expected, ATP and AEC decreased under conditions of nutrient limitation, but there were important differences between the results from batch cultures and chemostats, which were due to the age structure of the community as a whole. Differences also occurred based on which nutrient was limiting. Fewer such studies have been carried out with animals. The ATP content of the copepod (Calanus finmarchicus) decreased with star-

vation (Balch 1972). Dickson and Giesy (1982b) found that the ATP content increased or remained constant in two species of crayfish that were starved over a 45-day period.

When measuring ATP concentrations or AEC in larger multicellular organisms, it is also important to consider differences between tissues with regard to natural concentration and sensitivity to change by a given pollutional stress. Wijsman (1976) measured adenylate concentrations and AEC in four tissues of the mussel (Mytilus edulis), and found ATP varied from 4.76 μmol g^{-1} wet wt. in adductor muscle to 1.31 μmol g^{-1} wet wt. in the hepatopancreas. Jorgensen and Mustafa (1980) compared the adenylate concentrations in four tissues of the flounder (Platichthys flesus). ATP concentrations varied from 4.63 μmol g^{-1} wet wt. in the muscle to 1.35 μmol g^{-1} wet wt. in the liver. The tissues were differentially sensitive to hypoxia, liver being the most sensitive. In the Gulf killifish (Fundulus grandis), ATP concentrations varied from 17.94 μg mg^{-1} protein in the muscle to 11.85 μg mg^{-1} protein in the liver. Brain and gill were more sensitive to low pH than muscle or liver (MacFarlane 1981).

It is clear that changes in adenylate concentration can occur with changes in season, reproductive state, nutritional state and between different tissues. While there have been attempts to relate differences in adenylate concentration to various aspects of activity, these have all been ex post facto explanations of results. Research is needed that explicitly addresses the question of why different species have different adenylate concentrations. It is also important to establish that the units used to express adenylate concentration are not changing during the course of the experiment. For example, it may be true that ATP concentration increased during starvation (Dickson and Giesy 1982) but this may result from decreases in tissue weight as energy reserves are consumed rather than from an increase in ATP. Differences in the amount of a tissue that are composed of relatively inert material such as collagen or stored lipid may also explain apparent between-tissue differences. However, tissue differences persist even when expressed on a protein basis (MacFarlane 1981). While muscle tissue is often chosen for study because of its homogeneity, ease of removal and high adenylate concentration, the results from multiple-tissue studies suggest that, for some stressors, muscle is less sensitive than other tissues such as gill, brain or liver (Jorgensen and Mustafa 1980; MacFarlane 1981). While natural variations in adenylate concentrations should be considered in any long term field study of pollution effects, the variation in adenylate concentrations are less than variations in growth rate, respiration rate, energy reserves, productivity, community structure or other routinely used field measurements of pollution effects.

AN EXPANDED ENERGY PROFILE FOR THE MEASUREMENT OF STRESS

Many investigators have hoped to find single specific indicators of specific pollutant effects that can be used to show direct cause-and-effect relationships in real field monitoring situations. Since the work of Selye (1952) long ago demonstrated the generalized nature of organismal responses to stress, the above goal, however desirable, is probably unattainable. The determination of pollutant effects has much in common with the much older discipline of diagnosing diseases in man and a similar diagnostic approach may be the most consistently productive approach to pollution assessment. While a single measurement such as body temperature may indicate that a patient is sick, it is the careful combination of multiple measurements such as heart rate, blood pressure, respiration rate and blood chemistry that ultimately pinpoints a specific cause. Thus adenylate measurements, at best, indicate that the organism or community is stressed. In a controlled laboratory experiment with one variable, this may be sufficient. In a field situation where the cause of the stress is unknown, a series of general measurements determining the level of stress, followed by more specific measurements such as mixed function oxidase activity (hydrocarbons) or metallothionine levels (metals) may offer the best hope of consistently determining a cause-and-effect relationship between a pollutant and an impacted community.

In our studies, we have extended the basic adenylate measurements to include measurements of the energy reserves of the cell, so that changes or the lack thereof in adenylate concentration can be viewed within the larger context of cellular metabolism. Our "energy profile" approach includes measurement of neutral lipid and glycogen, the two most important energy storage compounds in both vertebrates and invertebrates. We also consider changes in structural compounds such as protein which can act as energy reserves. The methods used for determining different energy reserves within the same sample are published in Vetter et al. (1983). An advantage of the energy profiling concept is that different measurements, including respiration, can be interconverted into common units such as calories (e.g., Table 3), and the results combined into predictive equations or simulation models. By way of example, Figure 5 is a dynamic simulation model of energy flow through a tissue. The model, as illustrated, is parameterized with values obtained from the literature for concentrations, caloric content and turnover times of neutral lipid, glycogen, creatine phosphate and ATP pools in fish muscle. While protein can be an important energy reserve, its turnover time is similar to lipid and has therefore been excluded from the examples below. By altering input and output functions of different compartments, we can simulate toxicant induced lesions at different sites in intermediary

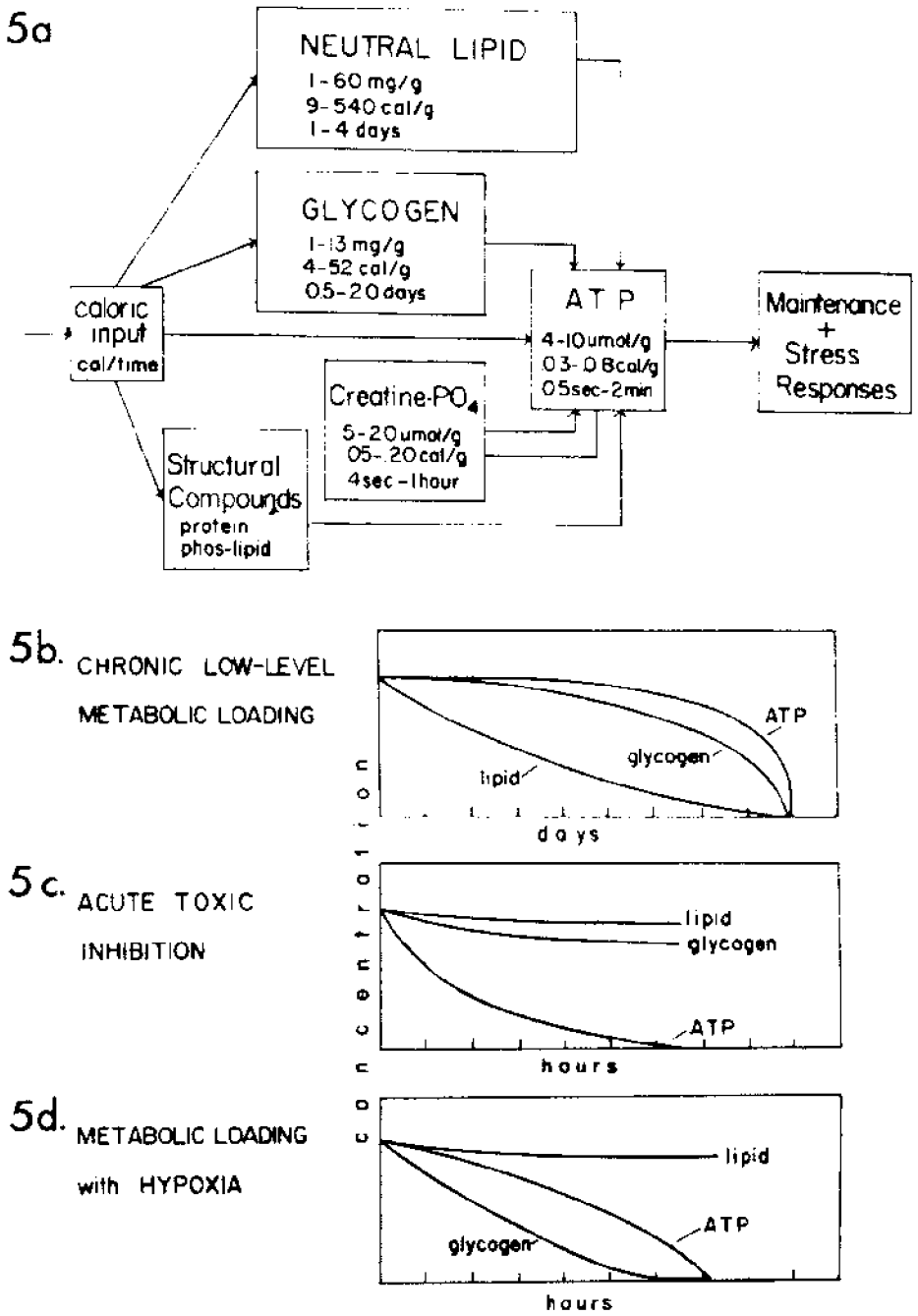

Figure 5. (a) Simulation model of energy flow (cal time^{-1}) through energy storage and transfer pools. Nutrient limitation can be simulated by decreasing input, metabolic loading by increasing ATP demand, and various sites of toxic inhibition or hypoxia by altering input and output functions to different pools. The model as shown was parameterized with values for fish muscle. Protein has a turnover and time response similar to lipid and was not included in the examples below; (b) effect on tissue levels of lipid, glycogen and ATP during long term chronic stress; (c) acute toxic inhibition results in rapid decreases in ATP despite ample energy reserves; (d) added stress of hypoxia results in rapid glycogen depletion.

metabolism. By increasing the energy flow to "stress responses" we can simulate the effects of a metabolic loading type stress. Creatine phosphate serves as a short term energy buffer which acts as a time delay but does not affect the ultimate outcomes. Although many pollutants are a combination of stress types, sample outcomes of the model illustrate the general conditions under which either lipid, glycogen or ATP would be the most sensitive indicators of stress. For example, a long-term chronic stress, such as elevated respiration resulting from a heated water discharge or perhaps a pulp mill effluent (metabolic loading), might result in a long-term decline in lipid, while glycogen and ATP reserves could remain relatively high until lipid is depleted (Figure 5b). An acutely toxic chemical which disrupts cell integrity or enzyme function (toxic inhibition) might result in a rapid decrease in ATP regardless of lipid or glycogen stores (Figure 5c). A pollutant which resulted in reduced oxygen concentrations or a reduced ability to transport oxygen would result in a steep decrease in glycogen due to the inherent inefficiency of anaerobic respiration, while lipid would probably remain constant since it is virtually unavailable for metabolism under aerobic conditions (Figure 5d). The above examples illustrate that the type, intensity and duration of a stress can determine which changes most rapidly and which would be the best indicator of stress. The above predictions have been tested with the saltmarsh killifish (Fundulus heteroclitus), using pulp mill effluent, pulp mill effluent combined with low oxygen, and DDT. Our work so far indicates that the basic predictions are valid. Table 4 shows the outcome of one such experiment, the effect of a 4-hour exposure to pulp mill effluent on white muscle adenylate and glycogen concentrations. Neutral lipid was below detection in the white muscle. When exposed to pulp mill effluent alone, respiration increased, glycogen was utilized, while adenylate concentrations were not significantly affected (e.g., Figure 5b). Pulp mill effluent combined with low oxygen resulted in a very large decrease in glycogen and a significant decrease in ATP and total adenylates (e.g., Figure 5d). There was a synergistic effect of pulp mill effluent plus low oxygen on glycogen concentrations (data not shown). Combining adenylate measurements into an overall "energy profile" allows us to determine not only the presence of stress but the mechanism by which the pollutant has its effect.

A similar multiple biochemical measurement approach is being used by White and co-workers to assess the biological structure of and perturbation effects on natural microbial assemblages. Specific compounds produced by specific taxa are used to estimate changes in the biomass of different components of the community (e.g., muramic acid for bacterial biomass and lipid galactose for photosynthetic biomass). Physiological condition of the community

Table 4. Fundulus heteroclitus. Adenylate concentrations, μ mol g^{-1} wet tissue (± 1 SD); Glycogen concentrations, mg glucose equivalent g^{-1} wet tissue (± 1 SD), of fish exposed to 1% (v/v) unbleached Kraft pulp mill effluent for 4 h. N=8 individuals for every group (from Hwang, Vetter and Hodson in prep).

	Control High O_2 (6.45 ± 0.18 mg O_2 L^{-1})	Pulp Effluent High O_2 High O_2 (6.36 ± 0.18 mg O_2 L^{-1})	Pulp Effluent Low O_2 Low O_2 (0.88 ± 0.20 mg O_2 L^{-1})
ATP	5.62 (0.93)	4.80 (0.88)	4.24 (0.77)*
ADP	0.70 (0.31)	1.19 (0.55)	0.88 (0.27)
AMP	0.34 (0.40)	0.02 (0.05)	0.31 (0.29)
Total Adenylates	6.66 (0.94)	6.01 (1.02)	5.42 (0.94)*
AEC	0.90 (0.03)	0.90 (0.03)	0.86 (0.03)*
Glycogen	10.25 (1.62)	7.27 (2.22)*	4.74 (2.05)*

* Significantly different from controls at $p < .05$

is measured by short term indicators such as AEC and ATP/adenosine ratio in which long term condition is indicated by neutral lipid glycerol (Bobbie and White 1980; Davis and White 1980; White et al. 1980; Bobbie et al. 1981; Nickels et al. 1981; Smith et al. 1982).

CONCLUSION

Adenine nucleotides are present in all living organisms and are the central compounds in the transfer of energy consumed as food or captured from sunlight into the energy requiring pathways of biosynthesis, growth and reproduction. They are attractive as indicators of pollutional stress because of the "physiological relevance" and because the low levels of inter- and intra- specific variability allow similar analytical techniques for all organisms and invite broad intra-specific comparison of stress effects.

Changes in adenine nucleotide concentrations are most likely to occur under conditions of acute stress where energy is consumed rapidly or where the ability to resynthesize ATP is somehow inhibited. AEC is particularly insensitive since large changes in total adenylate concentration can occur prior to a significant change in adenylate ratios. When changes in adenylate concentrations are observed, they are usually accompanied by obvious visible signs of

stress such as shell closure, mucus secretion and elevated ventilation rate and should be regarded as evidence of serious pollution effects (Ivanovici 1979; MacFarlane 1981; Vetter and Hodson 1982). Adenylate measurements alone fail to pinpoint a specific cause-and-effect relationship between a type of pollutant and an observed effect. This is not a failure of adenylate measurements in particular but an unavoidable consequence of the generalized stress response as it has evolved in most organisms. Combining adenylate measurements with other measures of energy reserves, such as lipid and glycogen, can improve detection of low-level chronic stress and begin to indicate the mechanism of toxic action.

The continued pursuit of single measurements to demonstrate direct cause and effect relationships will not yield satisfactory results. A multiple hierarchical approach, similar to medical diagnostics, in which general metabolic indicators are used to determine the degree to which a community of organisms is stressed, followed by more specific measurements characteristic of a specific pollutant group, will yield consistent and thus valuable results.

REFERENCES

Atkinson, D.E. 1968. The energy charge of the adenylate pool as a regulatory parameter: Interaction with feedback modifiers. Biochem. 7: 4030-4034.

Atkinson, D.E. 1977. Cellular energy metabolism and its regulation. Academic Press, New York.

Atkinson, D.W. and G.K. Walton. 1967. Adenosine triphosphate conservation in metabolic regulation; rat liver citrate cleavage enzyme. J. Biol. Chem. 24: 3239-3241.

Ansell, A.D. 1977. The adenosine triphosphate content of some marine bivalve molluscs. J. Exp. Mar. Biol. Ecol. 28: 269-283.

Azam, F., R.F. Vaccaro, P.A. Gillespie, E.I. Moussalli and R.E. Hodson. 1977. Controlled ecosystem experiment: Effect of mercury on enclosed water columns. II. Marine bacterioplankton. Mar. Sci. Commun. 3: 313-329.

Ball, W.J. and D.E. Atkinson. 1975. Adenylate energy charge in Saccharomyces cerevisiae during starvation. J. Bacteriol. 121: 975-982.

Bamstedt, U. and H.R. Skjoldal. 1976. Studies on the deep-water pelagic community of Korsfjorden, Western Norway. Adenosine phosphates and nucleic acids in Euchaeta norvigica (Copepoda) in relation to its life cycle. Sarsia 60: 63-80.

Bayne, B.L. 1976. Marine mussels, their ecology and physiology. Cambridge Univ. Press, London.

Bobbie, R. and White, D.C. 1980. Characterization of benthic microbial community structure by high resolution gas chromatography. Appl. Environ. Microbiol. 39: 1212-1222.

Boulekbache, H. 1981. Energy metabolism in fish development. Am. Zool. 21: 377-389.

Chapman, A., L. Fall and D. Atkinson. 1971. Adenylate energy charge in Escherichia coli during growth and starvation. J. Bacteriol. 108: 1982-1086.

Chapman, A.G. and D.E. Atkinson. 1973. Stabilization of energy charge by the adenylate deaminase reaction. J.. Biol. Chem. 248: 8309-8312.

Chapman, A.G., A. Miller and D.E. Atkinson. 1976. Role of the adenylate deaminase reaction in regulation of adenine nucleotide metabolism in Ehrlich ascites tumor cells. Cancer Res. 36: 1144-1150.

Christensen, G.M. 1975. Biochemical effects of methylmercuric chloride, cadmium chloride and lead nitrate on embryos and alevins of the brook trout, Salvelinus fontinalis. Toxicol. Appl. Pharmacol. 32: 191-197.

Christian, R.R., K. Bancroft, and W.J. Wiebe. 1975. Distribution of microbial adenosine triphosphate in salt marsh sediments at Sapelo Island, Georgia. Soil Sci. 119: 89-97.

Davis, W.M. and D.C. White. 1980. Fluorometric determination of adenosine nucleotide derivatives as measures of the microfouling, detrital, and sedimentary microbial biomass and physiological status. Appl. Environ. Microbiol. 40: 539-548.

Dickson, G.W. and R. Franz. 1980. Respiration rates, ATP turnover and adenylate energy charge in excised gills of surface and cave crayfish. Comp. Biochem. Physiol. 65A: 375-379.

Dickson, G.W. and J.P. Giesy. 1981. Variation of phosphoadenylates and adenylate energy charge in crayfish (Decapoda:Astacidae) tail muscle due to habitat differences. Comp. Biochem. Physiol. 70A: 421-425.

Dickson, G.W. and J.P. Giesy. 1982a. Seasonal variation of phosphoadenylate concentrations and adenylate energy charge in dorsal tail muscle of the crayfish, Procambarus acutus acutus e(Decapoda:Astacidae). Comp. Biochem. Physiol. 72A: 295-299.

Dickson, G.W. and J.P. Giesy. 1982b. The effects of starvation on muscle phosphoadenylate concentrations and adenylate energy charge of surface and cave crayfish. Comp. Biochem. Physiol. 71A: 357-361.

Dickson, G.W., J.P. Giesy and L.A. Briese. 1982. The effect of chronic cadmium exposure on phosphoadenylate concentrations and adenylate energy charge in gills and muscle tissue of crayfish. J. Environ. Toxicol. Chem. 1: 147-156.

Driedzic, W.R. and P.W. Hochachka. 1976. Control of energy metabolism in fish white muscle. Am. J. Physiol. 230: 579-582.

Driedzic, W.R. and P.W. Hochachka. 1978. Metabolism in fish during exercise. In: W.S. Hoar and D.J. Randall (eds.), Fish Physiology, Vol. 8, pp. 503-543. Academic Press, New York.

Edington, D.W., G.R. Ward and W.A. Saville. 1973. Energy metabolism of working muscle: Concentration profiles of selected metabolites. Am. J. Physiol. 224: 1375-1380.

Faupel, R.P., H.J Seitz, W Tranowski, V. Thiemann and C Weiss. 1972. The problem of tissue sampling from experimental samples with respect to freezing technique, anoxia, stress and narcosis. Arch. Biochem Biophys. 148: 509-522.

Fry, F.E.J. 1971. The effects of environmental factors on the physiology of fish. In: W.S. Hoar and D.J. Randall (eds.), Fish Physiology, Vol. 6, pp. 1-98. Academic Press, New York.

Giesy, J.P., C.S. Duke, R.D. Bingham, G.W. Dickson and G.J. Leversee. In press: Changes in phosphoadenylate concentrations and energy charge in the Asian clam, Corbicula fluminea due to starvation or chronic cadmium exposure. J. Environ. Toxicol. Chem.

Hodson, R.E., O. Holm-Hansen and F. Azam 1976 Improved methodology for ATP determination in marine environments. Mar. Biol. 34: 143-149.

Holm-Hansen, O. and C.R. Booth. 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnol. Oceanogr. 11: 510-519.

Ivanovici, A.M. 1979. Adenylate energy charge: Potential value as a tool for rapid determination of toxicity effects. In: Proc. 5th Annual Aquatic Toxicology Workshop, Hamilton, Ontario, Nov. 7-9, 1978, pp. 241-255. Fish. Mar. Ser. Tech. Rep. 582, (publ., place of publ.)

Ivanovici, A.M. 1980. The adenylate energy charge in the estuarine mollusc, Pyrazus ebeninus: Laboratory studies of responses to salinity and temperature. Comp. Biochem. Physiol. 66A: 43-55.

Jones, N.R. and J. Murray. 1960. The acid-soluble nucleotides of codling (Gadus callarias) muscle. Biochem. J. 77: 567-575.

Jorgensen, J.B. and T. Mustafa. 1980. The effect of hypoxia on carbohydrate metabolism in flounder (Platichthys flesus L.). II. High energy phosphate compounds and the role of glycolytic and gluconeogenic enzymes. Comp. Biochem. Physiol. 67B: 249-256.

Karl, D.M. 1980. Cellular necleotide measurements and applications in microbial ecology. Microbiol. Rev. 44: 739-796.

Karl, D.M. and P.A. LaRoch. 1975. Adenosine triphosphate measurements in soil and marine sediments. J. Fish. Res. Board Can. 32: 599-607.

Krogh, A. 1959. The Comparative Physiology of Respiratory Mechanisms. Univ. of Philadelphia Press, Philadelphia.

MacFarlane, R.B. 1981. Alterations in adenine nucleotide metabolism in the Gulf killifish (Fundulus grandis) induced by low pH water. Comp. Biochem. Physiol. 68B: 193-202.

Montague, M.D. and E.A. Dawes. 1974. The survival of Peptococcus prevotii in relation to the adenylate energy charge. J. Gen. Microbiol. 80: 291-299.

Newell, R.C. 1981. The maintenance of energy balance in marine invertebrates exposed to changes in environmental temperature. In: R. Gilles (ed.), Animals and Environmental Fitness: Physiological and Biochemical Aspects of Adaptation and Ecology, 1st Conference, European Society for Comparative Physiology and Biochemistry, Liege, Belgium.

Nickels, J.S. R.J. Bobbie, D.F. Lott, R.F. Martz, P.H. Benson, and D.C. White. 1981. Effect of manual brush cleaning on biomass and community structure of microfouling film formed on aluminum and titanium surfaces exposed to rapidly flowing seawater. Appl. Environ. Microbiol. 41: 1442-1453.

Precht, H. 1973. Temperature and Life. Springer-Verlag, Berlin.

Quammen, M.L., P.A. LaRock and J.A. Calder. 1973. Environmental effects of pulp mill wastes. In: L.H. Stevenson and R.R. Colwell (eds.), Estuarine Microbial Ecology. Univ. of South Carolina Press, Columbia.

Rainer, S.F., A.M. Ivanovici and V.A. Wadley. 1979. Effect of reduced salinity on adenylate energy charge in three estuarine molluscs. Mar. Biol. 54: 91-99.

Ridge, J.W. 1972. Hypoxia and the energy charge of the cerebral adenylate pool. Biochem. J. 127: 351-355.

Schöttler, U. 1979. On the anaerobic metabolism of three species of Nereis (Anelida). Mar. Ecol. Prog. Ser. 1: 249-254.

Selye, H. 1959. The Story of the Adaptation Syndrome, Told in the Form of Informal Illustrated Lectures. Acta Montreal.

Skjoldal, H.R. and U. Bamstedt. 1976. Studies on the deep-water pelagic community of Korsfjorden, Western Norway. Adenosine phosphates and nucleic acids in Meganyctiphanes norvigica (Euphausiacea) in relation to the life cycle. Sarsia 61: 1-14.

Skjoldal, H.R. and U. Bamsted. 1977. Ecobiochemical studies on the deep-water pelagic community of Korsfjorden, Western Norway. Adenine nucleotides in zooplankton. Mar. Biol. 42: 197-211.

Thomas, W.H., O. Holm-Hansen, D.I.R. Seibert, F. Azam, R.E. Hodson and M. Takahashi. 1977. Effects of coppeer on phytoplankton standing crop and productivity: Controlled ecosystem pollution experiment. Bull. Mar. Sci. 27: 34-43.

Van Den Thillart, G., F. Kesheke and A. Van Waarde. 1979. Influence of Anoxia on the energy metabolism of goldfish Carassius auratus (L.). Comp. Biochem. Physiol. 55: 329-336.

Vetter, R.D. and R.E. Hodson. 1982. Use of adenylate concentrations and adenylate energy charge as indicators of hypoxia stress in estuarine fish. Can. J. Fish. Aquat. Sci 39: 535-541.

Vetter, R.D., R. E. Hodson and C. Arnold. 1983. Energy metabolism in a rapidly developing marine fish egg (Sciaenops ocellata). Can. J. Fish. Aquat. Sci. 40: 627-634.

Walker-Simmons, M. and D.E. Atkinson. 1977. Functional capacities and the adenylate energy charge in Escherichia coli under conditions of nutritional stress. J. Bacteriol. 130: 676-683.

White, D.C., W.M. Davis, J.S. Nickels, J.D. King and R.J. Bobbie. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40: 51-62.

White, D.C., R.J. Bobbie, J.S. Nickels, S.D. Fazio and W.M. Davis. 1980. Non-selective biochemical methods for the determination of fungal biomass and community structure in estuarine detrital microflora. Bot. Mar. 23: 239-250.

Wiebe, W.J. and K. Bancroft. 1975. Use of adenylate energy charge ratio to measure growth rate of natural microbial communities. Proc. Nat. Acad. Sci. 72: 2112-2115.

Wijsman, T.C.M. 1976a. Adenosine phosphates and adenosine energy charge in different tissues of Mytilus edulis L. and under aerobic and anaerobic conditions. J. Comp. Physiol. 107: 129-140.

Wijsman, T.C.M. 1976. ATP content and mortality in Mytilus edulis from different habitats in relation to anaerobiosis. Neth. J Sea Res. 10: 140-148.

Zaroogian, G.E., J.H. Gentile, J.F. Heltshe, M. Johnson and A.M. Ivanovici. 1982. Application of adenine nucleotide measurements for the evaluation of stress in Mytilus edulis and Crassostrea virginica. Comp. Biochem. Physiol. 71B: 643-649.

Zotin, A.I., W.S. Faustov, L.I. Radzinskaja and N.D. Ozurnyuk. 1967. ATP level and respiration of embryos. Embryol. Exp. Morphol. 18: 1-12.

Zs-Nagy, I. 1973. Adenosine phosphate concentrations and carbohydrate consumption in the tissues of Anodonta cygnea L. (Mollusca, Pelecypoda) under normal and anoxic conditions. Acta Biochim. et Biophys. Acad. Sci. Hung. 8: 143-151.

Zs-Nagy, I. and M. Ermini. 1972. ATP production in the tissues of the bivalve Mytilus galloprovincialis (Pelecypoda) under normal and anoxic conditions. Comp. Biochem. Physiol. 43B: 593-600.

Zwaan, A. de. and T.C.M. Wijsman. 1976. Anaerobic metabolism in bivalvia (Mollusca). Characteristics of anaerobic metabolism. Comp. Biochem. Physiol. 4B: 313-324.

Effects of Toxic Substances on Decapod Larvae

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INTRODUCTION

The period from 1965 to 1975 was a time of increased public awareness of the environmental problems associated with intensive industrial and agricultural activities. This elevated environmental awareness was manifested in the scientific community as well, as the technical literature swelled with reports of investigations of the effects of numerous chemicals on the various life processes of terrestrial, aquatic and marine organisms. Until about 1970, work with marine organisms was concentrated largely on the adult forms, but it soon became apparent that inference concerning the effect of a given toxin on a species would be severely limited without information on the larval stages as well (Epifanio 1979). Work involving larval forms of decapod crustaceans has been especially popular because of the highly developed techniques for larval culture and well-defined phases of larval development. In the present paper, the toxicological literature on larval decapods is reviewed from the perspective of (1) the type of response tested, (2) the validity of experimental design and (3) the ecological significance of the response. The term "acute toxicity" is used to refer to 48 h or 96 h LC50 determined by standard methods. "Long-term toxicity" refers to experimental conditions where at least 10% of the larvae exposed to a toxin survive to metamorphosis (Epifanio 1971).

RESPONSE OF DECAPOD LARVAE TO TOXIC SUBSTANCES

Responses that have been most commonly studied in decapod larvae include mortality, rate of development, sequence of development, morphological traits, and behavioral traits; and among the various substances tested, insecticides have generally been the most toxic (Table 1). Acute toxicities with chlorinated hydrocarbons have been noted at concentrations in the range of 1.0 - 10.0 $\mu g\ L^{-1}$ while long-term toxicities have been detected at concentrations ranging from 0.01 - 1.0 $\mu g\ L^{-1}$ (Epifanio 1971; Bookhout et al. 1972; Bookhout and Costlow 1975; Bookhout et al. 1976). Common long-term effects include increased mortality in experimental groups relative to controls and decreased rates of larval development. Less commonly there have been reports of abnormal sequences of development (Bookhout and Costlow 1975; Cucci and Epifanio 1979) and morphological aberrations in larval stages (Bookhout and Costlow 1970). The stage of development that is most sensitive to a pesticide appears to vary with the particular toxin and species tested, but waterborne material is considerably more toxic to all stages than is material presented as a food contaminant (Epifanio 1972). This is apparently related to the much more rapid uptake of pesticide from the water (Epifanio 1973), even though no direct relationship has been established between the concentration of pesticide in the tissues of a larva and apparent toxicity (Bookhout and Costlow 1975).

There has been less study of other types of insecticides, and only the carbamate sevin has shown a level of toxicity similar to that of chlorinated hydrocarbons (Buchanan et al. 1970). Work with the organophosphate Malathion suggests that this material is considerably less toxic (Bookhout and Monroe 1977), but behavioral studies with another organophosphate, fenitrothion, have shown subtle effects on lobster larvae (Homarus americanus) at concentrations as low as 0.1 $\mu g\ L^{-1}$ (McLeese 1974). The only other pesticides that have been tested extensively with decapod larvae are the juvenile hormone mimics, and these have been less toxic than other insecticides (Christiansen et al. 1977a,b, 1978).

Among the more toxic non-insecticides are the heavy metals. However, there have been few studies of their effects on decapod larvae. Vernberg et al. (1973) found that mercury increased mortality and affected the behavior of crab larvae at concentrations as low as 1.8 $\mu g\ L^{-1}$ while the results of Rosenberg and Costlow (1976) suggest that cadmium is considerably less toxic to crab larvae. Glickstein (1978) studied the interactive toxicities of mercury and selenium to crab larvae, but his study was restricted to acute toxicity.

Table 1. Summary of toxicological studies using larvae of decapod crustaceans.

Category	Toxin	Species	Reference
Insecticide	DDT	Callinectes sapidus Hexapanopeus angustifrons Libinia emerginata Rhithropanopeus harrisii	Bookhout and Costlow (1970)
Insecticide	Sevin	Cancer magister	Buchanan et al. (1970)
Insecticide	Dieldrin	Leptodius floridanus	Epifanio (1971, 1972, 1973)
Insecticide	Mirex	Menippe mercenaria Rhithropanopeus harrisii	Bookhout et al. (1972)
Insecticide	Fenitrothion	Homarus americanus	McLeese (1974)
Insecticide	Mirex	Callinectes sapidus	Bookhout and Costlow (1975)
Insecticide	Methoxychlor	Callinectes sapidus Rhithropanopeus harrisii	Bookhout et al. (1976)
Insecticide	Malathion	Callinectes sapidus Rhithropanopeus harrisii	Bookhout and Monroe (1977)
Insecticide	Methoprene	Rhithropanopeus harrisii	Christiansen et al. (1977a)
Insecticide	Hydroprene	Rhithropanopeus harrisii	Christiansen et al. (1977b)
Insecticide	Dimilin	Rhithropanopeus harrisii Sesarma reticulatum	Christiansen et al. (1978)
Petroleum oil	Crude oil	Homarus americanus	Wells (1972)
Petroleum oil	Crude oil	Neopanope texana sayi	Katz (1973)
Petroleum oil	Crude oil	Homarus americanus	Wells and Sprague (1976)
Petroleum oil	Crude oil	Cancer magister	Caldwell et al. (1977)
Petroleum oil	Refined oil	Rhithropanopeus harrisii	Laughlin et al. (1978)
Petroleum oil	Crude oil	Eurypanopeus depressus	Cucci and Epifanio (1979)
Petroleum oil	Refined oil	Rhithropanopeus harrisii	Laughlin (1981)
Heavy metal	Mercury	Uca pugilator	DeCoursey and Vernberg (1975)
Heavy metal	Mercury	Uca pugilator	Vernberg et al. (1973)
Heavy metal	Cadmium	Callinectes sapidus Rhithropanopeus harrisii	Rosenberg and Costlow (1976)
Heavy metal	Zinc Lead	Rhithropanopeus harrisii	Benijts and Benijts (1975)
Detergent	Detergent	Rhithropanopeus harrisii	Czyzewska (1976)
PCP	Aroclor	Palaemonetes pugio	Roesijadi et al. (1976)
PCB	Aroclor 1016	Rhithropanopeus harrisii	Neff et al. (1977)
Dredge spoil	Unknown	Palaemonetes pugio	DeCoursey and Vernberg (1975)

In contrast to the limited study of heavy metals, there has been considerable work with petroleum hydrocarbons (e.g., Mironov 1969; Wells 1972; Wells and Sprague 1976; Katz 1973; Anderson et al. 1975). Results of these investigations have shown that petroleum oils are much less toxic to decapod larvae than pesticides and heavy metals, and that among petroleum oils, refined products with their increased percentage of medium-boiling-point aromatic compounds are more toxic than crude oils. Additionally, the polycyclic aromatic components of petroleum oils are much more toxic to crab larvae than are the whole oils (Laughlin et al. 1978). But in all cases early stage larvae appear to be more sensitive than later stages to petroleum or its derivatives, and a decrease in the rate of larval development is almost always associated with the long-term toxicity of petroleum products.

Several studies with petroleum hydrocarbons are of additional interest because they have investigated effects of continued exposure of the organisms after they have passed through their complete larval development and have metamorphosed into juveniles. For example, Cucci and Epifanio (1979) found that crab larvae that were exposed to crude oil continuously from hatching showed reduced rates of development through the first four juvenile molts while those crabs that were first exposed at Zoea Stage II or later showed no effects during continued exposure as juveniles. In a similar experiment with refined oils, Laughlin et al. (1978) and Laughlin (1981) reported effects of exposure during early development on subsequent growth of late stage larvae and juveniles.

Other pollutants studied include detergents (Czyzewska 1976), chlorinated polycylic aromatic compounds (Roesijadi et al. 1976; Neff et al. 1977) and dredge spoils (DeCoursey and Vernberg 1975).

EXPERIMENTAL DESIGN

The design of toxicological experiments with decapod larvae has been greatly influenced by the culture techniques available. Such techniques were well developed for culturing those species with very large larvae (e.g., the lobster Homarus americanus) by the early 20th century (Barnes 1911) but the lack of suitable foods made culture of other decapod larvae very difficult. In fact it was not until the work of Costlow and Bookhout (1959) that standard techniques for larval culture became generally available. These techniques involve holding gravid females under constant conditions of salinity and temperature until hatching occurs, or alternatively removing the eggs from the female and incubating them in vitro (Costlow and Bookhout 1960). The resultant larvae are cultured in small finger bowls at some specified number of larvae per bowl and the water is changed daily with concurrent addition of food—usually freshly hatched brine shrimp nauplii (Artemia sa-

lina). Each day the number of living and newly molted larvae are determined and the dead larvae removed. Upon molting to the post-larval or megalopal stage, larvae are separated to individual containers until molting to Crab Stage 1.

Recent innovations in culture technology include utilization of alternative foods for species whose early stages are too small to ingest brine shrimp nauplii (Sulkin and Epifanio 1975) and development of automated devices for the mass culture of large numbers of larvae (see, e.g., Rice and Williamson 1970). However, automated culture devices have not been used extensively in toxicological work with decapods.

Tests of acute toxicity are generally done by exposing replicate bowls of larvae of a known stage of development to a logarithmic series of concentrations of a toxin. Larvae of any stage can be produced by rearing groups of individuals in uncontaminated sea water until the desired stage is reached. In this way, values for LC50 (or other measures of acute toxicity) can be determined explicitly for each developmental stage. Long-term toxicities can be determined by continual exposure of larvae to a toxin from hatching through complete development. A typical experiment might involve 20 bowls of ten larvae at each of several concentrations of a toxin (usually a geometric series within a logarithmic interval of concentrations).

The strength of such a program of testing lies in the relative ease with which strong statistical inference can be drawn. For example, the acute toxicity of a compound can be determined unequivocally. Likewise, the stages that are most sensitive can be statistically determined in long-term tests where larvae are exposed continuously from hatching (e.g., Bookhout et al. 1976). But there are problems with this latter approach, as there is no way to account for the possibility of selection of more resistant larvae as development progresses. For example, if increased mortality is first observed in Zoea Stage II and again in Megalopa, there is no way to infer how those larvae that did not survive to Stage II might have responded in Stages III and IV (Epifanio 1979). An alternate technique (Epifanio 1971; Cucci and Epifanio 1979) allows direct assessment of the sublethal toxicity at each stage. This design involves mass culture of a large number of larvae in the absence of a toxin with initial exposure as the larvae reach a given developmental stage. The drawback with this design is the very large culturing effort that is necessary to provide the number of larvae required for each experiment.

Regardless of the details of experimental design, virtually all toxicological studies with decapod larvae have been done under static culture conditions. Generally, the test media are replenished every 24 h, and there have been no studies of the change in concentration of any toxin over that period of time. However, it might be expected that, with at least some compounds, there would

be loss due to adsorption, volatility or uptake by the test organisms (Neff et al. 1977). Flow-through systems have been used in toxicological studies with other small planktonic organisms (e.g., Redmond 1981), and these techniques could be applied to decapod larvae, but monitoring of responses other than mortality and morphological abnormality would be very difficult.

Another complication in long-term studies is the necessity of maintaining a population of food organisms in the cultures with the larvae. Clearly there is potential for contamination of the food organisms with the test toxin, and this would certainly complicate the interpretation of an experiment where the toxin was primarily dissolved or suspended in the water. Studies with the pesticide dieldrin suggest that this is not a severe problem (less than 5% of the measured bioaccumulation came from incidental contamination of the food), but there are few data for other toxins (Epifanio 1972 1973).

That many toxins of interest are not highly soluble in sea water creates additional methodological problems. Organochloride pesticides, for example, are generally insoluble in sea water and must be dissolved in a carrier solution (e.g., acetone or polyethylene glycol) to facilitate their dissolution in a test medium. It is quite simple to include appropriate controls for the toxicity of the carrier compound itself, but it is impossible to account for the possibility of synergistic interactions of the carrier and the pesticide.

Other slightly soluble substances such as petroleum oils are dissolved directly in sea water. Wells and Sprague (1976) found that a combination of stirring and ultrasonic vibration yielded higher concentrations of oil in test media than simple stirring; Anderson et al. (1975) have suggested a more simple stirring technique under controlled conditions. Test media prepared in these two different ways might well yield different results. An additional problem with petroleum studies is the vast difference in the composition of crude oils from different locations. The availability of four standard oils provided by the American Petroleum Institute has facilitated comparison among studies employing those oils, but these standard oils are in limited quantity and their continued availability is not certain.

Methodological problems with heavy metals largely center on the differential toxicity of chelated and non-chelated metal ions. None of the investigations of heavy metals with decapod larvae have addressed this problem, and as the degree of chelation is proportional to the concentration of organic material in the water, it is difficult to compare results of studies done with water taken from different areas.

ECOLOGICAL SIGNIFICANCE OF MEASURED RESPONSES

The responses that have been measured for decapod larvae have obvious ecological significance; it would be difficult to argue that factors such as increased mortality in a population, changes in rates of development, or behavior abnormalities would not have large effects on a species. However, the concentration at which most toxins have been shown to cause even long-term, sublethal effects is considerably higher than that found in all but the most polluted of natural environments (Epifanio 1979). One could then conclude that few of the toxins tested affect decapod larvae in natural systems. Alternatively, one could suppose that synergistic interactions occur among the many pollutants in natural systems or that sublethal effects more subtle than those observed in the laboratory occur at lower, more environmentally realistic levels of a toxin. Neither argument is very satisfying.

Nevertheless, the results of laboratory studies constitute an important tool in environmental management. In the ideal situation, laboratory experiments would simulate natural conditions, and results would be directly applicable to field situations. But, in fact, this has proved difficult, and the majority of toxicological studies with marine organisms (indeed, all of the studies with decapod larvae) have simply measured the toxicity of a given compound under some standard, controlled conditions. Therefore, direct inferences from the results of such studies are limited to comparisons of the toxicities of various compounds to a particular type of organism. For example, we can conclude that insecticides are more toxic to crab larvae than are suspensions of crude petroleum oil, but we cannot conclude much about the actual effects of insecticides in the natural environment. So, while the setting of an exact limit for the concentration of a particular toxin in a body of water is, to a large degree, arbitrary, differences in the limits for compounds of greatly differing toxicities are well-defined by laboratory experiments. Clearly, the situation is similar to that found in other areas of toxicology where limits on the quantities of a given compound that may occur in a food or drug are extrapolated from laboratory-based studies with artifically high levels of the material. In both the environmental and the food-and-drug situations, the investigator infers effects at lower concentrations rather than measuring them directly. It is unlikely that these types of studies will ever yield direct correlations between a laboratory response and an actual effect in the field. But in this limited context, decapods can serve as useful organisms in bioassays. Research to date has defined those responses that provide appropriate information, and the literature allows a base for comparison. The fact that decapod development includes discrete stages allows detection of differential sensitivity throughout development, and comparison of similar responses by larvae and adults of the same species allows yet another perspective on the potential effects of a toxin.

REFERENCES

Anderson, J.W., J.M. Neff, B.A. Cox, H.E. Tatem and G.M. Hightown. 1975. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustaceans and fish. Mar. Biol. 27: 75-83.

Barnes, E.W. 1911. Revised edition of the methods of protecting and propagating the lobster, with a brief outline of its natural history. Rep. R.I. Comm. Inland Fish. 41: 83-127.

Benijts, C.C. and F. Benijts. 1975. The effect of low lead and zinc concentrations on the larval development of the mud crab Rhithropanopeus harrisii Gould. In: J.J. Koeman and J.J.T.W.A. Stric (eds.), Sublethal Effects of Toxic Chemicals on Aquatic Chemicals, pp. 43-52. Elsevier, Amsterdam, Netherlands.

Bookhout, C.G. and J.D. Costlow, Jr. 1970. Nutritional effects of Artemia from different locations on larval development of crabs. Helgol. Wiss. Meeresunters. 20: 435-442.

Bookhout, C.G., and J.D. Costlow, Jr. 1975. Effects of mirex on the larval development of blue crab. Water Air Soil Pollut. 4: 113-126.

Bookhout, C.G. and R. Monroe. 1977. Effects of malathion on the development of crabs. In: F.J. Vernberg, A. Calabrese, F. P. Thurberg and W. B. Vernberg (eds.), Physiological Responses of Marine Biota to Pollutants, pp. 3-19. Academic Press, New York.

Bookhout, C.G., A.J. Wilson, Jr., T.W. Duke and J.I. Lowe. 1972. Effects of mirex on the larval development of two crabs. Water Air Soil Pollut. 1: 165-180.

Bookhout, C.G., J.D. Costlow, Jr. and R. Monroe. 1976. Effects of methoxychlor on larval development of mud crab and blue crab. Water Air Soil Pollut. 5: 349-365.

Buchanan, D.V., R.E. Millman and N.E. Stewart. 1970. Effects of the insecticide sevin on various stages of the Dungeness crab, Cancer magister. J. Fish. Res. Board Can. 27: 93-104.

Caldwell, R.S., E.M. Calderane and H.M. Mallon. 1977. Effects of a seawater-soluble fraction of Cook inlet crude oil and its major aromatic components on larval stages of the Dungeness crab, Cancer magister Dona. In: D.A. Wolfe (ed.), Fates and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems, pp. 210-220. Pergamon Press, New York.

Christiansen, M.E., J.D. Costlow, Jr. and R. Monroe. 1977a. Effects of the juvenile hormone mimic ZR-515 (Altosid) on larval development of the mud crab Rhithropanopeus harrisii in various salinities and cyclic temperatures. Mar. Biol. 39: 269-279.

Christiansen, M.E., J.D. Costlow, Jr. and R. Monroe. 1977b. Effects of the juvenile hormone mimic ZR-512 (Altozar) on larval development of the mud crab Rhithropanopeus harrisii at various cyclic temperatures. Mar. Biol. 39: 281-288.

Christiansen, M.E., J.D. Costlow, Jr. and R.J. Monroe. 1978. Effects of the insect growth regulator Dimilin (TH 6040) on larval development of two estuarine crabs. Mar. Biol. 50: 29-36.

Costlow, J.D., Jr. and C.G. Bookhout. 1959. The larval development of Callinectes sapidus Rathbun reared in the laboratory. Biol. Bull. 116: 373-396.

Costlow, J.D., Jr. and C.G. Bookhout. 1960. A method for developing brachyuran eggs in vitro. Limnol. Oceanogr. 5: 212-215.

Cucci, T.L. and C.E. Epifanio. 1979. Long-term effects of water-soluble fractions of Kuwait crude oil on the larval and juvenile development of the mud crab Eurypanopeus depressus (Smith). Mar. Biol. 55: 215-220.

Czyzewska, K. 1976. The effects of detergents on larval development of crab. Mar. Pollut. Bull. 7: 108-112.

DeCoursey, P.J. and W.B. Vernberg. 1975. The effect of dredging in a polluted estuary on the physiology of larval zooplankton. Water Res. 9: 149-154.

Epifanio, C.E. 1971. Effects of dieldrin in seawater on the development of two species of crab larvae, Leptodius floridanus and Panopeus herbstii. Mar. Biol. 11: 356-362.

Epifanio, C.E. 1972. Effects of dieldrin-contaminated food on the development of Leptodius floridanus larvae. Mar. Biol. 13: 292-297.

Epifanio, C.E. 1973. Dieldrin uptake by larvae of the crab Leptodius floridanus. Mar. Biol. 19: 320-322.

Epifanio, C.E. 1979. Larval decapod crustaceans. In: C.W. Hart and S.L.H. Fuller (eds.), Pollution Ecology of Estuarine Invertebrates, pp. 259-292. Academic Press, New York.

Glickstein, N. 1978. Acute toxicity of mercury and selenium to Crassostrea gigas embryos and Cancer magister larvae. Mar. Biol. 49: 113-117.

Katz, L.M. 1973. The effects of water soluble fraction of crude oil on larvae of the decapod crustacean Neopanope texona sayi. Environ. Poliut. 5: 199-204.

Laughlin, R.B., Jr. 1981. Hormesis: A response of low environmental concentrations of petroleum hydrocarbons. Science 211: 706-707.

Laughlin, R.B., Jr., L.G.L. Young and J.M. Neff. 1978. A long-term study of the effects of water-soluble fractions of No. 2 fuel oil on the survival, development rate, and growth of the mud crab Rhithropanopeus harrisii. Mar. Biol. 47: 87-95.

McLeese, D.W. 1974. Olfactory response and fenitrothion toxicity in American lobsters (Homarus americanus). J. Fish. Res. Board Can. 31: 1127-1131.

Mironov, O.G. 1969. On the influence of oil pollution on some representative of Black Sea zooplankton. Zool. Zh. 48: 1734-1736.

Neff, J.M., R.B. Laughlin, Jr. and G.S. Giam. 1977. Effects of polychlorinated biphenyls, polychlorinated naphthalenes and phthalate esters on larval development of the mud crab Rhithropanopeus harrisii. In: G.S. Giam (ed.), Pollutant Effects on Marine Organisms, pp. 95-110. Lexington Books, Lexington, Mass.

Redmond, Michele S. 1981. The effects of bunker C oil on the survival, reproduction, and growth of the mysid shrimp Mysidopsis bahia (crustacea: mysidacea). M.S. Thesis, Univ. of Delaware, Newark, Del.

Rice, A.L. and D.J. Williamson. 1970. Methods for rearing larval decapod crustacea. Helgol. Wiss. Meeresunters. 20: 417-434.

Roesijadi, G., S.R. Petrocelli, J.W. Anderson, C.S. Giam and G.E. Neff. 1976. Toxicity of polychlorinated biphenyls (Arochlor 1254) to adult, juvenile and larval stages of the shrimp Palaemonetes pugio. Bull. Environ. Contam. Toxicol. 15: 297-304.

Rosenberg, R. and J.D. Costlow, Jr. 1976. Synergistic efforts of cadmium and salinity combined with constant and cycling temperatures on the larval development of two estuarine crab species. Mar. Biol. 38: 291-303.

Sulkin, S.D. and C.E. Epifanio. 1975. Comparison of rotifers and other diets for early larvae of the blue crab Callinectes sapidus Rathbun. Estuar. Coast. Mar. Sci. 3: 109-113.

Vernberg, W.B., P.J. DeCoursey and W.S. Padgett. 1973. Synergistic effects of environmental variables on larvae of Uca pugilator. Mar. Biol. 22: 307-312.

Waterman, T.H. and F.A. Chase, Jr. 1960. General Crustacean biology. In: T.H. Waterman (ed.), The Physiology of Crustacea, Vol. I, pp. 1-30, Academic Press, New York.

Wells, P.G. 1972. Influence of Venezulan crude oil on lobster larvae. Mar. Pollut. Bull. 3: 105-106.

Wells, P.G. and J.B. Sprague. 1976. Effects of crude oil on American Lobster (Homarus americanus) larvae in the laboratory. J. Fish. Res. Board Can. 33: 1604-1614.

Can Tissue Anomalies that Occur in Marine Fish Implicate Specific Pollutant Chemicals?

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INTRODUCTION

The advantage of using tissue abnormalities in wild fish as a measure of fish health is that the abnormality, unlike sensitive biochemical anomalies, cannot be said to have occurred during capture or transport to the lab. It usually takes hours, days, weeks and sometimes even months for abnormal tissue pathologies to develop. The researcher can be confident that some factor in the field caused the abnormality. When an abnormal fish is captured, logical questions appear at four different levels: (1) What is the structure or morphology of the abnormality? Many scholarly articles by histopathologists describe in detail tissue anomalies observed in field specimens. At this level the focus is on the pathology itself. (2) What is the incidence of the pathology in the population? How many are afflicted, old or young, male or female? Here the focus is on the species population. (3) Can the incidence of a fish disease be linked to environmental pollution? Here the focus is on correlating pathology with pollution. (4) What factor or factors (chemical, physical and/or biological) in the polluted waters caused the pathology? This is probably the most difficult question to answer and is the subject of this paper. Answers to the first three questions must be found before attempts can be made to answer the fourth. If a specific fish disease can be linked to a specific xenobi-

otic, then another tier of questions arises: (5) What is the significance of this to human health and well being? Is a food source diminished? Are humans ingesting fish containing toxic chemicals? What is the aesthetic/economic cost versus the industrial/economic gain of having continued pollution? Answers to these questions are beyond the scope of this paper, which will focus on the question of whether there are pollutant-specific pathologies in marine fish.

CAUSES OF TISSUE ABNORMALITIES IN FISH

From the results of voluminous laboratory studies it is known that many chemicals and other agents can cause abnormalities in fish. But what are the natural causes of anomalies in the field that will complicate designation of a lesion as anthropogenic? Some of the most significant causes of disease in marine fish are biological agents such as viruses, bacteria, fungi, protozoa, helminthes and parasitic copepods (Sindermann 1966, 1979; Love et al. 1981). Many of these infections result in tumors and lesions that persist in the living fish. Fish are also subject to epizootics or plagues in which large percentages of a population are simultaneously killed or infected by a single infectious pathogen. Outbreaks of the fungal pathogen, Ichthyophonus hoferi, in herring of the western Atlantic have been known since 1898, and Sindermann (1966) reports that in 1954-55 an estimated one-half of the entire herring population of the Gulf of St. Lawrence was killed by the disease.

Another disease with a long history of epizootic prevalence is the "red disease" of eels caused by Vibrio anguillarum. Italian literature dating back to 1718 records repeated and severe outbreaks on the coast of Italy throughout the eighteenth and nineteenth centuries (Sindermann 1966). There is evidence that epizootics occur when fish populations reach high densities and when summer temperatures allow the rapid proliferation of bacteria (Sindermann 1966). The possibility that fish pathogens are also human pathogens cannot be ruled out. For example, Love et al. (1981) isolated a Vibrio from naturally occurring skin ulcers on a damselfish and from human wounds.

Inherited abnormalities and inherited propensities for disease undoubtedly also exist in the field. Unfortunately data in this area are scant. Gordon (1954) reviewed the genetics of fish diseases.

Another cause of deformed fish in the field is starvation. O'Connell (1980) found irregular shaped anchovy larvae off the coast of San Diego, Calif., and concluded that their emaciated condition was very similar to that induced in laboratory animals by total food deprivation. A condition known as "jellied" flesh that occurs in several flatfish species in cold water (Templeman and Andrews 1956) may also be caused by starvation. Other causes of anomalies in wild fish can be grouped under the heading of physical

environmental factors such as salinity, temperature, pH or O_2 concentration. Table 1 summarizes the categories of disease-causing factors in wild fish. It must be emphasized that multiple factors can be involved in any disease occurrence. For example, warm water may induce a microbial infection of a genetically predisposed population. A toxic chemical may cause immunosuppression in a population which then contracts a viral disease. A starving population may be killed by low oxygen when a well-fed population would survive.

Table 1. Causes of anomalies in wild fish.

1. Nutritional (starvation, etc.)
2. Biological (bacteria, viruses, fungi, protozoans, helminthes, etc.)
3. Genetic
4. Physical environmental (pH, temperature, salinity, O_2, etc.)
5. Chemical (red tide, xenobiotics, petroleum, etc.)

VARIATION IN DISEASE PREVALENCE IN MARINE FISH

The prevalence of anomalies in fish populations may vary with geography, time, age, sex and diet. Figure 1 shows the geographic incidence of two diseases of flatfish. Skin papillomas, a disfiguring protozoan disease of flatfish, occurs in the Pacific but not in the Atlantic. By contrast, the well-studied viral disease lymphocystis occurs primarily in the North Atlantic flatfish with no recordings in the Pacific south of Alaska. Superimposed on the global distribution pattern, however, are local variations in tumor presence from 58 to 0.01% of a population (Stich et al. 1977). Temporal variation in prevalence of disease is best illustrated by studies of epizootics. Sindermann (1966) lists a sequence of events in the Ichthyophonus disease of herring which includes a long enzootic phase of very low prevalence (< 0.1%), several years of rapidly increasing incidence which reaches an epizootic peak when about 25% of the individuals are infected and acute infections are common, then several years of rapidly declining incidence and return to the enzootic phase.

Disease in Pristine Alaskan Waters

An excellent example of a comprehensive survey of disease prevalence in wild fish is that of McCain et al. (1979) in Alaskan waters. A review of this study will further illustrate some of the kinds of variance that exist in disease prevalence. Presumably the Bering Sea stations used in this study (Figure 2) were relatively

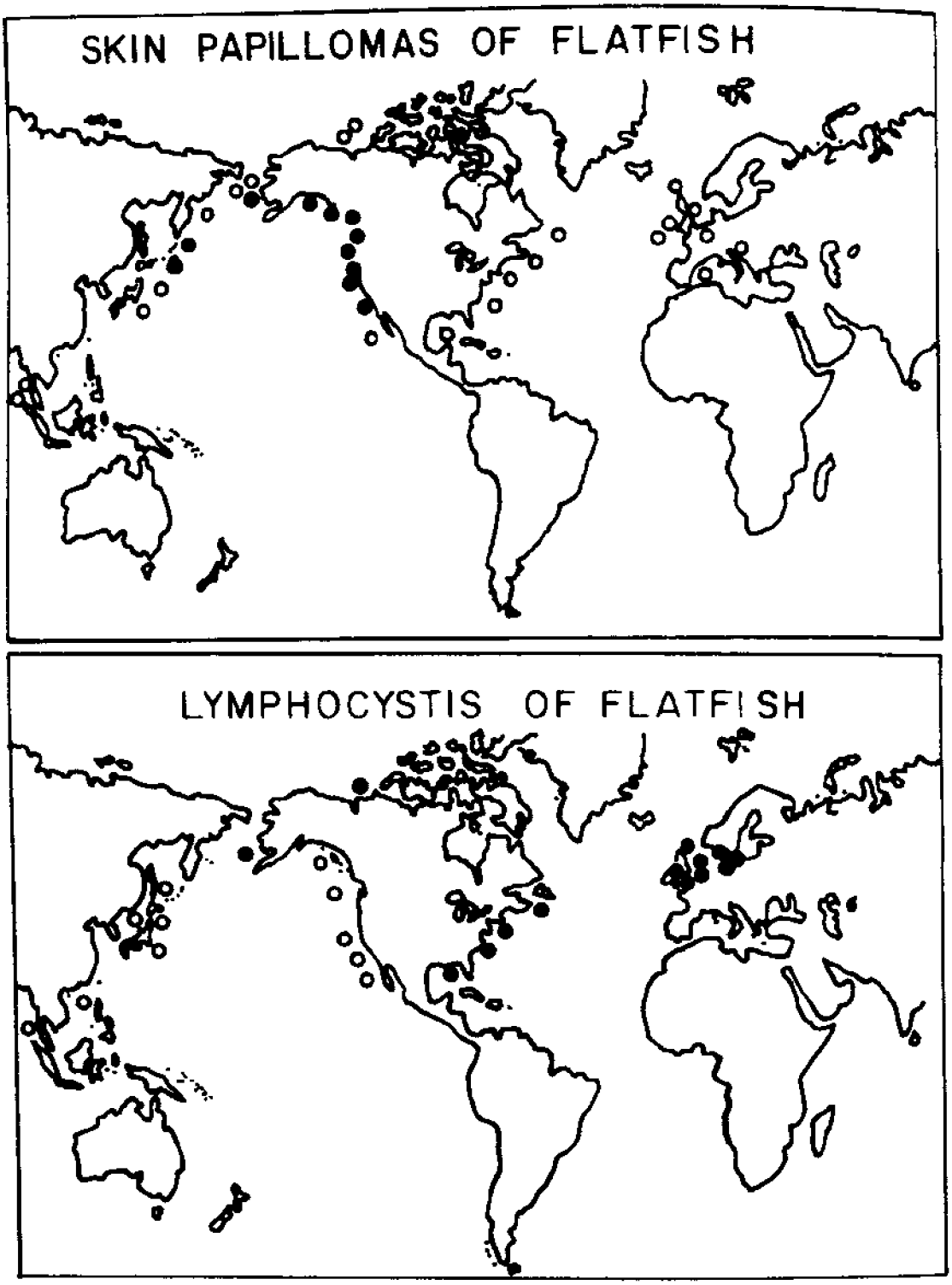

Figure 1. The distribution of two microbial diseases of flatfish. Black dots show sample stations where the disease was found. Open circles show stations where disease was absent. (After Stich et al. 1977).

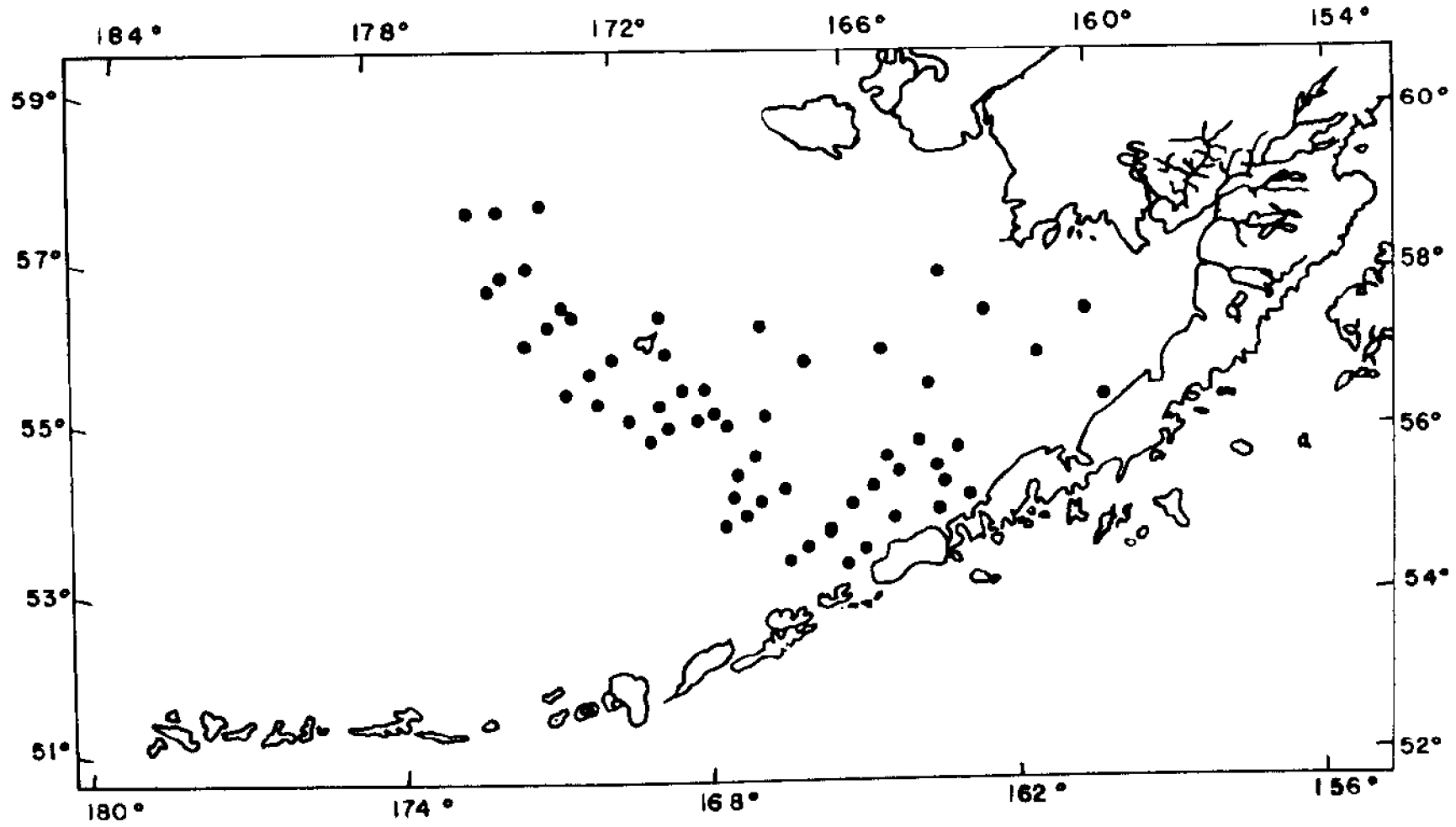

Figure 2. Location of sample sites (black dots) that were used in the McCain study of diseases of marine fishes in Alaskan waters. (After McCain et al. 1979).

pristine. A total of 36,618 fish were captured and examined for pathological conditions. Of the 26 species examined, only four had detectable abnormalities. Among Pacific cod, 8.7% had pseudobranchial tumors, and 1.6% had skin ulcers. Among walleye pollock, 1.7% had pseudobranchial tumors. Of yellowfin sole, 2.8% had lymphocystis and 1.4% of rock sole had epidermal papillomas. Pseudobranchial tumors, like the papillomas, are caused by protozoans (Dawe 1979). The tumors of the cod and pollock were distributed throughout the sampling area. The highest frequencies of sole with lymphocystis were in the southeastern Bering Sea. Figure 3 shows a composite of frequency-length profiles for four of the species along with lesion incidence. Note that a higher percentage of the small pollock (young) had tumors than the large. Also note that young male rock sole had a higher frequency of papillomas than young female sole. McCain et al. (1979) suggested that the occurrence of papillomas was depth related since the young individuals were captured in shallow water and the old in deeper water. This study illustrates the importance of patchiness, sex and age differences and species specificity and the need for large numbers of fish and sample sites for understanding variance in disease prevalence.

Deformities Near Los Angeles

Valentine's (1975) survey of gill raker deformities in barred sand bass from Southern Calif. presents data on a field anomaly that may have been associated with pollution. Here there were two sample areas. One area included six stations located between Los Angeles and San Diego; the other was 500-700 miles south of San Diego in Baja, California. The incidence of severely deformed gill rakers in Southern Calfornia was 30.5%; the incidence in Baja was 0%. Figure 4 shows the relationship between size and incidence of the deformity in California fish. Valentine asserts that sand bass are not migratory and that two additional species, the California grunion and the barred surf perch, also exhibited gill raker abnormalities when captured in California waters. Unfortunately we do not know whether the grunion and surf perch from Baja had lesions. During the time that this study was conducted (1967-1971), hundreds of kilograms of PCBs and DDT were being discharged into Los Angeles coastal waters (Schmidt et al. 1971) along with tens of thousands of kilograms of phosphates, nitrates, coliforms, metals and other substances. Valentine speculated that the abnormalities were either of genetic origin or caused by pollution and cited references showing that heavy metals, DDT and PCBs all can interfere with calcium metabolism: here was a study that comes as close as most to implicating pollution. Unfortunately, the concentrations of pollutants in the sample areas were not fully known. The concentrations of toxicants in the fish were not determined and the distribution of sample sites was too restricted.

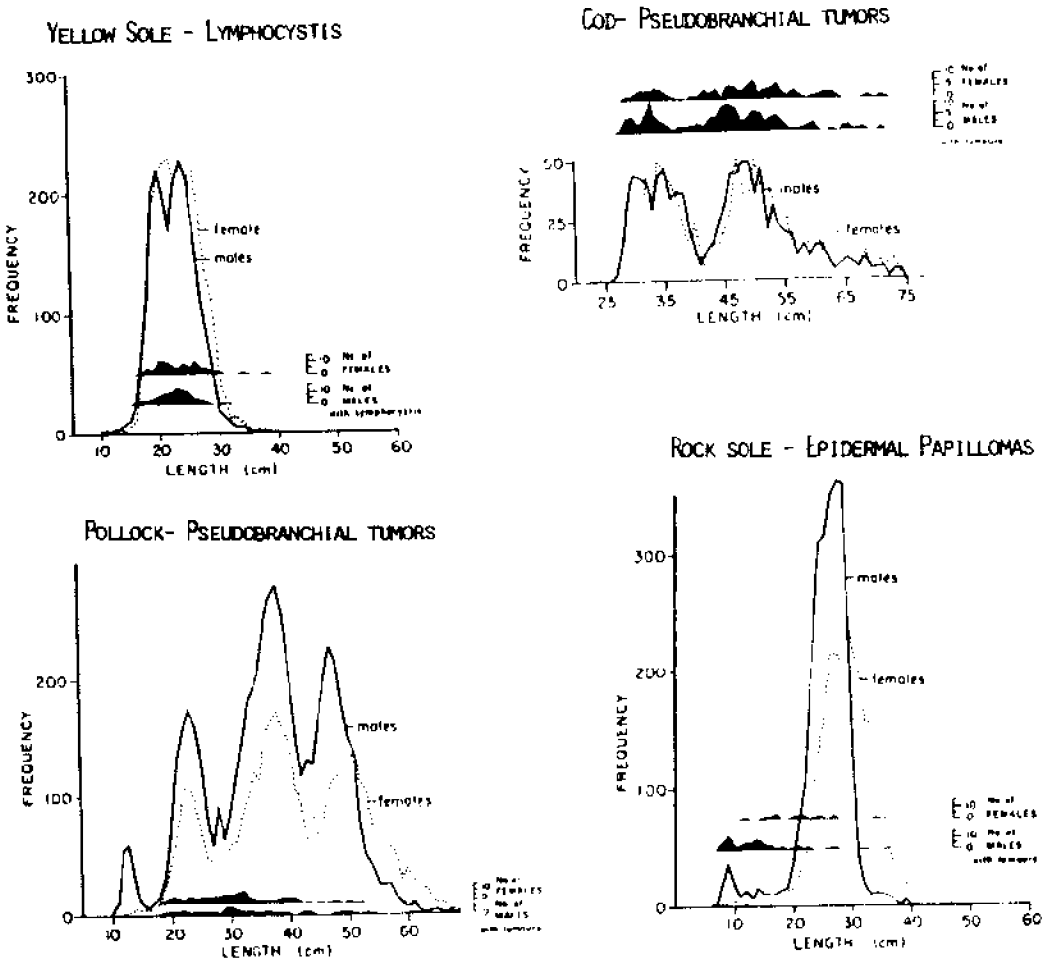

Figure 3. Frequency-length graphs for four of the species caught in the Alaskan study (in white). The incidence of a specific disease is shown in black. (From McCain et al. 1979, with permission of copyright holders, Blackwell Scientific Publications Limited)

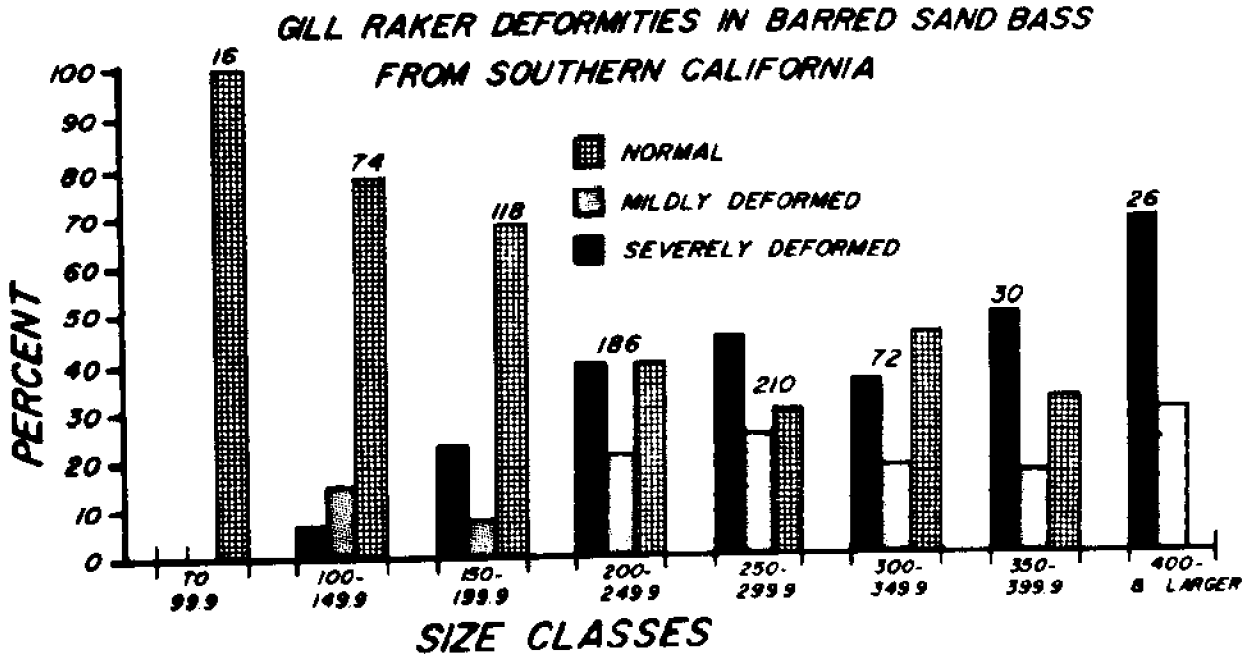

Figure 4. Bar graph of the incidence of normal, mildly deformed, and severely deformed gill rakers in barred sand bass from southern California. With increasing size (age), expressed in standard length (mm), the percentage of normal gill rakers decreases while the percentage of deformed gill rakers increases. All specimens represented in this figure were captured between 1967 and 1971, inclusive. The percentages of severely and mildly deformed and normal gill rakers respectively are as follows (size classes followed by three percentages): To 99.9 mm, 0.0, 0.0, 100.0; 100-149.9 mm, 6.8, 14.9, 78.3; 150-199.9 mm, 22.9, 8.5, 68.6; 200-249.9 mm, 39.2, 21.0, 39.8; 250-299.9 mm, 45.2, 24.8, 30.0; 300-349.9 mm, 36.1, 18.0, 46.0; 350-399.9 mm, 50.0, 16.7, 32.3; 400 mm and larger, 69.2, 30.8, 0.0.(From Valentine 1975 with permission of copyright holders University of Wisconsin Press and the Board of Regents of the University of Wisconsin System.)

There are many other examples in the literature that indirectly implicate anthropogenically contaminated environments with anomalies in feral fish, without direct proof of the association.

Liver Cancer in the Duwamish River

An example of a single species survey in which pollution effects are suggested but not proved is that of Pierce et al. (1978) using data for fish in the Duwamish River Estuary, Seattle, Wash. Figure 5 shows the four stations used: West Point and Alki Point are both sewage outfalls and the Duwamish River is a highly polluted ship channel. Point Bully was the single control site. Figure 6 summarizes the results of the analyses of English sole livers collected from the four sites. Eleven types of liver lesion were distinguished; for example, type 1 was fatty liver, type 11 was hepatoma. Clearly, most of the flatfish in the Duwamish River possessed diseased livers. Fish from both Alki and West Points also exhibited high incidence of lesions (although no cancer). Even fish from the control area (Point Bully) exhibited lesions.

Duwamish River sole contained 1-5 ppm PCB as a body burden. The authors cited laboratory studies showing PCBs caused lesions in fish similar to those seen in the sole, with the exception of hepatomas and concluded that "although environmental chemical contaminants were suspected as being the etiologic agents for the liver lesions, the establishment of cause must await additional investigations. . . ." Although inclusion of more control sites with documentably distinct populations would have strengthened the study, a correlation between pollution and fish tissue anomalies does appear to exist. Taking the next step (answering question #4) and determining the precise cause of liver lesions presents a challenge to the researchers. Like most cities, Seattle waters are polluted by a highly complex mixture of chemicals which interact with each other, the environment and biota in numerous ways.

Fin Erosion or Fin Rot

This abnormality is the best example of a disease of fish inhabiting polluted waters. Sindermann's (1979) review covers the relevant literature. An association between occurrences of fin rot and high sediment coliform counts (Mahoney et al. 1973) and high concentrations of heavy metal in sediments (Caromody et al. 1973) has been reported. The signs of disease can be produced experimentally by exposure of fish to polluted sediments, by laboratory exposure to 3-5 $\mu g\ L^{-1}$ of Aroclor 1254 (Couch and Nimmo 1975), or by exposure to 4-5 ppm crude oil (Minchew and Yarbrough 1977). Fin erosion has been observed in striped bass overwintering in heated effluents of power plants in the Middle Atlantic states (Sindermann 1979). At least 50 species of fish have been captured with fin rot. Many, on the other hand, appear to be immune to the disease. For example, the English sole with the very high levels of liver abnor-

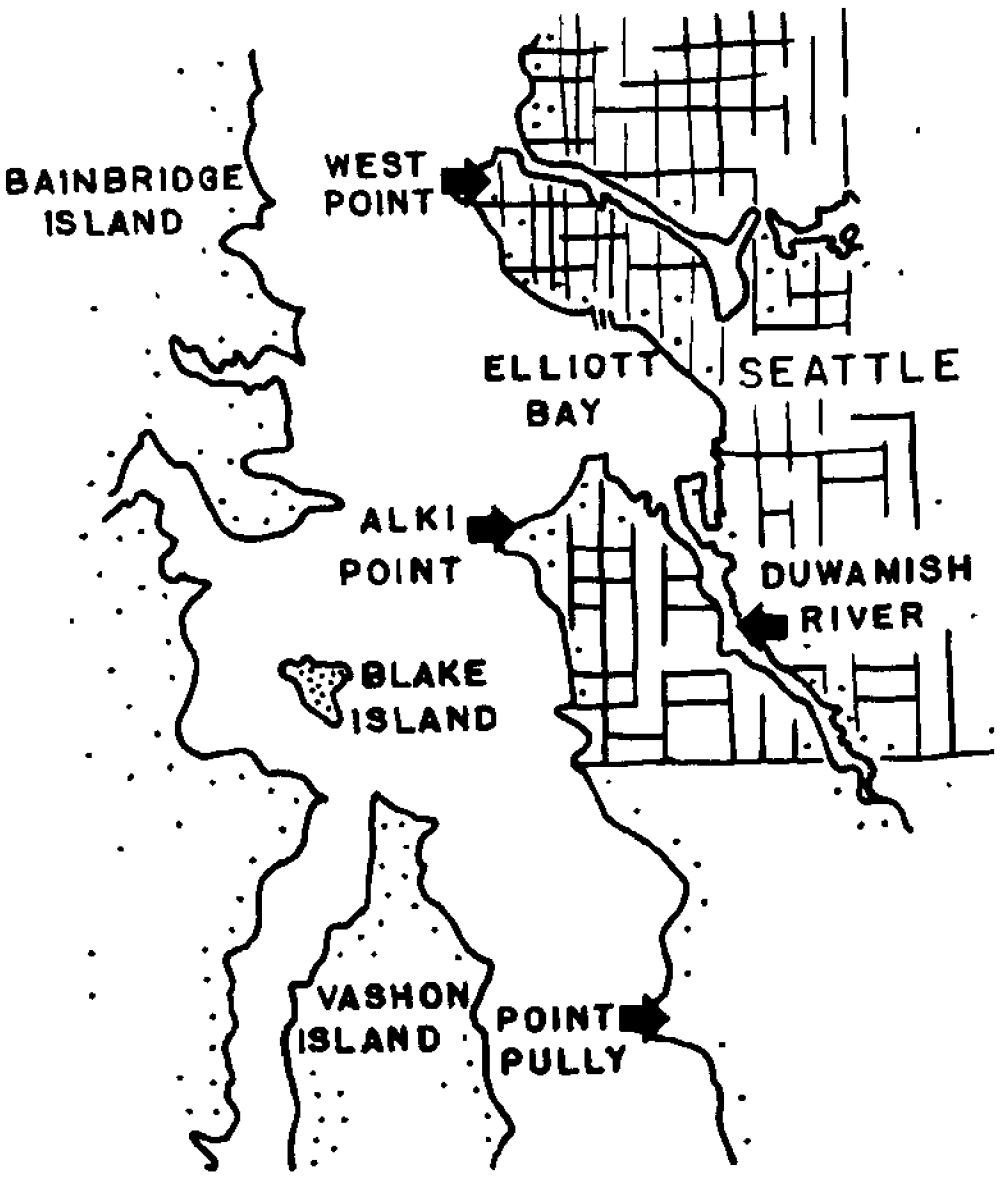

Figure 5. Locations of the four sample stations used in the Duwamish River study. (After Pierce et al. 1978)

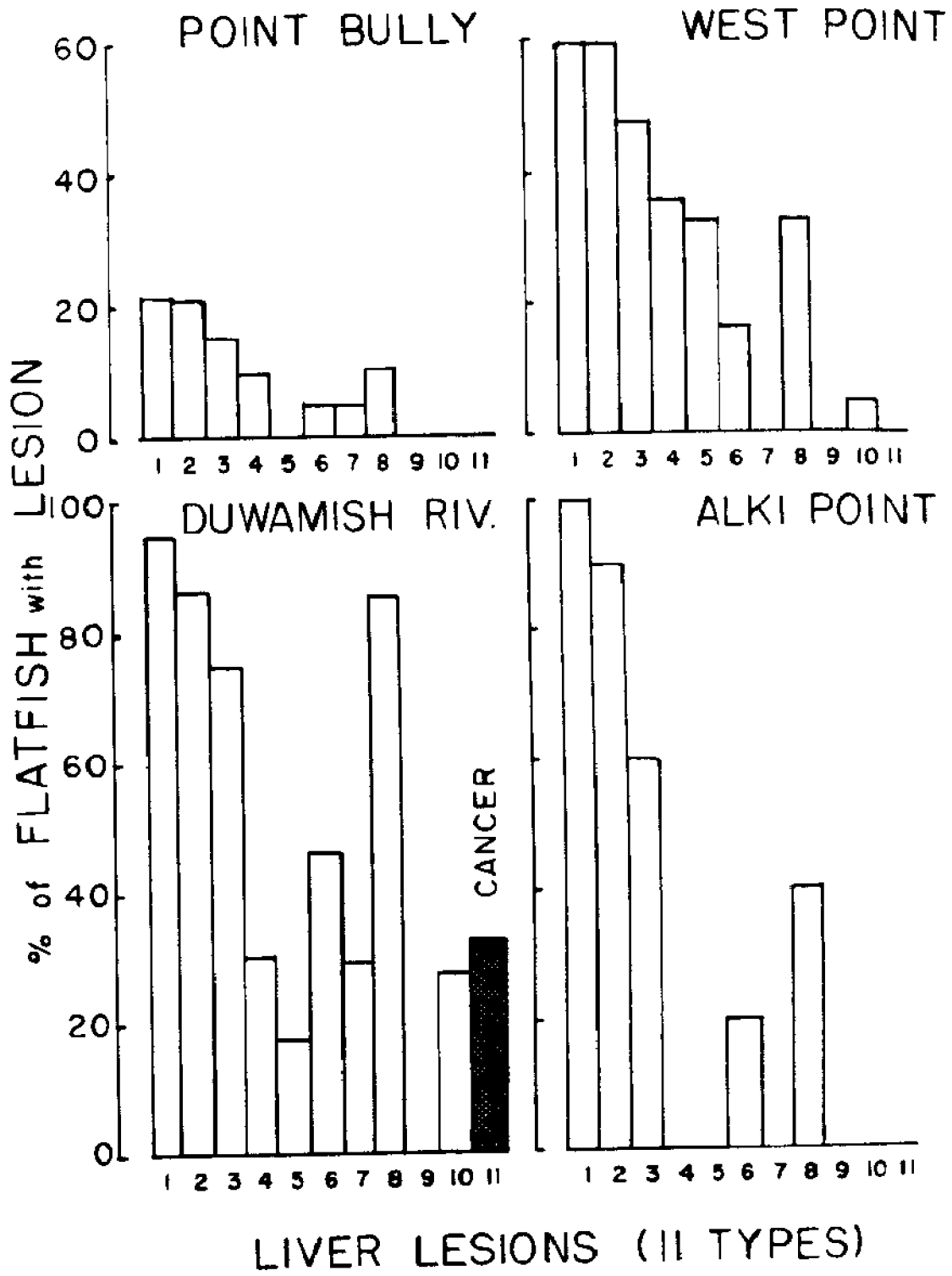

Figure 6. Percentages of English sole with different types of liver lesions from the four sample stations in Figure 5. Each number from 1 to 11 represents a different liver lesion (e.g., 1 is fatty liver, 11 is liver cancer). (Redrawn from Pierce et al. 1978)

malities in the polluted Duwamish River (Pierce et al. 1978) had only a 0.5% incidence of fin erosion while as many as 60% of all stargazers (Uranoscopus japonicus) from Suruga Bay, Japan, had eroded fins (Nakai et al. 1973). Bascom (1982) rejects bacteria, abrasion, macroparasites, fin nipping, H_2S in interstitial water and high metal concentrations as causes of fin erosion and suggests a principal chemical cause. This favoring of a principal chemical cause is also put forth by Sindermann (1979). And Wellings et al. (1976) also suggest "that the incidence of fin erosion in a particular population is related to an interaction between genetic constitution and multiple environmental variables, including a variety of chemical pollutants." Thus, the specific cause of the best known pollutant-associated fish disease remains unknown.

Hypothetical Correlations of Lesions and Pollution

Before specific chemicals can be implicated as causative agents of fish lesions (question 4), a positive correlation between incidence of a lesion and incidence of anthropogenic pollution must be established. Figure 7 illustrates four potential hypothetical relationships that could exist between lesion occurrence in a fish population and a site of pollution. There are, of course, many ways to plot the data. The curves presented do not allow for patchiness and water born pollution probably would not emanate from a city in a symmetrical fashion. Nevertheless, Figure 7 demonstrates that pollution can have opposite effects on disease incidence. In case A, disease in a given species exhibits no change in incidence among individuals in the polluted region; a possible example could be the case of the anisakine roundworm infection of fishes off California, where visceral tissue of 41.6% of the fish examined (2268 fish, 68 species) contained worms (Dailey et al. 1980). In case B the incidence of disease decreases among individuals in a polluted area; this could be interpreted as the additional stress of the degraded environment killing off many of the diseased animals. In case C, the incidence of disease increases in a polluted area, where the immune system of normally uninfected individuals may be repressed in the polluted area so that a larger percentage of individuals contract the disease. Finally, in case D, the disease is found only in fish inhabiting a polluted area, with fin erosion apparently one of the few known examples. Relationships among most of the diseases of wild fish and environmental contamination with chemicals are largely unknown. To establish such a correlation, the pollution source must be well within the range of fish sampling sites and must include appropriate control sites. Furthermore, fish must not migrate among the sites. In addition, the fish sampling sites should serve as chemical sampling sites, where levels in tissue, sediment and water of indicator pollutants are measured. Ideally, pathology and chemistry are done in the same laboratory.

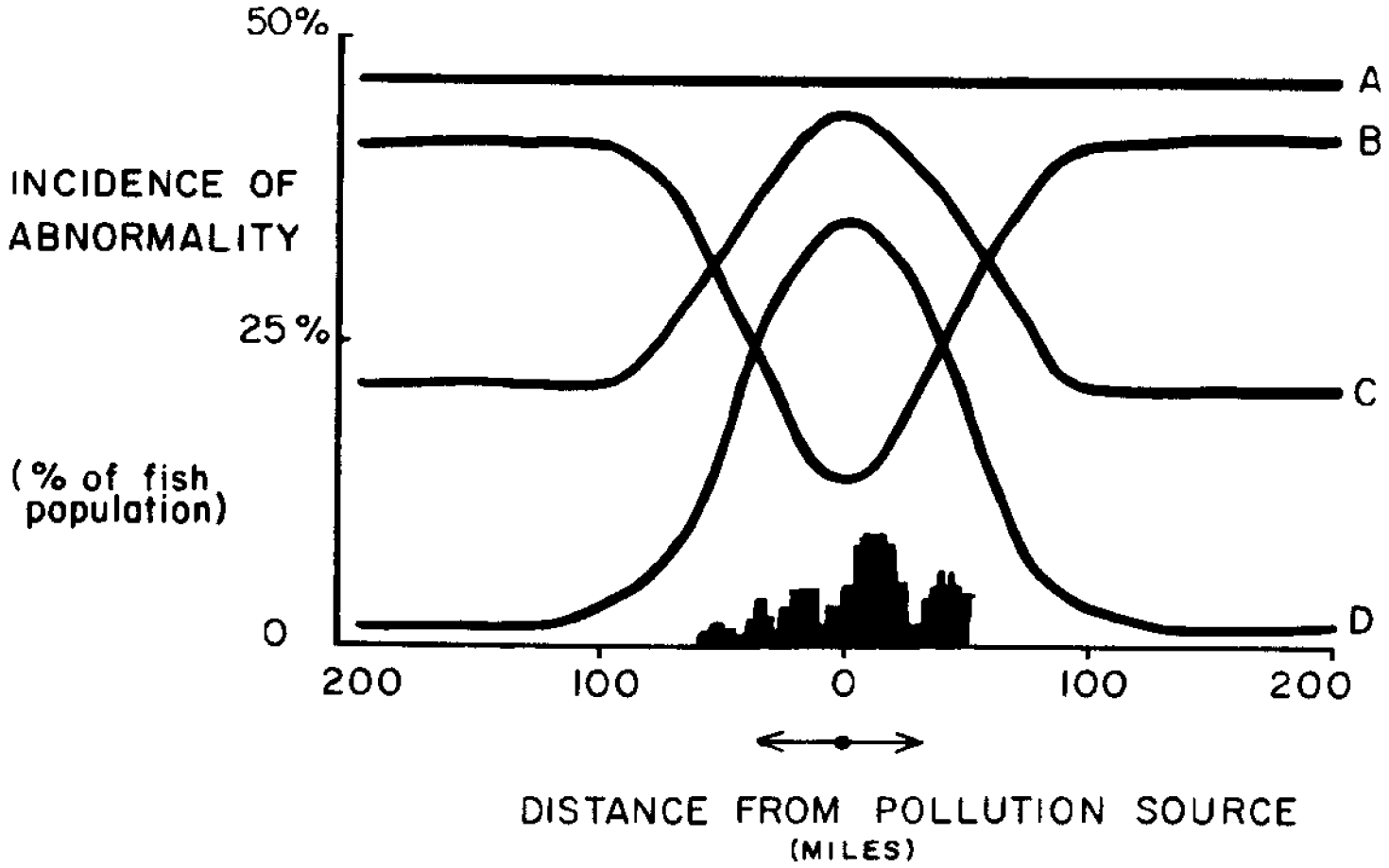

Figure 7. Four hypothetical relationships between disease incidence in marine fish and a site of chronic anthropogenic pollution. (A) No change in disease incidence with pollution. (B) Negative correlation--disease incidence decreases near pollution. (C) Positive correlation--widespread disease increases in incidence near pollution. (D) Positive correlation--disease and pollution occur together.

PATHOLOGIES INDUCED IN FISH BY CHEMICALS IN THE LABORATORY

Interspecies differences in the metabolism of xenobiotics can be dramatic. Figure 8 summarizes results of a PCB bioaccumulation study in which the same five PCB mixtures were fed to different vertebrates and each species accumulated a different amount of isomers (Sparling and Safe 1980).

Some species show no reaction to doses of toxicants that are lethal for other species. When a chemical causes a lesion in a species, the question to be asked is whether that lesion is specific to the chemical. There have been many laboratory studies of chemically-induced histopathological changes in fish. The impact of petroleum exposure on fish has received attention, and such studies illustrate the difficulty in gaining an understanding of pollution effects when a polluting material contains thousands of different chemicals (Malins and Hodgins 1981; Malins 1982). Often the relevance of these studies to the field are questionable because the doses of toxicants employed were higher than most feral fish ever encounter. Single chemicals are often used when, in the environment, pollution is usually a multi-chemical bombardment. Also, fish avoidance behavior can be blocked by confinement. Whether relevant in the field or not, laboratory exposure studies have clearly shown that many chemical agents may cause similar tissue pathologies. This apparent difficulty in using tissue responses to specific chemicals may be overcome, perhaps by relying on syndromes of responses and not on single, limited effects. Responses obtained in multiple end point studies can then lead to a holistic linking of cause and effect, in certain cases (Couch et al. 1977).

Fatty Liver

Known causes of triglyceride accumulation in liver (fatty liver) are many and these include starvation, low protein diet, high fat diet, hormone imbalance, anoxic factors, bacterial toxins and chemical poisons (Popper and Shaffner 1957; Alpers and Isselbacher 1975). The best studied of the chemical poisons are carbon tetrachloride, chloroform, phosphorus and trinitrotoluene. Agents that cause fatty liver in captive fish include Denison tags (Saddler and Cardwell 1971), rancid fat (Smith 1979), hydrogenated fat (Cowey et al. 1976), 1 ppb PCB in the diet (Jensen et al. 1970) and crude oil in aquarium sediments (700 $\mu g\ g^{-1}$ dry sediment) (McCain et al. 1978). In an extensive review of the effects of pesticides on livers of fishes, Couch (1975) reported that the most commonly encountered liver lesion was abnormal fatty accumulation. Thus, fatty liver is a useful, nonspecific indicator of stress in fish, but it is a questionable indicator of the presence of a specific chemical. The ultrastructure of fish liver cells is nearly identical to that of mammals (Welsch and Storch 1973; Peute et al. 1978; Schoor and Couch

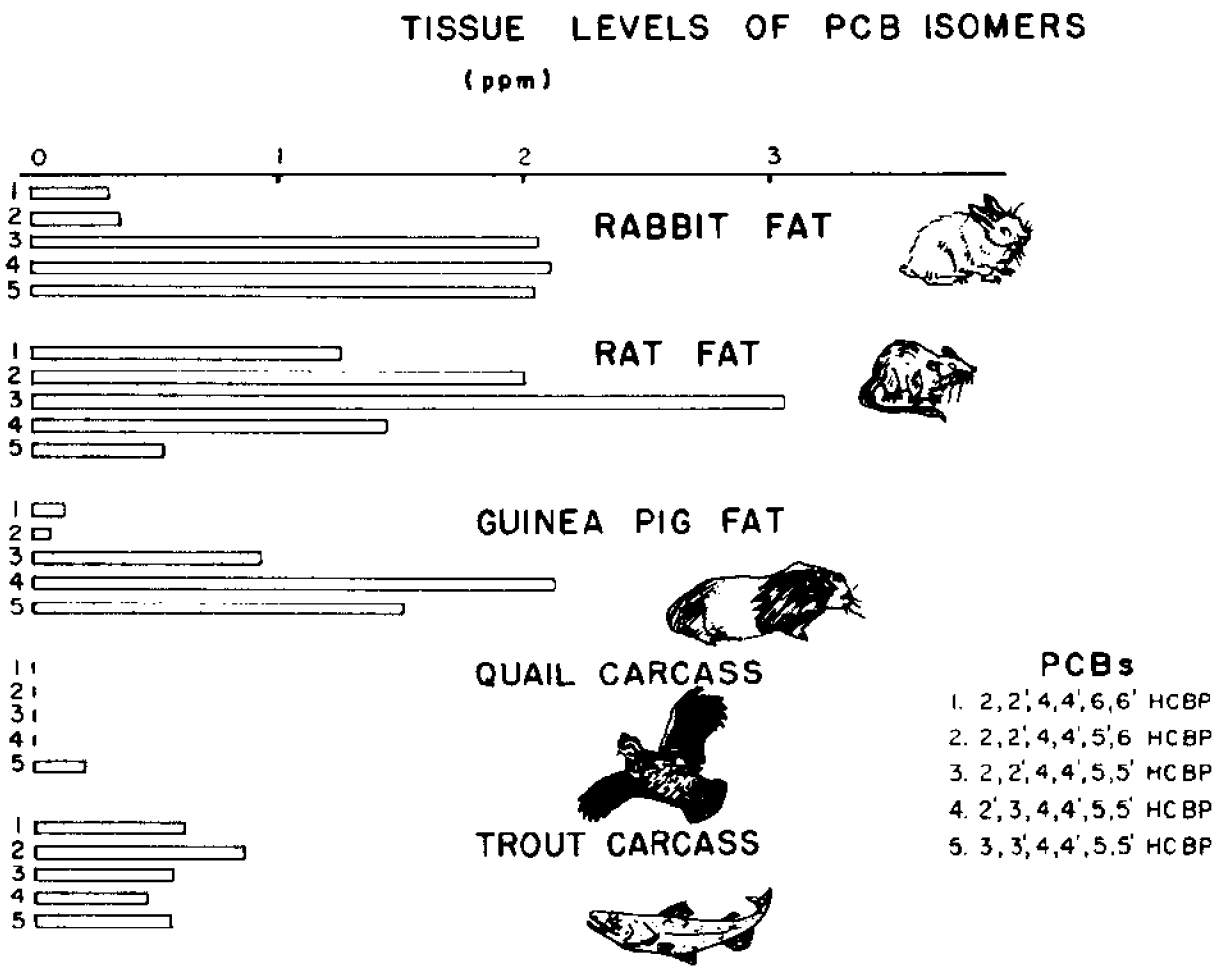

Figure 8. The concentration of PCB isomers in five animal species 29 days after a single feeding of an equimolar mixture of the isomers (5 mg kg^{-1}). Only fatty tissue was examined in the rat, guinea pig, and rabbit experiments. Whole carcasses were used in the trout and quail experiments. (From Sparling and Safe 1980)

1979). Some species, however, store reserve fat in their livers naturally (45%-67%) (Aure 1967). These species have lean flesh and are generally sluggish bottom dwellers, such as cod, snapper, haddock, perch and whiting. Other species store fat in muscle and possess lean livers identical to mammals (3%-5%), for example, salmon, mackerel, herring, shad, sturgeon, and tuna (Bilinski 1979; Welsch and Storch 1973; Bishop et al. 1976). Thus, what may be a pathological condition in one species can be normal in another. For a more complete discussion of hepatic toxicology in fish, see the excellent review by Gingerich (1982).

Gill Hyperplasia

Another nonspecific chemically induced lesion in fish is gill hyperplasia and hypertrophy which can lead to fusion of gill lamellae. Solangi and Overstreet (1982) found that two estuarine fish exposed to south Louisiana whole crude oil and/or its water soluble fractions developed gill epithelial hyperplasia. They noted, however, that similar lesions occur frequently in a variety of fishes exposed to various irritants such as heavy metals, pesticides, insecticides, ammonia and suspended sediments (Eller 1975; Smith and Piper 1975; Walsh and Ribelin 1975; Hodgins et al. 1977; and O'Connor et al. 1977).

Hepatoma (Liver Cancer)

Fish appear to be susceptible to all the types of cancers that humans get, and metastasis (spreading of the tumor to other tissues) appears to take place, although somewhat more slowly than in homeotherms (Lucke and Schlumberger 1949; Ashley 1969). Hepatoma formation has been induced in several teleost species by exposure to known hepatocarcinogens, including dimethyl-nitrosamine, acetylaminofluorene, urethan, thiourea, p-dimethylaminoazobenzene (Ashley 1969; Dollar et al. 1967; and Halver 1967). Hepatoma formation in hatchery-raised rainbow trout caused by contamination of their food supply with aflatoxin has been well documented (Yasutake and Rucker 1967; Ashley 1967; Dollar et al. 1967; Hendricks 1981). In addition to the study by Pierce et al. (1978) cited above, liver neoplasia has been found in feral hagfish (Falkmer et al. 1976, 1977) and feral white suckers (Dawe et al. 1964). Thus, like other nonspecific lesions cited above, i.e., fatty liver and gill hyperplasia, liver cancer occurs in wild fish and a variety of agents may cause it.

Inclusion Bodies

When metals and organics are bioaccumulated, they can be stored in accumulation or inclusion bodies that may be at least diagnostic of classes of toxicants if not specific chemicals. However, a harmful effect is not necessarily indicated. Lead, gold, iron, bismuth, uranium, beryllium, mercury, copper and arsenic are

a few of the metals that can be deposited intracellularly (Sorenson and Smith 1981). Intranuclear arsenic inclusions have been observed in hepatocytes of the green sunfish exposed to arsenic but not in channel catfish (Sorenson and Smith 1981). In the channel catfish 15 ppm arsenic exposure for 6 months caused a significant increase in the number and size of liver hemosiderin granules. Presumably these iron rich particles resulted from arsenic-induced erythrocyte lysis. Electron probe X-ray analysis data showed that about 30% of the hemosiderin granules contained small amounts of arsenic (Sorenson and Smith 1981). Thus, arsenic is related to the presence of different inclusion bodies (lesions) in different fish species and only in the green sunfish, Lepomis cyanellus, is the lesion an arsenic rich inclusion body.

Hawkes et al. (1980) examined the effects of petroleum hydrocarbons and chlorinated biphenyls on the morphology of Chinook salmon intestine. They found that after dietary exposure to petroleum hydrocarbons, the intestines contained inclusion bodies morphologically distinct from inclusion bodies that occurred when chlorinated biphenyls were fed to the fish. Unfortunately the composition of the inclusion bodies was not determined.

One of the best known of the chemically induced inclusion bodies in mammals is the "membrane whorl" or multilamellar body (Figure 9) that occurs in liver and other tissues. Recent electron micrographs of tissue from rats exposed to DDT and PCB show that these whorls are endocytotically expelled from the hepatocyte into the bile canaliculi (Jonsson et al. 1981). This is the most convincing evidence to date supporting the hypothesis that membrane whorls may be one vehicle whereby cells discharge specific hepatotoxic substances. More than 30 amphiphilic cationic drugs cause membrane whorls in mammals (Lüllman et al. 1978), and these lysosomally derived bodies appear to be the major site of toxicant accumulation (Matsuzawa and Hostetler 1980). Proliferation of rough endoplasmic reticulum occurs in fish liver following exposure to arsenic (Sorenson et al. 1980), petroleum (McCain et al. 1978) and benzo(a)pyrene (Schoor and Couch 1979). And proliferation of smooth endoplasmic reticulum occurs in Chinook salmon liver following dietary exposure to 5 ppm chlorobiphenyls for 28 days (Hawkes 1980). Characteristic ER whorls (myelin figures) also occur in hepatopancreatic cells of shrimp exposed to the PCB, aroclor 1254 (Couch and Nimmo 1974). Indeed, these findings of PCB effects on the ultrastructure of cells in an invertebrate (shrimp) anticipated the later findings in fishes cited above. Unfortunately membrane whorls have not been isolated from toxicant-exposed fish and compositions of the whorls determined. They appear, however, to be lesions suitable for further study since they may contain the causative chemical agent. It is not suggested, however, that these whorls necessarily constitute a harmful effect.

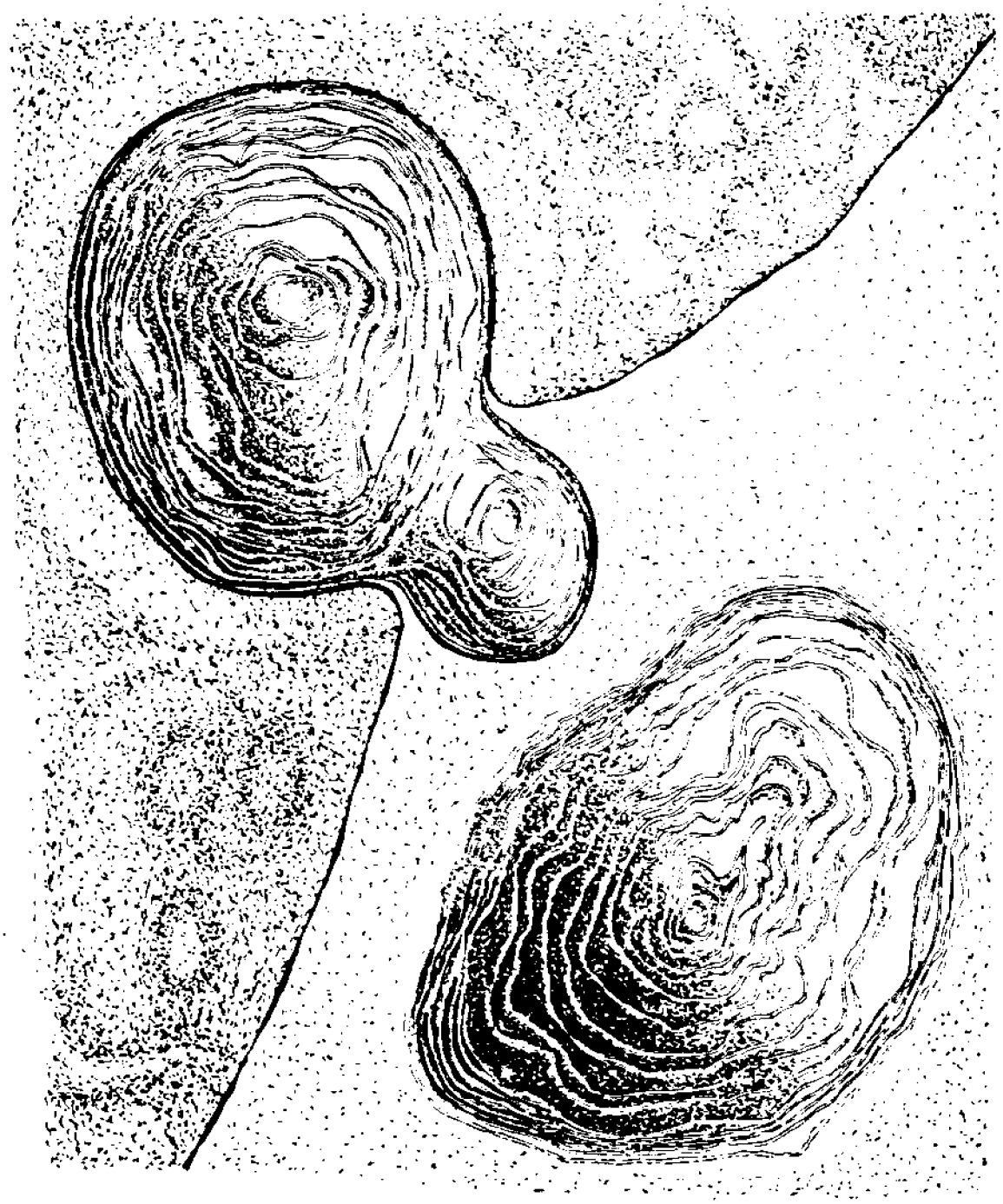

Figure 9. A liver membrane whorl (induced by DDT and PCB) that is undergoing exocytosis into the bile canaliculus. (Drawn from Jonsson et al. 1981)

From laboratory studies it can be concluded that many nonspecific lesions occur in organisms in response to environmental chemicals and few, if any, lesions have been proven to be toxicant specific. Isolation and chemical characterization of inclusion body lesions in wild fish may be the best means of determining causes of abnormalities in field specimens.

SCOLIOSIS, KEPONE AND THE JAMES RIVER FISH KILLS—COMBINED FIELD AND LABORATORY STUDIES

The following case study clearly illustrates the usefulness of experimental pathology in determining the probable cause of an abnormality in field specimens. In the spring of 1973 and 1974, massive kills of bluefish, spot and croaker entering the James River from the ocean occurred. R.J. Huggett (personal communication) observed that when sewage chlorinators draining into the river were turned off, the fish kills ceased. When they were turned on again, the fish kills occurred. Many of the species that were killed had broken backs (Figure 10C). It was then demonstrated in laboratory studies that chlorine could kill juvenile croaker, though no broken backs were observed in the chlorine-killed fish. Recently, Couch (1982) at the U.S. EPA Laboratory examined specimens of Cyprinodon variegatus exposed to chlorine (sodium hypochlorite) in laboratory toxicity tests. Fish that died after exposure to lethal concentration to chlorine showed no vertebral damage (Figure 10A) nor did fish swimming in water containing sublethal concentrations. Couch believes that the evidence strongly suggests that Kepone, alone, is a sufficiently specific toxic chemical to elicit the vertebral effect. Specimens of dead fish with broken backs taken from the James River were preserved in alcohol and stored. In 1973-74 the discharge of Kepone into the James River had not been thoroughly investigated. In 1976, Couch studied in the laboratory the effect of Kepone on the sheepshead minnow and found among other observations that Kepone caused scoliosis, a lateral deviation of the spine; at high doses Kepone caused the back to break during tetanic convulsions (Couch et al. 1977). This breakage was established as a dose dependent, time dependent, predictable response. The spinal break occurred (Figure 10B) after exposure of fish to parts per billion concentrations of Kepone. Subsequently, the James River specimens that had been stored in alcohol for 3-4 years were analyzed for Kepone, and the fish muscle and preservative alcohol were found to contain parts per million concentrations of Kepone. Scoliosis has been observed in a number of the fish species as a result of parasitic infections, dietary deficiencies, exposure to heavy metal and after organochlorine, organophosphate, and carbonate poisoning (Couch et al. 1979). What killed the James River fish? Probably a combination of events and causes, with Kepone poisoning a major factor. Perhaps other factors, such

Figure 10. (A) Original X-ray of several Cyprinodon variegatus exposed to 89 µg/L chlorine (sodium hypochlorite). Exposure was lethal to fish; however X-ray shows no vertebral breaks or other abnormalities. (B) Original X-ray of a bluefish collected from the James River in 1974. Note break of vertebrae column. Concentrations of 2.8 to 4.8 mg kg^{-1} were found in bluefish tissue samples analyzed in 1977. (C) Whole longitudinal horizontal section of Cyprinodon variegatus exposed to Kepone. Fish had advanced scoliosis; note fracture in spine.

as chlorine poisoning, also acted to trigger the lethal events. The James River is polluted with many chemicals, as are many rivers and estuaries in the United States. Sorting out causes of anomalies in field specimens thus remains a challenge.

CONCLUSIONS

1. Severe pathologies occur in some marine fish populations, sometimes tentatively associated with pollution, but usually the association is tenuous and difficult to draw conclusively.
2. In almost all cases the etiologies of the lesions, including cancer, are unknown because nonspecific lesions normally occur, field specimens are collected from regions that are contaminated by many different chemicals, and the site of exposure of mobile species is often unknown.
3. The type of lesion offering the best potential diagnosis is the nonspecific inclusion body type of lesion which may contain the toxicant eliciting its formation.

RECOMMENDATIONS

If an anomaly in a field specimen is thought to be caused by a specific toxicant, it is necessary to:

1. Establish a strong correlation between pathology in the fish in the wild and degree of anthropogenic pollution.
2. A test species from the pollution region must be found that survives the highest pollutant concentration, exhibits the severest pathologies (e.g., cancer), and can be cultured in aquaria or pools.

   The best test species appear to be the flatfish, which are easy to maintain in aquaria and often enter the most polluted environments where they are in direct contact with the sediment and feed on contaminated benthic organisms. Although flatfish often do not die immediately or become repelled by heavy pollution, their tissues, particularly liver, appear to be quite sensitive to pollution stress. Thus, flatfish represent an underutilized organism with significance in the field.

   The approach would be to introduce the test species (caged or tagged) into a polluted region and collect and examine specimens periodically for progressive pathological changes, and to induce the pathology in laboratory tests

employing sediment or food from the polluted area. The sediment or food can be fractionated and the agent causing the lesion isolated and tested in a bioassay.

This approach has been successfully applied by Ishio et al. (1972 a, b) to isolate the cause of tumors in the commercially important marine alga Porphyra tenura in Japan. In bioassays carried out with the alga over a 38-day period, they isolated two chemical carcinogens from sea bottom muds. These carcinogens caused algal abnormalities and were subsequently traced to a coal chemical industry. Thus, although there are undoubtedly additive and synergistic factors involved in any pollution-induced lesion, there may be only a few specific toxicants that act as primary agents in the etiology of a given lesion. This study by Ishio et al. (1972 a, b) shows that the cause of tumors in a marine plant population can indeed be traced to specific chemicals in the environment of that plant, a useful model for similar studies in the future.

ACKNOWLEDGMENT

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REFERENCES

Alpers, D.A. and K.J. Isselbacheer. 1975. Fatty liver: Biochemical and clinical aspects. In: L. Schiff (ed.), Diseases of the liver, pp. 515-832. Lippincott, New York.

Ashley, L.A. 1967. Histopathology of rainbow trout aflatoxicostsis. In: J.E. Halver and I.A. Mitchell (eds.), Trout Hepatoma Research Conference Papers, pp. 103-120. Res. Rep. 70, Publ. No. 15, U.S. Dept. Health, Education and Welfare, U.S. Dept. Interior. U.S. Government Printing Office, Washington, D.C.

Ashley, L.A. 1969. Experimental fish neoplasia. In: O.W. Neuhaus and J.E. Halver (eds.), Fish in Research, pp. 45-86. Academic Press, New York.

Aure, L. 1967. Manufacture of fish-liver oil. In: M.E. Stansby (ed.), Fish Oils, pp. 193-205. Avi Publ., Westport, Conn.

Bascom, W. 1982. The effects of waste disposal on the coastal waters of Southern California. Environ. Sci. Technol. 18: 226A-236A.

Bilinski, E. 1969. Lipid catabolism in fish muscle. In: O.W. Neuhaus and J.E. Halver (eds.), Fish in Research, pp. 135-147. Academic Press, New York.

Bishop, P.G., D.G. Jones and J. Olleg. 1976. Lipid composition of slender tuna as related to lipid composition of their feed. J. Fish. Res. Board Can. 33: 1156-1161.

Carmody, D.J., J.B. Pearce and W.E. Yasso. 1973. Trace metals in sediments of New York Bight. Mar. Pollut. Bull. 4: 132-135.

Couch, J.A. and D.R. Nimmo. 1974. Ultrastructural studies of shrimp exposed to the pollutant chemical polychlorinated biphenyl (Aroclor 1254). Bull. Soc. Pharm. Environ. Pathol. 1: 17-20.

Couch, J.A. and D.R. Nimmo. 1975. Detection of interactions between natural pathogens and pollutants in aquatic animals. In: R.L. Amborsk, M. Hood and R.R. Miller (eds.), Proceedings Gulf Coast Regional Symposium on Diseases of Aquatic Animals, pp. 261-268. Louisiana State Univ., Center for Wetland Resources, Baton Rouge, La.

Couch, J.A. 1975. Histopathological effects of pesticides and related chemicals on the livers of fishes. In: W.E. Ribelin and G. Migaki (eds.), The Pathology of Fishes, pp. 559-584. Univ. of Wisconsin Press, Madison, Wisc.

Couch, J.A., J.T. Winstead and L.R. Goodman. 1977. Kepone-induced scoliosis and its histological consequences in fish. Science 197: 585-587.

Couch, J.A., J.T. Winstead, D.J. Hansen and L.R. Goodman. 1979. Vertebral dysplasis in young fish exposed to the herbicide trifluralin. J. Fish. Dis. 2: 35-42.

Cowey, C.B., J.W. Adron, J.M. Owen and R.J. Roberts. 1976. The effect of different dietary oils on tissue fatty acids and tissue pathology in turbat Scophthalmus maximus. Comp. Biochem. Physiol. 53B: 399-403.

Dailey, M.D., O.A. Jensen and B. Westerhof-Hill. 1980. Larval anisakine round worms of marine fishes from southern and central California, with comments on public health significance. Calif. Fish Game 67: 240-245.

Dawe, C.J., M.F. Stanton and F.J. Scwartz. 1964. Hepatic neoplasms in native bottom-feeding fish of Deep Creek Lake, Maryland. Cancer Res. 24: 1194-1199.

Dawe, C.J., J. Bagshaw and C.M. Poore. 1979. Amebic pseudobranches of pacific cod, Gadus macrocephalus. Proc. Amer. Assoc. Cancer Res. 20: 245-253.

Dollar, A.M., F.A. Smuckler and R.C. Simon. 1967. Etiology and epidemiology of trout hepatoma. In: J.E. Halver and I.A. Mitchell (eds.), Trout Hepatoma Research Conference Papers, pp. 1-17. Res. Rep. 70, Publ. No. 15, U.S. Dept. Health, Education and Welfare and U.S. Dept. Interior. U.S. Government Printing Office, Washington, D.C.

Eller, L.L. 1975. Gill Lesions in freshwater teleosts. In: W.E. Ribelin and G. Migaki (eds.), The Pathology of Fishes, pp. 305-330. Univ. of Wisconsin Press, Madison, Wisc.

Falkmer, S., S.O. Emdin, Y. Ostberg, A. Mattisson, M.L. Johansson Sjöbeck and R. Faüge. 1976. Tumor pathology of the hagfish Myxine glutinosa and the river lamprey, Lampetra flaviatilis. Prog. Exp. Tumor Res. 20: 217-250.

Falkmer, S., S Markland, PE. Mattsson and C. Rappe. 1977. Hepatomas and other neoplasms in the Atlantic hagfish: A histopathologic and chemical study. Ann. N.Y. Acad. Sci. 298: 342-355.

Gingerich, W.H. 1982. Hepatic toxicity of fishes. In: L.J. Weber (ed.), Aquatic Toxicology, Vol. 1, pp. 55-105. Raven Press, New York.

Gordon, M. 1954. The genetics of fish diseases. Trans. Am. Fish. Soc. 83: 229-240.

Halver, J.E. 1967. Crystalline aflatoxin and other vectors for trout hepatoma. In: J.E. Halver and I.A. Mitchell (eds.), Trout Hepatoma Research Conference Papers, pp. 78-102. Res. Rep. 70, Publ. No. 15, U.S. Dept. Health, Education and Welfare and U.S. Dept. Interior. U.S. Government Printing Office, Washington, D.C.

Hawkes, J.W. 1980. The effects of xenobiotics on fish tissues: Morphological studies. Fed. Am. Soc. Exper. Biol. Proc. 39: 3230-3236.

Hawkes, J.W., E.H. Grager, Jr. and O.P. Olson. 1980. Effects of petroleum hydrocarbons and chlorinated biphenyls on the morphology of the intestine of Chinook salmon (Oncorhynchus tshawytscha). Environ. Res. 23: 149-161.

Hendricks, J. 1981. The use of rainbow trout in carcinogen bioassay, with special emphasis on embryo exposure. In: Proceedings of the 11th International Symposium of the Princess Takamatsu Cancer Research Fund, Japanese Scientific Society Press, Japan.

Hodgins, H.O., B.B. McCain and J.W. Hawkes. 1977. Marine fish and invertebrate diseases, host disease resistance, and pathological effect of petroleum. In: D.C. Malins (ed.), Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms, Vol. 11, pp. 95-173. Academic Press, New York.

Ishio, S., K. Kawabe and T. Tomiyama. 1972a. Algal cancer and its causes. I. Carcinogenic potencies of water and suspended solids discharged to the River Ohmuta. Bull. Jap. Soc. Sci. Fish. 38: 17-24.

Ishio, S., H. Nakagawa and T. Tomiyama. 1972b. Algal cancer and its causes. II. Separation of carcinogenic compounds from sea bottom and polluted by wastes of the coal chemical industry. Bull. Jap. Soc. Sci. Fish. 38: 571-576.

Jensen, S., N. Johansson and M. Olsen. 1970. PCB--indications of effects on salmon. Rep. LFI Meed, Swedish Salmon Research Inst., Sweden.

Jonsson, H.T., E.M. Walker, Jr., W.B. Greene, M.D. Hughson and G.R. Hennigar. 1981. Effects of prolonged exposure to dietary DDT and PCB on rat liver morphology. Arch. Environ. Contam. Toxicol. 10: 171-183.

Love, M., D. Teebken-Fisher, J.E. Hose, J.J. Farmer III, F.W. Hickman and G.R. Fanning. 1981. Vibrio damsela, a marine bacterium, causes skin ulcers on the damselfish, Chromis punctipinnis. Science 214: 1139-1140.

Lucke, B. and H.G. Schlumberger. 1949. Neoplasia in cold-blooded vertebrates. Physiol. Rev. 29: 91-126.

Lüllman, H., R. Lüllman-Ranch and O. Wasserman. 1978. Lipidosis induced by amphiphilic cationic drugs. Biochem. Pharmacol. 27: 1103-1108.

Mahoney, J.B., F.H. Midlige and D.G. Deuel. 1973. A fin rot disease of marine and euryhaline fishes in the New York Bight. Trans. Am. Fish. Soc. 102: 596-605.

Malins, D.C. 1982. Alterations in the cellular and subcellular structure of marine teleosts and invertebrates exposed to petroleum in the laboratory and field: A critical review. Can. J. Fish. Aquat. Sci. 39.

Malins, D.C. and H.O. Hodgins. 1981. Petroleum and marine fishes: A review of uptake, disposition and effects. Environ. Sci. Technol. 15: 1272-1280.

Matsuzawa, Y. and K.Y. Hostetler. 1980. Studies on drug-induced lipidosis: Subcellular localization of phospholipid and cholesterol in the liver of rats treated with chloroquine or 4,4'-bis (diethylaminoethoxy) α, ∞-diethyl-diphenylethane. J. Lipid Res. 21: 202-214.

McCain, B.B., H.O. Hodgins, W.D. Gronlund, J.W. Hawkes, D.W. Brown, M.S. Myers and J.H. Vandermenlen. 1978. Bioavailability of crude oil from experimentally oiled sediments to English sole (Parophrys vetulus), and pathological consequences. J. Fish. Res. Board Can. 35: 657-664.

McCain, B.B., W.D. Gronlund, M.S. Myers and S.R. Wellings. 1979. Tumors and microbial diseases of marine fishes in Alaskan waters. J. Fish. Dis. 2: 111-130.

Minchew, C.D. and J.D. Yarbrough. 1977. The occurence of fin rot in mullet (Mugil cephalus) associated with crude oil contamination of an estuarine pond-ecosystem. J. Fish Biol. 10: 319-323.

Nakai, Z., M. Kosaka, S. Kudoh, A. Nagai, F. Hayashida, T. Kubota, M. Ogura, T. Mizushima and I. Uotani. 1973. Summary report on marine biological studies of Suruga Bay accomplished by Tokai University 1964-'72. J. Fac. Mar. Sci. Technol. Tokai Univ. 7: 63-117.

O'Connell, C.P. 1980. Percentage of starving northern anchovy, Engralis mordax, larvae in the sea as estimated by histological methods. Fish. Bull. 78: 475-489.

O'Connor, J.M., D.A. Newmann and J.A. Sherk. 1977. Sublethal effects of suspended sediments on estuarine fish. Technical Paper No. 77-3, U.S. Army Corps of Engineers, Fort Belvoir, Va.

Peute, J., M.A. van der Gaag and J.G.D. Lambert. 1978. Ultrastructure and lipid content of the liver of the zebrafish, Brachydanio rerio, related to vitellogenin synthesis. Cell. Tiss. Res. 186: 297-308.

Pierce, K.V., B.B. McCain and S.R. Wellings. 1978. Pathology of hepatomas and other liver abnormalities in English sole (Parophrys vetulus) from the Duwamish River Estuary, Seattle, Washington. J. Nat. Cancer Inst. 60: 1445-1453.

Popper, H. and F. Schaffner. 1957. Liver: Structure and Function. McGraw-Hill. New York.

Saddler, J.B. and R. Cardwell. 1971. The effect of tagging upon the fatty acid metabolism of juvenile pink salmon. Comp. Biochem. Physiol. 39A: 709-721.

Schmidt, T.T., R.W. Risebrough and R. Gress. 1971. Input of polychlorinated biphenyls into California coastal waters from urban sewage outfalls. Bull. Environ. Contam. Toxicol. 6: 235-243.

Schoor, W.P. and J.A. Couch. 1979. Correlation of mixed-function oxidase activity with ultrastructural changes in the liver of a marine fish. Cancer Biochem. Biophys. 4: 95-103.

Sindermann, C.J. 1966. Diseases of marine fish. In: F.S. Russell (ed.), Advances in Marine Biology, Vol. 4, pp. 1-89. Academic Press, New York.

Sindermann, C.J. 1979. Pollution-associated diseases and abnormalities of fish and shellfish: A review. Fish. Bull. 76: 717-749.

Smith, C.E. 1979. The prevention of liver lipoid degeneration (ceroidosis) and microcytic anemia in rainbow trout, Salmo gaindneri Richardson, fed rancid diets--A preliminary report. J. Fish. Dis. 2: 249-437.

Smith, C.E. and R.G. Piper. 1975. Lesions associated with chronic exposure to ammonia. In: W.E. Ribelin and G. Migaki (eds.), The Pathology of Fishes, pp. 497-514. Univ. of Wisconsin Press, Madison, Wisc.

Solangi, M.A. and R.M. Overstreet. 1982. Histopathological changes in two estuarine fishes, Menida beryllina (Cope) and Trinectes maculatus (Bloch and Schneider), exposed to crude oil and its water-soluble fractions. J. Fish Diseases 5: 13-35.

Sorenson, E.M.B. and N.K.R. Smith. 1981. Hemosiderin granules: Cytotoxic response to arsenic exposure in channel catfish, Ictalurns punctatus. Bull. Environ. Contam. Toxicol. 27: 645-653.

Sorenson, E.M.B., R. Ramirez-Mitchell, C.W. Harlan and J.S. Bell. 1980. Cytological changes in the fish liver following chronic, environmental arsenic exposure. Bull. Environ. Contam. Toxicol. 25: 93-99.

Sparling, J. and S. Safe. 1980. The effects of ortho chloro substituents on the retention of PCB isomers in rat, rabbit, Japanese quail, guinea pig, and trout. Toxicol. Lett. 7: 23-28.

Stich, H.F., A.B. Acton, B.P. Dunn. K. Oishi, F. Yamazaki, T. Harada, G. Peters and N. Peters. 1977. Geographic variations in tumor prevalence among marine fish populations. Int. J. Cancer. 20: 780-791.

Templeman, W. and G.L. Andrews. 1956. Jellied condition in the American plaice Hippoglossoides platessoides. J. Fish. Res. Bd. Can. 13: 147-182.

Valentine, D.W. 1975. Skeletal anomalies in marine teleosts. In: W.E. Ribelin and G. Migaki (eds.), The Pathology of Fishes, pp. 695-718. Univ. of Wisconsin Press, Madison, Wisc.

Walsh, A.H. and W.E. Ribelin. 1975. The pathology of pesticide poisoning. In: W.E. Ribelin and G. Migaki (eds.), The Pathology of Fishes, pp. 515-558. Univ. of Wisconsin Press, Madison, Wisc.

Wellings, S.R., C.E. Alpers, B.B. McCain and B.S. Miller. 1976. Fin erosion diseases of starry founder (Platichthys stellatus) and English sole (Parophyrs vetulus) in the estuary of the Duwamish River, Seattle, Washington. J. Fish. Res. Board Canada 33: 2577-2586.

Welsch, U.N. and U.N. Storch. 1973. Enzyme histochemical and ultrastructural observations on the liver of teleost fishes. Arch Histol. Jap. 36: 21-37.

Yasutake, W.T. and R.R. Rucker. 1967. Nutritionally induced hepatomagenesis of rainbow trout (Salmo gairdneri). In: J.E. Halver and I.A. Mitchell (eds.), Trout Hepatoma Research Conference Papers, pp. 39-47. Res. Rep. 70, Publ. No. 15, U.S. Dept. Health, Education and Welfare and U.S. Dept. Interior. U.S. Government Printing Office, Washington, D.C.

Chapter 7. Mesocosms and Field Systems

Introduction: Ecology as an Experimental Science and Management Tool

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Mesocosms and field enclosures enable us to experiment with whole ecosystems. This approach provides a new dimension to marine ecology. Most field ecology has been descriptive. Much of the laboratory work has been directed to the study of single processes of single species. The best ecological mathematical simulation modeling has synthesized both types of information yet has remained descriptive rather than predictive. Ecology as an experimental predictive science is so new that we have much to learn. We are still dealing with basic questions such as how to formulate meaningful hypotheses, to state assumptions, to make use of controls and to know statistical requirements. All of these are the first steps of experimental science.

Whole ecosystem experiments have comprehensive applicability to environmental management and yet remain intellectually challenging to scientists. Experiments can be performed on routes, rates, metabolic products, reservoirs and effects of whatever pollutant is of interest to management: hydrocarbons, metals, toxic organics, sewage effluents, and so forth. The design of the whole ecosystem will test the experimenter's knowledge of natural systems. We were amazed at how well the Marine Ecosystems Research Laboratory (MERL) mesocosms duplicated the behavior of the natural system (Table 1, Figure 1). One of the main findings from examining a controlled system and comparing it with the natural system has been that the chemical functioning of a marine ecosystem is not dependent on and does not go through economically important large species such as bluefish. The mesocosm data from Figure 1 were derived from a sediment gradient experiment in which three tanks contained sediments from the polluted Providence River, three tanks contained sediments from measurably polluted mid-Narragansett Bay and three tanks contained sediments from relatively clean Rhode Island Sound. These data compare fa-

Table 1. Annual time-weighted means from three control mesocosms (C) and the lower West Passage (D). 1972-73 variables were averaged over Julian days 207-354 of 1972 and Julian days 5-198 of 1973. The 1976-77 variables were averaged over Julian days 229-363 of 1976 and Julian days 4-234 of 1977. The 1978 variables were averaged over Julian days 17-349 of 1978. 1978-79 variables were averaged over Julian days 227-353 of 1978 and Julian days 3-197 of 1979. The 1979 variables were averaged over Julian days 3-197 and 226-337.

Variable		1972-73	1976-77	1978	1978-79	1979	$\bar{x} \pm s$	C.V.
C-14	C	-	84	90	109	102	96 ± 11	11%
$g\ m^{-2}\ yr^{-1}$	D	-	-	145	129	97	123 ± 24	20%
Chla	C	-	6.3	3.1	4.1	4.5	4.5 ± 1.3	28%
$\mu g\ L^{-1}$	D	6.2	9.4	4.5	4.0	2.5	5.3 ± 2.6	49%
NH_3	C	-	2.4	1.2	1.2	1.1	1.5 ± .6	40%
$\mu g\ L^{-1}$	D	2.3	1.6	1.7	1.8	3.0	2.1 ± .6	29%
NO_2+NO_3	C	-	1.3	1.3	1.2	1.4	1.3 ± .1	6%
$\mu g\ L^{-1}$	D	3.3	1.6	2.3	2.3	4.1	2.7 ± 1.0	37%

PO_4	C	-	1.3	0.8	0.9	1.0	1.0 ± .2	20%
$\mu g\ L^{-1}$	D	1.1	1.2	1.3	1.2	1.4	1.2 ± .1	8%
SiO_4	C	-	9.4	9.8	10.3	7.1	9.2 ± 1.4	15%
$\mu g\ L^{-1}$	D	10.7	7.5	7.5	7.4	10.5	8.7 ± 1.7	20%
Zoopl. bio	C	-	35	45	34	25	35 ± 8	23%
$mg\ m^{-3}$	D	-	70	48	36	26	60 ± 38	63%
Zoopl. nos	C	-	33,500	29,600	28,600	27,600	29,800 ± 2,600	9%
$no\ m^{-3}$	D	-	30,000	17,900	13,200	12,500	18,400 ± 8,100	44%
Acartia tonsa	C	-	2,200	1,200	1,700	2,500	1,900 ± 570	30%
$no\ m^{-3}$	C	-	4,700	1,200	900	1,400	2,100 ± 1,800	86%
Acartia clausi	D	-	3,000	8,700	4,800	4,000	5,100 ± 2,500	49%
$no\ m^{-3}$	D	-	12,900	8,600	6,300	5,900	8,400 ± 3,200	38%

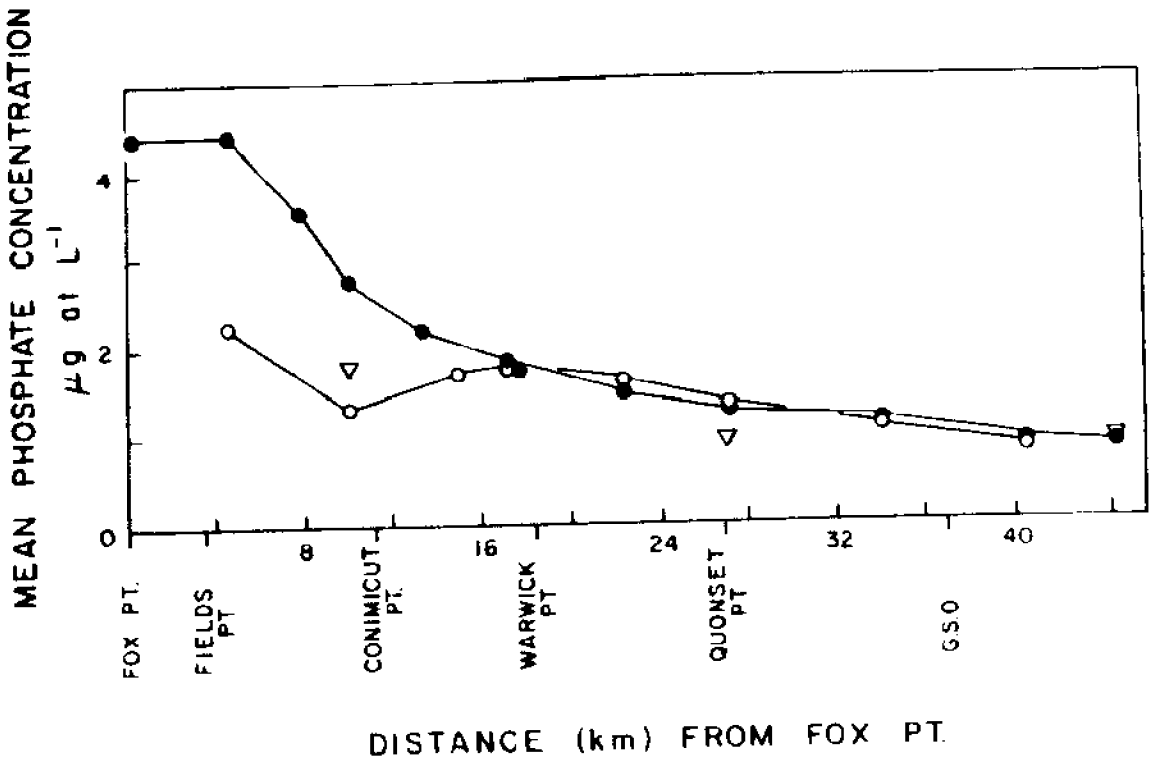

Figure 1. Time weighted annual means of phosphate concentration collected biweekly from surface and bottom water averaged in a 1972-73 survey (o) and a 1979-80 survey (●) in a transect from the upper Providence River down the West Passage of Narragansett Bay to Rhode Island Sound. The differences between the two years in the Providence River can be explained by greater rainfall and flushing in the earlier survey year. Time weighted annual means of phosphate concentration collected weekly in mesocosms (mean of 3 in each treatment) (▽) containing sediment from the Providence River, mid-Bay and Rhode Island Sound are plotted for comparison.

vorably with two field survey data sets. In a nutrient addition experiment which started in June 1981, three tanks acted as controls and six tanks received increased levels of nutrients in sewage derived ratios. The nutrient inputs spanned the known range of sewage nutrient loading to estuaries from clean lower Narragansett Bay to the inner New York Bight. A strength of these gradient approaches is that we can see where compounds will go and how different processes occur under different environmental conditions. With all perturbants we can observe how systems will redesign themselves to accommodate new substances. In the same manner physical parameters and ecosystem components can be manipulated to change food chain dynamics to answer, for example, questions on fish larval survival and recruitment. Interactive laboratory experiments (crossover experiments, diel studies, bioassays) inform us of detailed mechanisms of change. For example, how do pH changes along a eutrophication gradient affect copper speciation and therefore toxicity to phytoplankton? Field studies inform us of the predictability of mesocosm results. (For example, under eutrophic conditions those same pH changes have been observed in the field). This interactive approach among laboratory, mesocosm and field ecosystem experiments will simultaneously provide management with estimates of "assimilative capacity," and permit scientists to investigate "ecosystem behavior."

The science challenges experimenters to integrate results. The ecosystem experiment by its nature provides integrated answers which no one scientific discipline can reveal. Specialists in many aspects of ecology, biogeochemistry and engineering must work together to reveal those answers. This requires a discipline foreign to the reductive mind. Also one group such as engineering must not dominate over scientific output. Thus the interdisciplinary team that designs, operates and maintains the ecosystems, conducts the experiments and writes the papers must work together and be stable over long periods of time. In this context, whole ecosystem experiments provide integrated answers to problems in environmental management.

ADVANTAGES AND DISADVANTAGES

While there are a number of advantages of ecosystem experiments, attention is often directed at disadvantages. Among the latter are high cost, lack of higher trophic levels, wall effects, field validation requirements, results not of public concern and the fact that complexity obscures causal relationships (Table 2).

Neither initial nor running costs are high compared with other field and laboratory studies. For example, in comparison with laboratory studies the cost to buy and operate a gas chromatograph or a nutrient autoanalyzer is the same. In comparison with field

Table 2. The perceived disadvantages and advantages of using experimental ecosystems to answer questions for environmental management.

A.	Disadvantage
	Initial capital costs are high.
	Need stable funding to maintain multidisciplinary team of scientists.
	Large animals are too scarce to sample routinely.
	Wall effects.
	Horizontal advection is ignored.
	Field validation is a requirement.
	Time to produce integrated papers is long.
	Experimental results cannot be extrapolated to areas of public concern: commercial species, aesthetics, human health.
	Complexity obscures causal relationships.
B.	Advantages
	Inexpensive to operate.
	Simple engineering; flexible design; physical boundaries and parameters defined.
	Biogeochemical complexity of the natural system and natural system behavior.
	Multidisciplinary.
	Simple sampling logistics.
	Biogeochemical budgeting ability.
	The same populations can be re-sampled repeatedly over annual cycles.
	Crossover experiments, diel studies, tracer studies and similar add-ons multiply information gained.
	Results can be integrated.
	Experimental results are extrapolatable to the natural systems.
	Field validation tool.
	Experimental science.

studies, the cost to manage and operate a mesocosm facility such as MERL is $500 a day compared with $6000–$7000 a day for ship-time. Small boat operations cost about the same as MERL but the potential sampling intensity is much greater in mesocosms.

The investigation of higher trophic levels in mesocosms is a lively area of research. The Controlled Ecosystem Populations (formerly Pollution) Experiment (CEPEX) bags were large enough to conduct fish larval studies. At MERL larger animals have been placed in the tanks for short periods of time, and tank effluents

have been used in downstream studies of fish and larval growth. Large predatory animals such as a solitary crab or moon snail in the tanks have been controlled. However, ctenophores, juvenile fish and benthic infaunal predators are simply monitored or enumerated at the end of experiments.

Field validation of mesocosms is required and very informative (Table 1, Figure 1). First, the behavior of control mesocosms can be shown to be within the range of variability of the natural system over the annual cycle for all parameters of interest: productivity, zooplankton, nutrient levels, benthic fauna, respiration and so forth. Second, the results from perturbation experiments can be compared with laboratory and field studies to prove causal relationships and predictive capability. These comparisons demonstrate credibility, reliability, replicability and predictability.

In discussing disadvantages many advantages have revealed themselves. One is the fact that simple, flexible engineering designed with defined physical boundaries and parameters can develop into complex living models of natural systems. In these systems substances of interest can be budgeted, the same water column and benthic population can be sampled for 2 years or more and the data can be used to validate field and laboratory experiments and mathematical simulation models. The environmental gradient experiments can be used to generate key rate terms or functions for mathematical models of different kinds of environmental conditions. Conceptual and mathematical models should be tied to mesocosm definition of processes. If the processes are unknown, and if the relationships between major components of the ecosystem are unknown, it is impossible to develop a model.

Mesocosms are an integrative tool in and of themselves and provide integrated answers for information on indirect effects. Most of the scientific community still has difficulty trying to address the regulator's need. The fact that the mesocosms exist and interdisciplinary teams are there compels a degree of integration that would not otherwise exist. Environmental gradient experiments define assimilative capacity and can be used to develop marine dumpsite criteria. These experiments avoid the confrontation situation--all-or-nothing effects. They forecast at what levels effects are likely to begin for different environmental conditions.

APPLICATIONS: MESOCOSMS AND FIELD SYSTEMS

The use of enclosures for tracer studies has applicability to both aquatic and estuarine systems, as Santschi's and Pilson's papers indicate in this section. In lakes, tracer studies have been conducted to study questions related to eutrophication, acid rain, industrial and power plant emission and release from fossil fuel combustion. At MERL tracer studies have been conducted on

about 30 metal isotopes and C-14 labeled organic compounds: benzanthracene, dimethybenzanthracene, pentachlorophenol and toluene. Both groups of studies indicate that the enclosure technology is adequate to answer many questions for management, such as particle and non-particle transport rates for generic classes of compounds through the water column and into sediments. The resuspension of sediment dominates the removal behavior of particle reactive substances with similar rate constants in mesocosms and natural systems. In terms of fates many substances are controlled by benthic bioturbation. The rate constants, including biodegradation, photochemical degradation, volatilization, sedimentation and sediment back diffusion, modeled together help predict the field exposure concentrations of substances. However, as Santschi points out, pollutant concentrations as opposed to tracer concentrations may affect biological processes and therefore rate constants.

One of the frustrations of plankton ecologists is the problem of resampling the same population in the field. Attempts to observe the effects of perturbants must take into account horizontal advection. As Grice's paper points out, large in situ enclosures avoid that problem. Whole water columns of various dimensions can be captured, and various experimental manipulations can be carried out. Three general observations emerged from the CEPEX experiments:

1. The susceptibility to pollutant stress was related to the generation time of the organism.
2. The levels of pollutants that caused mortalities in zooplankton were well below those from laboratory tests.
3. Pollutant and natural stress effects were similar in that phytoplankton tended toward small species.

The strength of the CEPEX design is the fact that it is big enough to directly study larval and juvenile fish recruitment under different environmental conditions and different pelagic food chain dynamics.

Donaghay's contribution considers five different experimental designs for mesocosms with regard to system configuration, usage and statistics. Four of these have been used at MERL: tracer studies in single tanks, paired system without replication as in the thermocline experiments, replicate controls and treatments and simple gradients such as the nutrient addition experiment.

The advantage of the gradient design is that it directly defines assimilative capacity in terms of dose-response. An entire range of conditions can be simulated. Boundary conditions can be established. It defines at what level problems occur for various components of the system. Since a range of conditions is being observed, processes become more clearly defined. For a material that has both beneficial and harmful effects, the management question of how best to dispose of it in the marine environment can be addressed.

Regression analysis rather than analysis of variance is used to detail effects. This analysis evaluates potential cause-and-effect relationships.

For the future, complex gradient analysis has been proposed for determining fate and effects over a range of pollutant conditions (e.g., the behavior and effect of a trace metal from oligotrophic to eutrophic conditions). This approach should increase the applicability of results to various field sites. Other designs for mimicking site specific conditions increase in engineering complexity.

Interactive laboratory, mesocosm, and field studies multiply the strength of each approach. The Grassles conclude the chapter with a call for this interactive approach. They suggest that determining the dynamics of the most abundant infaunal species in mesocosms and the field is more useful than measuring derived indices. Laboratory studies on processes such as feeding and reproduction increase our understanding of how a species adapts to its environment. Some numerically dominant species such as Nucula are not sensitive indicators of pollution. Another dominant such as Mediomastus, which is more opportunistic, is a sensitive indicator of acute disturbance or pollution. Interactive studies of dominant species enlarge our understanding of pollution impacts.

The Enclosure as a Tool for the Assessment of Transport and Effects of Pollutants in Lakes

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INTRODUCTION

Many pollutants of concern today are natural chemical substances at elevated concentrations. The effects of pollutants on biological processes in lakes cannot be studied without a well-founded knowledge of the rates and mechanisms governing chemical cycles in natural waters. Enclosed ecosystems have been used in ecological studies for several decades, but only in the early 1970s did environmental scientists recognize the value of enclosures to study questions related to chemical transport. At that time scientists from the Freshwater Institute (Winnipeg, Manitoba) and from L-DGO of Columbia University started to use experimental ecosystems in the Experimental Lakes Area (ELA), Northern Ontario, Canada (Schindler 1973, 1980). Processes investigated include nutrient limitation of primary productivity, rates of gas exchange and vertical mixing, the measurement of primary productivity, and mechanisms and rates of trace metal removal. Their pioneering efforts to make the "limnocorral" a valuable tool for lake management resulted in many comparisons between responses to perturbations by whole lakes and responses to similar perturbations in enclosures. Many of these were drastic perturbations of the lake (such as additions of nutrients, heavy metals, radio-nuclides, nitric and sulfuric acid), almost impossible in any other area of the world. Enclosed water columns coupled to the benthos were often used as test systems first, and then if the study was success-

ful, whole lake experiments followed. Eventually, 1-10 m size (both in diameter and depth) limnocorrals became accepted tools for chemical limnologists all over the world to study questions related to eutrophication, acid rain, industrial and power plant emissions and releases from fossil fuel combustion. A description of different enclosure designs can be found in Schindler et al. 1980, Gachter 1979, Grice and Reeve 1982, Banse 1982 and references therein. The use of the enclosure as a test system to study the fate and effects of pollutants requires an understanding of biological interactions and geochemical processes (e.g., gas exchange, vertical mixing, primary production, adsorption rates on sediments). In order to evaluate the usefulness, advantages and disadvantages of enclosures for lake management, results from various studies of geochemical or biological nature were compared.

ADVANTAGES OF ENCLOSURES

Enclosed ecosystems, as living models, can give more reliable answers than can be obtained from laboratory-based studies such as 48 hour bioassays, single species tests or chemical studies. This is because responses of complete ecosystems (as well as individual compartments) to manipulation can be investigated. Enclosures have a great advantage over field studies because of the ability to accurately budget relevant chemical fluxes from and to all important reservoirs and because of the relatively high degree of experimental controls. Further advantages of enclosure experiments include the possibility of experimentation at near natural conditions by adding isotopic tracers to an ecosystem and the opportunity to test numerical transport models under near natural conditions.

DISADVANTAGES AND PITFALLS

Enclosures, however, are not without their problems. Enclosure effects on plankton, due to changes in turbulence, predation pressure, light quality and quantity, or due to contamination and/or adsorption by wall materials, have been reported by biological and chemical limnologists. Some of these potential problems could be corrected after they became quantified. For example, results from enclosure experiments should be interpreted with caution when trace metal contamination from enclosure materials occurs. Parts-per-billion levels of Zn^{2+} or Cd^{2+} can already affect zooplankton abundance in lakes (Marshall and Mellinger 1980; Marshall et al. 1981). However, our work shows that increased levels of Zn^{2+} in short term experiments did not significantly alter the

Table 1. Comparison of calculated transport parameters derived from various radiochemical and chemical methods in enclosures and in whole lakes.

Year of Study	Lake	Mean Depth (m)	Enclosure Type	Volume (m^3)	Method	Reference	Transport Parameters: Settling Velocity (cm/day)	Transport Parameters: Stagnant Film Thickness (cm)
1981	ELA-L302	1.2	tube	1	^{134}Cs,^{65}Zn,^{60}Co removal	this work	30-40	0.04-0.1
	ELA-L114	1.2	tube	1	"	"	30-40	0.04-0.1
	ELA-L302	1.2	tube	1	$CaSO_4 \cdot 2H_2O$ dissolution	Santschi et al. 1982	-	0.11
	ELA-L302	1.2	Lake Epilimnion		"	"	-	0.07
1977	Baldegg Switzerland	10	limnocorral	1130	Zn,Cu,Cd Pb removal	Baccini et al. 1979	20-30	-
1976	ELA-L223	2.2	limnocorral tube D,B	180	^{134}Cs,^{65}Zn ^{54}Mn, ^{60}Co -removal	Schindler et al. 1980	25-30	0.08
1976	ELA-L224	6(17)*	Lake Epilimnion	1.3×10^6	"	Hesslein et al. 1980	26	0.04

* The mean depth of the Lake Epilimnion is 6 m, the ratio of volume to surface area of sediments is 17 m.

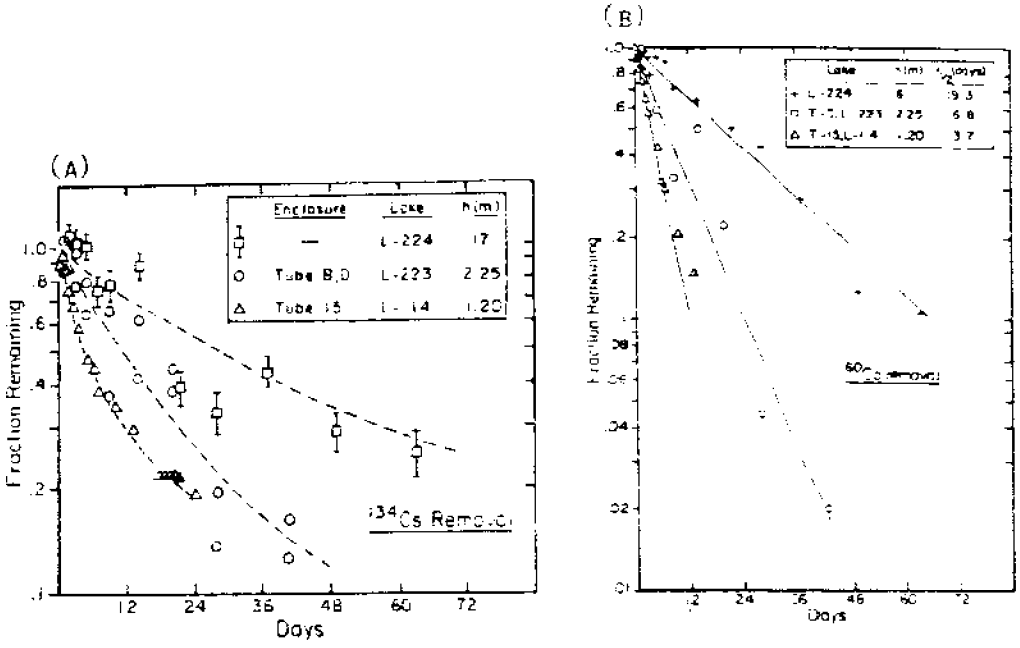


Figure 1. (A) The removal of ^{134}Cs in one of our tubes (T15, L114) is compared with that in larger tubes of Schindler et al. (1980) and in a whole lake experiment (Hesslein et al. 1980). The model curves predicted from the numerical transport model of Nyffeler et al. (1983) are shown in dashed lines. The initial removal rate from the water column (λ_i) is the sum of the rates for direct sediment uptake and particle settling, as follows: $\lambda_i = (1-f)D/(z \cdot h) + S \cdot f/h'$, with h=volume/sediment surface area, h'=mean depth of water column, f=fraction on particles ($=(1+(K_D \cdot pmc)^{-1})^{-1}$), D=molecular diffusion coefficient, z=thickness of the stagnant boundary film (="diffusive sublayer"), S=settling velocity, K_D = distribution coefficient, pmc=particulate matter concentration. Since K_D for ^{134}Cs is usually $0.5\text{-}1\times10^4$, and the particulate matter concentration is in the range of 1-3mg L^{-1}, the fraction of ^{134}Cs adsorbed to particles is quite small (<<10%). Thus, the initial removal rate of ^{134}Cs is mainly controlled by the value of z. Soon after spike addition, the rate of ^{134}Cs removal is slowing down because of control by the rate of diffusion and particle mixing (D_B) within the surface sediments. The model fit is thus initially fixed by the value of z and later by the value of D_B (here $3.5\times10^{-6}cm^2\ s^{-1}$). Since the magnitude of z is controlled by the wind induced turbulence in the water overlying the epilimnetic sediments, the variations of a factor of two in values of z needed to model fit the different data are understandable (0.04 cm in L114 and L224, 0.08 cm in L223).

(B) Comparison of removal of particle-reactive ^{60}Co ($K_D>10^5$) in enclosures of different sizes and in a whole lake. Since ^{60}Co rapidly adsorbs to particles (fraction on particles >90%), its removal is controlled by the rate of particle settling. Its initial removal rate $\lambda_i \cong S \cdot f/h' = (\ln 2)/t_{1/2}$. Thus the half removal time from the water column $t_{1/2}$ is directly proportional to the mean depth if the rate of particle settling is constant. The data in this figure demonstrate this to be the case. The value of S is 25-30 cm d^{-1}.

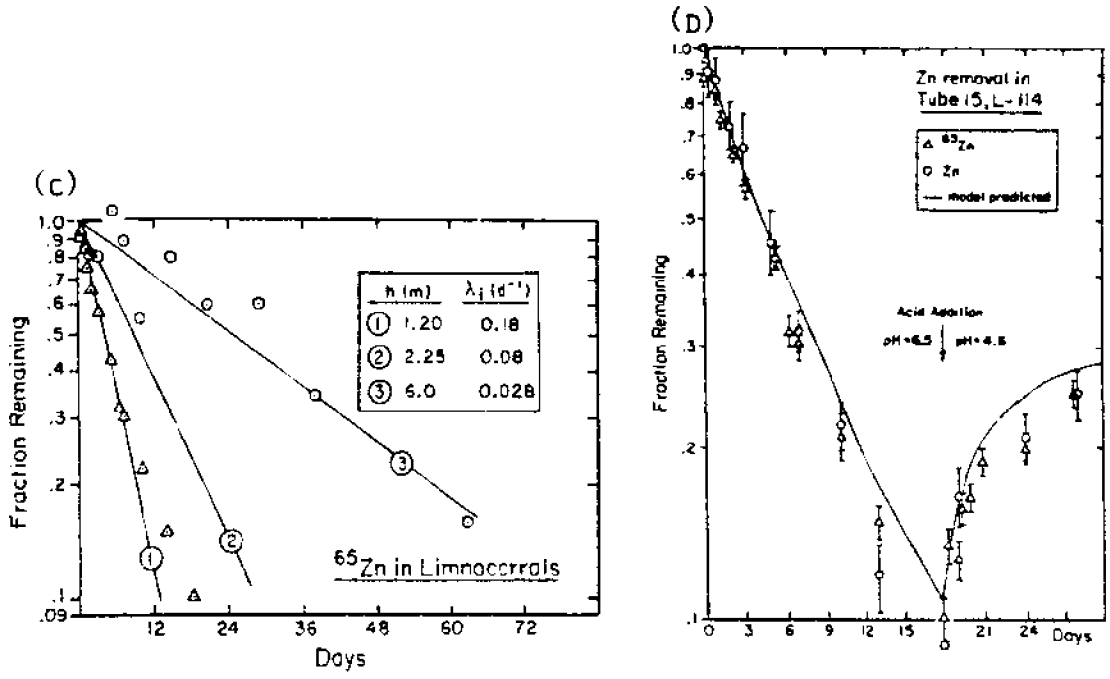


Figure 1 (C). The removal of ^{65}Zn in enclosures of 1.2 m (Tube 15, L114), 2.2 m (tube D, L223, Schindler et al. 1980) and whole lake (L224, Hesslein et al. 1980) is shown. Since K_D for ^{65}Zn is intermediate in these ELA lakes ($K_D \approx 10^4$ to 10^5), the removal of ^{65}Zn is controlled both by particle settling and by direct adsorption at the sediment-water interface. The indirect proportionality of λ_i to h implies that the values for both S and z are relatively constant in these lakes.

(D) The removal of radioactive ^{65}Zn in tubes 15 and 16 of Lake 114 reflects closely the removal of stable Zn, which was added simultaneously with the radiotracers to the enclosures in Lake 114. No effect of stable element addition on radiotracer removal was noticeable since the results of the control tube did not deviate from those in the trace metal tube. Both removal and back-diffusion (after HCl addition) curves are predicted by the numerical transport model of Nyffeler et al. (in preparation) if the same physical parameters (z, S, D_B) as those obtained from soluble ^{134}Cs and particle-reactive ^{60}Co, and the pH sensitivity of K_D as experimentally determined in the laboratory, are used.

transport parameters of different radioisotopes (Table 1, Figure 1) compared with control tubes.

Physical (e.g., light intensity or water turbulence), chemical (e.g., levels of nutrients or trace metals) or biological (e.g., phyto- or zooplankton biomass) conditions can be different inside and outside enclosures. Sometimes, however, the differences appear to be related to the size of the enclosure, for example, differences in turbulence levels. Turbulence levels appear to deviate from lake levels more in larger and deeper enclosures (12.5 m depth) than in shallow ones of 1-2 m depth (Table 2). Very early on it became apparent that scaling the size of the enclosure to the nature of the problem is crucial to the success of the study. Where the responses sought must develop over at least a few days, such as species succession of plankton or the transport of pollutant trace metals, minimizing container effects becomes of utmost importance. Only large enclosures, such as bags, large tubes or limnocorrals, or continuous in-situ cultures have proved adequate. If, however, variations in many experimental parameters and sufficient statistical replication are necessary, and if results develop over short time scales (i.e., less than a few days), then smaller containers such as small tubes and in-situ bottles are more appropriate.

A possible pitfall is the extrapolation of the enclosure results to a natural environment which is considerably larger and more complex. Very rarely can an enclosed water column be totally representative of the behavior of a whole water body where conditions at times may be locally quite extreme. The simulation of extreme conditions such as hypolimnetic processes which are often characterized by seasonal anoxia, or the resuspension of epilimnetic surface sediments caused by storms, and the effects of different lake morphologies on the ecosystem are much more difficult to predict from average enclosure experiments. Short term in-situ incubations, or the coupling of different experimental containers having different treatment conditions might then be a better approach. Nonetheless, average conditions in average epilimnetic waters, which are more closely linked to human needs and health, are relatively easy to simulate in enclosures.

VALIDATION OF ENCLOSURE RESULTS

How to validate insights gained from studies in enclosed water bodies always remains an important question. There are basically three different approaches for the validation of enclosure results that have been used in the past. One obviously is to compare results with those obtained from studies of equivalent processes in the whole lake. Another is to compare enclosure results with those obtained from model predictions based on laboratory data. A third

Table 2. Vertical mixing (K_v) and gas exchange (K_g): Comparisons between enclosures and whole lake.

Mean depth (m)	K(enclosure)/K(lake)	Conditions	Reasons	References
1 - 4	≥ 1 (K_g)	low wind stress	internal waves generated by (flexible) "wall pumping"	Hesslein and Quay, 1973 Quay, 1977 Hesslein et al., 1980 Bower et al., 1980 Torgersen et al., 1982
12.5	~ 1 (K_v)	low wind stress (lake thermocline ~5m)	—	Imboden et al. 1979
12.5	< 1 (K_v)	high wind stress (lake thermocline ~10m)	shielding effects of rims and walls	Imboden et al. 1979

is to compare results gained from enclosures of different sizes, which incorporate varying degrees of complexities. Examples of the first approach include (1) trace metal removal in limnocorrals and whole lake studies; Figure 1 shows that removal of radioactive Zn^{2+} and Cs^{+} in Lake 224 with a 6.5 m deep epilimnion (Hesslein et al. 1980) is consistent with that observed in enclosures of Lake 223 (Schindler et al. 1980) and Lake 114 (from our unpublished work); (2) vertical mixing studies of Quay (1977), Hesslein and Quay (1973), Imboden et al. (1979) and (3) gas exchange studies of Emerson et al. (1973), Bower and McCorkle (1980), Bower (1981) and Torgersen et al. (1982). The examples given strongly suggest similar rates in enclosures and small lakes (Table 2). The second approach is more difficult and there are fewer examples: (1) Bower and McCorkle (1980) compare the primary productivity inside a limnocorral with that predicted from the incubation method of Fee (1973), and (2) Nyffeler et al. (1982) compare removal rates of Mn and Cs isotopes and stable Mn from the enclosed water column and response to acidification to pH 4.6 with predictions based on a numerical transport model using distribution coefficient measurements in the laboratory. Both examples show that laboratory based predictions can be verified in enclosures. Examples for the third approach can be found in Marshall and Mellinger (1980) and Marshall et al. (1981), where the responses of zoo- and phytoplankton to additions of various amounts of Cd^{2+} or Zn^{2+} in different sizes of containers and hung in different lakes are compared. Similar effects on zooplankton were observed in in situ bottles (8L Carboys) and large limnocorrals, in both lake Michigan and in an ELA lake. Examples of stress studies in various container configurations, compared for different lengths of time, can be found in Marshall and Mellinger (1980) and DeNoyelles et al. (1980). Good replication of effects of Cd^{2+} on plankton in various in situ enclosures was found, whereas similar stress tests in laboratory based continuous cultures using filtered lake water produced less than satisfying results because of the development of abnormal plankton species. Further examples of the third approach will be demonstrated by comparing the rates of transport of trace metals inside containers of different sizes, carried out at different times and in different lakes (Schindler et al. 1980; Baccini et al. 1979; and our work). The two main transport mechanisms, the removal of dissolved trace metals by adsorption or incorporation onto falling particles and by adsorption at the sediment-water interface, appear to operate at similar rates in different types of environments when corrected for the different mean depths. The initial removal rate from the water column (λ_i) is then as follows:

$$\lambda_i = (1-f)\, D/(z \cdot h) + S \cdot f/h' \qquad (1)$$

where h = volume/sediment area, h' = mean depth of water column (cm), D = molecular diffusion coefficient ($cm^2 d^{-1}$) as taken from Li and Gregory (1974), f = fraction on particles, z_1 = stagnant boundary film thickness (cm), S = settling velocity (cm d^{-1}). The fraction on particles (f) is either predicted from laboratory adsorption experiments or is measured in situ. The settling velocity on particles (S) can be determined either from (1) measurements of primary production and algal standing crops or (2) from particle flux measurements in sediment traps (or particulate element flux) and particulate matter concentrations (or particulate element concentrations), as

$$S = \text{flux (g cm}^{-2}\ d^{-1})/\text{concentration (g cm}^{-3}) \qquad (2)$$

or (3) from measurements of initial removal rates (λ_i:) of a particle-bound radioisotope (such as ^{60}Co) where f is independently determined, as

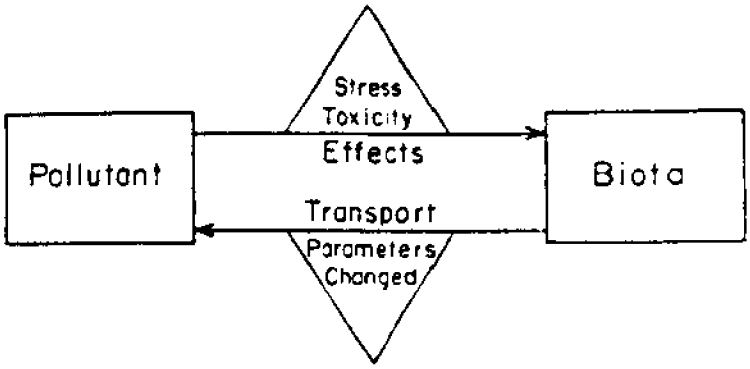
$$S = \lambda_i \cdot h'/f. \qquad (3)$$

Removal of heavy metals in lake enclosures in Canada and Switzerland indicates settling velocities (S) of 25-40 cm d^{-1} in the epilimnion of different lakes, which are typical for 5-15 µm plankton (Table 1 and Figure 1). The film thickness (z) can be determined from numerically modeling the removal of a more soluble tracer such as ^{134}Cs (Figure 1) or from the rate of dissolution of gypsum mounted on a flat plate at the sediment-water interface (Table 1, and Santschi et al. 1982). Film thicknesses ranging from 0.04 to 0.1 cm fit all tracer profiles inside 1-2 m enclosures and in a whole lake (Table 1 and Figure 1). Radioactive and stable Zn^{2+} are almost equally affected by these two mechanisms of removal. Since their initial removal rates in enclosures and whole lakes appear to be inversely related to the mean depth, this implies that both S and z are relatively constant in these lakes.

INTERRELATION OF FATES AND EFFECTS

Fates and effects of pollutants are interrelated. Whereas the initial statement that effects of a particular pollutant cannot be effectively studied without considering its fate in the ecosystem is certainly justified, its reverse is also true. Gachter (1979) and his co-workers describe an enclosure experiment in Lake Baldegg in Switzerland for investigating effects and fates of various trace metals at concentrations close to the maximum permitted for flowing water bodies. The observed effects on the living biota described in a series of papers (Gachter 1979; Gachter and Geiger 1979; Lang and Lang-Dobler 1979) could change the different

Table 3. Ecosystem response.



Examples of pollutant trace metal effects, taken from Gächter, 1979:

Effects	Changes in transport parameters
Growth of heavy metal tolerant phytoplankton organisms with lower sorption capacity	$K_D \downarrow$
Primary productivity decrease	Particle flux $\downarrow$ Organic C flux $\downarrow$
Zooplankton biomass decrease	Nutrient & trace metal recycling efficiency $\downarrow$
Zooplankton grazing decrease	$S \downarrow$
Benthic polychaete biomass increase	$D_B \uparrow$ (short term)
Benthic polychaete delayed reproduction	$D_B \downarrow$ (long term)

transport parameters in the ecosystem (see Table 3). Increased concentrations of pollutants such as heavy metals appear to favor growth of phytoplankton organisms with lower metal sorption capacity (Gachter and Geiger 1979), thus lowering the rate at which a pollutant is removed from the water column, and adversely affecting most strongly the organisms at higher trophic levels, such as zooplankton, benthic macrofauna and fish. Decrease in zooplankton biomass and filtering rate could negatively affect nutrient and trace metal recycling efficiency and could, at times when zooplankton control the phytoplankton biomass by grazing, diminish the flux of fast settling fecal pellets and thus decrease the overall settling velocity of particles. Owing to better oxygen conditions of the sediment water interface, caused by a decreased organic carbon flux to the sediments, the benthic oligochaete biomass increased over the course of the experiment. This would probably increase the bioturbation rate (D_B) of the surface sediment thus increasing the storage and assimilation capacity of the system for pollutants. Since the oligochaetes showed delayed reproduction as a sign of stress, their biomass might have diminished again over longer time periods, and thus caused a decrease in D_B .

The potential for altering the community structure by higher levels of pollutants can thus make the prediction of rates and fates at low concentrations a difficult task. The same can be said, of course, for the reverse situation.

CONCLUDING REMARKS

Present day enclosure technology is adequate to answer many questions for successful lake management. The assessment of enclosures in lakes is also applicable to estuarine and coastal marine environments, with some exceptions. Strong tidal currents and more frequent storms make long term, large scale, controlled ecosystem experiments more difficult in the near shore marine environment. Various types of enclosures, strong enough to withstand the more energetic marine environment, have been used in North American and European coastal waters. They are reviewed in Grice and Reeve (1982) and Banse (1982).

ACKNOWLEDGMENTS

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REFERENCES

Baccini, P., T. Ruchti, O. Wanner and E. Grieder. 1979. MELIMEX, an experimental heavy metal pollution study: Regulation of trace metal concentrations in limnocorrals. Schweiz. Z. Hydrol. 41(2): 202-227.

Banse, K. 1982. Experimental marine ecosystem enclosures in a historical perspective. In: G. D. Grice and M. R. Reeve (eds.), Marine Mesocosms: Biological and Chemical Reseach in Experimental Ecosystems, pp. 11-24. Springer-Verlag, New York.

Bower, P. 1981. Addition of radiocarbon to the mixed-layers of two small lakes: Primary production, gas exchange, sedimentation and carbon budget. Ph.D. Thesis, Columbia Univ., New York.

Bower, P. and D. McCorkle. 1980. Gas exchange, photosynthetic uptake, and carbon budget for a radiocarbon addition to a small enclosure in a stratified lake. Can. J. Fish. Aquat. Sci. 37(3): 464-471.

DeNoyelles, F., Jr., R. Knoechel, D. Reinke, D. Treanor and C. Altenhofen 1980. Continuous culturing of natural phytoplankton communities in the Experimental Lakes Area: Effects of enclosure, in situ incubation, light, phosphorus and cadmium. Can. J. Fish. Aquat. Sci. 37: 424-433.

Emerson, S., W.S. Broecker and D.W. Schindler 1973. Gas exchange rates in a small lake as determined by the radon method. J. Fish. Res. Board Can. 30: 1475-1484.

Fee, E.J. 1973. Modelling primary production in water bodies: A numerical approach that allows vertical inhomogeneities. J. Fish. Res. Board Can. 30: 1469-1473.

Gachter, R. 1979. MELIMEX, an experimental heavy metal pollution study: Goals, experimental design and major findings. Schweiz. Z. Hydrol. 41: 169-176.

Gachter, R. and W. Geiger. 1979. MELIMEX, an experimental heavy metal pollution study: Behavior of heavy metals in an aquatic food chain. Schweiz. Z. Hydrol. 41: 277-290.

Grice, G.D. and M.R. Reeve (eds.). 1982. Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems. Springer-Verlag, New York.

Hesslein, R. and P. Quay 1973. Vertical eddy-diffusion studies in the thermocline of a small stratified lake. J. Fish. Res. Board Can. 30: 1491-1500.

Hesslein, R.H., D.W. Schindler, W. S. Broecker and G. Kipphut 1979. Fates of metal radiotracer additions in experimental enclosures in lakes and in a whole lake. In: Proceedings of Advisory Group on the Application of Nuclear Techniques to the Study of Lake Dynamics pp. 261-271. International Atomic Energy Agency, Vienna.

Hesslein, R. H., W. S. Broecker and D. W. Schindler 1980. Fates of metal radiotracers added to a whole lake: Sediment-water interactions. Can. J. Fish. Aquat. Sci. 37: 378-386.

Imboden, D.M., B.S. F. Eid, T. Toller, M. Schurter and J. Wetzel 1979. MELIMEX, an experimental heavy metal pollution study: Vertical mixing in a large limno-corral. Schweiz. Z. Hydrol. 41: 177-189.

Knoechel, R. and F. DeNoyelles. 1980. Analysis of the response of hypolimnetic phyto-plankton communities to increased light or phosphorus using track autoradiography. Can. J. Fish. Aquat. Sci. 37: 434-441.

Lang, C. and B. Lang-Dobler. 1979. MELIMEX, an experimental heavy metal pollution study: Oligochaetes and chironomid larvae in heavy metal loaded and control limno-corrals. Schweiz Z. Hydrol. 41: 271-276.

Li, Y.-H. and S. Gregory 1974. Diffusion of ions in sea water and in deep-sea sediments. Geochim. Cosmochim. Acta 38: 703-714.

Marshall, J.S. and D.L. Mellinger 1980. Dynamics of cadmium-stressed plankton communities. Can. J. Fish. Aquat. Sci. 37: 403-414.

Marshall, J.S., J.I. Parker, D. L. Mellinger and C. Lei 1981. Bioaccumumulation and effects of cadmium and zinc in a Lake Michigan plankton community. Submitted.

Nyffeler, U.P., P.H. Santschi, R. F. Anderson and S. L. Schiff 1982. Trace metal freshwater lakes. EOS 6: 67.

Nyffeler, U. P., Y.-H. Li and P. H. Santschi. 1983. Adsorption kinetics and diffusion in marine and freshwater sediments. (In preparation).

Quay, P. 1977. An experimental study of turbulent diffusion in lakes. Ph.D. Thesis, Columbia Univ., New York.

Santschi, P.H., P. Bower, U.P. Nyffeler, A. Azevedo and W.S. Broecker 1982. Measurements of the resistance to chemical transport posed by the deep sea benthic boundary layer and their significance to benthic fluxes. (Submitted to Limnology and Oceanography.)

Schindler, D.W. 1973. Experimental approaches to limnology--An overview. J. Fish. Res. Board Can. 30: 1409-1413.

Schindler, D.W. 1980. Evolution of the Experimental Lakes Project. Can. J. Fish. Aquat. Sci. 37(3): 313-319.

Schindler, D.W., R.H. Hesslein, R. Wagemann and W.S. Broecker 1980. Effects of acidification on mobilization of heavy metals and radionuclides from the sediments of a freshwater lake. Can. J. Fish. Aquat. Sci. 37(3): 373-377.

Torgersen, T., G. Mathieu, R.H. Hesslein and W.S. Broecker 1982. Gas Exchange dependency on diffusion coefficient: Direct ^{222}Rn and ^{3}He comparisons in a small lake. J. Geophys. Res. 87(C1): 546-556.

Use of Enclosures in Studying Stress on Plankton Communities

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INTRODUCTION

The increasing concerns about oceanic pollution in the late 1960s led to the development of large experimental systems (mesocosms) in which natural communities could be enclosed, maintained and experimentally manipulated. Large plastic enclosures ("bags") suspended from modules floating at the sea surface were developed and used by three programs whose objectives were to evaluate the response of pelagic ecosystems to pollution. These programs were located in Den Helder, Holland (Kuiper 1977), Loch Ewe, Scotland (Davies and Gamble 1979), and Saanich Inlet, Canada, (Menzel and Case 1977). Drawing especially on the results of the Controlled Ecosystem Pollution Experiment (CEPEX) in Saanich Inlet, Canada, this review (1) describes the three types of enclosures, (2) considers the advantages and limitations of large enclosure systems for pollution research, (3) reviews major results of pollution experiments on plankton and (4) indicates areas of research on natural plankton communities that can be carried out in large enclosures.

STRUCTURE AND OPERATION OF DEN HELDER, LOCH EWE AND CEPEX ENCLOSURES

Size

The sizes of the enclosures (Figure 1) are 1.5 and 16 m^3 (0.75, 1.5 m in diameter; 3.5, 20 m deep) at Den Helder; 100 and 300 m^3 (3, 4.7 m in diameter; 17 m deep) at Loch Ewe and 68 and 1300 m^3 (2.4, 9.5 m in diameter; 16.1, 23.5 m deep) at Saanich Inlet. The enclosure fabric consists of one to two layers of translucent polyethylene or vinyl reinforced with nylon. The lower end of the Loch

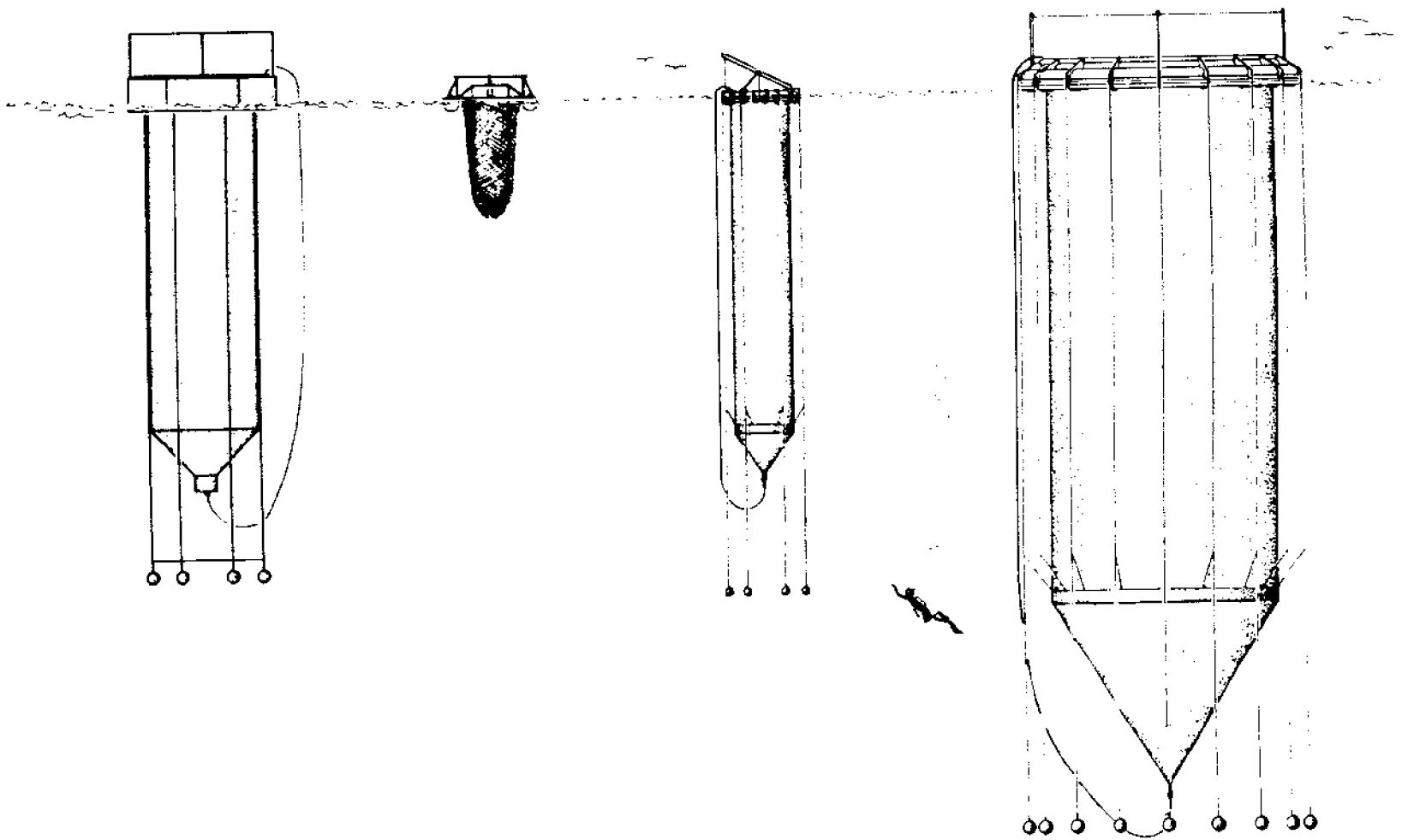

Figure 1. Some enclosures used for plankton pollution research (from left to right) at Loch Ewe (300 m^3), Den Helder (1.5 m^3) and CEPEX Saanich Inlet (68 m^3, 1300 m^3).

Ewe and CEPEX (Saanich) enclosures are cone shaped and terminate in sediment collectors. Sediment and its contained diatom spores can be pumped back into the top of the water column by means of a hose that extends from the collector to the surface. Den Helder enclosures have rounded lower portions and no sediment collectors. The tops of all the enclosures are attached to flotation collars and are open to the atmosphere.

Deployment

The usual method of filling enclosures consists of first lowering the plastic into the water column through the center of the flotation collar until the top of it reaches a depth at least equivalent to the length of the water column to be enclosed (i.e., the length of the bag). It is then raised to the surface manually or automatically by means of a counterweight system. Generally, more than 90% of the water column is captured in less than 3 minutes. Pumps are used to add what water is required to fill the enclosure. In this way several enclosures can be raised and filled simultaneously at any time of the day or night. In early Loch Ewe experiments and in some Den Helder experiments, bags were entirely filled by use of a diaphragm pump. Aprons of polyethylene are suspended 8 m deep inside the large CEPEX enclosures and serve as fouling skirts. They are scrubbed once a week to control the amount of fouling. At Loch Ewe the outer surfaces of the bags are scrubbed weekly.

Management

The management of enclosures depends on the objectives of the experiment. In general, periodic nutrient additions are required, especially in the larger bags, to prevent depletion or serious reduction in phytoplankton. Nutrients are usually introduced through a diffuser which is raised and lowered within the upper few meters in CEPEX bags. In Loch Ewe, nutrients were added near the bottom of the bags. A light screen can be placed over an enclosure to reduce the growth rate of phytoplankton, and a continous air bubbling system or mechanical stirrer can be provided to promote mixing.

Manipulations

Following the confinement of natural plankton communities, a number of experimental manipulations can be imposed on the populations and their responses compared with unstressed or control populations. Most manipulations consist of exposing populations to selected pollutants, those frequently used being mercury, copper, cadmium and petroleum hydrocarbons. The pollutants can be introduced in single doses or periodically to maintain a prescribed concentration.

Manipulations can also mimic natural stresses. For instance, upwelling can be imposed within the enclosures and differing quantities and proportions of nutrients or predators (fish larvae) can be

introduced. These stresses directly or indirectly influence the structure of the plankton communities, and subsequent ecosystem changes are reflections of the upward ramification of physical/chemical manipulations or the downward ramification of predation on prey and their food.

OPEN OCEAN VS. ENCLOSED SYSTEMS FOR EVALUATING STRESS ON PLANKTON

Enclosure systems were developed largely because of the many difficulties of determining the effects of pollutants in the open ocean. Population assessment in the ocean, a necessary part of any pollution program, is a major task because of patchiness, organism migration and advection. Moreover, estimates of production rates, population fluctuations and trophic interactions within the study area must be known and predictable. Yet the acquisition of this information is contingent upon the ability to quantify patchiness and vertical distribution, an ability that requires elaborate collection and analysis programs. Observed population fluctuations could be due to a variety of causes including climate, fishing pressure or pollution; and distinguishing among them would probably require years of comparably collected data. There is no easy way to measure zooplankton production. Finally, the ocean cannot be experimentally manipulated.

Given the above limitations and constraints of assessing the effects of pollutants in the field, the use of enclosures for pollution research offers the following advantages:

1. More than two interacting trophic levels, each having more than one species, can be maintained for periods up to 6 or more weeks.
2. The same natural populations can be revisited and sampled.
3. Communities can be physically, biologically and chemically manipulated.
4. Spatial (vertical) and temporal distributions of species can be examined.
5. Trace quantities of polluting substances can be introduced, and their biologically mediated fate can be observed over time.
6. Vertical biological and chemical transport in a natural system free from effects of advection or turbulent diffusion can be examined.
7. Mathematical models can be compared with actual observations obtained from enclosures.
8. Research within enclosures imposes considerable rigor on investigators in that major population fluctuations must

be explained because they cannot be related to advection or migration of organisms.

There are, of course, limitations in using enclosures for pollution research. Once physical barriers are erected to isolate columns of water from the natural environment, horizontal and vertical mixing will differ from those mixing outside, thereby creating stress on the contained populations. It is difficult to estimate the magnitude of this stress, but it must be considered in both the design of experiments and interpretation of results. Using enclosures has these limitations:

1. Reduction of vertical and horizontal mixing.
2. Impossibility of controlling initial conditions as bags capture the biota in the water column at the time they are raised to the surface.
3. Wall effects, especially in bags with large ratios of surface area to volume.
4. Inability to use present enclosures in open ocean because of fragility of structures.
5. High costs of enclosure construction, deployment and maintenance may limit the number of replicate units.

POLLUTANT STRESS STUDIES

Many pollution experiments have been conducted in Den Helder, Loch Ewe and CEPEX enclosures over the last 10 years, and experiments continue at Den Helder and Loch Ewe. These enclosure studies were supported by laboratory studies on fecundity, feeding, digestion time, pollutant incorporation rates, and so forth as such experiments are an essential part of enclosure research programs. Summaries of Den Helder experiments with mercury are provided by Kuiper (1977, 1982), on Loch Ewe experiments with copper by Gamble et al. (1977) and with mercury by Davies and Gamble (1979), and on CEPEX experiments with copper and petroleum by Menzel (1977) and with mercury by Grice and Menzel (1978). From observations made in enclosures and in laboratory studies several hypotheses emerged from the CEPEX experiments:

1. The susceptibility of pollutant stress is related to generation time of the organisms, those with faster generation time generally being more sensitive than those with slower generation time (Menzel 1980). Although bacteria were impacted first, their rapid generation time (hours to few days), numerous species or strains and mutational ability resulted in rapid recovery (i.e., return to control levels) of heterotrophic activity (Azam et al. 1977;

Vaccaro 1977). Zooplankton, having few species and long generation time, recover most slowly (Grice et al. 1977). Phytoplankton with intermediate characteristics were intermediate in their recovery rates (Thomas et al. 1977).

2. In mixed trophic level assemblages that occur in enclosures, the levels of pollutants that cause significant mortalities in zooplankton are well below those that result from traditional short-term toxicity tests conducted in the laboratory (Reeve et al. 1976; Gibson and Grice 1977).
3. The events produced by pollutant stress in phytoplankton do not appear to differ from those that occur over much longer periods of time in natural environments in response to natural stresses (Menzel 1980). The natural sequence manifests itself first at the primary producer level following a reduction of nutrient levels in the water column by forcing the succession of phytoplankton populations from relatively large centric diatoms to small phytoplankton. A very similar course of events is induced either by pollutant stress, by reduced vertical mixing in the enclosures, or during times when nutrients are depleted or during low light conditions in the natural environment.

Although these hypotheses suggest that we have gained, through the use of enclosures, some general ability to predict the effects of certain pollutants, the actual mechanisms controlling the observed changes are still inadquately understood. This inadequacy is related to the fact that many pollutants, once introduced into enclosures, act simultaneously on all components of the system, making it difficult to establish specific causes for the observed changes. For example, it could not be determined whether the detrimental effect of mercury on herbivorous zooplankton was a direct effect of the pollutant on the copepods or was due to changes in their food which also resulted from the pollutant (Sonntag and Greve 1977; Grice et al. 1977; Beers et al. 1977). Similarly, observed changes in phytoplankton species composition following pollutant introduction may be a direct effect on the phytoplankton or it may be a result of differing grazing pressure due to the pollutant's effect on herbivores.

There is another consideration in interpreting observed population changes in enclosures, following their exposure to pollutants. This relates to the difficulty of distinguishing population change due to stresses caused by the reduction of advection and turbulence from changes due to stresses caused by the pollutant (von Bröckel 1982).

NATURAL COMMUNITIES

As indicated earlier, many of the observed effects of pollutants on plankton communities in enclosures are similar to those that occur in nature in response to changes in light, nutrient availability, turbulence or possibly predation. Thus, to explain fully the responses of plankton to pollutants that were observed in previous experiments and in future enclosure experiments, major efforts should be directed at understanding natural mechanisms that control the structure and functioning of pelagic food webs. Predators, such as larvae and juvenile fish, should be integral parts of these experiments because recruitment processes and the success of year classes of commercial species are related to both trophic interaction and physical processes.

Because enclosed water columns can be well characterized biologically, they offer unique opportunities to examine natural stresses that are exerted at the bottom and at the top of plankton communities in nature. Manipulations of the lower trophic level (phytoplankton) can be induced by physical (light, turbulence) or chemical (nutrients) means while alteration of higher trophic levels can be carried out by introduction or removal of predators (fish larvae, invertebrate predators). Thus, food chain responses to "bottom up" and "top down" control strategies can be evaluated. Some preliminary experiments using natural stresses have already been conducted in CEPEX (Grice et al. 1980; Harris et al. 1982) and Loch Ewe (Steele and Gamble 1982) enclosures. Not only will these contribute to pollution related experiments, they will also provide insights into topics of current biological oceanographic importance such as food chain efficiency, fisheries recruitment and levels of harvestable yield.

The types of work that can be conducted on natural plankton include the following:

1. The ability to experiment with a multi-trophic and interacting food chain provides opportunities of investigating production processes, food chain efficiencies, species interaction, predator-prey dynamics--all in a well-characterized biological environment. As an example, the spring bloom of phytoplankton with its resulting food chain ramifications can be simulated (Figure 2). This can be seen in the sequential upward propagation of a biomass maximum over a 30-day period.
2. Cohorts of copepods and other zooplankton can be generated by appropriate stimulation of phytoplankton, thus permitting the estimation of population parameters such as recruitment, stage residence time, mortality, generation time and secondary production.

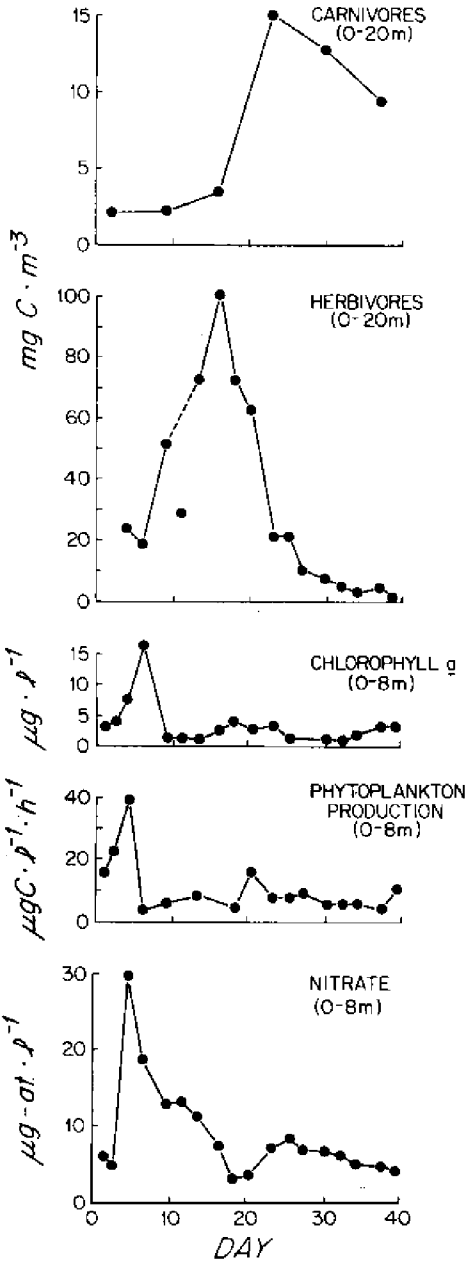

Figure 2. Results of simulation of spring bloom and subsequent food chain events in a CEPEX bag through nutrient addition on day three. (Modified from Figure 27-1 in Harris et al. 1982.)

3. Trophic factors relating to cause and persistence of microscale patches of plankton can be evaluated.
4. Growth and processes affecting recruitment of fish larvae under defined conditions can be quantified.
5. Plankton models can be tested and predictive models validated.

REFERENCES

Azam, F., R.F. Vaccaro, P.A. Gillespie, E.I. Moussalli and R.E. Hodson. 1977. Controlled Ecosystem Pollution Experiment: Effect of mercury on enclosed water columns. II. Marine bacterioplankton. Mar. Sci. Commun. 3: 313-329.

Beers, J.R., M.R. Reeve and G.D. Grice. 1977. Controlled Ecosystem Pollution Experiment: Effect of mercury on enclosed water columns. IV. Zooplankton population dynamics and production. Mar. Sci. Commun. 3: 355-394.

Davies, J.M. and J.C. Gamble. 1979. Experiments with large enclosed ecosystems. Phil. Trans. R. Soc. Lond. B. 286: 523-544.

Gamble, J.C., J.M. Davies and J. H. Steele. 1977. Loch Ewe bag experiment, 1974. Bull. Mar. Sci. 27(1): 145-175.

Gibson, V.R. and G.D. Grice. 1977. Response of macro-zooplankton populations to copper: Controlled Ecosystem Pollution Experiment. Bull. Mar. Sci. 27(1): 85-91.

Grice, G.D. and D.W. Menzel. 1978. Controlled Ecosystem Pollution Experiment. Effect of mercury on enclosed water columns. VIII. Summary of results. Mar. Sci. Commun. 4: 23-31.

Grice, G.D., M.R. Reeve, P. Koeller and D.W. Menzel. 1977. The use of large volume, transparent, enclosed sea surface water columns in the study of stress on plankton ecosystems. Helgol. Wiss. Meeresunters. 30: 118-133.

Grice, G.D., R.P. Harris, H.R. Reeve, J.F. Heinbokel and C.O. Davis. 1980. Large scale enclosed water column ecosystems: An overview of Foodweb I, the final CEPEX experiment. J. Mar. Biol. Assoc. U. K. 60: 401-414.

Harris, R.P., M.R. Reeve, G.D. Grice, G.T. Evans, V.R. Gibson, J.R. Beers and B.K. Sullivan. 1982. Trophic interactions and production processes in natural zooplankton communities in enclosed water columns. In: G.D. Grice and M.R. Reeve (eds.) Marine Mescosms: Biological and Chemical Research in Experimental Ecosystems, pp. 353-387. Springer-Verlag, New York.

Kuiper, J. 1977. An experimental approach in studying the influence of mercury on a North Sea coastal plankton community. Helgol. Wiss. Meeresunters. 30: 652-665.

Kuiper, J. 1982. Exotoxicological experiments with marine plankton communities in plastic bags. In: G.D. Grice and M.R. Reeve (eds.), Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems, pp. 182-193. Springer-Verlag, New York.

Menzel, D.W. 1977. Summary of experimental results: Controlled Ecosystem Pollution Experiment. Bull. Mar. Sci. 27: 142-145.

Menzel, D.W. 1980. Applying results derived from experimental microcosms to the study of natural pelagic marine ecosystems. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-11, 1978, pp. 742-752. (CONF.-781101, National Technical Information Service, Springfield, VA.)

Menzel, D.W. and J. Case. 1977. Concept and design: Controlled Ecosystem Pollution Experiment. Bull. Mar. Sci. 27: 1-7.

Reeve, M.R., G.D. Grice, V.R. Gibson, M.A. Walter, K. Darcy and I. Ikeda. 1976. A Controlled Environmental Pollution Experiment (CEPEX) and its usefulness in the study of larger marine zooplankton under toxic stress. In: A.P. Lockwood (ed.), Effects of Pollutants on Aquatic Organisms, pp. 145-162. Cambridge University Press, Cambridge, England.

Sonntag, N.C. and W. Greve. 1977. Investigation of the impact of mercury on enclosed water columns using a zooplankton simulation model. J. Fish. Res. Board Can. 34: 2295-2307.

Steele, J.H. and J.C. Gamble. 1982. Predator control in enclosures. In: G.D. Grice and M.R. Reeve (eds.), Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems, pp. 227-237, Springer-Verlag, New York.

Thomas, W.H., O. Holm-Hansen, D.L.R. Seibert, F. Azam, R. Hodson and M. Takahashi. 1977. Effects of copper on phytoplankton standing crop and productivity: Controlled Ecosystem Pollution Experiment. Bull. Mar. Sci. 27: 34-43.

Vaccaro, R., F. Azam, and R.E. Hodson. 1977. Response of natural marine bacterial population to copper: Controlled Ecosystem Pollution Experiment. Bull. Mar. Sci. 27: 17-22.

von Bröckel, K. 1982. Sedimentation of phytoplankton cells within controlled experimental ecosystems following launching and implications for further enclosure studies. In: G.D. Grice and M.R. Reeve (eds.), Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems, pp. 251-259. Springer-Verlag, New York.

Should We Know the Fates of Pollutants?

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The title of this paper is a rhetorical question. The answer is YES.

My purpose in using such a title is to draw attention to what I believe has been a relatively neglected part of the general problem of attempting to understand or predict the effects of pollutants in the coastal and oceanic marine environment. While everyone professionally involved with this problem is aware that knowing the effects requires a prior knowledge of the exposure concentrations to be expected, it is my impression that most experimental research directed towards the study of pollution has focused only on the study of effects. In this category I place both conventional bioassays and the study of effects in whole ecosystems, as done at the Marine Ecosystems Research Laboratory (MERL), and by others in other ways. Much less effort has been devoted to an analysis of the fates of substances that might enter the marine environment, so that we are woefully weak in our ability to predict the exposure concentrations that might realistically exist for a given discharge into a body of seawater.

My aim is to point out the primary importance of determining the fates of pollutants. If we do not know what happens to a particular substance, we cannot design an appropriate bioassay, far less carry it out. The necessity of finding out where substances go, or may go, is well recognized (e.g., statement of Hurd in D'Amours 1982), but has not been adequately addressed, perhaps because of a perception that we do not have the technical capacity to do it very well.

It has been estimated (Gunkel and Gassmann 1980) that several tens of thousands of papers have addressed the effects of petroleum in the marine environment. Many of these, of course, have described the results of gross oiling of birds, etc., but a large and mercifully uncounted number have reported experimental results ("Effect of petroleum hydrocarbons on the photosynthetic rate

of...," etc.). Many hundreds or thousands more papers have reported the effects of numerous other pollutants on many species, life history stages and physiological processes.

In reviewing some portion of this literature (Olsen et al. 1982) we noticed a frequent neglect of the physiological and ecological relevance of the experimental concentrations employed. This is, of course, especially evident in bioassay experiments where the response is reported on the basis of the amount of petroleum poured into an aquarium, even though most of the oil floated on the surface or evaporated. The difficulties are seen to be more subtly pervasive, however, when it is realized that most of the petroleum entering the coastal marine environment is actually from urban runoff and discharge from sewage treatment plants (Olsen et al. 1982) and is generally in a partially weathered and particle-bound state, but that very little attention has been devoted to experimental study of the effects of hydrocarbons in this condition. I conclude from this evidence that, in the marine literature at least, there has been too frequently a lack of focus on the need to establish realistic exposure states and concentrations for the substances of interest.

No doubt there are many reasons why so little attention has been paid to the fates of substances in marine waters, in comparison with the situation in lakes and rivers. It is easy in concept (however difficult in practice) to construct mass balances of substances in lakes (Figure 1). One has measurements of the volume of the lake, the flows of water in and out, and the necessary concentrations, and can therefore estimate the inputs and outputs. The lake can be thought of first as a simple chemostat, and then one can add to the analysis such features as the development and temporal behavior of the thermocline, uptake and release by sediments and transport by organisms.

The coastal marine environment is much more difficult to model, because so much of it is open to the vast ocean adjacent (Figure 1). The exchange of water is affected by fresh and salt water mixing processes, winds, tides and ocean currents. Even in relatively enclosed bays and estuaries the combination of tides and two-layer flow makes it difficult or, at the very least, expensive to measure and calculate the parameters of the basic hydrologic regime, needed to assess accurately the dilution, mixing or transport of substances discharged into the bay. In favorable circumstances it is possible to use the salinity of the water as a tracer, so that substances coming in with the fresh water are assumed to be diluted by a factor calculated from the salt content. This works for substances showing "conservative" behavior; that is, they are not acted upon by any process except the mixing of salt and fresh water. Most substances of interest in the context of pollution studies show non-conservative behavior, however, caused by one or many processes acting on each substance (Table 1). In the last two decades it has been popular to spend large sums of money to develop math-

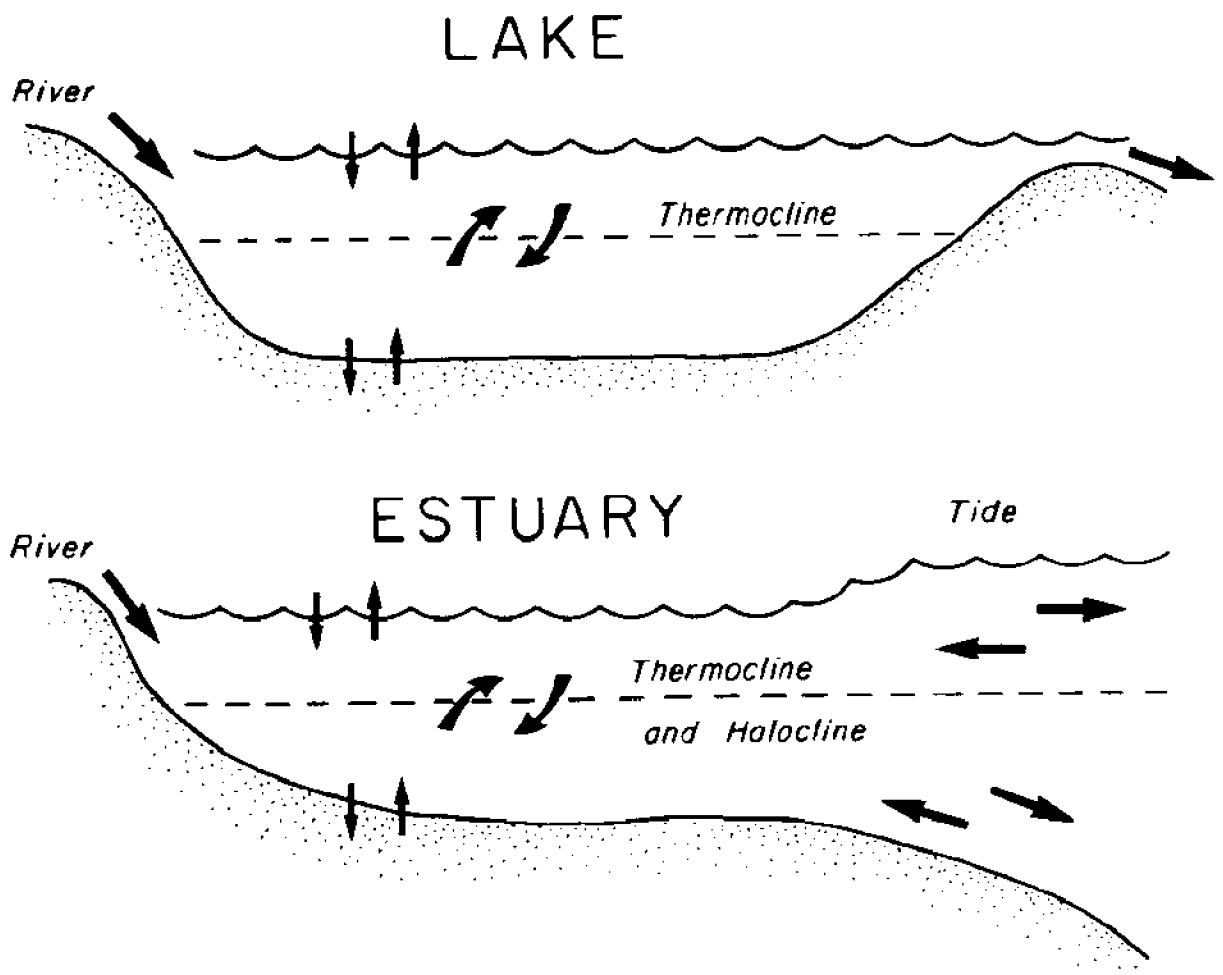


Figure 1. Comparison of lakes and estuaries with respect to major features of the hydrologic regimes and chemical exchanges.

ematical models, the intention being to predict the fates and effects of substances. According to Platt et al. (1981), whole ecosystem models have by and large not fulfilled the expectations of those who have funded them, and, with some limited exceptions, it seems we are not yet in a position to use them for predictions and management decisions. The building of such models is both useful and necessary; their greatest value is that they make us think of the important processes and interactions, and realize where it is that we most need quantitative data.

Table 1 lists processes that might act on some substance. Their complexity, their rates, their interactions, their dependence on temperature, salinity, sediment type or on the type of ecosystem involved, are simply staggering. The difficulty of obtaining quantitative information on the rates of each process measured in the laboratory one at a time, and on the many physical equilibria involved, and of extrapolation to the field, ensures that we can never have a perfect prediction of the behavior of any substance in coastal marine waters. However interesting and valuable in themselves, studies of individual processes in all their complexity will not easily enable us to make quantitative predictions of the trajectory of any average molecule in time and space.

Table 1. Processes that can affect the concentration of a substance entering the coastal marine environment.

Mixing and advection
Evaporation
Uptake by sediments, or release by sediments
(a) Directly
(b) Via particles in water column
Biological uptake and transport
(a) Within water column
(b) To sediment
Photochemical transformations
Chemical oxidations
Biochemical transformations, in water column or sediments
(a) Total oxidation
(b) Production of new structures

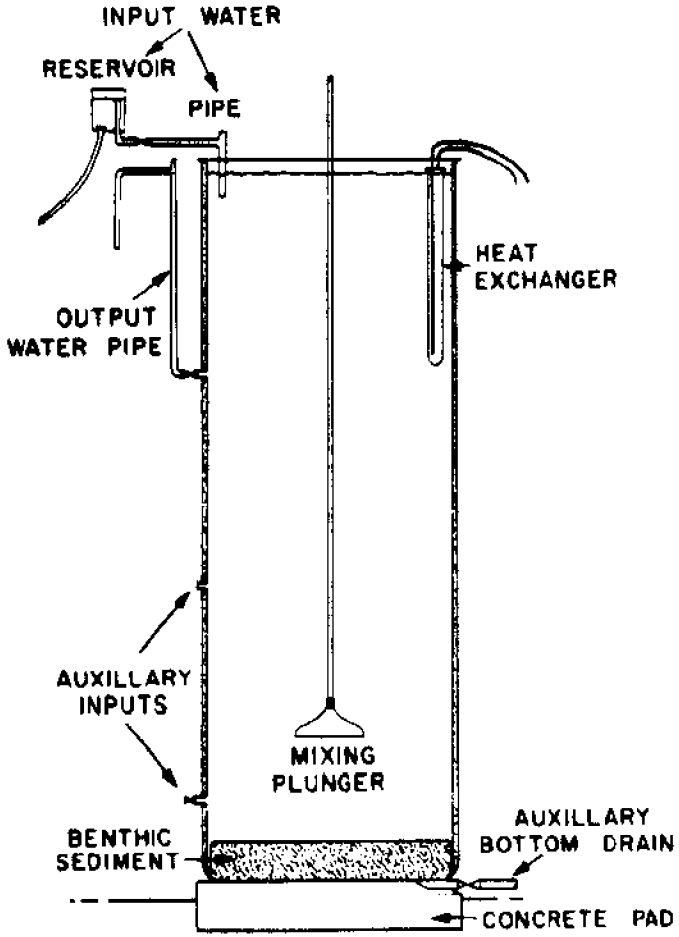
To know what will really happen to some substance discharged into marine waters, there is probably no perfect substitute for doing the real experiment. Discharge the substance. Then we will have a chance of knowing, provided that a great deal of effort is put into following the substance in question. The technical difficulty of this approach is not always appreciated. Despite decades of investigation, we still lack, for example, a well-constrained budget for total fixed nitrogen in any estuary or other body of marine water (Nixon and Pilson 1983). If we have so far not done better with a substance of such wide general interest, what hope do we have with some other individual pollutant, perhaps not yet studied at all?

An additional difficulty with doing the experiment in nature is that a number of our past experiments of this type (e.g., methyl mercury in Minamata Bay, Japan; DDT in California coastal waters) have brought us considerable grief. There were horrible illnesses and deaths of people who ate contaminated shellfish in Japan, and pelicans were nearly eliminated from the coast of southern California. Few people would advocate that these experiments be repeated.

Despite the many difficulties, we need some way to acquire at least a reasonable estimate of the possible fates of the many substances that are now discharged or may be discharged in the future. Without reasonably well-informed estimates of the fates of substances, it does not seem possible to predict exposure concentrations and consequent effects on the ecosystem. In the simplest case, that of a conservatively acting substance, the primary requirement is a good approximation to a three-dimensional hydrodynamic model. For the great majority of substances of interest, however, the other transport and transformation processes will often dominate. For these it seems that both guesses and estimates based on physical properties (quantitative estimates of particle reactivity, ease of metabolism, fat solubility, etc.) combined with direct experimentation are necessary. Since the experiment in nature is generally undesirable or unsatisfactory, the laboratory information needs to be supplemented by direct experimentation in living models of nature.

In the last several years it has been demonstrated that it is possible to maintain a considerable realism in various enclosed experimental marine ecosystems, and because of this I believe the approach has come of age, or nearly so. Microcosms (Giesy 1980) or mesocosms (Grice and Reeve 1982) of marine ecosystems can, in at least some circumstances, be used with confidence to examine chemical and biological processes in coastal waters. Following is a series of examples from the systems I know best.

At MERL, we have set up living models (mesocosms) of the coastal marine ecosystem (Figure 2). These systems have been studied extensively for several years (Pilson et al. 1980; Pilson and



Water		
	Depth	5.00 m
	Area	2.63 m^2
	Volume	13.1 m^3
	Salinity	29-30 o/oo
	Mass	13.4 tons
Sediment		
	Area	2.52 m^2
	Depth	30 cm
	Volume	0.76 m^3
	Mass (wet)	1.1 tons
	(dry)	0.57 tons

Figure 2. Cross section of one of the 14 MERL mesocosm tanks. The tanks are outdoors, exposed to natural sunlight, and can be run either in batch mode or flow-through. In the flow-through mode of operation, the usual practice is to add 120 liters of Narragansett Bay water each 6 hours, for the turnover time of 27 days.

Nixon 1980) and they have shown a remarkable fidelity to the adjacent reference system, Narragansett Bay, R.I. For example, the nutrient concentrations in the systems follow the same pronounced annual cycles that are observed in the bay. This is demonstrated for phosphate in Figure 3. The dynamics of nitrogen and of silicate are different, but bay-to-tank comparisons are equally convincing (Pilson 1982; Pilson et al. 1980).

From data such as those in Figure 3, I conclude that the sum total of all the processes that poise the phosphate concentration in the bay must operate in a remarkably similar and apparently normal way in the MERL tanks as well. Many processes are known or thought to be important in the cycling of phosphorus. These include uptake by each of the 70 or more species of algae identified in the systems, feeding and excretion by zooplankton, transport by fecal pellets to the sediments, remineralization by benthic organisms, transport in particulate form into the sediments by bioturbation, remineralization at depth, adsorption and desorption by sediment particles and release back to the water column. These processes must all be working at normal rates, or at least their net balance must be normal. These tank-enclosed systems are living models, and to a considerable extent they appear to recycle the nutrients as necessary.

It is not only the nutrient elements that are appropriately transported and recycled. Santschi and his co-workers have measured the half-removal times of several metals from the water column in the MERL tanks, compared with half-removal times predicted from investigations in Narragansett Bay (Figure 4). The predicted and observed values are remarkably similar. The removal of at least some particle-reactive substances is related to the flux of sediment particles through the water column. In Narragansett Bay this varies from time to time by a factor of nearly 100. It is somewhat less variable in the tanks, but in the tanks as well as in the bay it is much greater in the summer than in the winter, apparently because of the activities of benthic organisms. Some metals, of which manganese has been the most studied, exhibit a large transport in a dissolved form from the sediments to the overlying water and they are apparently recycled back to the sediments in a particulate form (Hunt and Smith 1980).

We have measured many parameters that define the state or function of the ecosystems in the MERL mesocosms. Although there are a few exceptions, which provide some insight into the ways that marine ecosystems operate, it has been for the most part difficult to distinguish the ecosystems in the tanks from the ecosystem in Narragansett Bay (Table 2).

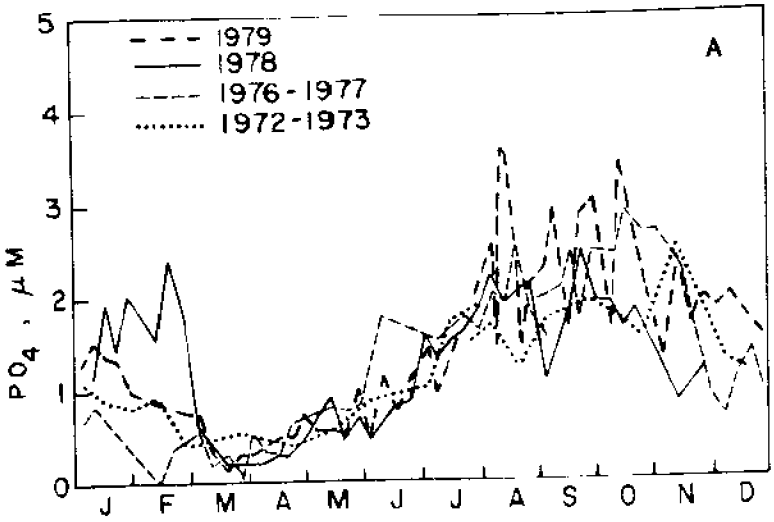


Figure 3A. Concentrations of inorganic phosphate measured weekly at or near the Graduate School of Oceanography (GSO) dock in lower Narragansett Bay, through the course of several annual cycles (1972-73 data from S. Nixon and J. Kremer, pers. communication). Our present understanding of phosphate dynamics is not sufficient to enable us to predict quantitatively the concentrations observed, but the annual cycle is fairly well defined and evidently is constrained by processes that show considerable seasonal regularity (Pilson et al. 1980).

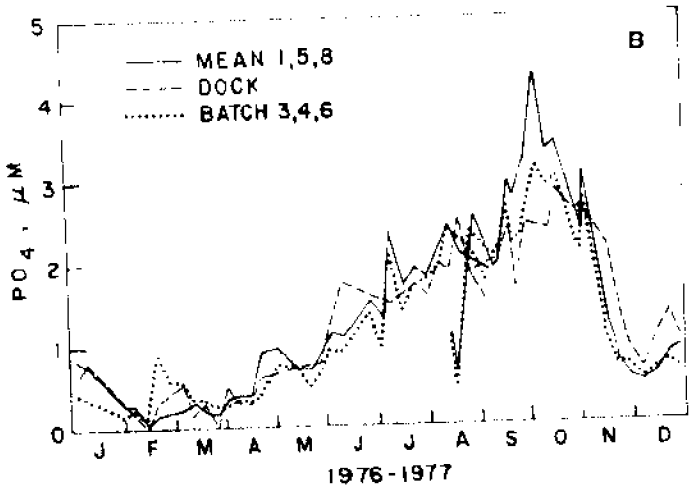


Figure 3B. Concentrations of inorganic phosphate observed weekly in MERL tanks, compared with concentrations observed at the GSO dock. Average values from three control tanks are plotted for each week. These tanks were run with a flow-through of bay water sufficient to replace the volume in 27 days. Average values for three tanks run without any flow-through from 1 Jan. to 30 July are also plotted. The same three tanks were on flow-through from August through September, and comparison with the controls provides an indication of inter-tank variability.

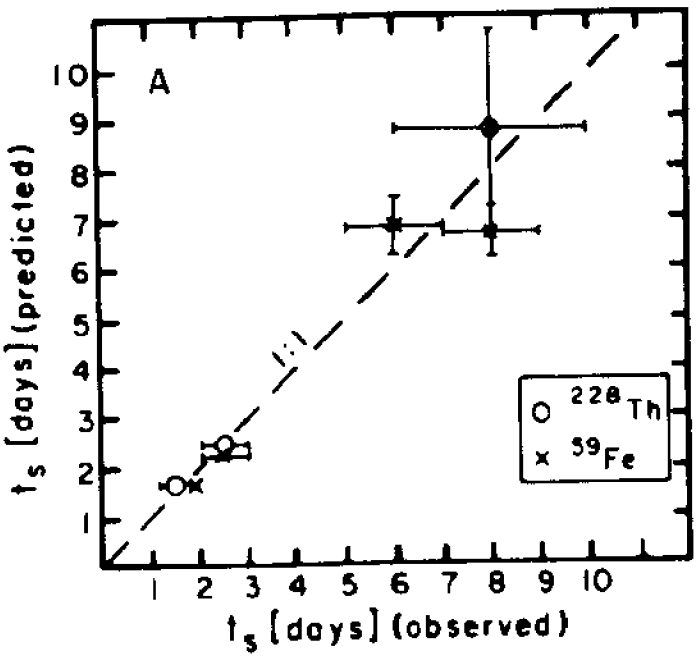


Figure 4A. The measured half-removal time t_s for ^{228}Th in the MERL tanks plotted vs. the predicted t_s' from ^{234}Th/^{238}U disequilibria in the bay. Since Fe appears to be as particle reactive as Th, the predicted half-removal times for Fe were assigned on the basis of a prediction for Th. Error bars indicate 1 standard deviation.

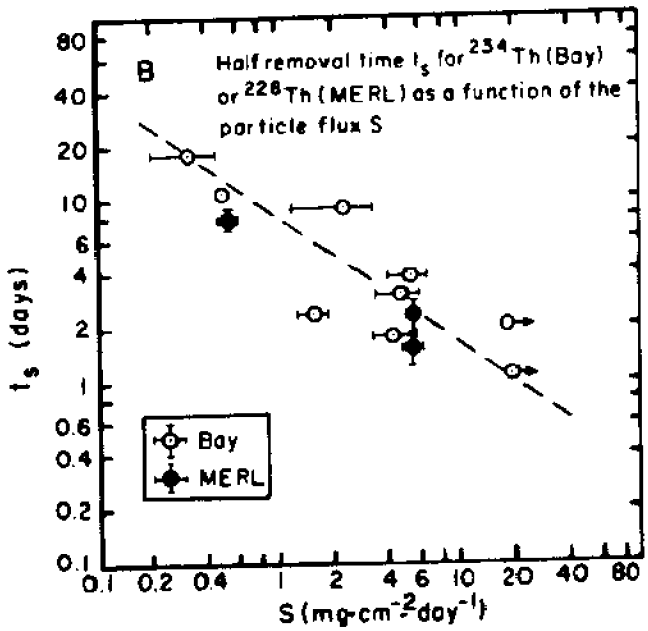


Figure 4B. Measured or calculated half removal time t_s or t_c' respectively, for removal of Th isotopes from the water column of the MERL systems or the bay is plotted vs. the particle flux, S, through the system. Error bars indicate 1 standard deviation.

Table 2. Features of the chemical and biological processes and concentrations observed in the MERL mesocosms and in Narragansett Bay. In every case noted the mesocosm values appear to lie within the range of values observed in the Bay, or the qualitative evidence does not seem easily distinguishable.

Nutrient concentrations
Annual nutrient cycles
Metal concentrations
Annual metal cycles
Photosynthetic production
Respiration - water column
Respiration - benthos
Phytoplankton species and biomass
Zooplankton species and biomass
Benthic species and biomass
Sediment mixing rates
Sediment-water exchange rates

If most of the natural geochemical rate processes that we know, including transport to the sediments, proceed in the mesocosms at rates within the observed range of rates in Narragansett Bay, then it seems reasonable to suppose that the transport and fates of more exotic pollutants can also be investigated in the mesocosms.

One example of such a study is an experiment we carried out using radiocarbon-labeled benzanthracene (Hinga et al. 1980). We added about 1 mg of the material to one tank, and followed the radio-tracer label for about 200 days (Figure 5). During the time it remained in the water column the benzanthracene was rapidly metabolized to CO_2. Some also appeared in other metabolic products, and the fraction of parent compound or other products was measured in phytoplankton and zooplankton as well. However, the total material not metabolized to CO_2 rapidly left the water column and

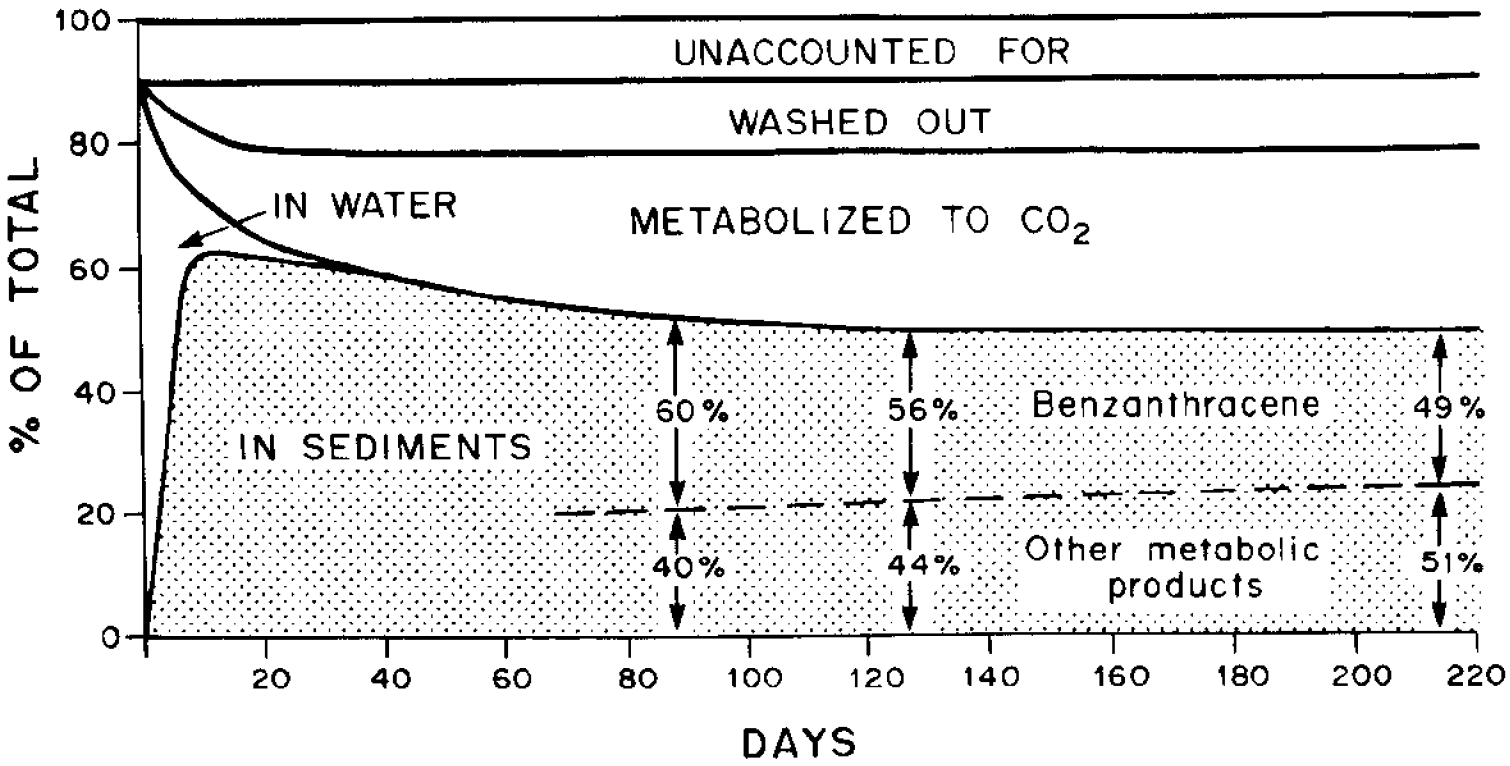


Figure 5. Fate of C-14 labeled benzanthracene in a MERL mesocosm. Approximately 1 mg of the labeled compound was added to the mesocosm on 29 May 1978. The distribution of label between parent compound, intermediate metabolic products (thought to be mostly oxygenated derivatives of benzanthracene) and CO_2 was determined at intervals over the next seven months. Conversion of labeled benzanthracene into intermediate metabolic products occurred both in the water column and in the sedimets, but the residence time of all non-CO_2 metabolic products in the water column (in phytoplankton, zooplankton and dissolved) was short. Once in the sediment, both parent compound and metabolic products were largely protected from further metabolic destruction. Adapted from Hinga et al. (1980) and Olsen et al. (1982).

entered the sediments. There both the original benzanthracene and the various non-CO_2 metabolic products appeared remarkably stable, having perhaps a very slow continuing conversion of parent compound to other products. The material moved gradually down to a depth of several centimeters in the bioturbated sediments. After 200 days about one-half was still parent benzanthracene, and one-half was in the form of various metabolic products that behaved chemically like oxygenated (and therefore more polar) derivatives of benzanthracene. It is not known whether there are likely to be ecological consequences associated with the sequestration of benzanthracene and its products in the sediments, but it should be possible to find out. Similarly, it is not known precisely what are the metabolic products of benzanthracene in such a naturally operating system, but it should be possible to find out. The same suggestions hold for each of the thousands of organic compounds of possible interest in the marine environment.

A review of the evidence given above leads me to the conclusion that we should in the future pay more attention to the fates of substances and, furthermore, that it is experimentally possible to do so. We should carry out MERL-type experiments in several locations. We should put more effort into observing the rates of destruction and the metabolic products of organic compounds.

I am often asked whether MERL-type experiments are not very expensive, and I further often detect a feeling that for some reason the systems just cannot work. In regard to the first question, historically the operation of the MERL systems has cost about 15% of the budget, the rest is the cost of the science. It is necessary to maintain a critical mass of investigators in order to carry out coordinated and focused whole-ecosystem experiments, but the same is true of field work as well. Also, it seems much more cost-effective to carry out simultaneous observations of many interacting species in whole systems than to try to put together the plausible effects from numerous individual studies carried out under varying conditions in different places. With regard to the question of whether living models of ecosystems in mesocosms can "work" well, it seems the burden of proof has to be on those who might show empirically that the results obtained are somehow misleading.

So far, however, living models have proved to be powerful tools for the analysis of the systems they represent. In them one can learn of the important processes that control the functioning of coastal marine ecosystems, and of the fates and effects of substances. The cost-effectiveness and technical value of simultaneous studies of fates and effects during the same experiment are additional attractive features.

SUMMARY

Studies of pollution in the coastal marine environment have emphasized the investigation of effects, to the considerable neglect of fates. Knowledge of fates is, however, a prerequisite to the study of effects.

The fates of substances can be predicted to some extent by an analysis of the physical, chemical and biochemical properties of the substances. In addition it is now technically possible to carry out apparently realistic studies in living models of the coastal marine ecosystem. The experimental study of model marine ecosystems is only in its infancy, and there is much to learn both of the processes that control the functioning of such systems, and of the fates and effects of substances in the coastal marine environment.

ACKNOWLEDGMENTS

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REFERENCES

D'Amours, N.E. 1982. Statement of the Honorable Norman E. D'Amours, Chairman, Subcommittee on Oceanography, U.S. House of Representatives Committee on Merchant Marine and Fisheries, Joint Hearing on the Reauthorization of the Marine Protection, Research and Sanctuaries Act, 18 March, 1982, and Attachments.

Giesy, J.P., Jr. (ed.). 1980. Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov 8-19, 1978. (CONF-781101, National Technical Information Service, Springfield, Va.)

Grice, G.D. and M.R. Reeve (eds.). 1982. Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems. Springer-Verlag, New York.

Gunkel, W. and G. Gassman. 1980. Oil, oil dispersants and related substances in the marine environment. Helgol. Wiss. Meeresunters. 33: 164-181.

Hinga, K.R., M.E.Q. Pilson, R.F. Lee, J.W. Farrington, K. Tjessem and A.C. Davis. 1980. Biogeochemistry of benzanthracene in an enclosed marine ecosystem. Environ. Sci. Technol. 14: 1136-1143.

Hunt, C.D. and D.L. Smith. 1980. Conversion of dissolved Mn to particulate Mn during a diatom bloom: Effects on the Mn cycle in the MERL microcosms. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga. Nov. 8-19, 1978, pp. 850-868. (CONF-781101, National Technical Information Service, Springfield, Va.)

Nixon, S.W. and M.E.Q. Pilson. 1983. Nitrogen in estuarine and coastal marine ecosystems. In: E.J. Carpenter and D.G. Capone (eds.), Nitrogen in the Marine Environment. Academic Press, New York.

Olsen, S., M.E.Q. Pilson, C. Oviatt and J.N. Gearing. 1982. Ecological consequences of low, sustained concentrations of petroleum hydrocarbons in temperate estuaries. Marine Ecosystems Research Laboratory, graduate School of Oceanography, Univ. of Rhode Island, Kingston.

Pilson, M.E.Q. 1982. Annual cycles of nutrient concentrations in Narragansett Bay, R.I. EOS, Trans. Am. Geophys. Union 63: 347.

Pilson, M.E.Q. and S.W. Nixon. 1980. Marine microcosms in ecological research. In: J.P. Geisy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp. 724-741. (CONF-781101, National Technical Information Service, Springfield, Va.)

Pilson, M.E.Q., C.A. Oviatt and S.W. Nixon. 1980. Annual nutrient cycles in a marine microcosm. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research, DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp. 753-778. (CONF-781101, National Technical Information Service, Springfield, Va.)

Platt, T., K.H. Mann and R.E. Ulanowicz (eds.). 1981. Mathematical Models in Biological oceanography. UNESCO Press, Paris.

Santschi, P.H., S. Carson and Y. -H. Li. 1982. Natural radionuclides as tracers for geochemical processes in MERL mesocosms and Narragansett Bay. In: G.D. Grice and M.R. Reeve (eds.), Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems, pp. 97-109. Springer-Verlag, New York.

Utility of Mesocosms to Assess Marine Pollution

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INTRODUCTION

Marine mesocosms have considerable potential for assessing pollution effects. In contrast to field studies, mesocosms provide sufficiently contained marine ecosystems to allow the experimental manipulation and repetitive sampling necessary to permit fate, exposure and effects assessements and to allow elucidation of controlling processes. The ability to manipulate and replicate these systems allows for valid experimental designs and the testing of models about pollutant fate and effect, and ecosystem function. The major disadvantage relative to field studies is the need to assure field validity of the systems. In contrast to laboratory studies, mesocosms permit simultaneous measurement of fate and effects at the ecosystem and all other levels of organization, ensuring that the response parameters can be significant at the ecosystem level. The general applicability and validity of the results can be increased by measuring fate and effect over seasonal cycles or by manipulating the mesocosm to enhance the diversity of environmental conditions. The major disadvantages of mesocosms relative to laboratory studies is how best to measure ecosystem response and how to detect effect against the background of natural variability (both are also field problems).

In attempting to evaluate how well mesocosms have achieved their potential, it has become clear that the utility of these systems has depended on selection of appropriate mesocosm systems and experimental designs, and the degree to which an interdisciplinary effort is used to elucidate the processes controlling the behavior of the system and pollutants within the system. Furthermore, it appears that there is considerable uncertainty on the part of the scientific and administrative communities as to the most appropriate match of system design, experimental design and inter-

disciplinary effort to solve a given pollution problem (Banse 1982). It is therefore the objective of this paper to try to define the advantages and limitations of existing systems and experimental designs, and subsequently to consider how combinations of existing and potential systems and experimental designs could be used to address unsolved marine pollution problems. It will not be our approach to review every mesocosm experiment, but rather to discuss those that illustrate a particular point.

SYSTEM DESIGN

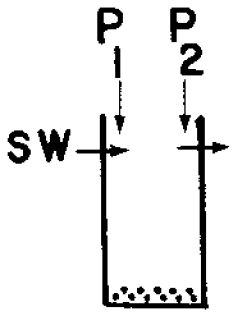
The term system design refers to the physical structure of the model ecosystem and the ability of water, organisms and pollutants to move from compartment to compartment within the system. Since the behavior of coastal ecosystems (Nixon 1982) and the fate and effect of pollutants within those systems (Santschi 1982, for review) appear to be strongly affected by the degree to which vertical physical structure or mixing depth limits the interaction of planktonic and benthic processes, our classification of system designs is based on the degree of planktonic-benthic coupling (Figure 1). It is our contention that this difference is more important than differences between land based or floating mesocosms. Throughout the following discussion of system design, we will consider physical system design, potential usage, the ability to define exposure and control loading, and the field applicability of the design.

Single Well-Mixed Benthic Coupled System

This system design involves a single mesocosm composed of a well-mixed water column in direct contact with sediment and benthos. The system can be run with or without seawater input and allows direct input of pollutants. The fate and effect of added pollutants and the behavior of the ecosystem are controlled by planktonic and benthic processes and their interactions. Planktonic organism exposure in this system is controlled by the rate of pollutant addition, the rate at which it is degraded or buried in the sediment and the rate of loss due to total degradation, volatilization or washout. Exposure for all elements of the plankton will be identical as long as adequate mixing is maintained to prevent spatial inhomogeneities from developing. Exposure for benthic organisms will vary depending on the route of exposure for a particular organism (via water column, sediment or interstitial waters) and the vertical profile of pollutant within the sediment.

This system has been standard at the Marine Ecosystems Research Laboratory (MERL) (Nixon et al. 1980). It has been used for modeling fates and effects of a variety of pollutants; it has also been used in a variety of experimental designs. The major advantages of this system are (1) the applicability of results to a wide

SINGLE
WELL MIXED
BENTHIC
COUPLED



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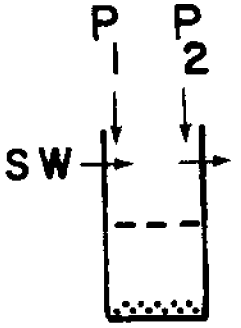

TOTALLY
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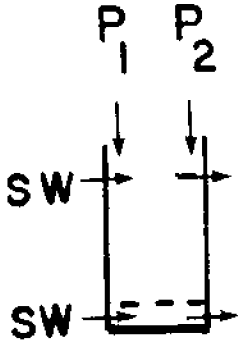


Figure 1. Existing mesocosm system designs schematically illustrated. These systems are termed single to differentiate them from systems connected in a series (see Figure 9). SW = sea water input (if zero, then system is in batch mode; if not zero, then system is flow-through mode). P_1 = input of contaminant of interest. P_1 may be a single concentration addition made only once (termed a spike addition) or made at regular intervals (termed constant loading factor) or P_1 may be continuously adjusted to maintain a fixed concentration of pollutant in the system (termed constant exposure). P_2 = input of a second contaminant or other material to alter the biotic or chemical activity of the system. Mixing in any layer may be continuous or periodic. The dashed line represents a thermocline, pycnocline or halocline. Stippled area represents benthos (sediment and associated benthic fauna).

variety of benthic coupled field sites as defined by extensive field validation; (2) the capability for precise definition of loading and exposure to all components of this system; (3) the demonstrated ability to define fates and effects, and (4) the ability to manipulate the system to define underlying processes.

The field validity of this system design has been extensively tested both in terms of the similarity to field systems of ecosystem function and in terms of the similarity of fates and effects of pollutants. Major ecosystem components such as seasonal cycles of nutrients and primary and secondary producer biomass and composition have been shown to be similar to those occurring in a benthic coupled field system, Narraganett Bay (Pilson et al. 1980; Oviatt, this section). More importantly, major ecosystem processes have been shown to be similar to the bay (Santschi, this volume). Experiments using this system design have been able to predict fate and effect of pollutants in both Narragansett Bay and other coastal systems (Gearing et al. 1979; Wade and Quinn 1980; Gearing et al. 1980; Elmgren and Frithsen 1981). For example, Elmgren and Frithsen (1982) have shown similarities between effects of the MERL oil experiment and the Tsesis oil spill (Table 1). The striking similarity of results is a good test of the field validity of these systems and indicates that mesocosms can be meaningful and operational tools for predicting environmental effects of pollutants. The oil mesocosm study was not only able to predict the qualitative ef-

Table 1. Comparison of effects of oil as measured in the MERL oil experiment and the Tsesis oil spill. Reprinted from Elmgren and Frithsen (1982).

Plankton	MERL 1077	Tsesis
Phytoplankton, as Chlorophyll a	Increased 120%	Increased
Bacterioplankton Abundance	Increased 200-300%	Increased
Ciliate Microzooplankton Biomass	Changed Species Composition	No Effect
Mesozooplankton Abundance	Decreased 43%	Decreased
Macrobenthos		
Total Abundance	Decreased 27%	Decreased
Amphipod Abundance	Decreased 98%	Decreased
Dominant Deposit	Slow Decrease 37%	Oil Contaminated
Bivalves Abundance		No Mortality
Meiofana		
Nematode Abundance	Decreased 47%	Decreased
Harpacticoid Abundance	Decreased 52%	Decreased
Ostracod Abundance	Decreased 100%	Decreased

fects observed in the spill, but also provided the quantitative estimation of effect that is not obtainable from any field spill study. The ability to estimate effect quantitatively was also dependent on using the appropriate replicate experimental design. In addition, as pointed out by Elmgren and Frithsen (1982), the mesocosm study suggested which elements in the ecosystem were most sensitive indicators of effects and thus which factors should have been monitored in the field. The abilities to predict effects quantitatively and to define sensitive indicators are two major advantages of conducting mesocosm studies as an integral part of pollution assessment programs.

Single Stratified System

This system design involves well-mixed top and bottom layers separated by an unmixed thermocline, halocline or pycnocline (Figure 1). Only the bottom layer is in contact with the sediment and benthos. This system can be run with or without seawater input to top or bottom layers. Input of pollutants may also occur to either layer. In contrast to the well-mixed benthic coupled system, the surface layer of this system is dominated by planktonic and cross-pycnocline transport processes. The bottom layer of this system is dominated by interactions with the benthos and sediment and by organic input from the surface layer (by settlement and vertical migration). Pollutants introduced into the surface layer are lost by flocculation, sedimentation and photodegradation, and by biotic uptake, degradation and transport to other layers. Pollutants directly introduced into or otherwise reaching the bottom layer will be partitioned between the lower water column and the sediments and benthos. Photodegradtion and volatilization should be much less significant in controlling fate in the lower than in the upper layer. Once reaching the lower layer, pollutants will be introduced into the surface layer at rates limited by cross-pycnocline mixing and upward transport of biota. Exposure of organisms living solely in the surface layer can be easily defined as in the well-mixed system. Estimates of exposure for organisms residing in the thermocline, or for organisms migrating through the thermocline are dependent on (1) detailed vertical profiling of pollutant distribution over time in terms of concentration and bioavailability and (2) detailed definition of the patterns of vertical movement of organisms.

The stratified system design has only recently been developed at MERL (Donaghay and Klos 1983) to address specific questions about the fate of ocean dumped acid waste (Brown and Kester 1983). Experience indicates that the stratified systems have considerable potential for (1) defining the fate and effect of pollutants in stratified coastal ecosystems, and (2) defining the importance of vertical stratification in controlling ecosystem and pollutant behavior. The ability to run comparative fate and effect studies in

stratified and well-mixed systems could become very useful in developing site selection criteria.

The field validity of these systems has not been as extensively tested as that of the well-mixed systems. The biotic, physical and chemical profiles developed in these systems are similar to those observed in stratified field systems (Figure 2) (Donaghay and Klos 1983). The field validity of the fate results from these systems has, however, been carefully evaluated by Brown and Kester (1983); half-lives calculated for the waste in the surface layers of the mesocosm were quite similar to the surface layer half-lives determined at the ocean disposal site. The similarity of the mesocosm and field results suggests that stratified mesocosms may have the field validity necessary for studying fate and effects of pollutants in stratified coastal ecosystems. Additional tests are needed.

In addition to providing evidence of the field validity of stratified mesocosms, the acid iron waste experiment illustrates the benefits of coupling field and mesocosm studies. Prior field studies of acid iron waste had provided estimates of rates of lateral dispersion not obtainable from mesocosm experiments (Brown et al. 1983). The mesocosm experiments provided more precise estimates of waste half-lives than were available from the short period the waste could be followed in the field (Brown and Kester 1983). More importantly, the mesocosm studies allowed definition of the behavior of the waste in the thermocline and below, thus answering questions raised in the field study as to whether vertical stratification could retard the settling of the waste and thus alter its fate (Brown and Kester 1983). The mesocosm study demonstrated that although vertical stratification could retard settling of the waste and thus lead to concentration on the pycnocline, the effect was transitory: most of the waste was gone from the water column in 164 hours (Brown and Kester 1983) (Figure 3). The complex layered structure of the waste in the pycnocline suggests that more than simple physical gradients are controlling the distribution of waste and therefore biological exposure in this zone. The steepness of the biotic and pollutant gradients in the pycnocline (compare Figure 2a and Figure 3 at 6 hours) emphasizes the importance of knowing the distribution of both pollutant and organisms in attempting to assess exposure in stratified systems. Similar gradients for organisms and pollutants also need to be examined in the field.

Single System Without Benthic Coupling

This system design is intended to model fates and effects in stratified deep water systems with no benthic coupling. Planktonic processes are intended to dominate such systems. In such systems, once a waste penetrates the pycnocline, it is effectively lost from the system. This system design has been used at CEPEX (Menzel and Case 1977; Grice et al. 1980), Loch Ewe (Davies and Gamble

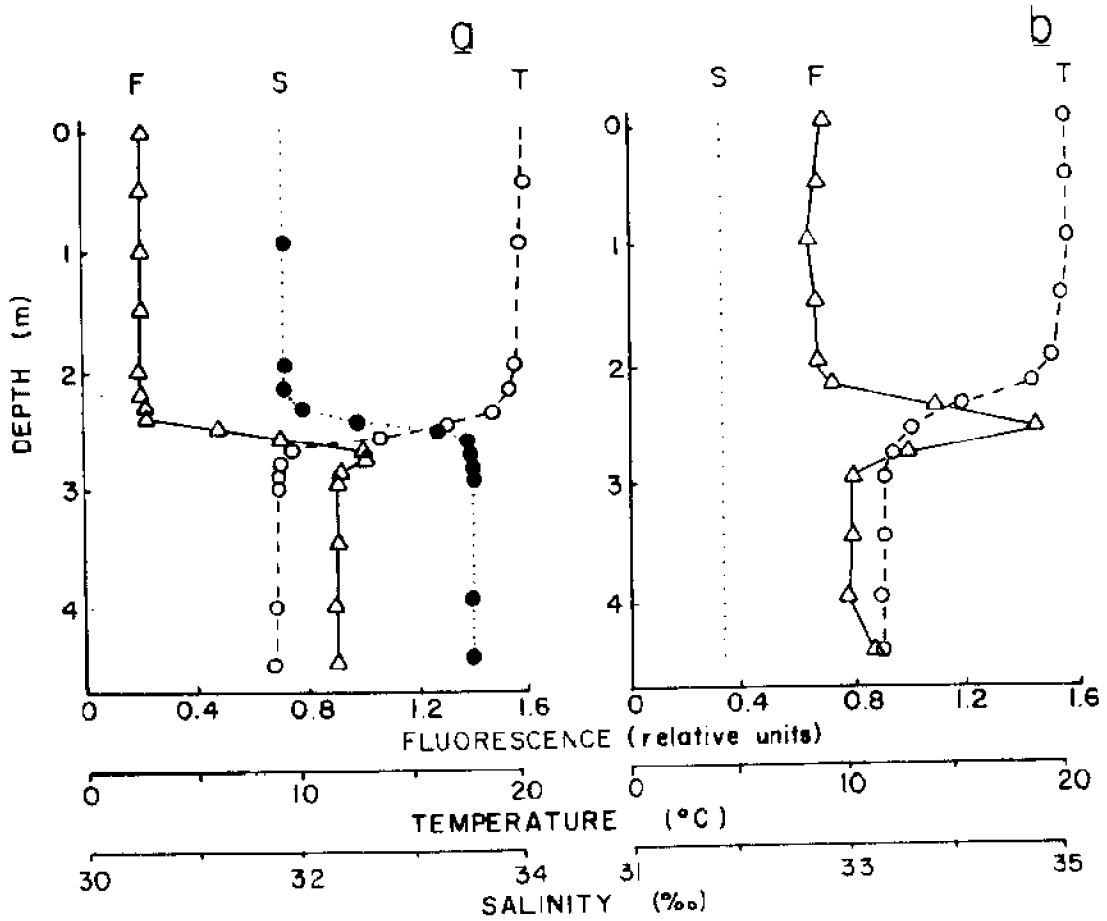


Figure 2. Vertical structure of temperature (T), salinity, (S), and phytoplankton biomass (measured as fluorescence) (F) in thermohaline stratified mesocosms (a) and thermally stratified mesocosms (b). (Redrawn from Donaghay and Klos 1983)

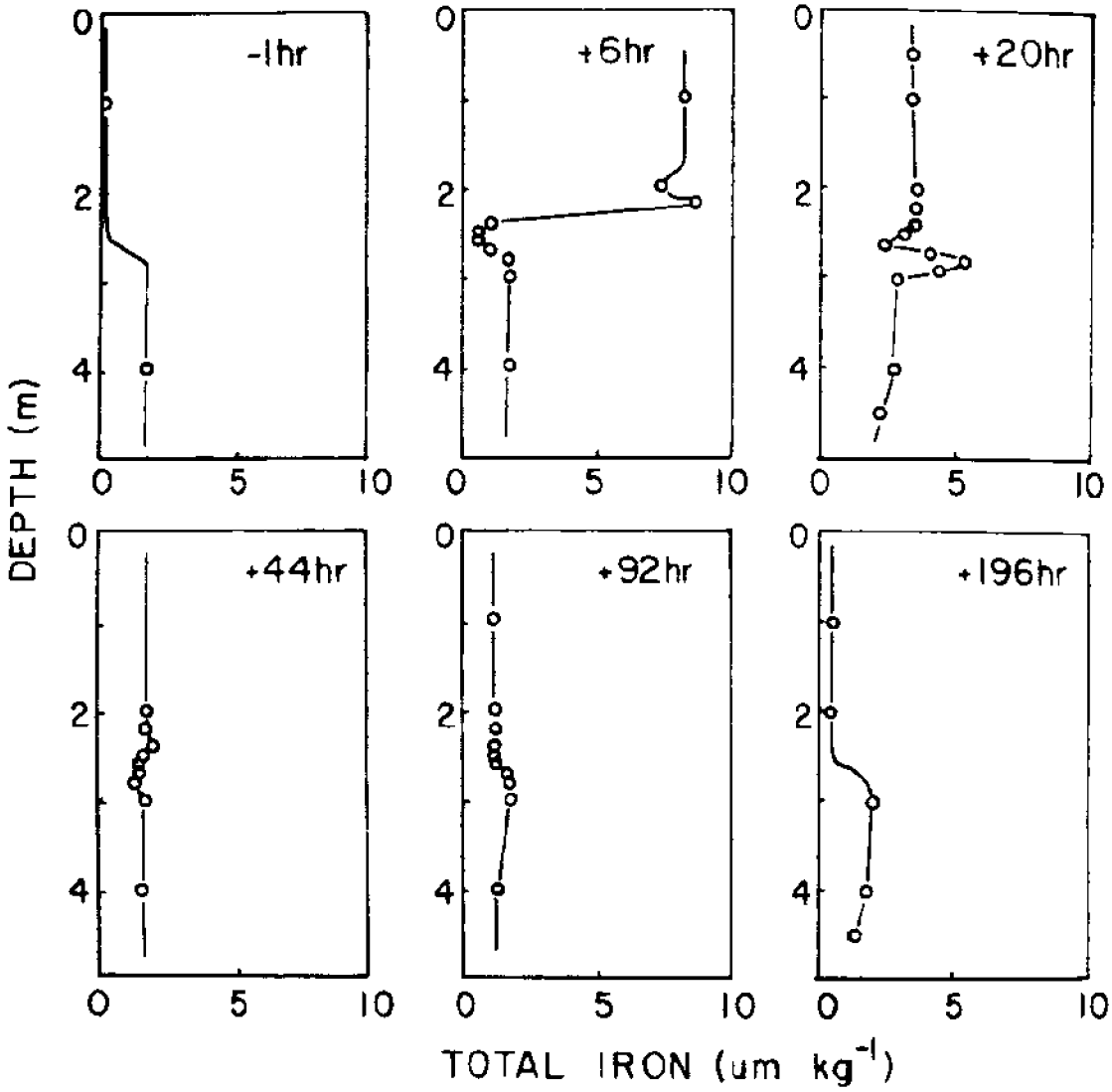


Figure 3. Vertical profiles of acid iron waste measured as total iron concentration, before and at varying intervals after dumping of waste into a stratified mesocosm. Density structure in the mesocosm is similar to that shown in Figure 2. Times represent hours before or after waste dump. Redrawn from Brown and Kester 1983 .

1979) and at MERL (Figure 1). The criteria for this design are met by existing systems only when great care is taken to ensure that all sedimented material is removed from the bottom of the system before recycling can occur. If this condition is met, the pollutant loading is extremely well defined since recycled products from the bottom will have no effect on loading. In practice, this condition can only be approached. As long as the system is well mixed, exposure will be highly defined. Development of vertical physical structures may result in altered exposure as in the vertically stratified systems. The results from systems that meet the design criteria are broadly applicable to non-benthic-coupled deep water systems.

The field validity of benthic decoupled systems varies considerably depending on the duration of the experiment, system design (bag or tank) and type of system simulated. The applicability of large bag enclosures is covered by Grice (this chapter).

EXPERIMENTAL DESIGN

The term experimental design refers to statistical design of the experiments. We consider here the advantages and disadvantages to mesocosm studies of different experimental designs. The underlying tenet will be that scientific and financial resources for mesocosm studies are limited and therefore efforts must be made to maximize the information gained and the statistical rigor of the analysis while minimizing the number of systems analyzed. (In the absence of this constraint, replication at all levels is always desirable.) Four different experimental designs have been used with marine mesocosms (Figure 4). We will consider the nature of each design, its most appropriate usage, the statistical assumptions underlying usage, and the kind of statistical analysis that can be applied. All the experimental designs are compatible with all the system designs described.

Single System

This design involves a single system without replication. Many mesocosm experiments have been run using a single tank, bag or other containment device. The most appropriate use of this design is to determine the fate of materials, that is, rates, routes and reservoirs (see Santschi 1982 for review). These experiments usually involve the spike addition of a material followed by a period of intensive sampling to define the fate of that material. Many of the experiments done in single systems have been tracer experiments involving either radioactive tracers or trace levels of materials (see Pilson this chapter, for review and examples). Such experiments assume that the contaminant input is a tracer that does not

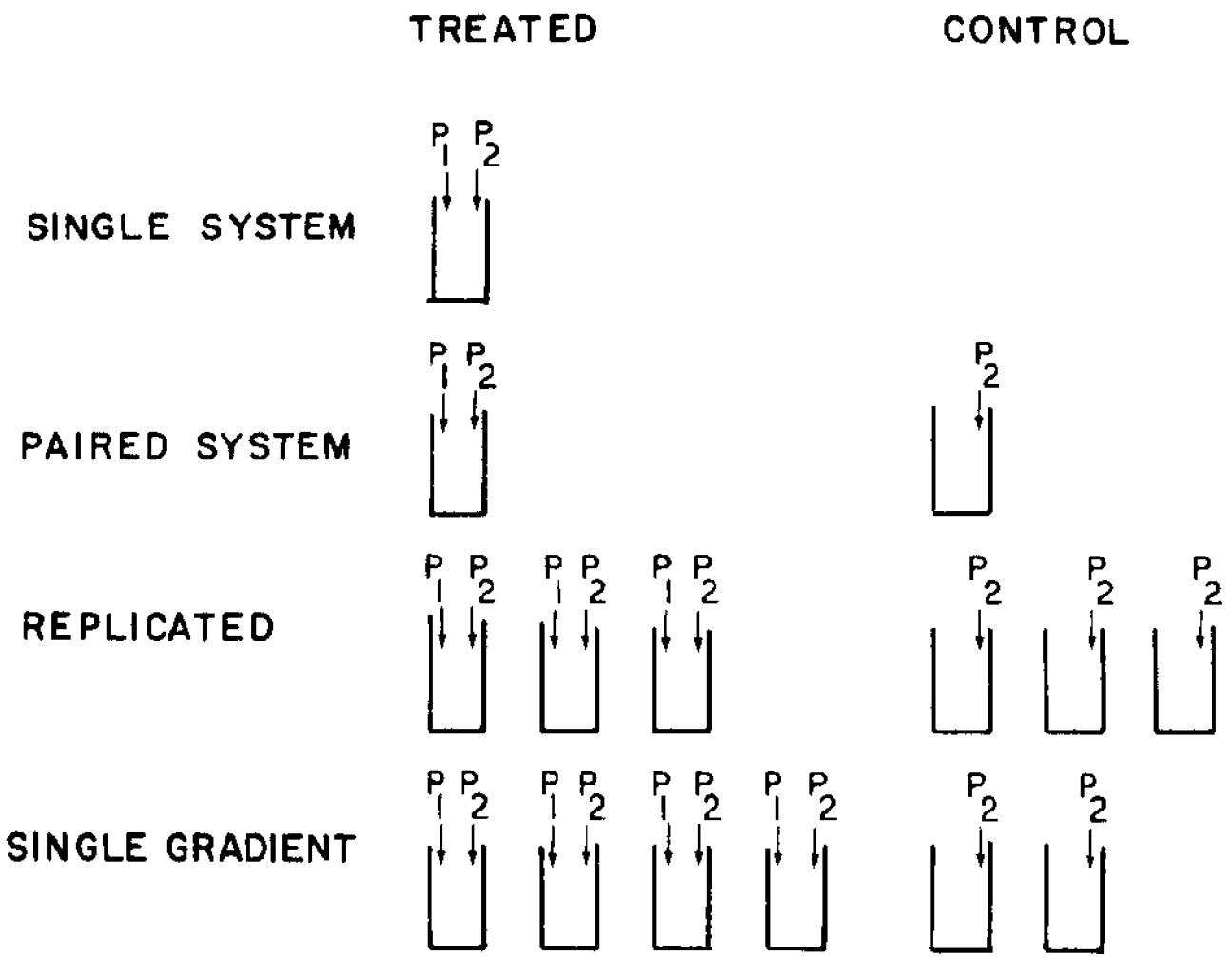


Figure 4. Existing experimental designs. The container represents any system design defined in Figure 1 or any potential system design defined in Figure 9. P_1 and P_2 are as defined in Figure 1.

perturb the balance of the ecosystem, and that the tracer rapidly reaches equilibrium with the existing components of that material and therefore its behavior is identical to that of the existing components of that material. This assumption is currently being tested by Santschi (personal communication). If this assumption is not valid, alternative experimental designs are required. Single system experiments can be replicated by repeating the spike addition at a later time. Without such replication, the results of single system experiments are purely descriptive. One can define the time dependent decay rates and pathways with detailed definition of sampling error statistics. Under optimal conditions these errors can be reduced to a few percent (see Santschi this chapter). Such sampling statistics should not be confused with the estimates of variability resulting from multiple experiments at one time or one experiment repeated multiple times. Variability in decay rates between experiments run at the same time tends to be less than a factor of 2; between experiments run at different times it tends to be less than a factor of 10 (Amdurer et al. 1982). Large numbers of temporal replications can provide excellent statistics as to the range of possible fates and rates of removal. Repeating these experiments under slightly different environmental conditions can provide information on what processes are dominant in controlling the fate of the material as well as an even better estimate of the range of possible fates and rates of removal (Gearing and Gearing 1982).

There have been a few attempts to use single systems to define pollutant effects. Although such experiments can be useful in providing preliminary insights, effects cannot be statistically defined because of the time dependent nature of biological variability. The statistical problems are identical to those of field spill or dump studies, but without the additional difficulty of following the exposed system in the field. As in the field, detailed before and after measurements cannot be used to solve this statistical problem. There are still no controls.

Paired System Without Replication

Fate studies have also been run with paired systems composed of a single treated system and a single control. In the oil fates experiment (Gearing and Gearing 1983), acid waste dump experiment (Brown and Kester 1983) and many of the CEPEX experiments (Grice, this chapter), the contaminant is often not present in tracer quantities, but the intent is still to define the fate of the material. For example, in the acid iron waste experiment (Brown and Kester 1983), the waste was purposely added at concentrations similar to those observed during ocean disposal of the material. The intent was not to trace the natural cycling of iron and other metals, but rather to determine the fate of the waste under current disposal practices. The objective of the single control in such

experiments is to define effects of covariant parameters (such as the effects of temperature, vertical structure, etc., on recycling and resuspension rates). The normal procedure in such cases is to follow in detail both systems over an initial period to demonstrate whether the two systems track in behavior, and then to dose one. The statistical validity of such a procedure assumes that divergence of the two systems would not increase in time.

As in the case of the single system, repeating the experiment in time would be required for the statistical variability in rates and fate assessment. With replication, the same statistical analyses that were applied to replicated single systems could be applied, and covariance analysis could be added. For example, in the acid iron waste experiment, iron was observed to accumulate along, and then gradually disappear from the thermocline following the dump of acid iron waste (see Figure 3 above). Since no similar accumulation occurred at the same time in the control tank and since the levels in the treatment tanks eventually returned to predump and control levels, it was clear that the accumulation on the thermocline was waste iron, and was not due to some other factor altering iron distribution in the tanks. The ratio of iron to chromium was also used to identify the origin of iron in the vertical profile (Brown and Kester 1983).

Despite the power of paired systems to define fates, their power to define effects is very weak and depends on repetition of the experiments. The statistics resulting from repeated sampling of a single experiment (with single control and treatment) cannot be substituted for repeating the entire experiment to define effects. For example, in the acid iron waste experiment, based on sampling statistics from 10 replicate benthic cores, there was a statistically significant lower level of benthic macrofauna in the waste treated tank (J. B. Frithsen personal communication). However, since these were not replicate tanks, the differences cannot be uniquely attributed to the waste dump.

Repeating the experiment in time is a less powerful method for detecting effects than simultaneous replicates. The ability to statistically detect effects is a direct function of the similarity of the replicates as compared to the differences between treatments and controls. Since mesocosms run sequentially are less likely to have similar biological and chemical character than those run at the same time, greater differences between treatments and controls are required to statistically detect effects. As a result, sequential replication will almost always result in the inability to detect any but the largest effects.

Replicate Controls and Treatments

Replication of controls and treatments provides the first statistically rigorous method that we have considered for defining both fates and effects. As pointed out in the earlier discussion of the

oil experiment (Table 1), such experiments provide clear and statistically quantifiable estimates of fate and effect. Because of the requirement for multiple containers, the use of such experimental designs has been limited. The major studies were the MERL oil effects (Oviatt et al. 1983 and references therein; Elmgren et al. 1980) and the MERL sediment gradient experiments (Kelly 1983; Hunt and Smith 1983). Both of these studies involved the comparison of three replicated treated mesocosms with three or more replicated controls.

Experimental designs using replicated controls and treatments have a minimum of statistical assumptions. Problems of system divergence and other types of variability are directly measured in both control and treated systems. Rigorous statistical testing of fates and effects can be accomplished using standard analysis. Additional statistical analyses can be run to determine whether the natural variability between systems evident in the controls has been constrained by the contaminant, as was observed in the oil effects study (Oviatt et al. 1982). For such experiments to be generally applicable, it is essential that they be run for at least one-half year to allow assessment of differences in fates and effects due to seasonal changes in biological and chemical activity. Despite the fact that this experimental design provides defined statistics for a given dose level, it provides no information about the sensitivity of the results to slightly different dose levels. For example, two dose levels were examined in successive years in the MERL oil effects experiments to provide some statistical evaluation of the steepness of the dose-response relationship. Evaluation of the dose-response relatonship can be more cost-effectively accomplished using gradient experimental designs (see below).

Despite the statistical advantages of replicated control and treatment designs, some practical problems are often encountered in evaluating the statistical results. These problems are usually the result of having to detect effects against a background of large fluctuations in biotic and chemical processes and concentrations. These fluctuations are not simply stochastic; they are the response of the systems to seasonal fluctuations in light, temperature, nutrients and other important environmental variables. Simple averaging techniques may obscure major effects. For example, lags in response of a few days between replicate tanks can result in such large error estimates that only the largest differences are statistically detectable. Solutions to these problems would appear to involve a variety of more sophistcated statistical techniques. The application of multivariate analysis techniques (multiple discriminate analysis and distance analysis) has been useful in statistically quantifying effects in spite of time lag problems. However, such procedures have not revealed any effects that were not already obvious from a visual examination of the raw time series data (Oviatt et al. 1980). The utility of such statistical techniques may be en-

hanced in the future (1) by selecting measures of ecosystem response that are less time variant than factors such as system structure or form of components, and (2) by defining the frequency of oscillation of important parameters to ensure that sufficiently frequent data are collected to allow appropriate filtering of the time series data before statistical tests are made. These problems are not unique to mesocosm work; they are the bane of most field work and of monitoring in particular (see discussion by Smayda, Chapter 8). Biological oceanographers are only now beginning to use such techniques.

Single Gradient Analysis

Single gradient analysis is a technique designed to determine the dose response relationship of a pollutant with statistical precision rather than the fate and effect of a single dose level. The technique depends on regression analysis to define the dose-response relationship and to define the statistical confidence limits. The statistical power of this analysis increases dramatically with range of dose levels and the number of treatments over that range. The range must be great enough that measured changes in fate or effect over that range are much greater than the inherent variability of the system at one level. At the same time, there must be a sufficient number of treatments at intermediate dose levels to define response between the extremes of no effect and massive ecosystem failure. Increasing the number of dose levels also increases the statistical power of regression analysis. Early application of single gradient analysis (Kuiper 1982; Grice et al. 1977; Menzel and Case 1977; Takahashi et al. 1977) usually had only two or three levels of dosing plus one or more controls. Regression analysis does not appear to have been extensively applied to interpret these data, probably because of its limited statistical power resulting from only a few treatment levels. In contrast, regression analysis has been used extensively to analyse the results of the MERL nutrient gradient experiment (currently in progress at MERL and described by Donaghay et al. in prep.). In this experiment six dose levels plus three controls were used to cover the entire range of anthropogenic nutrient loading to coastal waters (Figure 5). In gradient analysis, replication at any given treatment level is not statistically required. Replication at any given level may be employed to meet specific research objectives. For example, in the nutrient gradient experiment, multiple controls were used to achieve better statistical definition of the dose-response relationship at the lower end of the regression where effects might be harder to detect (Figure 6). In addition, multiple controls allow definition of differences at any given level from the controls.

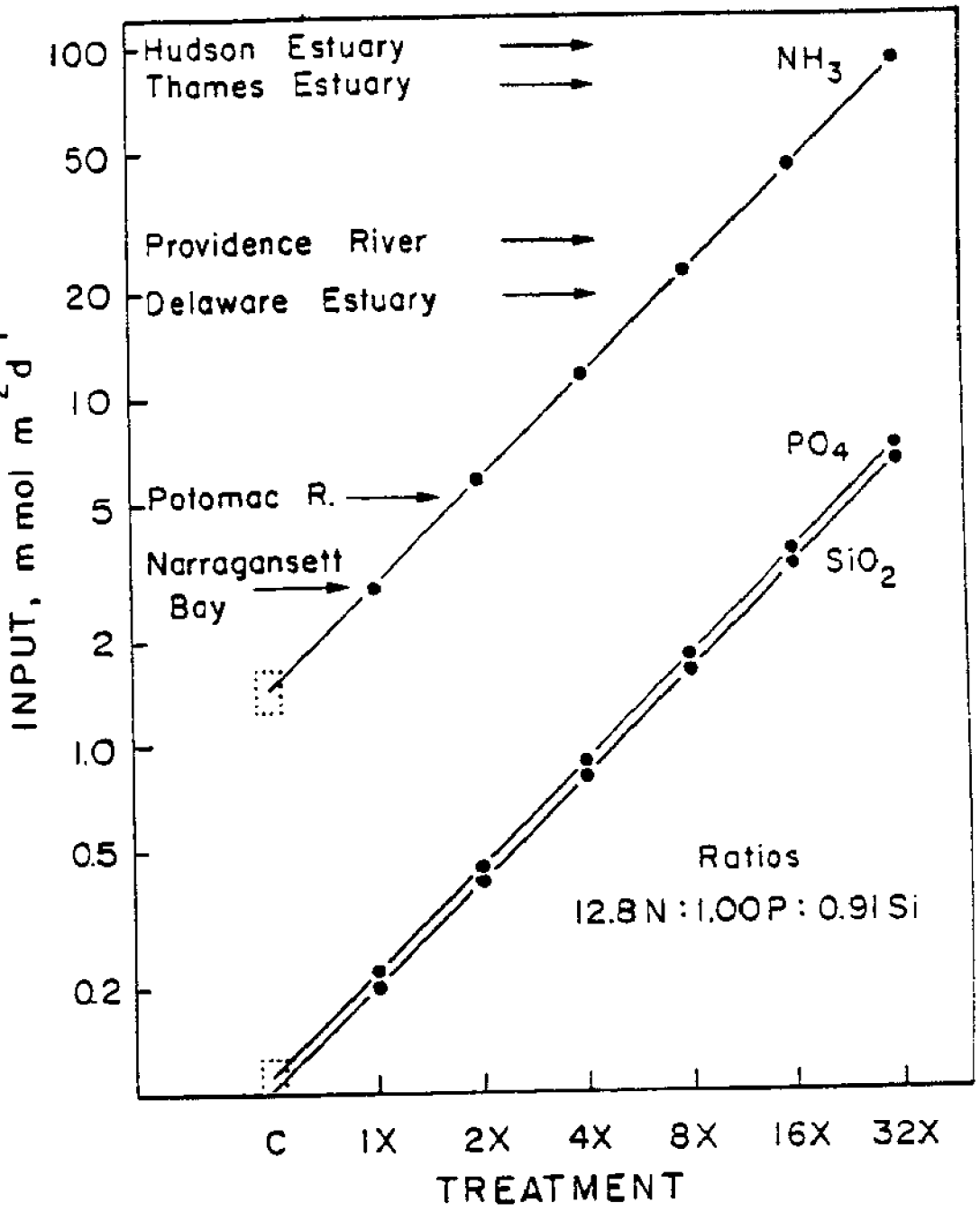


Figure 5. Comparison of nutrient loadings in MERL nutrient gradient experiment and loadings to several natural systems. Reprinted from Donaghay et al. (in prep.)

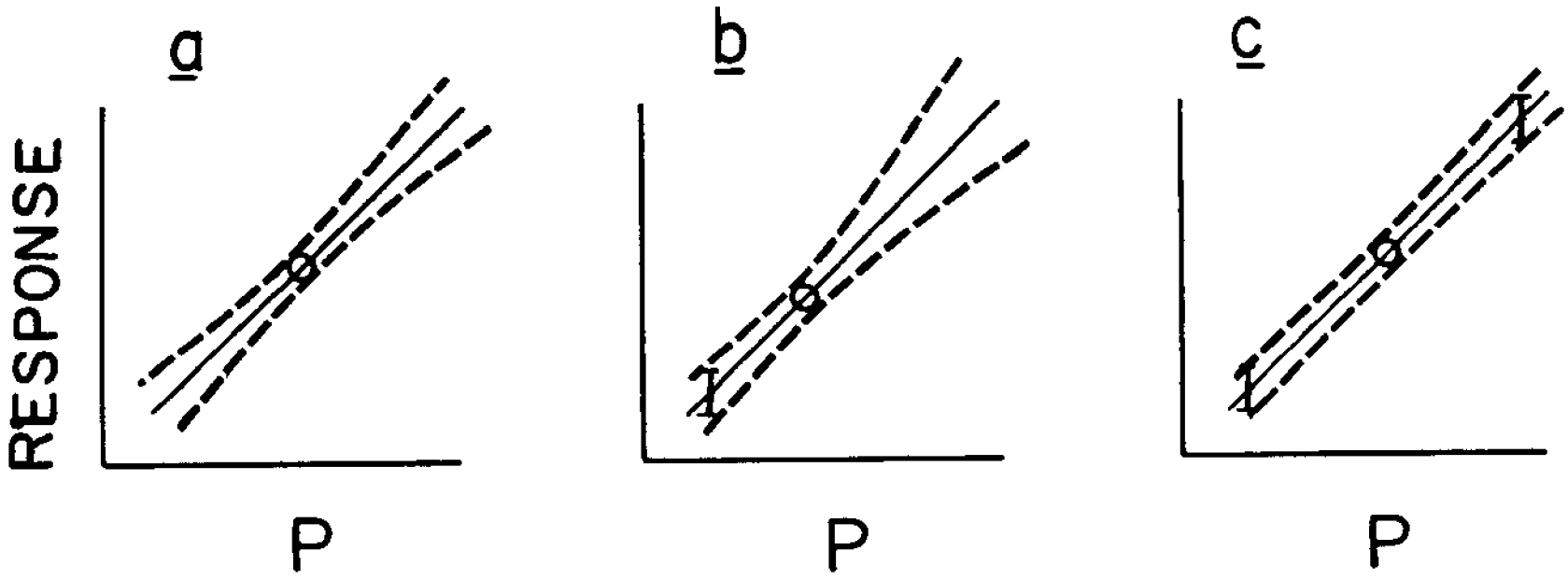


Figure 6. Statistical confidence limits expected around a dose-response relationship: (a) no replication at any dose level; (b) replication only of controls; (c) replication at both control and highest dose level.

The gradient approach requires the use of regression analysis rather than analysis of variance to detect effects. In order for regression analysis to be effectively employed, the respose over the range of loadings must be sufficiently good to exceed natural variability as defined in the controls. The response must also be some smooth linear or curvilinear function of loading factor in order to statistically define the coefficients of the dose-response relationship. Analysis of data for the first 4 months of the nutrient experiment (Donaghay et al. in prep.) indicates that both of these conditions may be met for a variety of measures of ecosystem response to nutrient loading. For example, total system respiration increased as linear function of the natural log of loading (r^2 = .965) (Figure 7a), while macrofaunal abundance increased as a linear function of loading (Figure 7b). In each case the variability between the controls is similar to that observed in previous experiments, but is a small fraction of the total response. In other cases such as zooplankton abundance, no clear response to loading can be defined (Figure 7c). The ability to define dose-response relationships for different processes or functional components of the ecosystems allows one to test statistically for differences in sensitivity to loading and to test hypotheses about dose-response relationships. For example, the differences between the responses of benthic macrofauna and zooplankton (Figures 7c and d) contributed to the statistical rejection of the hypothesis that increased nutrient loading would lead to proportional enhancement of production or biomass at all trophic levels. This hypothesis was also rejected because of differences between the dose-response relationships for metabolic activity of various ecosystem components. For example, planktonic respsiration increased much more rapidly with nutrient loading than with benthic respiration (compare Figures 7a and 8). Further, the difference between the slopes of the dose-response function for benthic metabolism as measured by O_2 consumpton and as measured by NH_4 regeneration clearly indicated a change in the fundamental nature of the metabolic processes occurring in the benthos at nutrient loading levels of 16 x (50 m moles N m^{-2} day^{-1}) and above (Figure 8). These abilities to define ecosystem level dose-response relationships at both the organism and process level and to test hypotheses concerning those relationships are the most important advantages of gradient analysis.

The above example of benthic metabolic activity illustrates an additional advantage of gradient analysis: the ability to define boundary conditions for the operation of critical ecosystem processes. From a comparison of the two measures of benthic metabolism, it appears that an important boundary occurs between 8 and 16 X (25-50 m moles N m^{-2} day^{-1}). Below that boundary, aerobic processes could consume all organic inputs; above that boundary anaerobic processes become very important, if not dominant. Both the location and temporal stability of such boundaries can be very

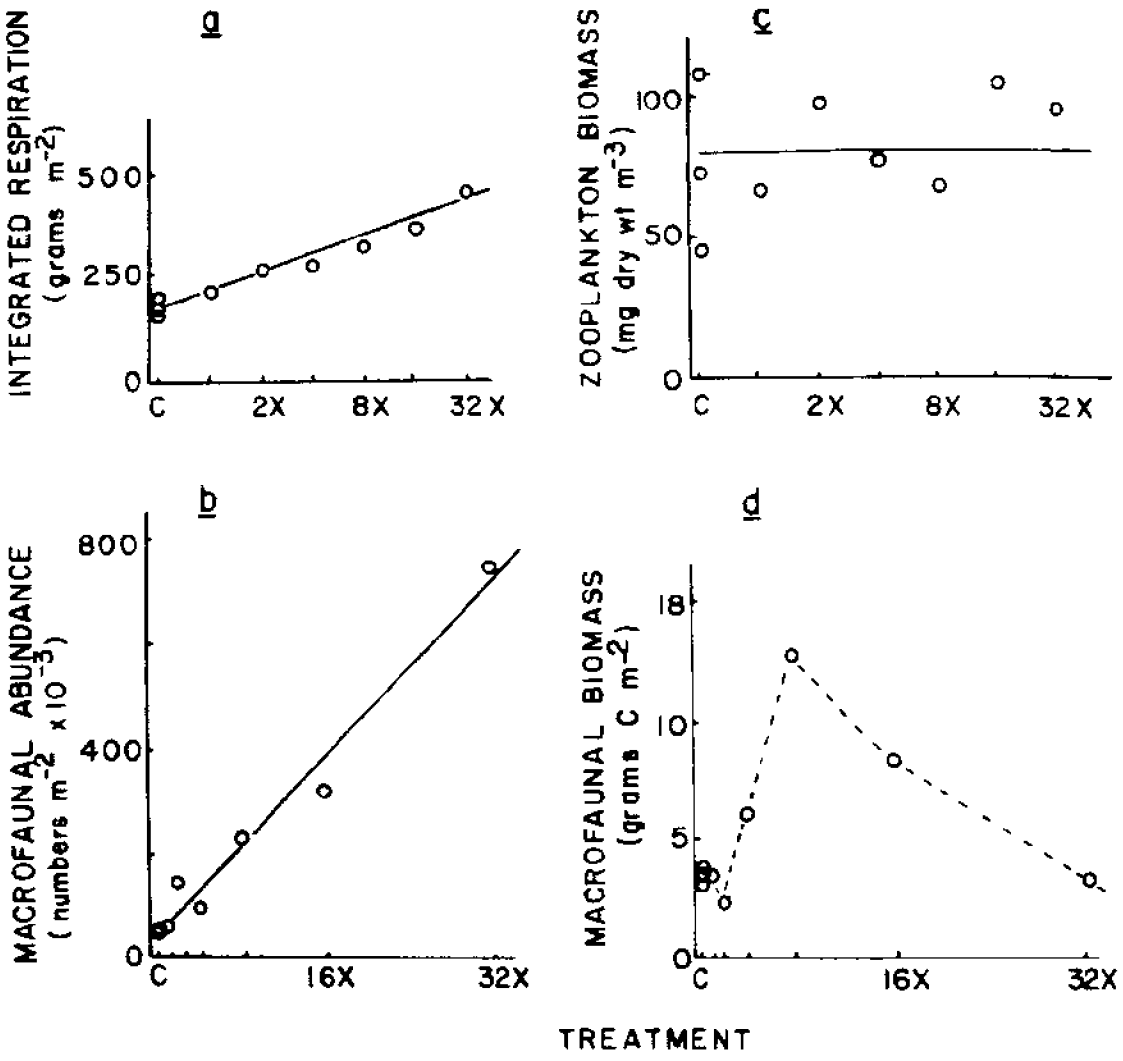


Figure 7. Response to nutrient loading as measured by (a) integrated total system respiration; (b) September macrofaunal abundance; (c) average zooplankton biomass (excluding benthic polychaete larvae); and (d) September macrofaunal biomass. All values are for the first four months of nutrient loading in a MERL nutrient gradient experiment (June 1 to September 30, 1982).

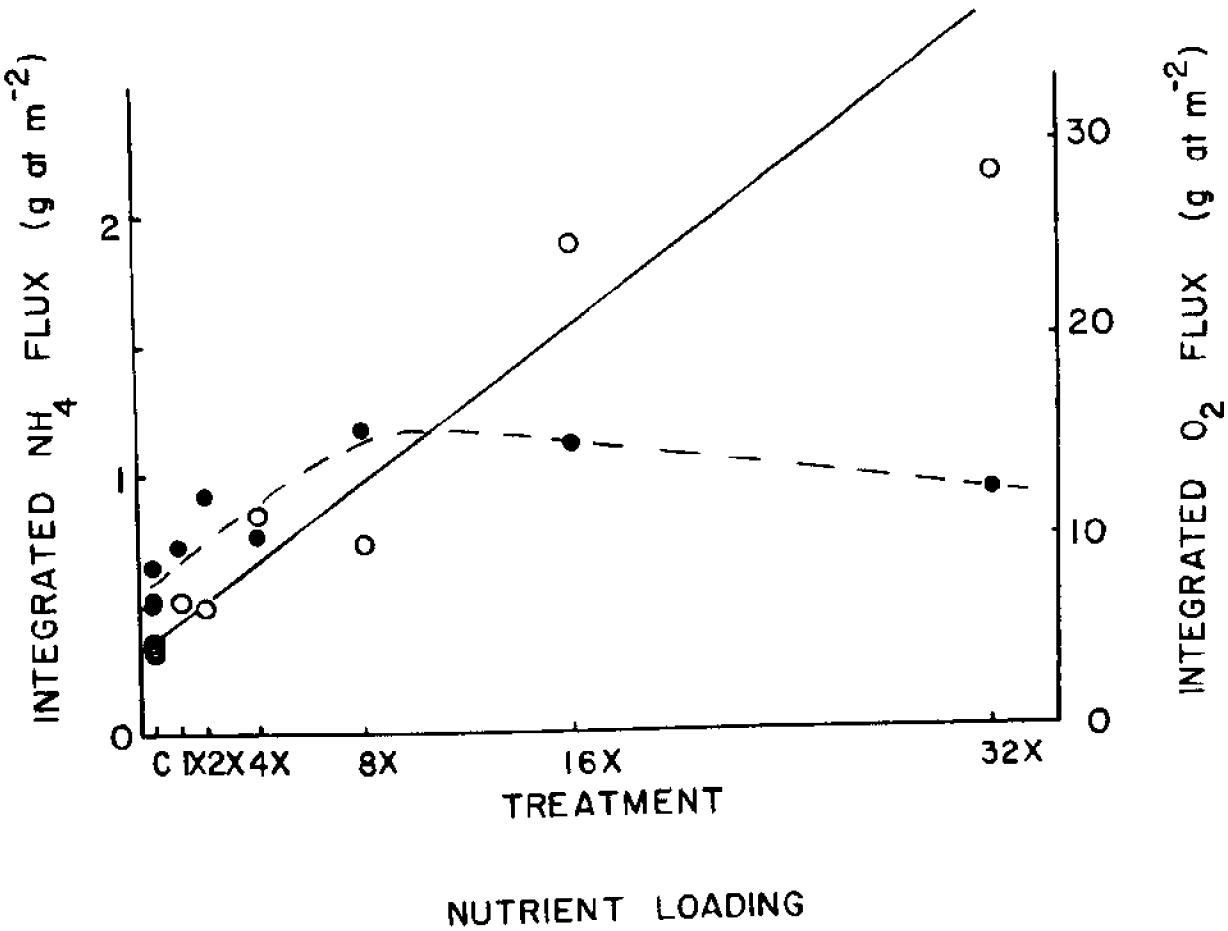


Figure 8. Integrated system metabolic response to nutrient loading (same period as in Figure 7) as measured by (a) integrated night respiration and (b) integrated benthic NH_4 flux. Redrawn from Donaghay et al. (in prep.)

important since they can affect the biotic and chemical behavior of the system. For example, as the importance of anerobic metabolism increases, the chance of anoxic events increases and the degree to which primary production is converted to fish decreases. If the effects associated with crossing these boundaries are sufficiently undesirable, such boundaries may serve as critical end points for managing loading. Any such management must also take into account the steepness of the dose-response relationship as the boundary is approached. For example, considerably greater risk would be involved in exceeding a boundary condition if a cubic rather than a linear dose-response relationship were involved. Such power function relationships have been defined both in the field (Mearns 1981) and in the nutrient gradient experiment. If properly designed, gradient analysis experiments can provide estimates of both the shape of dose-response relationships and the location of boundary conditions.

Hypotheses concerning system responses that cannot be defined by determining integrated or average values can be tested with gradient analysis if appropriate sampling design and interactive interdisciplinary research approaches are used. For example, at the beginning of the nutrient gradient experiment it was hypothesized that eutrophication would not enhance diel or longer term oscillations to the point where direct or indirect toxic effects would be induced. Routine sampling indicated that major oscillations were induced in phytoplankton biomass, oxygen, nutrient flux and pH (Donaghay et al. in prep.). In the case of oxygen, these oscillations were sufficient to exceed toxic levels for a variety of aquatic organisms and thereby supply sufficient evidence to reject the initial hypothesis on the basis of direct effects. Indirect effects were, however, demonstrated only by using routine sampling to identify possible effects, and then designing additional analyses and experiments to measure them. For example, by late in the first summer of the nutrient gradient experiment diatoms had been excluded from the intermediate level treatments and primary production was severely depressed (discussed in detail by Donaghay et al. in prep.). This depression could not be explained by a lack of nutrients, but was correlated with pH depression. Correlation suggested that the depressed pH had sufficiently altered metal availability to induce toxic or limitation effects. Non-routine chemical analyses (involving measurement and modeling of metal speciation) and bioassay and transfer experiments were initiated. These analyses rejected a variety of alternative explanations and supported the idea of pH induced changes of metal availability as the cause of the late summer phytoplankton changes.

The above example of detecting indirect effects illustrates one of the greatest challenges in executing mesocosm experiments in general and gradient designs in particular. The challenge is to be able to collect the routine data so necessary to defining average or

integrated responses and the presence or absence of oscillatory phenomena, yet at the same time remain sufficiently aware of changes in the experiment to design and execute additional sampling or experiments to define mechanisms and effects. It does not, at this point, appear reasonable to suggest that these additional analyses become routine because of the effort required for their execution. The gradient design tends to increase this challenge since it increases the number of systems on which the measurements must be made, and because it tends to increase the need for such sub experiments because of its ability to dramatize deviations from expected responses. The latter characteristic is the direct result of the broader range of environmental conditions inherent in using gradient analysis.

Experience with gradient analysis has thus far been very positive. However, it should be noted that experience is still very limited, both in number and in length of experiments. Problems with such a design might be expected to increase as an experiment continues since the divergence of replicate systems may increase with time (Smith et al. 1982). This tendency, however, may be largely neutralized if, as has thus far occurred, the tendency for nutrient loading to control ecosystem behavior continues to reinforce itself by radically constraining potential behavior. Only the continuation of this experiment and the conduct of similar experiments will permit a rigorous evaluation of gradient analysis.

POTENTIAL MODIFICATION OF SYSTEM AND EXPERIMENTAL DESIGNS

As we have seen from the preceding discussion, there is considerable flexibility in system and experimental design that can be taken advantage of to address specific questions. All of the mesocosm designs described in this section could be developed by connecting existing mesocosms in a series allowing flow of water, organisms and pollutants laterally from one compartment to another. The major technical problem in developing such serial systems will be to ensure that organisms as well as water will flow as desired from one system to another and that all interconnecting surfaces can be cleaned to prevent fouling. Here we consider the advantages and disadvantages of further modifications of system and experimental design. Many of these designs have been proposed (See Parsons 1982 for examples) to more accurately simulate particular marine environments. In evaluating the designs, the potential benefits of their use must be balanced against the costs in time and money for their development, testing and field validation. These costs can be expected to increase dramatically as new systems develop from existing systems.

Potential System Design

Benthic Coupled Serial Gradient System

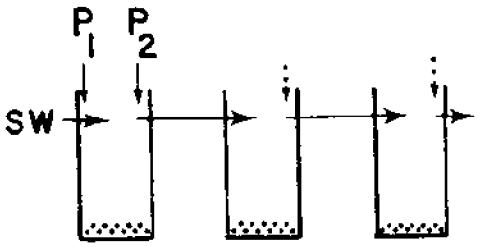
This is a slight modification of the simple benthic coupled system wherein the outflow from the first mesocosm in a series is the inflow to the next (Figure 9). This design can be used to examine the effect on higher trophic levels of changes in any mesocosm without allowing the activities of those higher trophic level organisms to alter the behavior of that mesocosm. This usage is currently being explored to examine the effects of eutrophication on fish larvae growth and survival without allowing the resultant juveniles the opportunity to exert excessive predation pressure on their food resources. Another potential usage of such a system would be to model the downstream effects of a pollutant away from the source. Such a system might also be very useful in determining if the degradation products of a waste are more toxic than the original waste or in investigating the process of waste differentiation. For example, if a complex waste were discharged into a coastal current, rapidly settling materials might affect the benthos in the first mesocosm of the series, but planktonic effects of dissolved fractions might not be observed until later in the series. For the first mesocosm in the series, contaminant loading and exposure are well defined; for subsequent mesocosms, loading is a function of unused contaminant plus degradation and recycling products from all preceding mesocosms. Exposure is thus more complex, but still definable. The field applicability of results from such systems should be broad to moderate in well-mixed benthic coupled systems. The flow rates used should begin to affect applicability only at extremes where dilution begins to alter the fate of the material. This latter property might be useful to study alternate disposal practices such as controlling initial dilution to regulate flocculent transport of waste to the bottom.

Serial Gradient Stratified System

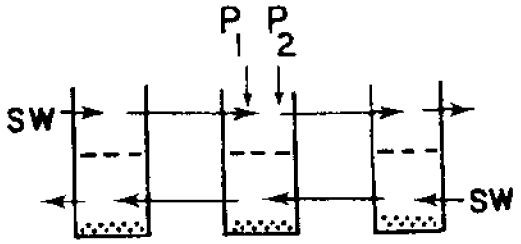
The stratified systems can be used to achieve the same objectives in a serial gradient mode as in the benthic coupled serial gradient. The design allows one to model fates and effects in stratified coastal systems dominated by strong longshore currents. Such systems potentially result in longshore separation of the effects on the benthos from those on the plankton. They also result in a strong longshore displacement from the input site of the effects of recycling through the thermocline. The field site applicability is very narrow since the flow rates in the top and bottom layers relative to the rate of waste penetrating the thermocline define the loadings to varous parts of the system. This dependence could, however, be very useful for understanding particular sites or for separating effects (as in the benthic coupled serial gradent system).

POTENTIAL SYSTEM DESIGNS

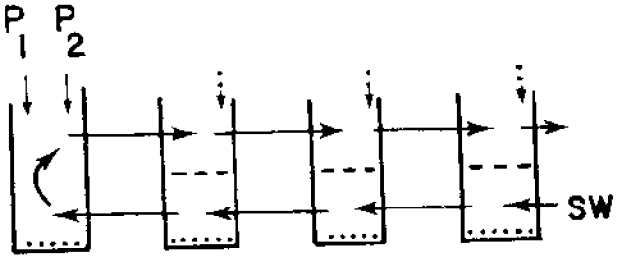
BENTHIC COUPLED SERIAL GRADIENT



STRATIFIED SERIAL GRADIENT



WIND DRIVEN SERIAL GRADIENT



THERMO-HALINE ESTUARINE SERIAL GRADIENT

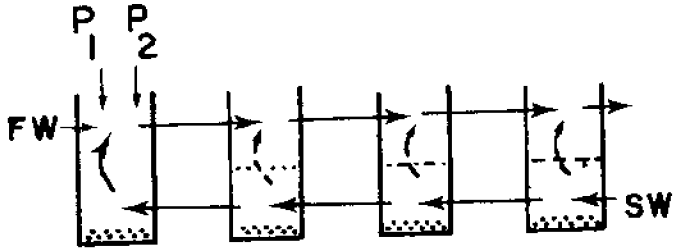


Figure 9. Potential system designs. Terms are defined as in Figure 1. Vertical arrow represents optimal sea water input to mimic downstream entrainment of water as may occur in effluent plumes; horizontal arrow represents flow from one mesocosm in a series to the next; dashed line represents strong vertical stratification; dot-dashed/dotted line represents moderate vertical stratification; dotted line represents weak vertical stratification. Mixing across density structure is indicated by a curved arrow; FW represents fresh water input.

Wind Driven Upwelling Serial Gradient System

This potential system is designed to model fates and effects of pollutants introduced into wind driven estuaries (Rattray 1965) or along coastlines dominated by upwelling. The system is composed of at least three tanks, one being well-mixed and the others strongly stratified by temperature. Sea water input would occur predominantly in the bottom layer farthest downstream from the well-mixed (or upwelling) tank. Additional inputs to the surface layer could occur if it were desirable to mimic entrainment of surface water along the length of an upwelling plume. The density structure in each of the stratified tanks would be maintained solely by temperature; cross thermocline mixing would be limited. Estimation of exposure and loading to individual parts of the system would be complex, but still simpler than in thermohaline systems (see below). Results from the upwelling serial gradient system would be broadly applicable to all such systems. Consideration of flow rate would become site specific only at the extreme; as with the benthic coupled serial gradient system, the only rationale for use (in preference to a single well-mixed or stratified design) would be to test for downstream effects.

Thermohaline Driven Estuarine Serial Gradient System

This system design is intended to model fates and effects along complex physical gradients typical of thermohaline driven estuaries. The major feature of such a system is a physical mixing gradient ranging from well-mixed to highly stratified as one moves downstream. This gradient is maintained by freshwater input at the upper end and seawater input into the bottom layer at the downstream end of the system. Such a system has been attempted (Cooper and Copeland 1973) but it is extremely complex in design, operation and interpretation. It has all the complexity for defining loading of the serial gradient plus recycling of contaminant from downstream. The increase of salinity as one moves downstream may radically alter the pollutant behavior in terms of both fate and effects. The behavior of the system and of a pollutant in the system will be very sensitive to flow rates, salinity, temperature and vertical stratification gradients downstream. As a result, the field site applicability of results from any given system will be extremely narrow and will be restricted to sites with the given set of characteristics. These problems could be overcome given sufficient time and financial resources.

Potential Experimental Design

Complex Gradient Analysis

Complex gradient analysis is a potentially powerful, yet untried, technique for determining fates and effects: it makes use of a pair of single gradients located at the boundary condition of a second input gradient. Complex gradient analysis is designed to

determine if the fates and effects of one contaminant are affected by differences in a second factor such as degree of stratification or eutrophication. These second factors often represent important differences between different ecosystem types or sites. For example, the results of the nutrient gradient experiment suggest that eutrophication might severely alter the sensitivity of ecosystems to heavy metal pollutants. Since the effects observed should be functions of both the system sensitivity and the loading of a metal such as copper, the most appropriate experimental design might be to apply a series of dosing levels of copper to one group of mesocosms receiving low nutrient loadings (P_2 = 1 X, Figure 10) and to a second group of mesocosms receiving high nutrient loadings (P_2 = 8 X, Figure 10). The results from such complex gradient experiments could not only be more broadly applicable, but could also contribute to the solution of the dual problem of field-to-field extrapolation and development of criteria for ocean disposal site selection. For example, repeating of a waste dump experiment (similar to the acid iron experiment of Brown and Kester 1983) along a mesocosm gradient of increasing degrees of stratification and at two levels of nutrient enrichment would allow description of the fate and effects of the waste at potential sites with varying degrees of stratification and euthrophication. In the northeast United States, most potential dumpsites differ principally in regard to these two parameters. The information could be critical to developing criteria for waste disposal sites. Complex gradient analysis could also be used in development of better models and field monitoring strategies. Such gains, however, must be balanced against the need for performing experiments with large numbers of mesocosms to achieve the same level of gradient resolution as in single gradient analysis.

The assumptions inherent in such an experimental approach are identical to those for a single gradient. In order to be the most effectively used, there is a need for information on the ecosystem response to the second variable P_2. This might generally be achieved by having first run a single gradient experiment with that variable. In the first example above, this would be the nutrient gradient experiment. If the ecosystem responses to the second variable are linear, or simple mathematical relationships, then appropriate selection of P_2 levels can reduce the variance in response that is normally associated with the uncontrolled variance in P_2 in the ecosystem. This could be extremely useful if the ecosystem response to P_2 is very steep relative to our ability to measure or control it in mesocosms. For example, variation in benthic nutrient flux between mesocosms might significantly contribute to differences in plankton responses. Any addition of nutrients will tend to reduce the magnitude of the effects of that inherent variability simply because it will reduce the relative importance of the natural variability in flux to the total flux perceived by the plankton.

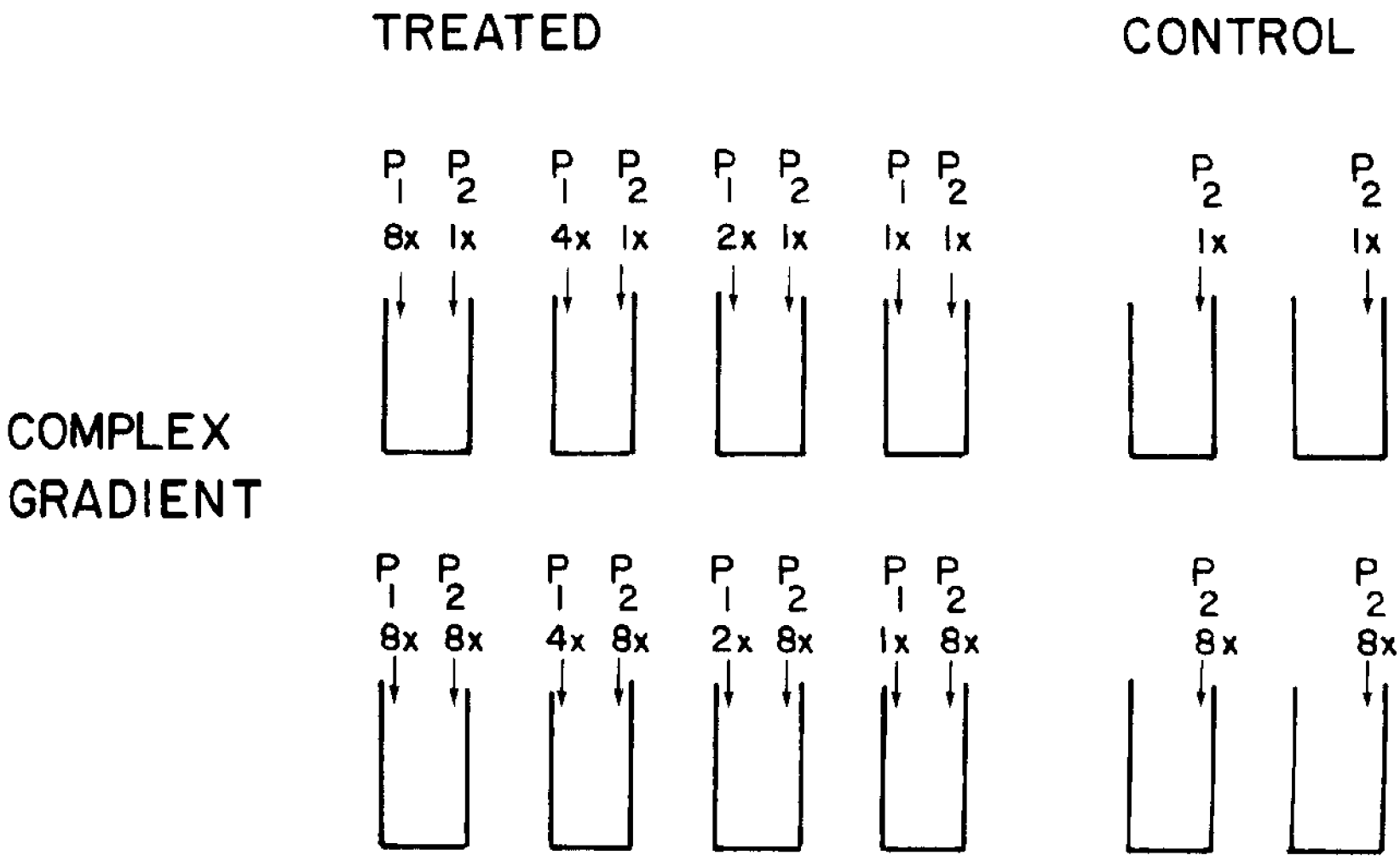


Figure 10. Potential experimental designs. Terms are defined as in Figure 4.

Statistical evaluation of fates and effect in complex gradients would be by regression analysis as in single gradients. Additional analytical power would come from use of factorial analysis and from the inherent increase in replication present in this experimental design. The use of these more sophisticated analytical tools coupled with any realized reduction in natural variability caused by P_2 (as above) could greatly increase the ability to define effects.

SUMMARY AND RECOMMENDATIONS

Eight recommendations can be made regarding the use of marine mesocosms for measuring marine pollution effects.

1. Field validated marine mesocosms have now developed to the point where they are ready to be used to measure marine pollution effects. If appropriate systems and experimental designs and research approaches are used, mesocosms can meet the three criteria of meaningful measures (valid experimental design, field validation and significant response parameters at the ecosystem level).
2. Mesocosms, which for many substances are the only tool, should be used to define the processes that control the fate of wastes and to identify classes of similarly behaving compounds. Such fate studies can have sufficiently rapid turnaround times (less than 6 months under good conditions) to allow them to become a part of both short and long term waste management decisions.
3. Field validated marine mesocosms should be used for defining both the fate and effect of materials that are highly toxic and/or will be discharged chronically and/or in large quantities into the marine environment. These systems are currently the only way to achieve a strict definition of both the fate and effect at the ecosystem level. Effects studies require significantly greater investment of time, funds and integrated research effort than fate studies.
4. Field validated marine mesocosms should not be substitutes for bioassays and chemical analysis as initial screening tools for large numbers of chemicals (i.e., pre-manufacturing licensing). They should, however, be used to test the validity of standard laboratory screening procedures for protecting both species and ecosystem function. Such testing can be achieved by comparing dose-response relationships defined for individual ecosystem components in the laboratory with the dose-response relationships for these same species in the mesocosm and for the ecosystem as a whole.

5. Field validated marine mesocosms should be used to help resolve major analytical questions that affect our ability to monitor for pollutants or to predict potential field sensitivities to those pollutants. For example, the ability to construct mass balances in mesocosms allows one to determine how important analytically undetectable degradation products are as fates for a given compound and therefore how much risk might be involved in using a monitoring strategy to protect the environment against that compound. The diversity of conditions possible in these systems might also allow rapid and cost effective (i.e., single site and time) intercalibration of analytical and/or biotic measurement techniques where significant disagreement between methods exists, for example, metal speciation.
6. Although mesocosms suitable for immediate use have been developed, three questions still need to be addressed that strongly affect the general applicability, analytical power and cost effectiveness: (1) How important is system size to the kinds of information derived? (2) How can we improve measurements of critical ecosystem processes (and what are those processes or elements)? (3) How broadly can the results of mesocosm studies at one location be applied? Question (1) can be answered by comparing results of fates and/or effects studies run on systems of different size. Questions (2) and (3) can be answered only by examining how the natural factors that vary between coastal ecosystems alter the behavior of those systems. The major factors are nutrient loading, temperature (average and extremes), predation and physical structure (stratification and mixing). These questions should be answered by carefully designed mesocosm experiments coordinated with field studies of these environments. Some level of funding should go to answering these questions while mesocosms are being used as operational tools to assess marine pollution problems. Our understanding of how these factors affect critical ecosystem processes will form an essential step to solving the dual problem of developing site selection criteria and extrapolating data from field site to field site. Such studies could also increase the cost effectiveness of both mesocosms and field sampling programs.
7. The need to compare the results of mesocosm studies with field results will continue even after the field fidelity of a given mesocosm design has been established. This should not be viewed just as a search for possible artifacts of mesocosm studies, but rather as a tool to allow reinterpretation of field data, to identify problem areas requiring further research or to identify more sensitive field monitoring tools. Such field validation should be a joint cooper-

ative effort of those running the field program and those running the mesocosm studies.

8. There is a need for better coupling of mesocosm studies with the development of models and other analytical tools to describe ecosystem response to pollutants and other factors. This will require more than the application of existing models and statistical techniques (which have proved inadequate). The application of more process oriented modeling and statistical time series analysis techniques may be helpful. Ultimately, such models and analytical tools will be invaluable in management of ocean pollution.

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REFERENCES

Amdurer, M., D.M. Adler and P.H. Santschi. 1982. Radiotraces in studies of trace metal behavior in meoscosms: Advantages and limitations. In: G.D. Grice and M.R. Reeve (eds.), Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems, pp. 81-95. Springer-Verlag, New York.

Banse, K. 1982. Experimental marine ecosystem enclosures in a historical perspective. In: G.D. Grice and M.R. Reeve (eds.), Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems, pp. 11-14. Springer-Verlag, New York.

Brown, M.F and D.R. Kester. 1983. Fate of ocean dumped acid-iron waste in a MERL stratified microcosm. In: I.W. Duedall, B.H. Ketchum, P.K. Park and D.R. Kester (eds.), Wastes in the Ocean, Wiley-Interscience, New York.

Brown, M.F., D.K. Kester and J.M. Dowd. 1982. Automated iron measurements after acid-iron waste disposal. In: I.W. Diedall. D.R. Kester, B.H. Ketchum and P.K. Park (eds.), Wastes in the Ocean, Vol. 1. Chemical and Sewage Wastes. Wiley-Interscience, New York.

Cooper, D.C. and B.J. Copeland. 1973. Responses of continuous series estuarine microecosystems to point source imput variations. Ecol. Monographs 43: 213-236.

Davies, J. M. and J.C. Gamble. 1979. Experiments with large enclosed ecosystems. Philos. Trans. R. Soc. Lond. B. 286: 523-544.

Donaghay, P.L. and E. Klos. 1983. Development and behavior of controlled stratified mesocosms. Submitted to Mar. Biol.

Donaghay, P.L., C.A. Oviatt, B.K. Sullivan, C.D. Hunt, M.E.Q. Pilson, D. Rudnick, J. Frithsen and A. Hanson. 1982. Fate and effect of nutrients along a simulated estuarine gradient.

Elmgren, R. and J.B. Frithsen. 1982. The use of experimental ecosystems for evaluating the environmental impact of pollutants: A comparison of an oil spill in the Baltic Sea and two long-term, low-level oil addition experiments in mesocosms. In: G.D. Grice and M.R. Reeve (eds.), Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems, pp. 153-165. Springer-Verlag, New York.

Elmgren, R., J.F. Grassle, J.P. Grassle, D.R. Heinle, G. Langlois, S.L. Vargo and G.A. Vargo. 1980. Trophic interactions in experimental marine ecosystems perturbed by oil. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov 8-10, 1978, pp. 779-800. (CONF-781101, National Technical Information Service, Springfield, Va.).

Gearing, J.N., P.J. Gearing, T. Wade, J.G. Quinn, H.B. McCarty, J. Farrington and R.F. Lee. 1979. The rates of transport and fates of petroleum hydrocarbons in a controlled marine ecosystem, and a note on analytical variability. In: Proceedings 1979 Oil Spill Conference. Publ. No. 4308, Mar. 8-10, 1979, pp. 555-564. Washington, D.C.

Gearing, P.J. and J.N. Gearing. 1982. Behavior of No. 2 fuel oil in the water column of controlled ecosystems. Mar. Environ. Res. 6: 115-132.

Gearing, P.J., J.N. Gearing, R.J. Pruell, T.L. Wade and J.G. Quinn. 1980. Partitioning of No. 2 fuel oil in controlled estuarine ecosystems: Sediments and suspended particulate matter. Environ. Sci. Technol. 14: 1129-1136.

Grice, G.D., M.R. Reeve, P. Koeller and D.W. Menzel. 1977. The use of large volume, transparent, enclosed sea surface water columns in the study of stress on plankton ecosystems. Helgol. Wiss. Meersunters 30: 118-133.

Grice, G.D., R.P. Harris, M.R. Reeve, J.F. Heinboken and C.O. Davis. 1980. Large scale enclosed water column ecosystem: An overview of Foodweb I, the final CEPEX experiment. J. Mar. Biol. U.K. 60: 401-414.

Hunt, C.D. and D.L. Smith. 1983. Remobilization of metals from polluted marine sediments. Can. J. Fish. and Aquatic Sci. 40(2).

Kelly, J.R. 1983. Benthic-pelagic coupling in Narragansett Bay. Ph.D. Thesis, University of Rhode Island, Kingston, R.I.

Kuiper, J. 1982. Ecotoxicological experiments with marine plankton communities in plastic bags. In: G.D. Grice and M.R. Reeve (eds.), Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems, pp. 181-194. Springer-Verlag, New York.

Mearns, A.J. 1981. Effects of municipal discharges on open coastal ecosystems. In: R.A. Geyer (ed.), Marine Environmental Pollution, Vol. 2, Dumping and Mning, pp. 25-66. Elsevier, Amsterdam, Netherlands.

Menzel, D.W. and J. Case. 1977. Concept and design: Controlled ecosystem pollution experiment. Bull. Mar. Sci. 27: 1-7.

Nixon, S.W. 1982. Remineralization and nutrient cycling in coastal marine ecosystems. In: J.B. Neilson and L.E. Cronin (eds.), Estuaries and Nutrients, pp. 111-138. Humana Press, Clifton, N. J.

Nixon, S.W., D. Alonzo, M.E.Q. Pilson and B.A. Buckley. 1980. Turbulent mixing in aquatic microcosms. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series, 52, Augusta, Ga., Nov. 8-10, 1978, pp. 818-840. (CONF-781101, National Technical Information Service, Springfield, Va.)

Oviatt, C.A., H. Walker and M.E.Q. Pilson. 1980. An exploratory analysis of microcosm and ecosystem behavior using multivariate techniques. Mar. Ecol. Prog. Ser. 2: 179-191.

Oviatt, C., J. Frithsen, J. Gearing and P. Gearing. 1982. Low chronic addition of No. 2 fuel oil: Chemical behavior, biological impact and recovery in a simulated estuarine environment. Mar. Ecol. Prog. 9: 121-136.

Parsons, T.R. 1982. The future of controlled ecosystems enclosure experiments. In: G.D. Grice and M.R. Reeve (eds.), Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems, pp. 411-418. Springer-Verlag, New York.

Pilson, M.E.Q., C.A. Oviatt and S.W. Nixon. 1980. Annual nutrient cycles in a marine microcosm. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. DOE Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp. 753-778. (CONF-781101, National Technical Information Service, Springfield, Va.).

Rattray, M. 1967. Some aspects of the dynamics of circulation in Fjords. In: G.H. Lauft (ed.), Estuaries, pp. 52-67. American Association for Advancement of Science, Washington, D.C.

Santschi, P.H. 1982. Application of enclosures to the study of ocean chemistry. In: G.D. Grice and M.R. Reeve (eds.), Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems, pp. 63-80. Springer-Verlag, New York.

Smith, W., V.R. Gibson and J.F. Grassle. 1982. Replication in controlled marine systems: Presenting the evidence. In: G.D. Grice and M.R. Reeve (eds.), Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems., pp. 217-225. Springer-Verlag, New York.

Takahaski, M., G.T. Wallace, F.A. Whitney and D.W. Menzel. 1977. Controlled ecosystem pollution experiment: Effect of mercury on enclosed water columns. I. Manipulation of experimental enclosures. Mar. Sci. Commun. 3: 313-329.

Wade, T.L. and J.G. Quinn. 1980. Incorporation, distribution and fate of saturated petroleum hydrocarbons in sediments from a controlled marine ecosystem. Mar. Environ. Res. 3: 15-33.

The Utility of Studying the Effects of Pollutants on Single Species Populations in Benthos of Mesocosms and Coastal Ecosystems

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INTRODUCTION

Pearson and Rosenberg (1978) have recently reviewed benthic field studies on the effects of organic enrichment and pollution in the marine environment. Particular species were singled out as useful in interpreting effects of pollutants (e.g., Capitella spp., Polydora spp., Streblospio benedicti, Scolelepis fuliginosa, Mediomastus ambiseta, Pygospio elegans, Mulinia lateralis). Generalizations derived from a comparison of field studies and field experiments were weakened by the confounding effects of organic enrichment as a source of food for benthic species, versus the deleterious effects of reduced oxygen concentrations resulting from high levels of organic matter in poorly flushed systems, in addition to the often poorly defined effects of pollutants from multiple sources, and of a variety of chemical species, that today characterize most coastal marine environments. For this reason studies at the Marine Ecosystems Research Laboratory (MERL) at the University of Rhode Island have used replicated mesocosms to study the effects of single pollutants on samples of the water column and benthos taken from the middle region of Narragansett Bay. This report discusses the separate effects of No. 2 fuel oil and organic enrichment on benthic populations in the mesocosms, and touches on parallel studies on fluctuations in the source field populations at Station 1 in mid-Narragansett Bay. The discussion focuses on fluc-

tuations in density in a single polychaete species Mediomastus ambiseta (Hartman 1947), the dominant macrofaunal benthic species at Station 1 and in the mesocosms. This focus demonstrates the utility of analyzing the results of such experimental studies, and of field studies as well, in terms of single species populations rather than by the use of derived indices. It also highlights the gaps in our knowledge of the biology of this species, a species that appears to be ubiquitous in shallow coastal environments on the eastern coast of the United States.

It may be important to emphasize at the beginning that the intention here is not to substitute Mediomastus ambiseta for "Capitella capitata" as a marine pollution indicator species in the sense that the term was formerly used (Grassle and Grassle 1976) but rather to point out the complexities in the responses of a single species to single pollutants within the context of the whole community. Single species populations are sensitive indicators of pollution only when one has a thorough knowledge of the biology of each species.

MEDIOMASTUS AMBISETA (HARTMAN 1947) SYSTEMATICS AND DISTRIBUTION

In our benthic studies in Wild Harbor, Buzzards Bay, New Bedford Harbor and Narragansett Bay we have followed Hobson (1971) in calling the Mediomastus found in almost all samples from mud and muddy sands Mediomastus ambiseta (Hartman 1947). The type species for the genus is Mediomastus californiensis (Hartman 1944) which Hartman (1969) described as having hooded hooks in all abdominal setigers. Hartmann-Schröder (1962) pointed out that Capitita ambiseta (Hartman 1947) should in fact be Mediomastus ambiseta. Hobson (1971) examined specimens from Station R (Sanders 1960) in Buzzards Bay collected in 1969, and from Wild Harbor (collected in October 1969 and June 1970). Hobson (1971, Figure 2c) showed M. ambiseta to have small subdermal eyes dorsally in the prostomium. The peristomium lacks setae, segments 2-5 bear capillary setae, and the remaining 6 thoracic segments bear hooded hooks. The largest specimens Hobson examined were 20 mm long and 0.3 mm wide and had 50 segments; a "digitate caudal appendage on the pygidium" was noted but not figured by Hobson. She noted that ripe females with eggs of maximum diameter 60 μm were present in June and July samples. Hartman (1969) described Mediomastus (Capitita) ambiseta with 70 segments and "a posterior end terminating in a midventral digitate process exceeding the length of the last three asetigerous segments. Last 25 or more setigers with single long spines."

Hartman (1969) illustrates a third species of this genus M. acutus, a generally smaller species, with 60 or more setigers, dis-

tinguished by having the parapodia at the very posterior end of the elongate abdominal segments, and a prostomium that is longer and more acute than that of M. californiensis. In this species too "the posterior end terminates in a long slender process." But Ewing and Dauer (1981) have stated that Amastigos caperatus n. sp. is partially synonymous with M. acutus. The genus Amastigos was first described by Piltz (1977). Ewing and Dauer (1981) provide a key to identification of capitellid species occurring in Chesapeake Bay. The species include both Mediomastus ambiseta and M. californiensis.

Rasmussen (1973) has described yet another species of Mediomastus, M. fragilis, from a variety of depths and sediment types from the Danish Isefjord. In 1956 Rasmussen referred to this species as Heteromastus filiformis (Claparède), a species that also occurs in the Isefjord. There seems no doubt that many of Rasmussen's remarks on M. fragilis (= H. filiformis in Rasmussen 1956) do indeed apply to a species of Mediomastus, and not Heteromastus, since the number of thoracic segments conforms to the definition of the genus Mediomastus. M. fragilis in the Isefjord reaches a length of 50-60 mm with more than 100 segments. Spawning occurs during a very circumscribed period in early April when the temperature reaches 6-7°C. Globular jelly egg masses (8 mm in diameter) containing greenish-yellow eggs 100 µm in diameter (notably larger than the coelomic eggs measured by Hobson [1971] in M. ambiseta) were attributed to M. fragilis. Rasmussen (1956, Figure 23) has described the larval and early postlarval morphology of the embryos contained in these egg masses; his figure shows a recently metamorphosed juvenile with four thoracic setigers with capillary setae, six setigers with hooded hooks, and a pygidium with a "finely wrinkled anal cirrus with dorsally placed anus". Rasmussen (1973) described M. fragilis as living in permanent vertical sandy tubes and forming mounds of fecal pellets--that is, he inferred a limited mobility for this deposit-feeder.

With the description of three similar species of Mediomastus from inshore areas in the southwest and western United States it is often difficult to know whether results of pollution studies reporting dominant or subdominant abundances of M. ambiseta or M. californiensis, or even Mediomastus sp., are referring to the same species or to closely related species (Barnard 1970; Flint 1981; Holland et al. 1973; Reish and Winter 1954; Reish 1956; Swartz et al. 1980). Most workers on the east coast have identified their Mediomastus as M. ambiseta (Appy et al. 1980; Dauer and Simon 1976a,b; Grassle and Grassle 1974; Hobson 1971; Lizarraga-Partida 1974; Maurer et al. 1979; Rowe et al. 1982; Sanders et al. 1980) but Ewing and Dauer (1981) have identified both M. ambiseta and M. californiensis from Chesapeake Bay. The sites represented in these field studies range from the Bay of Fundy in Maine into the Gulf of Mexico to Tampa Bay and Ensenada Bay. It is at present impossi-

ble to say what the affinities are between animals identified as M. ambiseta that have been reported, for example, from Passamaquoddy Bay in Maine (Appy et al. 1980) and from the San Gabriel River in California (Reish 1956), or among M. californiensis from Galveston Bay, Texas (Holland et al. 1973), from the type locality (Hartman 1944) and from Chesapeake Bay (Ewing and Dauer 1981).

Sanders (1960) reported small numbers of an unnamed capitellid in dredge samples from Station R in Buzzards Bay (the species ranked twenty-second in numerical abundance and formed 0.15% of the fauna). Voucher samples from that study have been reexamined and identified as M. ambiseta (J.F. Grassle unpublished data). Whitlach (personal communication) has resampled Station R and shown that there has been a general increase in the relative abundance of M. ambiseta over the last 20–30 years.

Studies on larval recruitment into azoic sediment in the shallow Mediterranean (Guérin and Massé 1978; Massé and Guérin 1978) have shown that an unnamed species of Mediomastus is almost always present in relatively high densities, with Notomastus latericeus, Pista cristata and Polycirrus sp. forming more than 50% of total individuals in all experiments regardless of the season at which the sediments were exposed or the length of the exposure period (Guérin and Massé 1978), although the peak period of larval recruitment was in May-June and June-August intervals (Massé and Guérin 1978). It is now known (Hannan 1981) that benthic species recruiting as larvae into off-bottom collectors of the kind used by Guérin and Massé may not do so in direct proportion to their availability in the plankton or to the number recruiting to the bottom. Cylindrical collectors may over-collect or under-collect certain particles depending on the hydrodynamic regime and the intrinsic properties of the particles, and certain species may survive preferentially in the collector where the azoic sediment is enriched by the fine particles of organic matter that also accumulate there.

This discussion of the systematics and distribution of M. ambiseta and other species in the genus is intended to highlight the need for giving priority to modern systematics studies as a key part of all environmental studies. Uncertainty about species identifications within a genus such as Mediomastus is the most obvious problem. Since the species name is the only retrievable way of entering information about an organism into the scientific literature, whether the information deals with physiology, biochemistry or changing temporal patterns of distribution and abundance, misidentifications mean that the efforts expended in gaining such information have been totally wasted. Those who regard "taxonomy" as too expensive should consider the profligate waste of time and money that has resulted from large scale environmental studies where too little support was provided for good taxonomy. But we need to go further in our systematics studies to determine the biological affinities between species that have been alloted the same

species name. Our own studies on sibling species within the genus Capitella (Polychaeta) (Grassle and Grassle 1976) have made it clear that extreme morphological similarities may conceal clusters of species that ecologically behave in extremely different ways. Such clusters of species may be rather common among the opportunistic polychaete genera (Grassle 1980), and their existence should be regarded as an opportunity for improving our understanding of how different species exploit disturbed habitats.

TWO RECENT FIELD STUDIES

West Falmouth, No. 2 Fuel Oil

Following the 1969 spill of No. 2 fuel oil in West Falmouth Harbor, M. ambiseta formed an increased percentage (45-95% of total individuals) of the benthic fauna at subtidal, but not intertidal, stations that received intermediate degrees of oiling in the summer immediately after the spill. At some of these stations the densities were very high indeed, for example, 600,000 m^{-2} at Station 10 (Sanders et al. 1980). This paralleled the dominance and high densities of Capitella sp. I at heavily oiled intertidal and subtidal stations. The dominance of M. ambiseta was still marked at some stations in the second summer following the oil spill, but was reduced still further the following year. This study suggests that M. ambiseta larvae, postlarvae and adults were tolerant of intermediate levels of components of No. 2 fuel oil in the sediments (Sanders et al. 1980) but also that the high densities of M. ambiseta were favored not only by the low densities of other species of benthic deposit feeders, but also by increased levels of organic matter resulting from the decomposition of dead fauna and flora. Although M. ambiseta was not recorded at intertidal Wild Harbor River stations from 1969 to 1973 (Michael et al. 1975) it now occurs at low densities at those same stations (J.P. Grassle unpublished). It is also common at stations in New Bedford Harbor (J.P. Grassle unpublished), an area that is heavily impacted by PCBs and heavy metal pollution.

Tampa Bay: Recolonization After Defaunation Following a Red Tide

Dauer and Simon (1976a,b), in their study of sandy intertidal sediments following substantial defaunation caused by a red tide of Gymnodinium breve, classified Mediomastus (Capitita) ambiseta with their Group IV species, polychaetes that became and remained dominant throughout the second year of recolonization, especially at the station just below mean low water. M. ambiseta first appeared in the samples 6 months after the defaunation occurred. Dauer and Simon (1976a) explained the earliest recolonization at the shallowest station by Polydora ligni as a result of early removal

by wave action of "detrimental conditions--decaying fish, anaerobic overlying water mass, sediments, etc.," but no assessment was made in this study of the possible positive effects of organic enrichment on facilitating the survival of postlarval juveniles of this and other species such as M. ambiseta. Earlier studies reviewed by Pearson and Rosenberg (1978) suggest that organic enrichment may often play a role when M. ambiseta reaches high densities (Barnard 1970; Lizarraga-Partida 1974; Reish 1956; Reish and Winter 1974).

MEDIOMASTUS AMBISETA IN NARRAGANSETT BAY

The source community for the benthos in the MERL mesocosms is brought from Station 1 in mid-Narragansett Bay. Monitoring of the communities there from 1976 to 1980 has confirmed that M. ambiseta was the dominant species (or co-dominant with Nucula annulata) for all 5 years and at all seasons. The sharp declines in M. ambiseta densities in the summers of 1977, 1978 and 1979 are noteworthy (Figure 1) and are discussed further below. Benthic communities in control mescocosms have been very similar to those at Station 1 and have replicated well, although densities of individual species are often lower than in the Bay (Figure 2). Samples from three stations spaced along Narragansett Bay (Providence River, Station 1 in the mid-Bay and a lower Bay station) indicated that while M. ambiseta is present at all three stations, its peak densities occur in the mid-Bay.

MERL MESOCOSM EXPERIMENTS

Effects of Low Levels of No. 2 Fuel Oil on M. ambiseta Populations

MERL mesocosms have been used to examine the effects of No. 2 fuel oil on the whole community (Elmgren et al. 1980; Grassle et al. 1981; Elmgren and Frithsen 1982; Vargo et al. 1982; Oviatt et al. 1982). In the first chronic addition experiment the average water column concentration was 180 ppb over 168 days; in the second the average concentration was 90 ppb over 122 days. In both experiments M. ambiseta densities were significantly reduced in the three oiled tanks compared with controls (Figure 3); there was no recovery even 1 year after oil additions had ceased, and total hydrocarbons in sediments were reduced to 10-20% of the total 40 gm added (Elmgren and Frithsen 1982). There was, however, a clear difference between the two experiments. In the first, M. ambiseta populations also declined in the control tanks. An examination of the slow-flow delivery of seawater designed to achieve a 30 day turnover time in the mesocosms showed that zooplankton individuals could avoid introduction to the mesocosms and escape through an overflow device, and that the amount of suspended par-

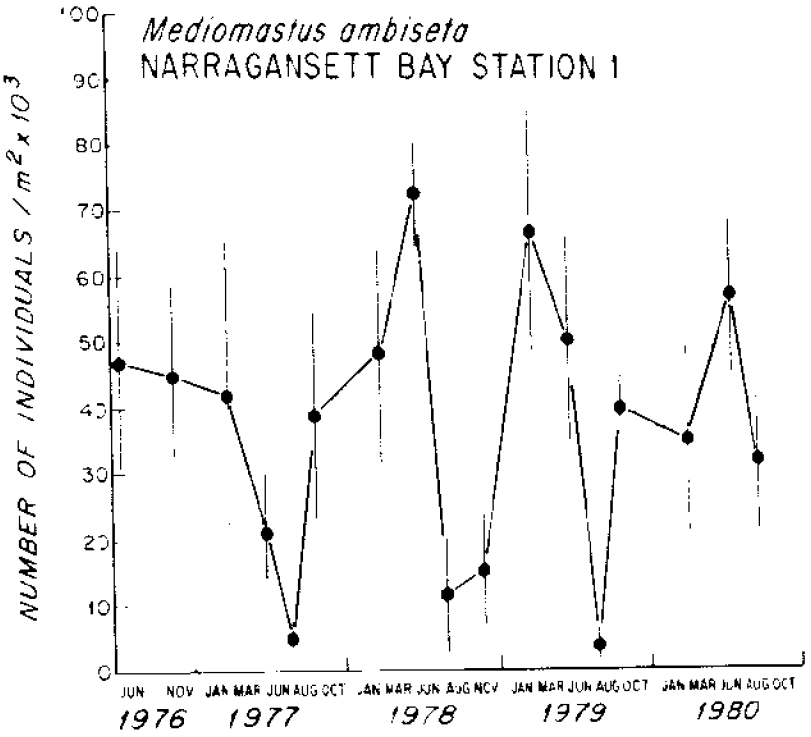

Figure 1. Density of Mediomastus ambiseta populations at Station 1, Narragansett Bay from June 1976 to August 1980. Error bars are ± 2 S.E.

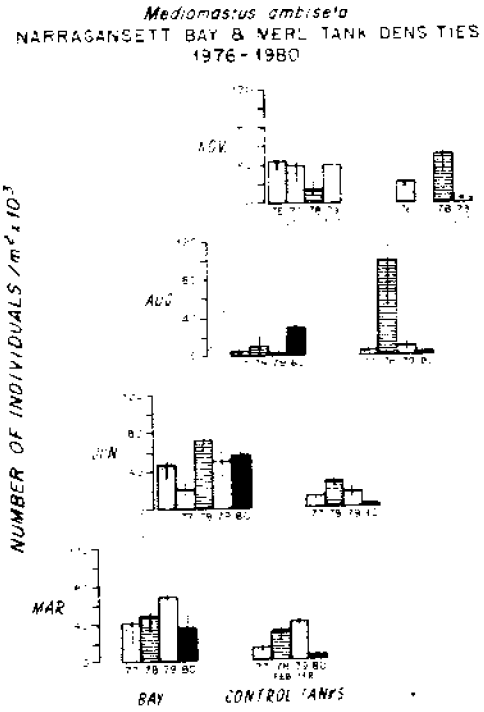

Figure 2. Comparison of Mediomastus ambiseta densities at Station 1, Narragansett Bay, and in control mesocosms from 1976 to 1980. Error bars are ± 2 S.E.

ticulate matter entering the tanks was also reduced. It was theorized that this additional source of organic matter was crucial for successful recruitment of larval M. ambiseta. In the second oil experiment in which seawater was introduced to the mesocosms by intermittent fast flow, no decline in M. ambiseta populations in control tanks was observed, dramatizing the significant effects of the lower oil concentration (90 ppb) on the M. ambiseta populations in the oiled tanks.

A Short Term Organic Addition Experiment

At the end of the second oil experiment we took the opportunity to examine the effect of organic additions to three tanks (dried Ascophyllum nodosum was added to the tanks from May 7 to July 18 at the rate of 1.43 g d^{-1}). We found increased recruitment of juvenile M. ambiseta in two out of three of the organic addition tanks compared with controls (Figure 4). There was also increased recruitment of Polydora ligni and amphipods to these tanks (Figure 5). This suggested that the periodic summer declines in M. ambiseta populations in Narragansett Bay (Figure 1) might occur as a result of respiration exceeding production during the high temperatures of August (C. Oviatt personal communication), and that this effect could be mitigated by an increased input of organic matter that could readily be assimilated by M. ambiseta postlarvae.

MERL MESOCOSM EXPERIMENTS: EUTROPHICATION

On June 1, 1981, a eutrophication experiment was initiated in the MERL mesocosms. The benthic community introduced to the mesocosms was collected in May from Station 1, Narragansett Bay, and was dominated by relatively low densities of M. ambiseta (the range in starting mean densities in nine experimental tanks was 10-29/5 cm^2 core = 20,000 - 58,000 m^{-2}). Three tanks were used as replicate controls; six tanks received six levels of nutrient loading in a logarithmic series (x1, x2, x4, x8, x16, and x32), the x1 level approximating anthropogenic nutrient loadings in Narragansett Bay (Donaghay, this chapter). This experiment is still in progress, and not all of the monthly benthic samples are analyzed. Here we report on the effects of these nutrient additions on M. ambiseta densities determined from just two 5 cm^2 cores taken monthly from each tank and immediately live-sorted. Previous sampling has shown that data obtained in this way for dominant and codominant species such as M. ambiseta and Nucula annulata are very similar to those obtained by the routine sampling and analysis of ten 5 cm^2 cores per tank per month. Figure 6 shows the almost linear relationship between M. ambiseta densities in August and September and the level of nutrient additions. This enhanced recruitment of Mediomastus in the x32 treatment was already evident in July samples taken only 63 days after the experiment was

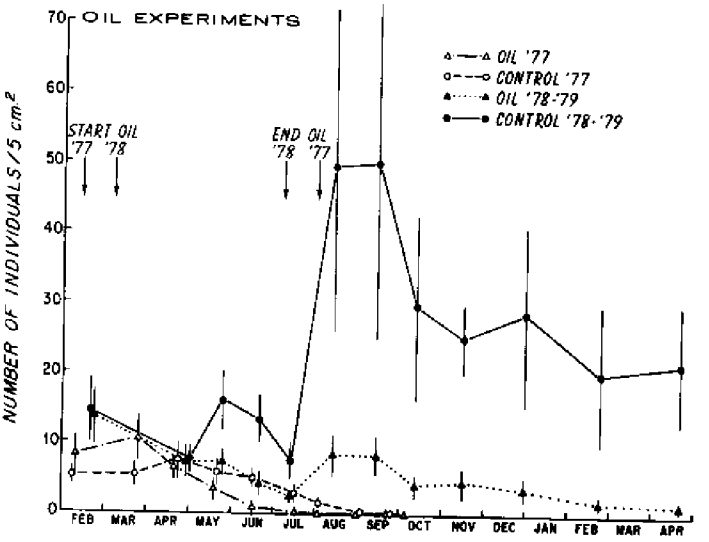

Figure 3. Mediomastus ambiseta densities in oiled and control mesocosms during the first (1977) and second (1978) oil experiments. Error bars are ±2 S.E.

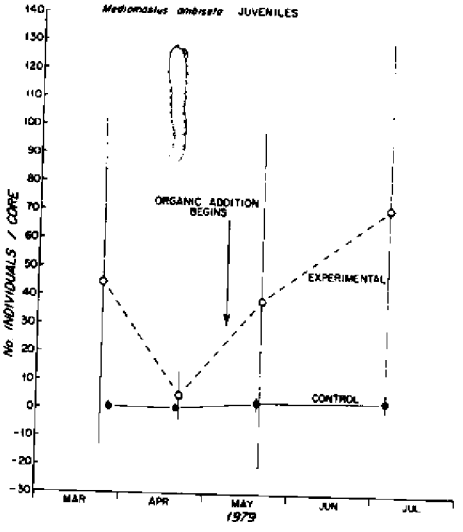

Figure 4. Organic addition (A. nodusum) experiment March-July 1979: density of M. ambiseta juveniles in control and experimental mesocosms. Error bars are ±2 S.E.

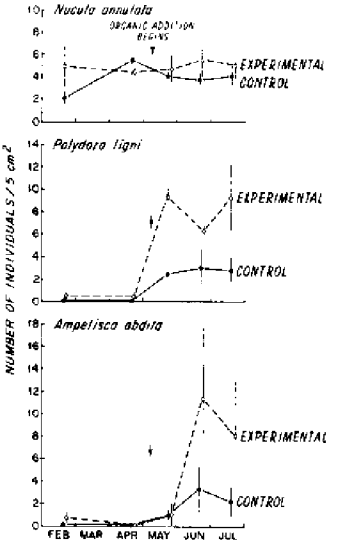

Figure 5. Organic addition (A. nodusum) experiment March-July 1979: densities of Nucula annulata (Bivalvia), Polydora ligni (Polychaeta), and Ampelisca abdita (Amphipoda) in control and experimental mesocosms. Error bars are ±2 S.E.

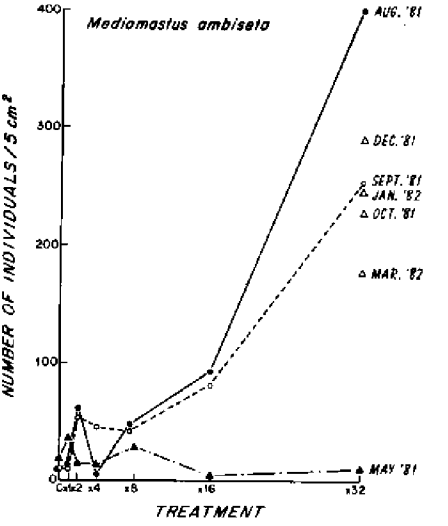

Figure 6. Eutrophication experiment: response of average M. ambiseta densities to six levels of nutrient addition (x1, x2, x4, x8, x16, x32).

initiated. That is, there was no delay in the translation of the effect of increased primary production in the water column to the increased recruitment and survival of M. ambiseta postlarvae. The elevated density of M. ambiseta in the x32 treatment persisted on into the fall and winter. The fraction of individuals in the largest size category was higher in this treatment, and many of these individuals were much bigger and contained more gametes than any M. ambiseta previously observed from Narragansett Bay or mesocosm samples. This increased individual growth and fecundity was achieved at densities equivalent to approximately 800,000 m^{-2}, higher than the highest densities reported for subtidal stations in the first summer after the West Falmouth oil spill. Figure 7 graphically illustrates that not all species respond in the same way to the increased nutrient levels. At the highest nutrient level (x32) the recruitment and survival of the two polychaete species Polydora ligni and Streblospio benedicti are enhanced. This was first evident in the August samples, 40 days after the effect on M. ambiseta was first noted. The elevated densities of S. benedicti in this treatment appear to have persisted through the winter. On the other hand there is no significant effect of nutrient level on recruitment, survival or growth rate of Nucula annulata. Previous sampling in Narragansett Bay at the three stations in the upper, middle, and lower Bay had shown that peak densities in N. annulata were found at the lower Bay station, and almost no individuals at the Providence River station. Moreover, N. annulata did not respond to the trial organic addition experiment (Figure 5) with increased juvenile recruitment.

In the current eutrophication experiment Mulinia lateralis has shown massive recruitment in a single tank with an intermediate level of nutrient addition (x8) (Figure 7). Although M. lateralis is well-known as a relatively opportunistic bivalve species that is believed to respond positively to the effects of eutrophication, and although a number of laboratory and field studies have been carried out on this species (Boesch et al. 1976; Calabrese 1969, 1970; Jackson 1968; Levinton 1970; Rhoads et al. 1978; Sanders 1956, 1981; Wass 1965), we have no good explanation for this response. It may be that the veliger larvae that were present in the tanks in July could not survive in the extremely dense phytoplankton blooms present in the x16 and x32 treatments. Previous sampling in Narragansett Bay showed that peak densities of M. lateralis were found at the upper Bay (Providence River) station where anthropogenic nutrient levels approximate the x8 experimental treatment. M. lateralis are patchily distributed in the mesocosm benthos. The apparently fluctuating densities in Figure 7 reflect this patchiness which is exaggerated in these two-core-per-tank data. It does appear however, that the high densities of M. lateralis in the x8 treatment have persisted at least until the end of January 1982.

LIFE HISTORY DATA ON M. AMBISETA FROM LABORATORY STUDIES

Mediomastus is a relatively fragile capitellid and needs careful manipulation in the laboratory. It is, however, readily maintained in static cultures under conditions similar to those used for Capitella sibling species (Grassle and Grassle 1976).

Rasmussen's (1973) description of Mediomastus fragilis forming vertical, semi-permanent sandy tubes and nearby mounds of fecal pellets also describes the life habit of M. ambiseta in the laboratory. The tubes of M. ambiseta that project above the sediment surface are flexible, thin-walled mucus tubes, often covered with fecal pellets. From this tube the caudal filament projects into the water. It is extremely extensible and well vascularized in life and fecal pellets are deposited in mounds a short distance from the tube (the anus is at the base of this caudal filament). In laboratory cultures the sediment is not more than 5 mm deep so the worms perforce must feed "at the surface." When the sediment around the tube has been exhausted (i.e., all the fine-grained sediment has been pelletized) the worms move to another part of the culture dish. Given the small size of M. ambiseta, their tube formation and habit of forming fecal mounds with a projecting caudal filament, it seems unlikely that they feed at any depth in the field.

In living mature worms it is possible to distinguish males and females (males with some difficulty). Mature females have the anterior abdominal segments crammed with eggs that are free in the coelom. When these eggs are released into seawater they have a diameter of 75 μ m. The number of ripe segments is very variable but at a maximum these segments occupy two-thirds to three-quarters of the total abdominal length. In fully ripe males stellate clusters of sperm can be seen in the dorso-lateral parts of the anterior abdominal segments. In cultures of ripe males and females in the laboratory we have never observed or been able to induce spawning. Nor have we observed any jelly egg masses of the kind Rasmussen (1956) has attributed to M. fragilis either in the laboratory, or in mesocosm samples.

It is possible to study fertilization and larval development in M. ambiseta by stripping eggs and sperm from mature worms. Fertilization and development to a swimming trochophore larval stage took 3 days at 15°C. Larvae fed a mixture of Isochrysis and Monochrysis completed their planktonic development, settled and metamorphosed after 13-18 days at 15°C. Some segment formation was observed in the larvae after 7 days, 8-day larvae had 5-6 metamers, 13-day larvae had 13 segments and some settled, while others delayed as long as 5 more days. At 18 days the early settling individuals had small caudal filaments. These juveniles grew rapidly in the laboratory. In one experiment we found a generation time of 74 days at 15°C.

Mature M. ambiseta are observed in the field in Narragansett Bay and in the mesocosms in small numbers in April in some years, but generally not until May. Recruitment first becomes evident in samples sieved on a 0.3 mm screen in July in most years. Settling larvae are about 250 µm in length, so many of these pass through the sieve used to separate the macrofauna and are first seen in the meiofaunal fraction. In 1978 some recruitment was seen as early as April in one control tank, suggesting that ambient temperature may not be the only cue triggering spawning. Attempts to induce spawning in the laboratory by small, acute temperature increases were never successful.

In the current eutrophication experiment recruitment into the smallest size class continued into the sampling of October 20, 1981, suggesting that spawning, development and settling may continue under conditions of organic enrichment until the end of September. No detailed growth studies at different temperatures have been carried out but the rapid growth of laboratory raised animals at 15° (a generation in 74 days) indicates that there is sufficient time each year for some early recruits to grow to maturity and spawn that same summer. Winter populations consist of animals that are very variable in size and show no macroscopic evidence of gametogenesis until April or May. These populations include a small number of "old" individuals; by analogy with observations on Capitella spp. individuals of known age, the blood of these animals has lost its bright red color, and instead, small accumulations of dark heme pigments are seen in the coelom.

Some samples of M. ambiseta from Narragansett Bay and from the mesocosms included a high percentage of animals showing evidence of regeneration at the posterior end, that is, foreshortened abdomens with small caudal filaments. Observations on the fraction of animals regenerating led us (Grassle et al. 1980, Table 3) to compare predation levels in the mesocosms and the field. In 1978 40-70% of mature M. ambiseta in June, July and August samples from three control tanks showed evidence of regeneration. In July 1979, only 4-8% of mature animals were found to be regenerating in mesocosm samples, and 9-11% in animals sampled from Station 1 Narragansett Bay. These differences in the fraction of M. ambiseta regenerating were taken to be some measure of the relative intensity of one kind of predation on M. ambiseta--heavy in the mesocosms in 1978 and light in both the mesocosms and the Bay in July 1979. Subsequent laboratory observations on M. ambiseta regeneration rates (Hill and Grassle, unpublished) have indicated that they are very rapi* indeed. At 25°C all worms, whether fed or starved, whether gravid or not, whether the head was also amputated or not, regenerated a new caudal filament within 48 hours. After 1 week the worms also regenerated a number of small setigers, more in fed than in starved worms (although the number of experimental animals was too small in these preliminary experi-

ments to state that this difference was significant) and one or two "segments" lacking setae (Figure 8). At 1 week the abrupt change in segment length in the regenerate makes it possible to identify the worm as regenerating--the segment length in the new segments is approximately equal to the width and approximately one-fifth of the length of the original abdominal segments. But the process is so rapid that it is unlikely that regenerating worms could be identified after 2 weeks at these temperatures. Our identifications of regenerating worms in 1978-79 were always conservative. For example, animals with possibly broken abdomens (i.e., with no positive evidence of regeneration) were classed as non-regenerating, so a finding of 40-70% of mature animals regenerating indicates intense predation by a "nipping" predator species such as Crangon septemspinosus or small crabs. Of course no estimates are available on the level of predation by predators that take whole worms. In the Wadden Sea Netherlands workers (DeVlas 1979) have estimated that posterior ends of another large capitellid, Heteromastus filiformis, provided a significant, renewable (i.e., regeneration occurs) food resource for flatfish.

Biomass of Mediomastus (measured as decalcified ash-free dry weight) was .54 ± .17 g m^{-2} in samples taken from four control tanks in June 1978 (mean density was low at 22,000 m^{-2} = 11 individuals/5 cm^2 core) and 1.71 ± .38 g m^{-2} in five 35 cm^2 Bay cores (mean density was somewhat higher at 74,000 m^{-2}). Biomass estimates for the current eutrophication experiment will be available from the MERL facility (Rudnick and Frithsen, personal communications). A species such as Mediomastus, that responds quickly to elevated nutrient levels with high recruitment, good postlarval survival, rapid growth, high fecundity and the capacity to quickly regenerate parts of the body that are readily available to predators because of its life habit, may be a significant food resource to juvenile fish.

CONCLUSION

In separate mesocosm experiments we have shown that Mediomastus ambiseta populations were severely reduced by components of No. 2 fuel oil reaching the sediment as a result of chronic low level additions, and that they did not recover within 1 year after oil additions ceased. We have also shown in two experiments that M. ambiseta responds rapidly to increased primary production resulting from elevated nutrient levels. The response includes enhanced recruitment and survival of postlarvae, rapid growth to a large maximum size, increased fecundity, and survival through the winter at densities much higher than have been observed in the source communities or in previous mesocosm experiments with ambient nutrient levels. These results suggest that when M. ambiseta

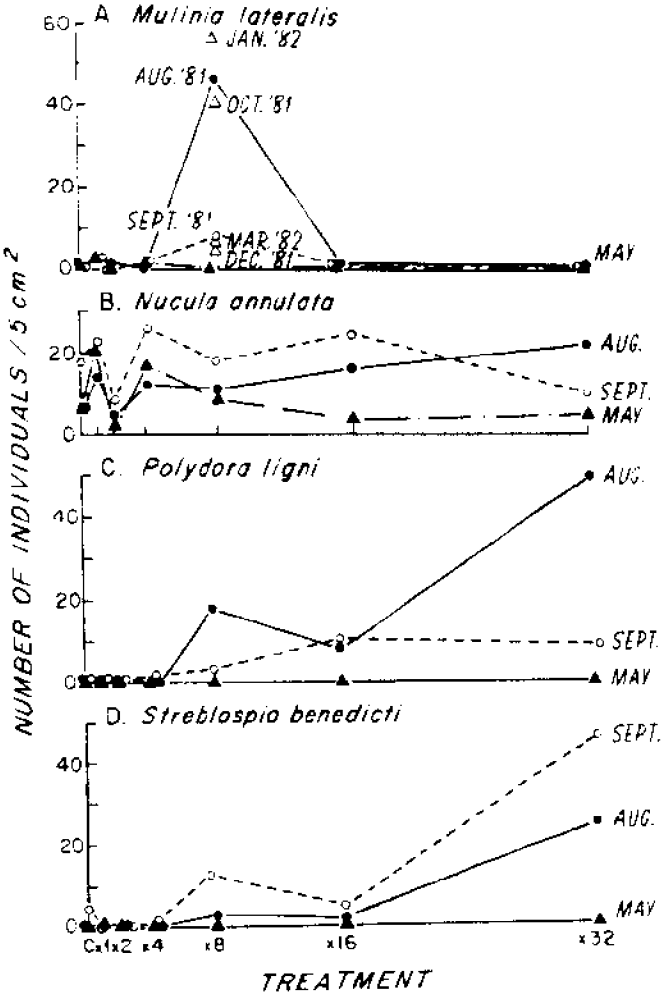

Figure 7. Eutrophication experiment: response of average densities of Mulinia lateralis and Nucula annulata (Bivalvia), Polydora ligni and Streblospio benedicti (Polychaeta) to six levels of nutrient addition.

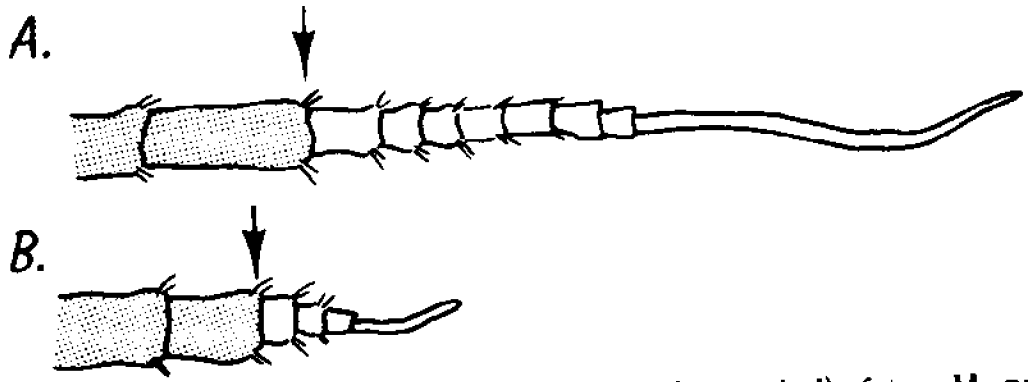

Figure 8. Regenerating posterior regions (unshaded) of two M. ambiseta individuals after one week at 25°C.

densities are observed to increase rapidly in field studies under disturbed conditions (e.g., following an oil spill, at a sewage outfall, or following a natural defaunation event that included some organic enrichment) that the primary cause is the increased amount of readily available food. Where the source of organic enrichment persists (e.g., in the vicinity of a sewage outfall) the dominance of M. ambiseta may be expected to persist. Where the source declines (e.g., following the West Falmouth oil spill) it is not surprising to find summer peaks in density that diminish over several years. The occasional "natural" low summer densities for Mediomastus conversely may in fact be attributed to a lack of these critical food supplies for the postlarvae that appear in the benthos in July, just as the time approaches when respiration often exceeds production in these sediments (the "August effect") (C. Oviatt personal communication). The gradual increase in Mediomastus abundance over the last several decades in mid-Narragansett Bay and at Station R in Buzzards Bay indicates gradual eutrophication. It seems paradoxical that since this species is now one of the most abundant macrofaunal species as a result of its response to increasing eutrophication, sudden reductions in its abundance are the most sensitive indicators of acute disturbance or pollution. Unlike Mediomastus ambiseta, Nucula annulata is historically an abundant element of shallow coastal communities in the northeast--its abundance has not changed under the nutrient additions that so favor M. ambiseta. Mesocosm experiments confirm the sensitivity of N. annulata to low levels of No. 2 fuel oil, and a lack of response to elevated nutrient levels.

This discussion has not touched on competition between infaunal benthic species for space or food. In the eutrophication experiment it is apparent that high densities of M. ambiseta and Streblospio benedicti have persisted together through the winter in the x32 treatment, while the initially high densities of Polydora ligni have declined. In the x8 treatment Nucula annulata persists in normal densities together with exceedingly high densities of Mulinia lateralis. We expect, as the nutrient additions continue through the summer of 1983, and the tanks are then allowed to recover, that it will be possible to examine the outcome with some interspecific interactions in mind.

In this report we have focused on experimental mesocosm studies on Mediomastus and how they help to explain the results of field studies. We have merely touched on the very different responses of some of the other species. Laboratory studies on live Mediomastus have increased our understanding of how this species makes its living although many gaps in our knowledge still exist. A much better understanding of the systematics of this genus is required, most particularly studies that will indicate the genetic relatedness of Mediomastus ambiseta populations over its whole reported range. We still know very little about the spawning cues

and actual mode of reproduction, although we have very good information about the time of year at which this occurs and the length of time available for larval dispersal. We especially need to know how M. ambiseta larvae and postlarvae are able to out-hustle almost all other benthic species for organic matter that has recently originated in the water column under conditions of eutrophication.

SUMMARY

Results of MERL mesocosm studies on the effects of No. 2 fuel oil and eutrophication on the dominant benthic species (Mediomastus ambieseta) in the mesocosms and in the source community in mid-Narragansett Bay are discussed in the light of previous field studies that documented the effects of several kinds of environmental disturbances on whole communities. M. ambiseta recruitment was shown to respond quickly and positively to increased nutrient levels in a way that suggests that increases in densities of M. ambiseta in the field in recent years are due to gradual eutrophication. M. ambiseta populations were shown to be reduced by low level, chronic additions of No. 2 fuel oil to the mesocosms. In situations where disturbance included both organic enrichment and the presence of No. 2 fuel oil, and M. ambiseta populations reached high densities (West Falmouth), it is suggested that the positive effects of organic enrichment temporarily outweighed the negative effects of the oil. Comparison of the responses of other species in experimental mesocosm studies and in the field indicates that consideration of species one at a time, when the biology of those species is well understood, is the most meaningful approach to studying pollution effects. Carefully coordinated experimental mesocosm and field studies will be meaningful only when accompanied by studies on modern systematics and population biology of the individual species.

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REFERENCES

Appy, T.D., L.E. Linkletter and M.J. Dadswell. 1980. A guide to the marine flora and fauna of the Bay of Fundy: Annelida: Polychaeta. Tech. Rep. No. 920, Fisheries and Marine Service of Canada, Biological Station, St. Andrews, New Brunswick.

Barnard, J.L. 1970. Benthic ecology of Bahia de San Quentin, Baja California. Smithson. Contrib. Zool. 44: 1-60.

Boesch, D.F., M.L. Wass, and R.W. Virnstein. 1976. The dynamics of estuarine benthic communities. Estuar. Proces. 1: 117-196.

Calabrese, A. 1969. Mulinia lateralis: molluscan fruit fly? Proc. Natl. Shellfish. Assoc. 59: 65-66.

Calabrese, A. 1970. Reproductive cycle of the coot clam, Mulinia lateralis (Say) in Long Island Sound. Veliger 12: 265-269.

Dauer, D.M. and J.L. Simon. 1976a. Repopulation of the polychaete fauna of an intertidal habitat following natural defaunation: species equilibrium. Oecologia 22: 99-117.

Dauer, D.M. and J.L. Simon. 1976b. Habitat expansion among polychaetous annelids repopulating a defaunated marine habitat. Mar. Biol. 37: 169-177.

DeVlas, J. 1979. Annual food intake by plaice and flounder in a tidal flat area in the Dutch Wadden Sea, with special reference to consumption of regenerating parts of macrobenthic prey. Neth. J. Sea Res. 13: 117-153.

Elmgren, R. and Frithsen, J.B. 1982. The use of experimental ecosystems for evaluating the environmental impact of pollutants: a comparison of an oil spill in the Baltic Sea and two long-term, low-level oil addition experiments in mesocosms. In: G.D. Grice and M.R. Reeve (eds.), Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems, pp. 153-165. Springer-Verlag, New York.

Elmgren, R., G.A. Vargo, J.F. Grassle, J.P. Grassle, D.R. Heinle, G. Langlois and S.L. Vargo. 1980. Trophic interactions in experimental marine ecosystems perturbed by oil. In: J.P. Giesy, Jr. (ed.), Microcosms in Ecological Research. Symposium Series 52, Augusta, Ga., Nov. 8-10, 1978, pp. 779-800 (CONF-781101, National Technical Information Service, Springfield, Va.)

Ewing, R.M. and D.M. Dauer. 1981. A new species of Amastigos (Polychaeta: Capitellidae) from the Chesapeake Bay and Atlantic Coast of the United States with notes on the Capitellidae of the Chesapeake Bay. Proc. Biol. Soc. Wash. 94: 163-168.

Flint, R.W. 1981. Gulf of Mexico Outer Continental Shelf benthos: macrofaunal-environmental relationships. Biol. Oceanogr. 1: 135-155.

Grassle, J.F., R. Elmgren and J.P. Grassle. 1981. Response of benthic communities in MERL experimental ecosystems to low level chronic additions of No. 2 fuel oil. Mar. Environ. Res. 4: 279-297.

Grassle, J.F. and J.P. Grassle. 1974. Opportunistic life histories and genetic systems in marine benthic polychaetes. J. Mar. Res. 32: 253-284.

Grassle, J.F., J.P. Grassle, L.S. Brown-Leger, N.J. Copley, and J.G. Smith Derby. 1980. Quantitative studies on benthic communities in experimental ecosystems. In: The Fate and Effects of Chronic Low Level Pollutants in Marine Ecosystems, pp. 144-168. Report for Year II of EPA Grant #80-6072020, Marine Ecosystems Research Laboratory, Univ. of Rhode Island, Kingston, R.I.

Grassle, J.P. 1980. Polychaete sibling species. In: R.O. Brinkhurst and D.G. Cook (eds.), Aquatic Oligochaete Biology, pp. 25-32. Plenum Press, New York.

Grassle, J.P. and J.F. Grassle. 1976. Sibling species in the marine pollution indicator Capitella (Polychaeta). Science 192: 567-569.

Guérin, J.-P. and H. Massé. 1978. Étude experimentale sur le recrutement des especes de la macrofaune benthique des substrats meubles. 1. Methodologie--donnees qualitative et quantitatives. Tethys 8: 151-168.

Hannan, C.A. 1981. Polychaete larval settlement: correspondence of patterns in suspended jar collectors and in the adjacent natural habitat in Monterey Bay, California. Limnol. Oceanogr. 26: 159-171.

Hartman, O. 1944. Polychaetous annelids from California including the description of two new genera and nine new species. Allan Hancock Pac. Exped. 10: 239-307.

Hartman, O. 1947. Polychaetous annelids. Pt. 7. Capitellidae. Allan Hancock Pac. Exped. 10: 391-481.

Hartman, O. 1969. Atlas of the Sedentariate Polychaetous Annelids from California. Allan Hancock Foundation, University of Southern California, Los Angeles, Calif. 812 pp.

Hartmann-Schröder, G. 1962. Zweiter Beitrag zur Polychaetenfauna von Peru. Kieler Meeresforsch. 18: 109-147.

Hobson, K.D. 1971. Polychaeta new to New England, with additions to the description of Aberranta enigmatica Hartman. Proc. Biol. Soc. Wash. 84: 245-252.

Holland, J.S., N.J. Maciolek and C.H. Oppenheimer. 1973. Galveston Bay benthic community structure as an indicator of water quality. Contrib. Mar. Sci. Univ. Texas 17: 169-188.

Jackson, J.B.C. 1968. Bivalves: spatial and size-frequency distribution of two intertidal species. Science 161: 479-480.

Levinton, J.S. 1970. The paleoecological significance of opportunistic species. Lethaia 3: 69-78.

Lizarraga-Partida, M.L. 1974. Organic pollution in Ensenada Bay, Mexico. Mar. Pollut. Bull. 5: 107-112.

Massé, H. and J.-P. Guérin. 1978. Étude experimentale sur le recrutement des especes de la macrofaune benthique des substrats meubles. 2. Données sur les cycles biologiques des polychetes et des bivalves. Tethys 8: 283-294.

Maurer, D., L. Watling, W. Leathem and P. Kinner. 1979. Seasonal changes in feeding types of estuarine benthic invertebrates from Delaware Bay. J. Exp. Mar. Biol. Ecol. 36: 125-155.

Michael, A.D., C.R. Van Raalte and L.S. Brown. 1975. Long-term effects of an oil spill at West Falmouth, Massachusetts. Proceedings 1975 Conference on Prevention and Control of Oil Pollution, Mar. 25-27, 1975, San Francisco, Calif., pp. 573-582. American Petroleum Inst., Washington, D.C.

Oviatt, C., J. Frithsen, J. Gearing and P. Gearing. 1982. Low chronic additions of No. 2 fuel oil: Chemical behavior, biological impact and recovery in a simulated ecosystem. Mar. Ecol. Prog. Ser. 9: 121-136.

Pearson, T.H. and R. Rosenberg. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. Mar. Biol. Ann. Rev. 16: 229-311.

Piltz, F.M. 1977. A new genus and species of polychaete (Family Capitellidae) from southern California. Bull. South Calif. Acad. Sci. 76: 57-60.

Rasmussen, E. 1956. Faunistic and biological notes on marine invertebrates. III. The reproduction and larval development of some polychaetes from the Isefjord, with some faunistic notes. Biol. Medd. Dan. Vid Selsk. 23: 1-84.

Rasmussen, E. 1973. Systematics and ecology of the Isefjord marine fauna (Denmark). Ophelia 11: 1-507.

Reish, D.J. 1956. An ecological study of Lower San Gabriel River, California, with special reference to pollution. Calif. Fish Game 42: 51-61.

Reish, D.J. and H.A. Winter. 1954. The ecology of Alamitos Bay, California, with special reference to pollution. Calif. Fish Game 40: 105-121.

Rhoads, D.C., P.L. McCall and J.Y. Yingst. 1978. Disturbance and production on the estuarine sea floor. Amer. Sci. 66: 577-586.

Rowe, G.T., P.T. Polloni and R.L. Haedrich. 1982. The deep-sea macrobenthos on the continental margin of the northwest Atlantic Ocean. Deep-Sea Res. 29: 257-278.

Sanders, H.L. 1956. Oceanography of Long Island Sound, 1952-1954: The biology of marine bottom communities. Bull. Bingham Oceanogr. Collect. Peabody Mus. Nat. Hist. (Yale University) 15: 345-414.

Sanders, H.L. 1960. Benthic studies in Buzzards Bay. III. The structure of the soft-bottom community. Limnol. Oceanogr. 5: 138-153.

Sanders, H.L. 1981. Environmental effects of oil in the marine environment. In: Safety and Offshore Oil: Background Papers of the Committee on Assessment of Safety of OCS Activities, pp. 117-146. National Academy Press. Washington, D.C.

Sanders, H.L., J.F. Grassle, G.R. Hampson, L.S. Morse, S. Garner-Price and C.C. Jones. 1980. Anatomy of an oil spill: long-term effects from the grounding of the barge Florida off West Falmouth, Massachusetts. J. Mar. Res. 3: 265-280.

Swartz, R.C., W.A. DeBen, F.A. Cole and L.C. Bentsen. 1980. Recovery of the macrobenthos at a dredge site in Yaquina Bay, Oregon. In: R.A. Baker (ed.), Contaminants and Sediments, pp. 391-408. Ann Arbor Science Publ., Ann Arbor, Mich.

Vargo, G.A., M. Hutchins and G. Almquist. 1982. The effect of low, chronic levels of No. 2 fuel oil on natural phytoplankton assemblages in microscosms. 1. Species composition and seasonal succession. Mar. Environ. Res. 6.

Wass, M.L. 1965. Study of a soft-bottom community in the lower York River, Virginia. Atlantic States Biology Meeting, Charlottesville, Va. (Abstract).

Chapter 8. Field Monitoring Programs

Introduction: Field Assessment of Marine Pollution Effects: The Agony and the Ecstasy

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Documented and quantified measures of marine pollution effects in the field (i.e., in nature) are ultimately the most conclusive and, therefore, meaningful measures of pollution. Resolution of doubts concerning the realism of experimental approaches in the laboratory and in simulations of various scales--microcosms, mesocosms and field experiments--relies implicitly on field data. However, the ecstatic appeal of such definitive resolution is tempered by agonizing limitations.

Not the least of these limitations is that field monitoring is inherently retrospective, allowing effects of an activity to be observed, but not predicted. Rolf Hartung discusses this dichotomy between prospective, experimental approaches and retrospective, observational approaches: there are distinct limitations to both, and clearly they can be complementary. Hartung endorses a holistic approach, integrating a variety of prospective and retrospective methods. No one would seriously disagree with that recommendation. But how does one implement it? The impediments to such integration are cultural as well as conceptual. I will leave these issues for the broader synthesis to follow. Rather, I will here outline some other sources of agony confronting field assessments in order to set the stage for the papers to follow, which, I believe, cast some rays of optimism on the briars of our passion for field study.

Specifically, field assessments have generally been criticized because of (1) generally weak or ineffective design (this is usually a retrospective evaluation); (2) the difficulties in relating observations to specific causes; (3) the difficulty in gauging the broader importance of an observed modification to an ecosystem component; and (4) the difficulty regulators have in codifying the products of even the best field studies in the context of existing regulations, and frequently even in subsequent actions.

Field assessments have been criticized as basically unscientific, in that they involve only observation and not hypothesis testing. Many field assessment programs have not been clearly planned to address explicit questions. Moreover, many field programs were not based on an appreciation of the dominant sources of natural spatial and temporal variation, sometimes resulting in "controls" that are in vastly different habitats. Once a monitoring program has begun, there is a reluctance to change the design because of the need for consistency, which may simply perpetuate initial mistakes. All too frequently, there is an implicit assumption that if enough biological and chemical parameters are measured at the same place and time, truth will mysteriously emerge from statistical analyses, liberally applied to bless the proceedings.

Roger Green provides sound advice regarding the need to identify the purpose, question, hypotheses and underlying model for the development of sampling and experimental designs. He asserts that biologically defined objectives should dominate and determine the statistics.

The variability of nature is legend. In simple terms, variations in biological populations and rates of processes in time and space constitute statistical noise from which the pollution effects must be deciphered. However, some understanding of the causes, or at least the patterns, of natural variation must be gained in order to properly design sampling. It is not sufficient to treat natural patterns merely as statistical variation. Nonetheless, there is always the lingering doubt whether causes other than the pollutant or human disturbance factor in question were responsible for an observed biological change or difference. The variability of natural systems and the resultant difficulty in relating observations to specific causes have brought into dispute the validity of the baseline and monitoring approach (Gray 1976). Such concern was one reason, for example, for the abrupt termination of the Bureau of Land Management's (BLM) extensive baseline studies of continental shelf environments prior to oil and gas development (Burroughs 1981). The lack of focus and simplistic assumptions of the BLM baseline studies were widely criticized by the scientific community, although, in fairness, the prematurely standardized design of the studies was based on the collective recommendations of the scientific community--a scientific "tragedy of the commons".

Notwithstanding its inherent limitations, the environmental monitoring approach remains our ultimate "ground truth," and better understanding of the causes of natural variations in space and over long time periods is urgently required. Therefore Smayda presents the results of his unusually long-term study of the plankton community in Narragansett Bay. Plankton communities are notoriously highly variable, and, for this reason, have often been discounted as a useful subject of applied environmental monitoring. Smayda's study illustrates that temporal variability of phytoplank-

ton is indeed the usual, but can be related to environmental factors operational over various time scales. Determination of meaningful biological alterations due to human effects depends on such understanding coupled with suitable experimentation.

The quantitative determination of human effects on a biological population is the objective of most field assessments. This would be sufficient if the underlying philosophy were that any resulting biological change is undesirable and, therefore, should dictate further control or elimination of the pollutant or activity. This is not practical, however, particularly given the current policy revisions that allow waste disposal as a legitimate use of the ocean. One needs to know to what degree these effects will reduce or interfere with other resources or uses. As marine environmental scientists, we need to be prepared to answer in quantitative terms the "so what" question and thus contribute to an assessment of whether the effect constitutes "unreasonable degradation." Unfortunately, very few marine environmental research programs are designed to answer this question. Sponsors consequently become upset with the analyst when they are told that their discharge is resulting in mortalities of larvae, for example, and demand conclusions that this effect is insignificant to the adult populations. However, they shortsightedly may not have specified a design or allowed flexibility adequate to address even the spatial extent of the effect. The analyst is often forced to borrow data and assume certain relationships that significantly reduce the quantitative rigor of the assessment (Boesch 1982).

Robert Livingston's studies of coastal ecosystems of the Florida Gulf Coast illustrate the necessary complexity of multifaceted research designed to place changes in biological populations in a broader ecosystem context. Alan Mearns and Thomas O'Connor seek to place documented alterations in the common perspective of spatial scale. Furthermore, Mearns and O'Connor are able to demonstrate relationships between the magnitude of waste inputs and the spatial scale of effect, which allow feedback to waste management decisions. This addresses, in part, the question of significance and also the fourth limitation I initially identified--the use of results in decision-making.

Although field assessment approaches require general improvement and continued development, disparate currency and timing of observations and regulations are at least as much the fault of the regulatory institutions and mechanisms as of researchers. The ecologist should not be blamed for the fact that environments deteriorate in complex and subtle ways as a result of human activities.

REFERENCES

Boesch, D.F. 1982. Ecosystem consequences of alterations of benthic community structure and function in the New York Bight region. In: G.F. Mayer (ed.), Ecological Stress in the New York Bight: Science and Management, pp. 543–568. Estuarine Research Foundation, Columbia, S.C.

Burroughs, R.H. 1981. OCS oil and gas: Relationships between resource management and environmental research. Coast. Zone Manage. J. 9: 77–88.

Gray, J. 1976. Are marine base-line surveys worthwhile? New Sci. 70: 219–221.

Some Guidelines for the Design of Biological Monitoring Programs in the Marine Environment

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INTRODUCTION

Statistics in environmental studies are overused and are often superficial. They have been too much used as an after-the-fact salvage operation, and as a window dressing of respectability for demonstrating the "significance" of studies that were not designed to test any clearly formulated hypotheses in the first place. What is needed is a priori design of environmental studies validated by some preliminary sampling of experimentation, so that the results--effectively displayed--can speak for themselves.

With hypothesis-testing statistics in particular, most of the statistics--including (1) estimates of the replication needed to obtain specified power in the test and (2) description of multivariate structure to provide a basis for choice of variables and good design--should be done on preliminary data. In the final report good graphic display should suffice to show what is going on. In descriptive studies, on the other hand, statistics can play a major role in the final report, and multivariate statistical methods are often appropriate.

NONSTATISTICAL CONSIDERATIONS

Many authors have emphasized that in the design of any study there should be a logical flow of purpose → question → hypotheses → model → sampling (or experimental) design → statistical analysis → tests of hypotheses → interpretation and presen-

tation of results. Biologically defined objectives should dominate and determine the statistics, although the reverse is too often the case. Statistical tests require biological hypotheses that are formulated in terms of models, because statistical tests have reality only as tests of hypotheses and they have validity only for specified models. (The same is true of "confidence limits," which are nothing but inverted significance tests.) As is true of any sequential process, the failure of any step will invalidate the steps following it. Of most concern to me in environmental studies is lack of clarity of purpose resulting in failure to formulate meaningful questions, and the concern is especially great because the validity of all the other steps depends on these first two.

Choose Appropriate Temporal and Spatial Scales

Environmental biologists spend much of their time running around "putting out fires," doing short-term research to provide short-term answers to problems that have just become urgent. History tells us that it is the long-term irreversible changes that affect man most seriously. There is increasing evidence that even in natural, unimpacted communities the structure we see is usually controlled by infrequent severe events that disrupt the community and return the successional process to an earlier stage. At any given time we see the mosaic of successional stages representing the evolving spatial pattern through time since the last severe event. Even the supposed stability of deep sea communities is mostly a matter of time scale. The most productive communities--located around volcanic vents--come and go unpredictably (from the organisms' point of view) as the vents become active and then after a period of years become inactive. On the abyssal plain away from the vents, experiments are suggesting the importance of opportunism in response to unpredictable availability of large chunks of food or of unusual substrates (see Sanders (1977) for discussion and references).

For these reasons ecologists should avoid the trap of becoming knee-jerk opponents of all change. Change is unavoidably part of history and--as should be obvious to any biologist--of organic evolution. Complete stasis is equivalent to absolute zero in a physical context, and to system-death in a biological context. Many ecologists do get trapped into spending most of their time fighting unwinnable fights against inevitable, perhaps even partially desirable, change. Far too many impact study designs are in effect testing null hypotheses of "no change" against an implicit alternative hypothesis of "any detectable change of any kind, which will be assumed to be a change for the worse unless someone (else) proves it otherwise." Ecologists to some extent deserve their image of being in principle against progress. It is of course possible, given enough money and sampling effort, to detect a change due to impact that is too small to be of concern to anyone.

What does constitute change of a kind and of a magnitude to cause concern? Who decides? The politician, the manager, the ecologist? Certainly it is one of these; it should not be the professional statistician, who if competent will tell the ecologist consulting him to begin by clearly stating his objectives, questions and hypotheses. Then and only then will he proceed to advise on design and analysis. If, however, ecologists claim--as many of us do these days--to be both competent ecologist and competent statistician rolled into one professional person, then they assume a responsibility to decide what the meaningful questions are for a given environmental problem as well as to design the study properly and to analyze the results and present them effectively. I think that we often do not realize what we take on when we agree, and even passionately argue, that we are capable of performing this dual role for society. For one thing we cannot blame poor results on a naive ecological conception of the problem, or on poor design, insufficient sampling effort, failure to do preliminary sampling, and the like. I would argue strongly that the role of the statistically competent ecologist, difficult as it may be, is vital to environmental research and management.

In attempting to answer the question "How big a change is ecologically significant?" one has to come back to the question "On what time scale?" Is the concern one of short term ugliness and the fouling of a few very visible and lovable animals such as birds and seals? Or is it long term deterioration of the environment in some sense? When gradual, long term, perhaps irreversible environmental impacts are not tied in with any sudden spectacular mess, it is most impossible to generate concern or research money. Perhaps the only solution to this problem is to have a core of internationally respected environmental scientists who receive steady year-to-year funding and who have a mandate to study the problems they believe to be most important in the long run. Such an approach to research funding is not at all in fashion these days; instead we have short term "impact studies" which must generate immediate results and fast decisions. Eutrophication, heavy metals, PCBs, oilspills--each comes and goes in pulses of urgency and financial support. Never mind that we lack a basic understanding of these natural communities and ecosystems. This may represent good politics, but it is certainly not a sound way to conduct environmental science.

For that matter what is environmental deterioration? Individual oil spills may cause short term messiness and deaths of some animals (usually not of whole populations), medium term increases of biological productivity in the region, and few demonstrable long term effects. A more important concern—as with nuclear power station accidents--may not be the average or typical incident at all, but the very improbable and very worst ones. Most important of all is the cumulative effect on the world's oceans of a century of

average incidents. If these things are what matters, then what role is there for short term environmental studies in general, or sophisticated statistical analyses in particular? Statistical estimation in the tails of distributions is notoriously imprecise and often biased, and here we are estimating for distributions whose parameters are unlikely to remain constant over time.

At the least we must convince the politicians and managers that significance of impact effects (represented by the numerator effect in an F-test, for example) must be tested against a meaningful null hypothesis error term (the denominator in the F-test). I would argue that the appropriate error term is usually among-year variation in the unimpacted situation. The common practice of using replicate sample observations within the impact and control area for this purpose is particularly inappropriate. This is an estimate of sampling error, not of meaningful natural variability in the system. Can we possibly convince those who approve and fund our studies that years of work, enough years to provide adequate among-years error degrees of freedom for robust tests, are not only legitimate but necessary? It will not be easy, but we can point to a trade-off: more time and money will be needed for a given study but any rejection of the null hypothesis (concluding that there is an impact effect) will be much more meaningful. Trivially "significant" impact effects, judged against inappropriate error terms with excessive pseudoreplication, will preoccupy the politicians and managers less frequently.

Within-year temporal variation can be of great importance (e.g., high discharge of nutrients, pollutants and sediment load from rivers into coastal areas in the spring) but this importance relates to the numerator in the test (the impact effect) rather than to the denominator (the null hypothesis error). Time of year can, and should, be controlled or stratified in the design (e.g., spring runoff time only, or each month). Use of yearly averages is rarely good practice. There is no way to stratify or control among-year variation, and there will not be unless predictable long term cycles in natural phenomena of interest to us are convincingly demonstrated.

What are Appropriate Criterion and Predictor Variables?

Criterion variables should represent a direct, robust linkage to the question and the hypotheses. There are three possible situations. In some cases, the criterion variable is defined as part of the question, for example, "Are lobsters in the vicinity of spoil sites picking up PCBs or heavy metals?" The design and execution may be difficult but it is at least clear what the subject of study is. Of course the question of temporal and spatial scale remains. For example, over what length of time (the maximum length of life of a lobster?) and what area (how far do lobsters disperse?) may accumulation of heavy metals occur? Against what temporal scale

of variation in "natural" heavy metal concentrations will any apparent increased levels be judged?

In other situations ecologists are presented with apparently value-free questions, for example, "Does the impact cause a change of any kind? If so, describe it." In such a case there are choices to be made but without any a priori rationale for doing so. Such studies are easily trivialized, in that some change of some kind can probably be demonstrated. The difficult and completely nonstatistical job of deciding what really matters has only been postponed and passed on to the person who receives the results of the study. Unfortunately, when the managers finally do decide what is important socially, economically and politically--in relation to a given problem--it usually turns out to be something that was not optimally treated by the sampling design or the choice of criterion variables.

These "Describe any change that occurs" problems often lend themselves to multivariate approaches. It is analogous to the situation in numerical taxonomy where any variation among specimens is of potential interest. A recommended strategy (Sneath and Sokal 1973) is to use a large and representative sample from the universe of possible variables, assuming that there will be little information in omitted variables that is not contained in (= correlated with = predicted by) included variables. A similar strategy may be employed by the environmental biologist faced with this kind of problem. He may choose as a criterion variable set an assemblage of organisms that is easily and reliably sampled (e.g., the benthic community in freshwater or marine studies). Then field sampling can be visualized as a random sample from that universe of variables, using numerical abundance, biomass, percentage cover or whatever measure seems most appropriate. There is usually high redundancy among taxa in the information they contain about spatial and temporal structure of both natural and impacted communities (Kaesler et al. 1974; Green 1979). Despite the passionate beliefs of devotees of particular measures (e.g., biomass as opposed to numerical abundance), there is also high redundancy among different measures of taxonomic variables. For example, in a recent study of marine benthic species (molluscs, crustaceans, polychaetes and others) in demersal fish diet, it was found that 94% of the spatial and temporal variation described by measures of numerical abundance, wet weight and frequency of occurrence was contained in one principal component. The best (= containing the most information about all measures and variables) measure, numerical abundance, contained 90% of the total information (Macdonald and Green, in press).

The process of choosing the best variable (in the above sense), then the next best, and so on, can be done by one of several multivariate "dimension reduction" techniques. The most efficient reduction (in the sense of the greatest percentage of the variation

being described by the fewest variables) will be accomplished by an eigenvalue-eigenvector technique such as principal components analysis. Another approach, which is usually only slightly less efficient and has the advantage of retaining the identities of the original variables (species, say) is to use a variable subset selection algorithm such as that proposed by Orloci (1973). Another approach to dimension reduction is to use diversity indices or cumulative species abundance curves, but such criterion variables are not clearly and logically related to any generalizable environmental reality. They are also not robust empirical indicators of any important "environmental health" correlates of biological systems, in that they are strongly influenced by naturally varying environmental parameters. Finally, they carry no information about species identities. See Green (1979) for further discussion of diversity indices.

Most often ecologists are handed problems that fall between these extremes of (a) criterion variable explicitly defined as part of the question, and (b) unspecified criterion variables, which can be chosen on purely statistical grounds without a priori constraints. For example we may be asked, "Does the power plant effluent cause deterioration of environmental quality in that estuary?" These in-between problems are the most difficult ones because they imply that the ecologist can and will make nonstatistical judgments about what the concern is, judgments that have ethical and political consequences and must precede choice of criterion variables. (They must also, of course, precede choice of appropriate spatial and temporal scales for the sampling and experimental design.) The ecologist usually accepts this responsibility, often implicitly rather than explicitly, but in my experience does not always effectively discharge it. This is where the greatest weakness in environmental studies lies, rather than in the statistical aftermath. Multivariate approaches can also be useful here, for example, in selecting a subset of the variables that are most informative about the natural spatial and/or temporal variation of the community (as described above) and then selecting from those the variables that previous studies or laboratory toxicological experiments have shown to be most sensitive to the impact in question. Ideally one wants (1) socioeconomically "meaningful" variables that are (2) sensitive to the impact and (3) relatively invariant in the unimpacted "natural" situation. However, criteria (2) and (3) may often be correlated, in that "sensitive" species may be sensitive to environmental variation whether natural or impact related.

What of the predictor variables? They must represent the presence/absence (the latter being a control) of the impact, or the intensity of the impact. Also they must represent any important non-impact environmental variables that might influence the criterion variable(s). Ideally the sampling design should hold constant any such extraneous environmental variables, or if that is not pos-

sible then it should explicitly include them as controlled levels of a fixed effect (e.g., as depth strata offshore from a power plant development). Less desirable is their inclusion as uncontrolled variables measured along with the criterion variables during the sampling; they will not then be Model I fixed effects, and their is no guarantee that the distribution of their values will represent a balanced design. If the result is a statistically significant impact effect, but level of impact is correlated with an uncontrolled evironmental variable, then interpretation of the result will be difficult. Ignoring potentially important environmental heterogeneity, and not including measures of it at all, is of course the worst approach.

SUMMARY

My theme has been that statistical methodology rests on knowing what the question is, and that environmental biologists worry too much about statistical methodology and too little about what they are monitoring for. To play the difficult role of statistically competent ecologist as honestly, as ethically and as effectively as possible, we must first choose our level of concern. Let those who wish to fight all change do so; we cannot afford the loss of energy, of time, or of credibility that such a pointless and unending fight requires. For example, we cannot man the trenches in the face of every threatened species extinction. Neither should we equate all environmental change with loss of environments. In thousands of years most of North America's ecosystems will inevitably be different from what they are now, just as long term natural processes have made them very different now from what they were in the late Pleistocene. To influence the eventual result of large scale processes of change (patterns over large areas changing over long times) is a reasonable and attainable ambition for ecologists; to stop them, or even to totally control them, is not.

Related to choosing our level of concern is the necessity of being aware that the time and space scales of greatest importance to our economic and political masters do not usually coincide with what an ecologist sees as being of greatest importance to the environment. In most environmental studies the concern, the money and the deadlines are roughly on the scale of the life of a government, the length of a term in office, or the period of a research grant. Too many ecologists accept such time scales willingly. The question "What is a simple, easily and cheaply determined index of damage to ecosystems?" is very much like the question "Have you stopped beating your wife?" The proper response in both cases is that the implied terms of reference are unacceptable. Perhaps the training of ecologists requires more emphasis on the large temporal and spatial scale arts and sciences, such as geology, geography, history and even the better quality science fiction, and less emphasis

on ever more sophisticated statistical methods for fine tuning answers to temporally and spatially coarse-grained questions. We must learn to look at environmental problems on a realistic time scale. On the scale of a human generation or, at the other extreme, on the scale of geological processes, the importance of human impact on the environment is relatively slight. It is on the time scale of half-centuries to millenia that the most important changes take place. The role of paleoecologists in environmental studies certainly should increase. Rhoads and Lutz (1980) provide a state-of-the-art review of methods and examples of application to marine organisms and environments.

I believe that ecologists are responsible, as a matter of professional ethics, for looking beyond the wording of the problem as it is given to us, to the real environmental problem on the time scale that really matters. We must argue for the importance of long term studies, to obtain reliable estimates of "baseline" variation on the among-years time scale and to determine the long term effects of impacts on complex systems. We should also insist that planning of short term studies include time and money for publication of the results in the refereed primary literature, so that it can be easily accessed by others later on. In the long term, this will provide genuine replication, though admittedly of a qualitative nature, of given types of impact (e.g., of impoundments, of ocean dumping, of heated effluents) and their lasting biological effects.

There is no one kind of study that is the answer. We need the mix: the long term sophisticated study at one location, the broad geographic survey over a short time period, and spatially and temporally restricted "impact studies." But the assessment of natural variation over years, and of long term effects of impact on natural ecosystems, must be included in the mix. Maturity is the acceptance that some things require long term solutions. We should hope that ecologists, politicians and the public will acquire the maturity to support studies that will yield rewards only on a time scale of decades or longer. The attitude of the last decade or two, that all can be solved by impact studies of 1 or 2 year duration, may--with luck--be an immature stage we will outgrow.

We must say that any single variable, whether it is a diversity index or the first principal component or the incidence of lesions or any other measure, cannot represent more than one thing that is going on in the system. It is a mathematical impossibility for it to represent more than that, and loosely coupled marine ecosystems cannot be effectively described by the single most important thing that is going on. Certainly techniques such as principal components analysis or nonmetric multidimensional scaling can and should more often be used on preliminary data to determine the minimum dimensionality for adequate representation of the system under study. However, no ecologist should encourage a non-biologist manager or politician who wants to search for that elusive

goal, the single variable that can be easily and inexpensively determined and that represents human impact on natural ecosystems.

Finally, we must insist that any statement of statistical significance include a statement (in simple English--not statistical jargon) of what null hypothesis error term is being used in the contrast. If it is claimed that the impact is "significant," then "relative to what" must also be stated and justified. Year-to-year natural variation? Replicate sampling error? Something else? Without that information the result cannot be evaluated.

REFERENCES

Green, R.H. 1979. Sampling Design and Statistical Methods for Environmental Biologists. John Wiley and Sons, New York.

Kaesler, R.L., J. Cairns, Jr. and J.S. Crossman. 1974. Redundancy in data from stream surveys. Water Res. 8: 637-642.

Macdonald, J.S. and R.H. Green. In press. Redundancy in environmental variables. Can. J. Fish Aquat. Sci.

Orloci, L. 1973. Ranking characters by a dispersion criterion. Nature 244: 371-373.

Rhoads, D.C. and R.A. Lutz. 1980. Skeletal Growth of Aquatic Organisms. Plenum Press, New York.

Sanders, H.L. 1977. Evolutionary ecology and the deep-sea benthos. In: The Changing Scenes in Natural Sciences 1776-1976, pp. 223-243. Spec. Publ. 12, Academy of Natural Sciences, Philadelphia, Pa.

Sneath, P.H.A. and R.R. Sokal. 1973. Numerical Taxonomy: The Principles and Practice of Numerical Classification. W. H. Freeman, San Francisco, Calif.

Holistic Assessments of Pollution Effects: The Interplay of Prospective and Retrospective Studies

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Assessments of the impact of pollutants in any system can be divided into two major categories. The first, prospective or protective assessment, tries to predict the immediate effects of a certain pollutant dose rate on organisms, as well as the eventual effects on an ecosystem. The basic goal is to prevent adverse effects by establishing acceptable release rates of chemicals subject to control. Characteristically, protective assessments compensate for any perceived errors in the estimation of "safe" exposure rates by the use of safety factors, uncertainty factors and/or conservatism built into the assessment methodology. The compensation is usually one-sided; that is, poor data result in the use of greater uncertainty factors.

The second category, retrospective assessment, tries to predict the impact of a known ongoing exposure, or the residual effects of an exposure that has been terminated only recently. This category actually embraces a continuum from obvious, major impact to minor or even undetectable impact. In situations of obvious impact, cause-effect relationships are not really in doubt, and thus the major goal of the assessment may be to determine the qualitative and quantitative aspects of the impact. At the other end of the continuum are situations where it may be known that certain pollutants are being released into the environment, but elucidation of an effect or a cause-effect relationship is difficult. Ideally, findings from retrospective studies can be used to assess the validity of predictions made as part of protective assessments.

The types of experimental designs used in these two assessment approaches can be very different, and by necessity the types of er-

rors that can be made are also very different. Each assessment category is plagued by different gaps in knowledge and understanding. In prospective or preventive assessment we use many laboratory tests which are readily executed using generally accepted statistical methodologies. These tests are understood in terms of their chemical and physical principles, their superficial biology, biochemistry, genetics, etc. The one exception is when we try to analyze the low end of the dose/response curve; in this, we are attempting to evaluate minimal responses in test species after long periods of exposure, and to utilize this information to predict what might happen to other species exposed to even lower doses for longer periods of time. Since relationships among various species sensitivities and exposures are often poorly understood, the predictive reliability of such analysis can be disappointingly poor.

Similarly, retrospective studies that attempt to evaluate ecosystem responses are usually hampered by a lack of understanding of the functioning of the ecosystem as a whole. Most studies that seek to evaluate impacts on ecosystems are based on an identification of the species present and an estimate of the number and/or mass of the individuals representing each species. The implied hope is that fluctuations in the arrays of individuals per species will reflect something about the structure or functioning of the ecosystem as a whole. It is usually perceived that an ecosystem is a level of organization that exhibits more phenomena than the populations of the species that make up the ecosystem. Thus any analysis based purely on species enumerations and abundances is likely to miss aspects of ecosystem structure that are as yet only poorly understood. Understanding in this area may be particularly difficult to come by.

While many of us enjoy and feel an affinity to coastal marine life, we are nevertheless poorly equipped visitors in a very foreign environment. We are not even equipped to register the inputs that are meaningful to individual marine organisms! How do we sense the low frequency pressure waves that fish experience through their lateral line system? How do we distinguish among the "smells" in ocean currents as the salmon may do to find their ancestral home? How can a terrestrial semi-urban human understand the grand design and functioning of a marine ecosystem? The human, including the marine biologist, is blind to some of the very important stimuli that shape behavior at the individual level, the species level and certainly at the ecosystem level. Thus our interpretations regarding marine ecosystems are likely to represent a curious mixture of truths, half-truths and wishful thinking. One of the biggest problems may well be that we often do not know whether we are dealing with truths or falsehoods. Our systems for collecting data and analyzing them may incorporate misconceptions that inherently invalidate the data, irrespective of sampling design or analytical precision.

Let us examine some of the problem areas of predictive assessments in greater detail. In almost all instances this type of assessment is based on laboratory studies involving single chemicals or perhaps single process streams tested on single species. The complex environmental situation, involving many individual compounds where duration of exposure and concentrations change continuously, is essentially never studied for predictive purposes. This is so for the the simple reason that the complexity of the varying parameters transcends our capabilities to analyze them properly. Another problem is that we are able to study only a few models, often under highly artificial conditions, as predictors for all the species present in the ecosystem. The species most commonly selected for such studies have not been chosen for their ecological importance. (I am not sure that we can objectively gauge ecological importance. Ecological predominance might be a better and more testable concept.) Species have commonly been selected for their adaptability to laboratory conditions, and for unstated subjective preferences on the part of the investigator. Characteristically, many phyla are not represented in the testing scheme.

Predictive assessments for ecosystems tend to contain more uncertainties than predictive assessments for the protection of human health. The latter assessments characteristically examine the responses of several species whose physiological and biochemical relationships to humans are backed by a vast amount of previously gathered information and experience. The responses are then extrapolated to the most likely response in the human species. We have spent comparatively vast resources in our own self-interest for the simple task of drawing conclusions from several species towards ourselves. The resources required to provide relatively reliable predictive and protective assessments of ecosystem responses may be even greater, unless we are willing to tolerate far larger uncertainties for the protection of ecosystems.

In the case of retrospective assessments we find ourselves in a situation similar to that of the human epidemiologist. While the methodology establishes associations, it cannot readily establish cause-effect relationships, and often has to rely on controlled laboratory experiments to establish those relationships. Dose levels and time courses of exposure are usually only poorly defined. Chemical analyses are usually few and far between.

The choice of analytical methodology is more often governed by expediency or regulatory demands than by toxicological considerations. For example, metal analyses usually do not differentiate between the chemical forms of the metal (i.e., whether the metal is ionic, chelated, adsorbed onto particles, or existing in mineral form in suspended particles). These various forms can have quite different dose effects when absorbed by the exposed organism. The form in which an organic chemical is present in the environment also is significant. An organic chemical may occur in true solution in

water, or as micelles, or dissolved in lipids inside other organisms, or adsorbed onto particles. Again, the bioavailability of these various forms differs.

Some of the same problems are found in experimental situations in laboratory testing for predictive purposes. For instance, metals are often added in the form of a soluble salt, but depending on treatment conditions, they end up as an unresolved mix of ionic metal, hydroxides, carbonates, oxides and organic complexes. It is highly questionable whether the responses of organisms exposed to a mix of metal forms in nature are comparable with responses when the organisms are exposed to an unknown mix of metal forms resulting from experimental expediencies.

Similar problems pertain to organic chemicals. Monitoring data usually measure total concentrations for each xenobiotic. This total is usually a mix of dissolved, adsorbed and bioconcentrated chemicals. During experimental testing, such chemicals are commonly dispersed into the test chamber in solvent and surfactant vehicles. The final solution contains a mixture of dissolved and micellar xenobiotics. The biological availability of the xenobiotics in the test chamber is again likely to be very different from that in nature.

One dilemma is that our present analytical techniques cannot determine various chemical and physical forms at very low concentrations. However, the state of the art of analytical chemistry is much further advanced than present routine environmental analyses would lead one to suspect. In this respect, regulatory requirements have stifled the introduction of state-of-the-art techniques.

The majority of laboratory tests employ single species. Such tests fail to explore interactions between species, such as symbiosis, parasitism and predation. A number of experiments have been conducted on species interactions, usually simple two-species interactions. Such studies have a long way to go before they can be verified in the field. Since each species normally is expected to interact with many other species, multi-species tests might be more applicable as an intermediate step of experimental complexity. A research technique that seeks to bridge the gap between single-species tests and the environment as a whole employs microcosms and mesocosms. Problems still unresolved in these systems include scaling and species selection for tests with specific purposes.

As problems are resolved there should be a convergence between the results of prospective and restrospective assessments. In summary, the validity of such assessments will strongly depend upon a proper understanding of (1) the environmental dynamics of the pollutants, (2) appropriate chemical analyses, (3) knowledge of ecosystem structure and function, (4) knowledge of the responses of individuals, populations and ecosystems to specific dose rates for specific durations, (5) knowledge of interactions of chemicals acting on biota at various levels of organization. Not all of these

problem areas seem to be susceptible to early solution, and for some our understanding is pathetically poor. But I believe it is possible to move towards a more holistic analysis of our present pollution problems in the marine environment and elsewhere, although we seem to be missing opportunities to do so. On the way towards that goal we may even find that our early attempts to reconcile predictive and retrospective assessments of pollution impacts will increase our understanding of ecosystem functions that currently escape our vision.

Variations and Long-Term Changes in Narragansett Bay, A Phytoplankton-Based Coastal Marine Ecosystem: Relevance to Field Monitoring for Pollution Assessment

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INTRODUCTION

Natural variation is the essential baseline against which any effects of man-induced changes in the ocean are to be detected, measured and predicted. A major problem in monitoring anthropogenic effects on life in the sea, however, is separation of these effects from those induced by natural climatic and hydrographic changes in the environment. Fishery biologists discovered this long ago. Natural variability, fluctuations and change are probably the least understood of the major characteristics of marine ecosystems, notwithstanding numerous descriptive and process-oriented studies and, more recently, mechanistic numerical modeling of the structure and functioning of marine ecosystems. Unfortunately, as Longhurst et al. (1972) have reported, natural fluctuations in certain animal stocks have already been attributed incorrectly to the effects of pollutants. Presumably similar errors have been made with regard to other components of the food web and environmental properties.

The natural variations which characterize plankton communities in terms of species composition, abundance, dynamics and trophic structure largely have been ignored by pollution analysts. This is so partly because the widely scattered literature (not reviewed here) indicative of such variation is usually not applicable to pollu-

tion assessment, either because the data time series was too short (the normal time series duration is usually one year or less) or relevant variables were not measured. For example, plankton community structure is often analyzed without attention to nutrients. Monitoring, therefore, usually cannot separate anthropogenic effects from those due to natural climatic and hydrographic variability.

Some insight into long-term variability is available from an ongoing 23-year process-oriented time series (1959-1981), based on weekly sampling, for the unpolluted waters of lower Narragansett Bay, Rhode Island. This data set (probably the most extensive continuous record of plankton dynamics in the coastal waters of the United States) illustrates that significant short-term and long-term variations and changes characterize phytoplankton abundance, species composition, seasonal cycles and primary production and, based on less extensive data, nutrients and zooplankton abundance. A synopsis of the pertinent results from this data set is presented here.

METHODS

Narragansett Bay (41°30' N, 71°20' W), a well-mixed, shallow estuary 451 km^2 and 9 m in mean depth, extends inland approximately 40 km from its connection with Rhode Island and Block Island Sounds. It is a phytoplankton-based ecosystem. Quantitative weekly samples were collected at 0, 4 and 8 m depths at a permanent station located (41°34'07" N, 71°23'31" W) in the lower Bay near its entrance, where the mean depth is 8 m. Weekly measurements included temperature, salinity, phytoplankton species composition and abundance as cell numbers, chlorophyll, ATP-carbon, phosphate, ammonia, nitrate, silicate, water column extinction coefficient, incident radiation, and zooplankton species composition and abundance as dry weight. Some of these observations are summarized here. Also measured, but not discussed here, were zooplankton abundance as carbon and nitrogen, ctenophore abundance and dry weight, phytoplankton primary production, phytoplankton carbon growth rate and generation time (d^{-1}), turnover of the nitrogen and phosphorus nutrient pools, nitrate reductase and alkaline phosphatase.

RESULTS

Climatologic Changes

Significant long-term climatological changes have occurred over the 23-year time series since 1959, with measurable effects on river runoff and in situ conditions of temperature and irradi-

ance, factors which influence plankton dynamics. Figures 1 and 2 show the progressive decrease in annual mean wind speed and incident light intensity. Note the significant decline in annual mean incident light (based on daily measurement). The years since 1973 can be characterized as the "dark" decade. This long-term pattern of decreasing irradiance available for photosynthesis is also evident in the annual mean in situ irradiance levels calculated from the incident irradiance and water column extinction coefficient (Figure 3). Note the considerable year-to-year (i.e., short term) variations within the long-term pattern. This is a general characteristic of the data set. Temperature shows the opposite trend (Figure 4). A significant warming period began in 1969, but note the relatively cool period during 1976-1978. Precipitation and river runoff have also varied, elevated river flow generally characterizing the years since 1972.

Clearly, monitoring programs must sort out short-term and long-term variations in both community dynamics and environmental conditions caused by environmental properties (i.e., mixing, light, temperature and runoff) which are naturally variable and under climatologic influence. These can exhibit considerable interannual variations and significant long-term trends. Moreover, statistically significant correlations were found between annual changes in phytoplankton abundance and annual temperature and light conditions. That is, the phytoplankton are indeed responsive to climatologic variability in these two key growth factors.

Nutrients

Nutrient data, although less extensive, also exhibited significant variations and trends. Annual mean phosphate levels exhibited significant year-to-year changes with an interesting pattern (Figure 5). A "phosphate-rich" year was followed usually by a "phosphate-poor" year. For example, the mean annual phosphate concentration of about 15 mg-at m^{-2} in 1971 decreased to 7 mg-at m^{-2} in 1973, then increased the following year. A similar pattern is evident for nitrate, although the data suggest that nitrate levels have increased significantly since the early 1960s (Figure 6). While variations in nutrient levels are also under direct climatologic regulation through precipitation and runoff, nutrient levels obviously are influenced significantly by the utilization and recycling mechanisms characteristic of marine ecosystems. That is, their levels and oscillations are under the dual regulation of climate and trophodynamics. This characteristic poses a particularly great problem to monitoring programs which attempt to sort out the eutrophic links and signals from the naturally variable ones.

Phytoplankton

Figure 7 shows the significant year-to-year variations (about threefold) in mean annual diatom abundance in lower Narragansett

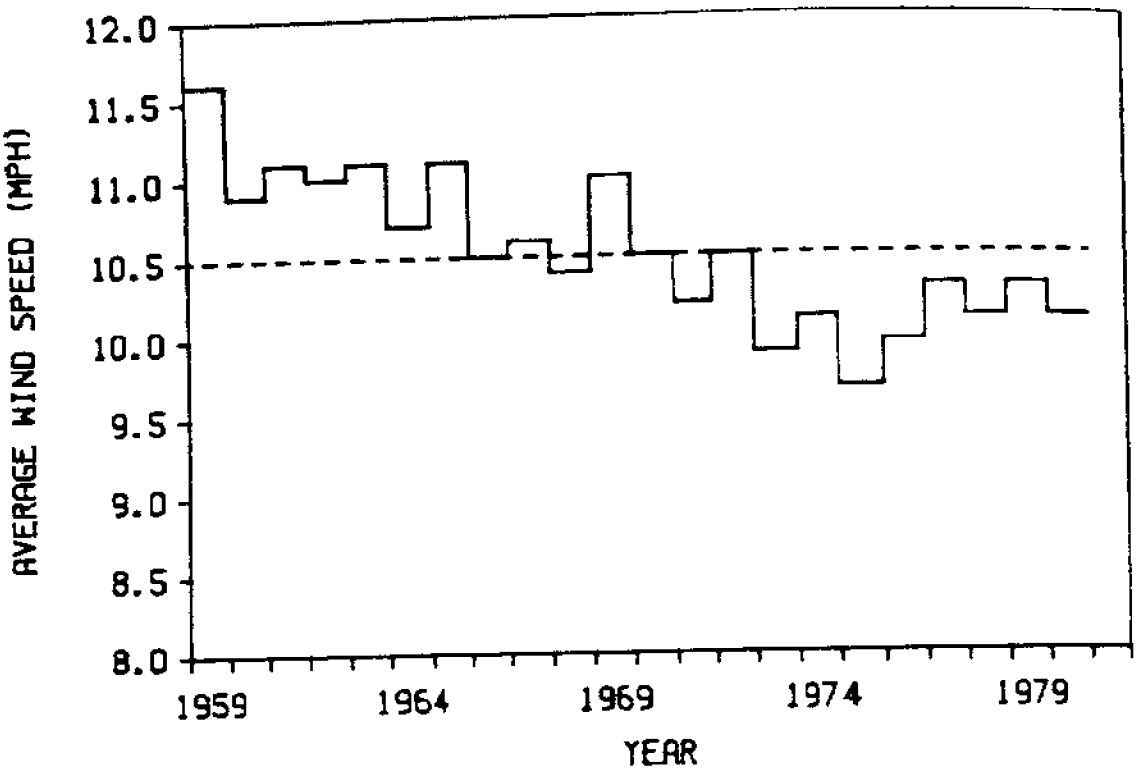

Figure 1. Annual mean wind speeds registered at Theodore Francis Green Airport, Providence, R.I. Dashed line represents mean wind speed for 1959-1980.

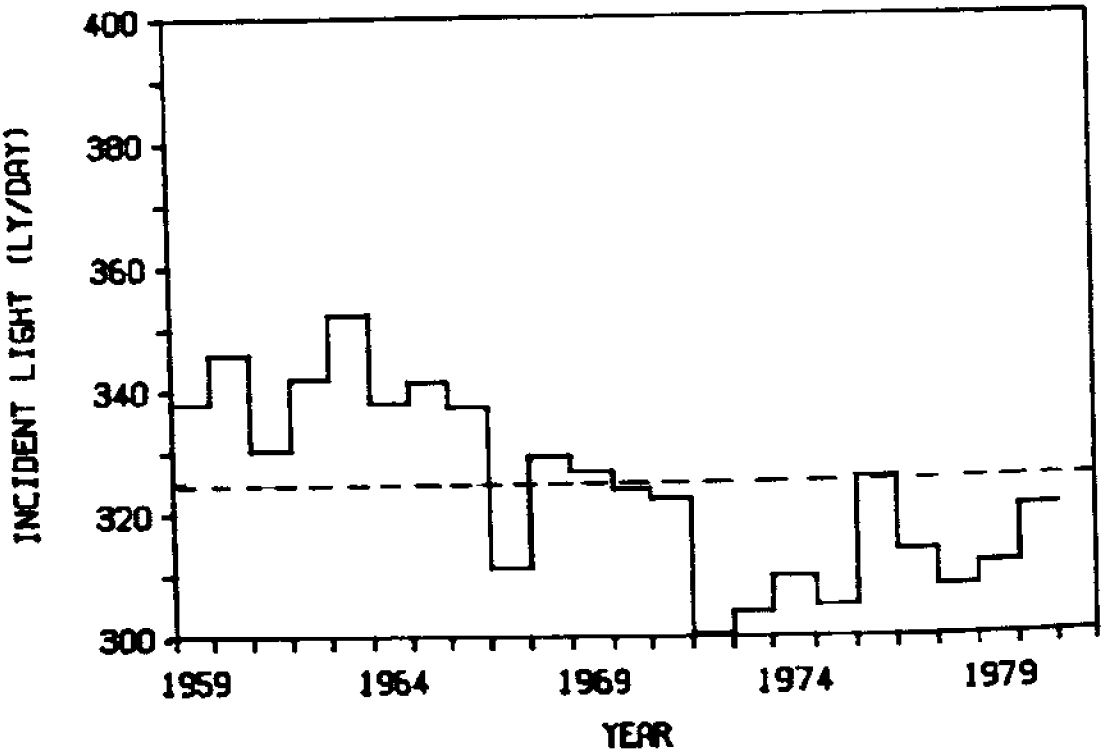

Figure 2. Annual mean incident light intensity measured at Newport, R.I. Dashed line represents 21-year mean.

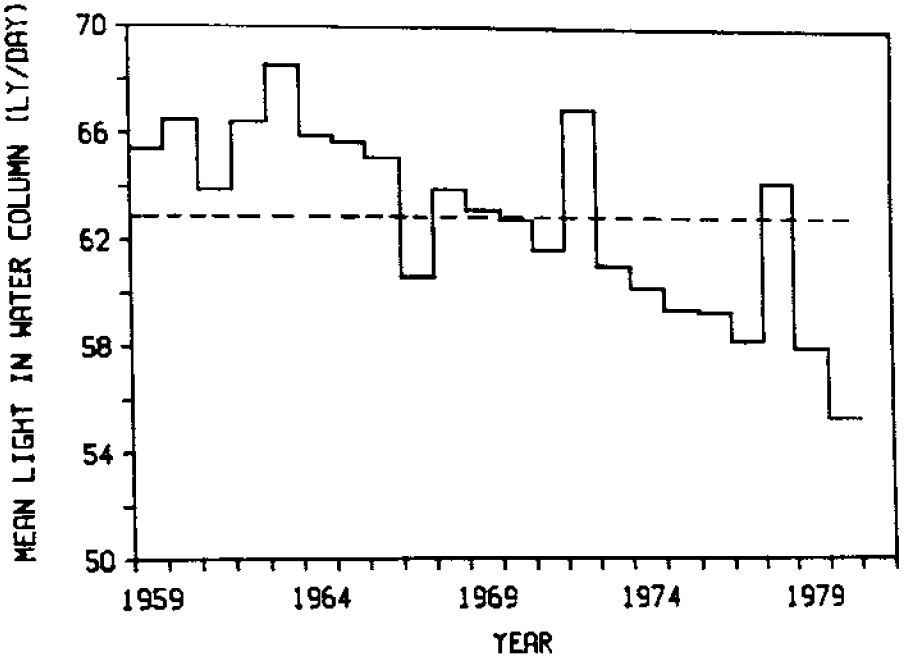

Figure 3. Annual mean in situ irradiance as ly day $^{-1}$ in lower Narragansett Bay calculated from

$$I = \frac{Io}{kz}(1 - e^{-kz})$$

where Io is incident irradiance, k the extinction coefficient (m^{-2}), z the depth (8 m) of the mixed layer which extends to the bottom. Dashed line is 21-year mean.

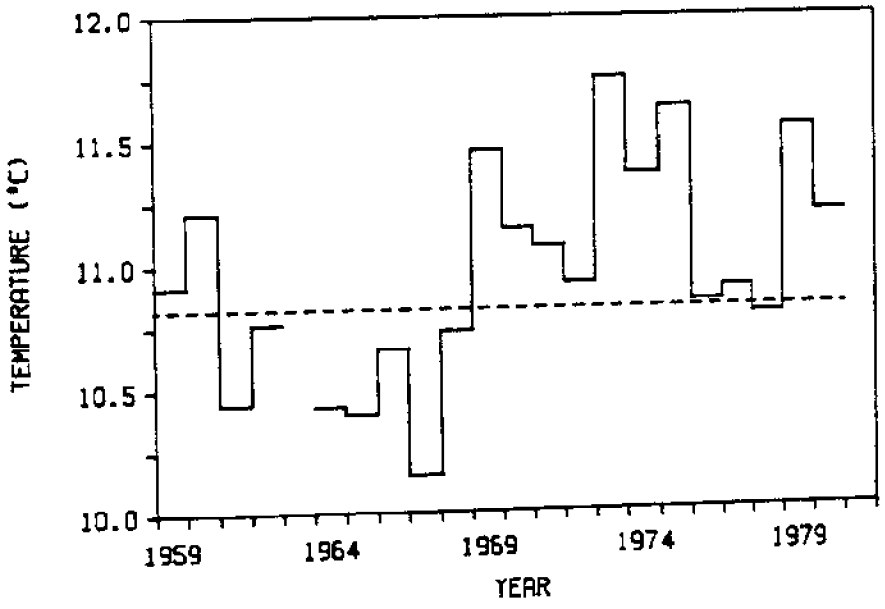

Figure 4. Annual mean sea-surface temperature in Narragansett Bay. Dashed line is 21-year mean.

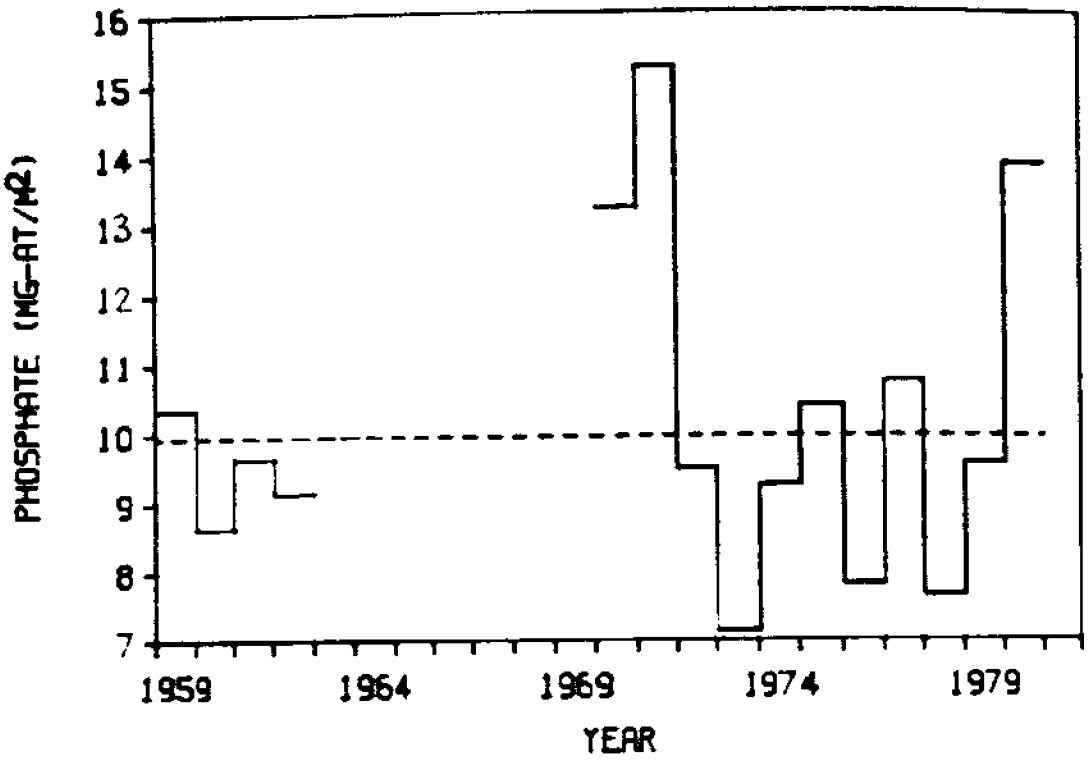

Figure 5. Annual mean phosphate concentrations in the water column of lower Narragansett Bay. Dashed line is mean for all years.

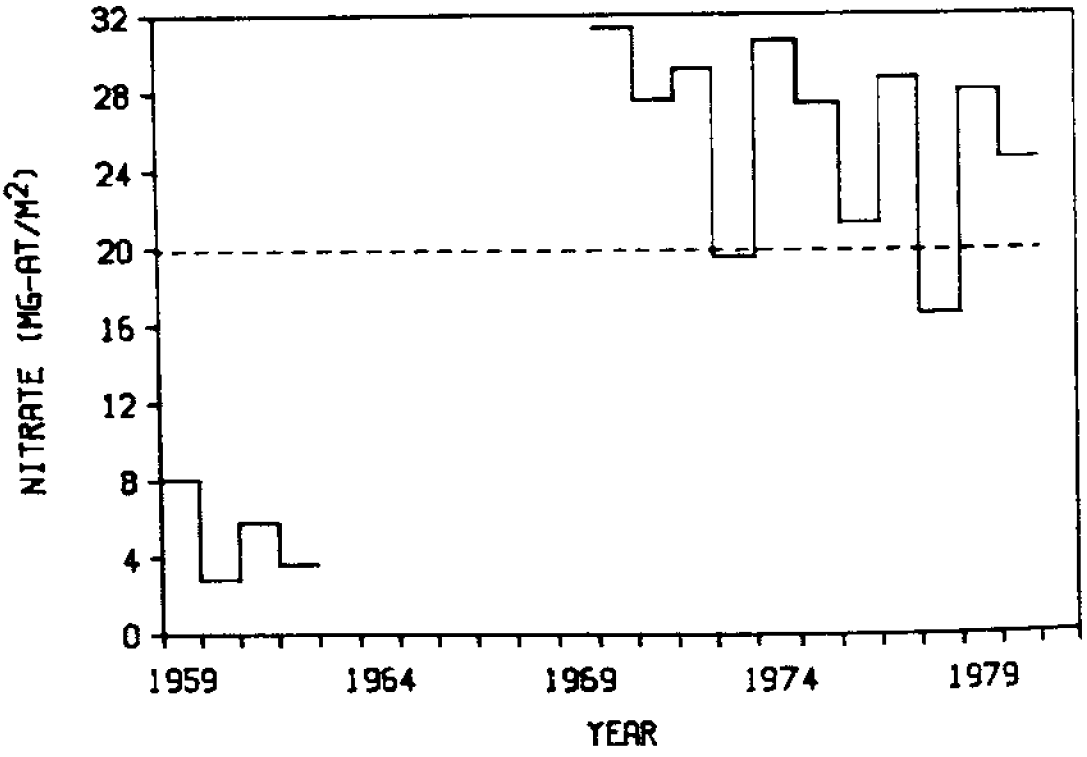

Figure 6. Annual mean nitrate concentrations in the water column of lower Narragansett Bay. Dashed line is mean for all years.

Bay. A clear long-term trend is not evident, but, as pointed out, these fluctuations and annual mean temperature levels are related statistically.

The interannual variations and annual successions in the occurrence and abundance of phytoplankton species have been subjected to Principal Components Analysis and Stepwise Discriminant Analysis (SDA). Multivariate analyses were also applied to the light, temperature and nutrient data to establish trends in these environmental variables which might relate to successional patterns, temporal variability and change in phytoplankton. These multivariate statistical analyses of the occurrence patterns of the phytoplankton species provide a numerical approach towards the identification of species groupings and their relationships to each other and to environmental conditions, and facilitate evaluation of the extent to which the taxonomic structure of the phytoplankton assemblages may have changed in Narragansett Bay during the 23-year time series. Approximately 1000 sample dates involving 49 significant phytoplankton species (using log-transformed cell counts) have been analyzed in 37 different treatments, two of which are shown.

Figure 8 illustrates the mean SDA values for each year between 1959 and 1980. The position of these mean values is a relative measure of the similarity of species occurrence patterns within each yearly group. The proximity of the points representing 1959-1968 indicates a high degree of repetition in species composition and succession during these years. Note that 1969 is distinctly separate from this cluster, and marks the beginning of a different phytoplankton pattern in the early 1970s, which continued to evolve in 1980. Notwithstanding this trend, a sequence of 5-year cycles prior to 1975 is evident. The years 1959-1963, 1964-1968 and 1970-1974 represent interannual periods of greater phytoplankton similarity, each period characterized by a similar 5-year pattern of variability.

A similar analysis based on the five most abundant species gives a very different pattern, however (Figure 9). A distinct separation between the 1960s and 1970s is not evident, nor the sequence of the three 5-year cycles prior to 1975. This indicates that the interannual differences in yearly phytoplankton successional sequence patterns in Narragansett Bay are primarily due to yearly differences in the frequency and magnitude of occurrence of the less abundant species of the 49 species in Figure 8. This result is consistent with other field and experimental observations that the major phytoplankton species simply become more abundant upon exposure to eutrophication. Hence, the search for marine ecosystem phytoplankton equivalents of the "miner's canary" as indicators of incipient or more advanced stress, prior to permanent ecosystem change, has not been a very productive approach. However, this finding that most of the annual variability in phytoplankton assemblage composition and response in Narragansett Bay was due to

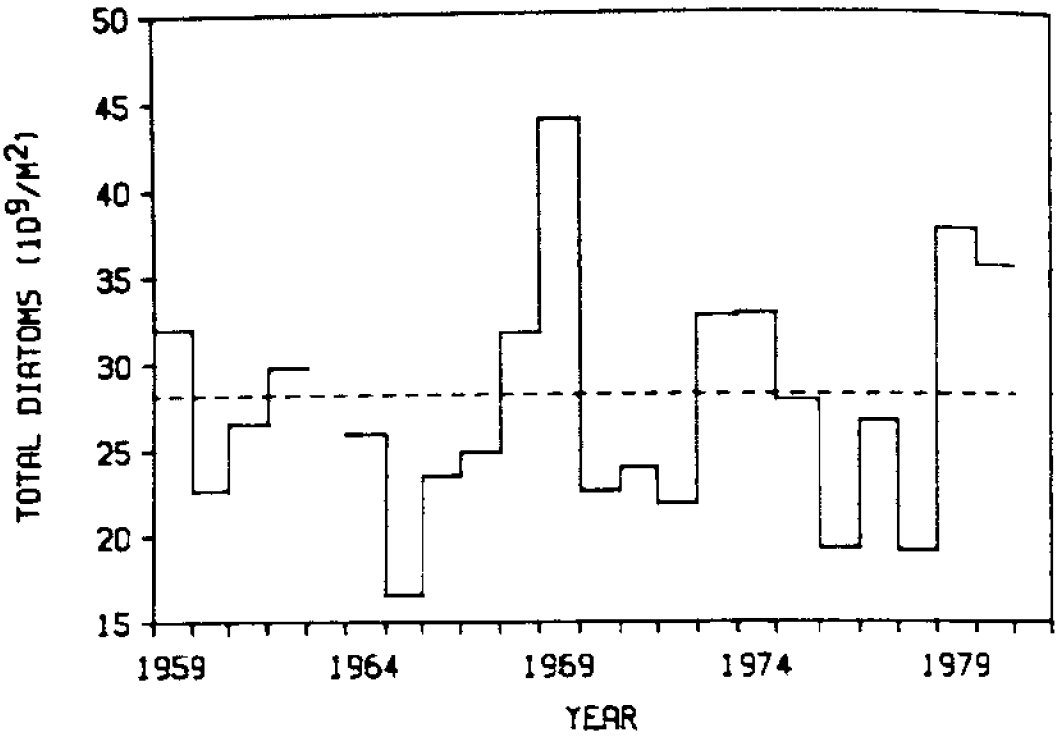

Figure 7. Annual mean diatom abundance as 10^9 cells m^{-2} in lower Narragansett Bay. Dashed line is 21-year mean.

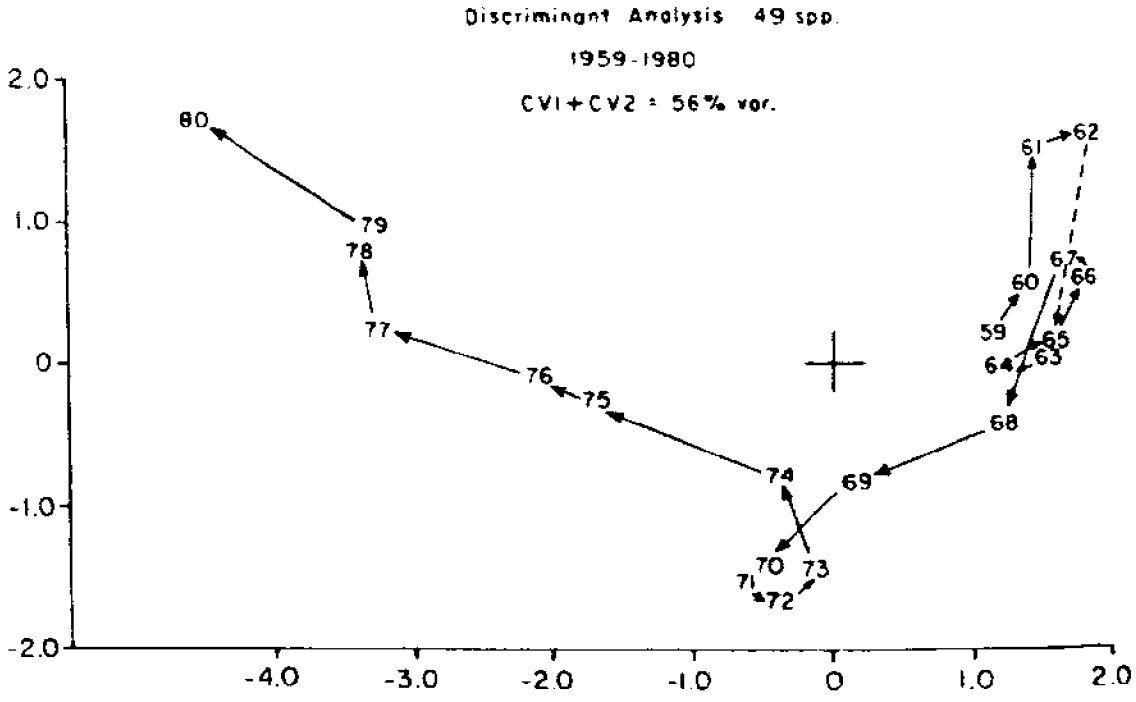

Figure 8. Stepwise discriminant analysis of annual mean abundance of 49 species of phytoplankton in lower Narragansett Bay from 1959 through 1980. Ordinate and abscissa represent canonical variables CV1 and CV2, respectively.

variations of the less abundant species requires assessment of the extent to which it reflects episodic species-introductions or variable responses of the resident community. Such assessment would be preliminary to more detailed evaluation of the applicability of the "indicator species" and "early warning community" concepts to marine pollution assessment. Should this variability primarily represent the response of the resident community, then in situ monitoring programs should take special care to assess the less abundant species, a group usually ignored.

Interannual variability also characterized the total zooplankton assemblage which exhibited a 9 to 10 year cycle and was due primarily to variations in the copepod component. The meroplanktonic component remained quite similar from year to year, however.

Thus, in addition to long-term changes in plankton and selected environmental variables characteristic of the Narragansett Bay time series, both the phytoplankton and zooplankton communities exhibited cycles in their structure and dynamics. Phytoplankton assemblages appear to cycle in 5-year units and zooplankton in 10-year units. That is, the annually varying phytoplankton assemblages and their dynamics are generally more similar to each other during a given 5-year cycle than to those in other 5-year cycles. The associated characteristic of this cyclical behavior is a precipitous annual shift into a new cycle, a phenomenon most notably exhibited by the 1969 year-class (Figure 8). Such cyclical variability during long-term environmental change complicates significantly the evaluation of short-term (= ephemeral) and long-term environmental effects of marine pollution.

Environmental Correlations

Theoretically, measurement of the relative importance of environmental parameters in regulating phytoplankton succession and community change can be evaluated from the correlations occurring between a successional rate parameter and the rates of change of various environmental factors (Smayda 1980). We have applied Lewis' (1978) summed difference index (σ_s) of succession rate, defined as

$$\sigma_s = \Sigma_i \mid d(b_i(t)/B(t)) \mid dt$$

which is estimated over the short time interval as

$$\sigma s = \Sigma_i \frac{\left|(b_i(t_1) / B(t_1)) - (b_i(t_2) / B(t_2))\right|}{t_2 - t_1}$$

where $b_i(t)$ is the abundance of the ith species at time t, and B(t) is the size of the community at time t. This index is insensitive to changes in community size unless there are accompanying changes in relative abundance of the species. A value of zero obtains for a

shrinking or expanding community, if all species are changing at an identical rate. The entire Narragansett Bay phytoplankton time series of weekly observations from 1959 through 1980 was analyzed.

Correlation of the 22-year time series succession rate indices with environmental variables revealed the following. The succession rate index was positively and significantly correlated with light and temperature in the 1960s, but not with nitrate and silicate concentrations. However, in the 1970s σ_s was positively and significantly correlated with nitrate and silicate concentrations, but not with light and temperature. σ_s was also positively and significantly correlated with phosphate concentrations during the entire time series. These correlations reveal that the apparent relative importance of an environmental variable, or combination of regulatory variables, in the regulation of phytoplankton can change over time.

Of the other variables, σ_s was positively correlated only with the rate of change in zooplankton biomass per unit time: $|\Delta \text{ dry weight}/\Delta t|$. It was not correlated with the abundance of total zooplankton, total copepods, Acartia hudsonica and Acartia tonsa (the dominant copepods), and ctenophores (Mnemiopsis leidyi), nor with their rates of change with time. Neither was the succession rate index correlated with phytoplankton production rates expressed in various ways, unlike that found for a tropical lake (Lewis 1978).

Multivariate statistical analyses clearly showed that the shift in yearly patterns of light and temperature from the 1960s to the 1970s (Figures 2-4) was primarily manifested during the winter months, and that winter temperatures were more repetitive during the 1960s than during the 1970s. Independently, the succession rate index, σ_s, correlated positively and significantly with light and temperature during the 1960s, but not during the 1970s. Multivariate statistical analyses also established that variations in nitrate concentrations were particularly pronounced, and exhibited distinctly different behavior between the 1960s and 1970s. Silicate, temperature, phosphate and light, in that order, followed in the degree to which they exhibited interannual variability. Interestingly, σ_s was significantly and positively correlated with nitrate and silicate levels during the 1970s but not during the 1960s. The fact that σ_s always correlated with phosphate concentrations, a nutrient rarely limiting to phytoplankton growth in Narragansett Bay, based on experimental evidence (Smayda 1974; Hitchcock and Smayda 1977), is notable and poses a problem: it suggests that σ_s correlates with nonlimiting variables. If this is correct, then the appropriate interpretation of the results is the converse, namely, that nutrients, and not temperature or light, were limiting (more often) in the 1960s, and that in the 1970s nutrients were no longer limiting and, thus, light and temperature were. Monitoring pro-

grams without benefit of experimental data are no less vulnerable to such potential misidentification of cause-and-effect combinations.

Nonetheless, the significant finding is that long-term variation and changes in phytoplankton assemblages are evident upon statistical analysis, and that two independent analyses suggest that these were accompanied by long-term changes in light, temperature and nutrients. Although statistical correlations were found, these are presumed to reflect parallel trends rather than cause-and-effect features. The data have also been subjected to rigorous statistical analysis, correlating between the variables when expressed in terms of their annual, quarterly and monthly means, and the weekly levels. These analyses confirmed that the relative importance of a given variable in regulating plankton dynamics varies considerably between seasons and years. Thus, plankton dynamics are under multivariate environmental regulation. While the growth factors remain the same, their importance and factor-interaction effects in regulating in situ growth are variable. This compromises in situ monitoring programs, which usually cannot evaluate factor interactions and which usually focus on a limited number of variables because of the incorrect assumption that they exert an unvarying and similar regulatory role.

Phytoplankton-Zooplankton-Phosphate Interactions

It has been emphasized that lower Narragansett Bay appears to be an unstressed embayment characterized by significant natural variability in its plankton dynamics and environmental properties. We have some data relevant to this and, especially, to the problems of eutrophication, assimilative capacity and in situ biological monitoring. Between 1973 and 1979, the annual mean phytoplankton and zooplankton biomass levels progressively increased. The relationship between these annual mean standing stocks between 1973 and 1981 is especially provocative (Figure 10). There is a strong, highly significant ($r = 0.62$) direct relationship between annual mean zooplankton (predator) and phytoplankton (prey) abundance, which follows the classic yield-dose response. Moreover, it suggests that there is an annual mean carrying capacity for zooplankton in lower Narragansett Bay of about 1.0 g dry weight m^{-2} and that annual mean phytoplankton standing stocks exceeding about $\geq$ 3.5 g C m^{-2} represent surplus production not accompanied by increased zooplankton biomass.

These variations and build-up in annual mean phytoplankton biomass as carbon were not correlated with temperature, light intensity, ammonia, nitrate or silicate, but correlated positively with annual phosphate level (Figure 11). (The markedly aberrant datum in Figure 11 represents 1979; excluding this point yields a highly significant correlation coefficient of $r = 0.67$; it would be 0.97 if the 1978 datum were also dropped.) Given this provocative and un-

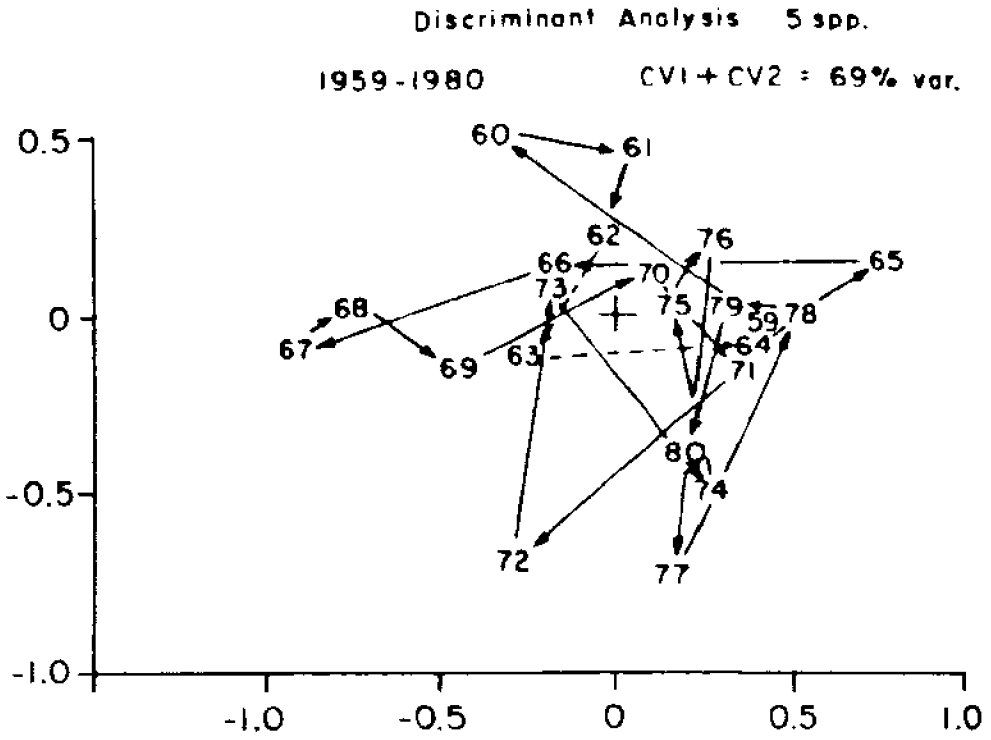

Figure 9. Stepwise discriminant analysis of annual mean abundance of five most dominant phytoplankton species in lower Narragansett Bay. See legend, Figure 8.

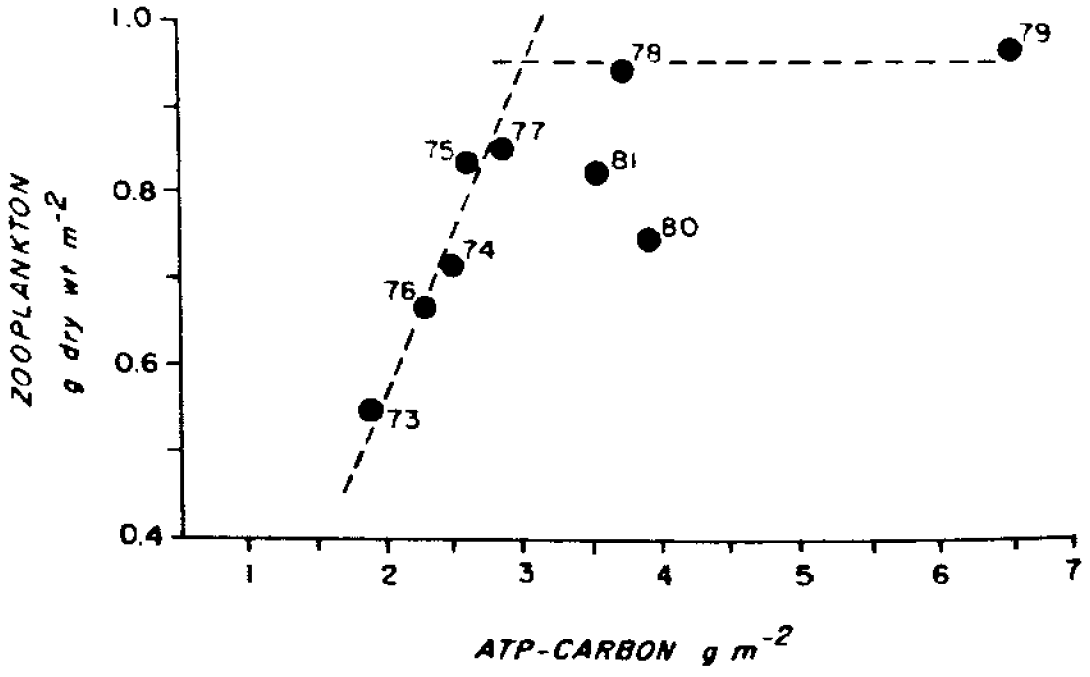

Figure 10. Relationship between annual mean standing stocks of zooplankton dry weight and phytoplankton carbon in lower Narragansett Bay for the years 1973-1981. Dashed line, drawn by eye, goes to origin.

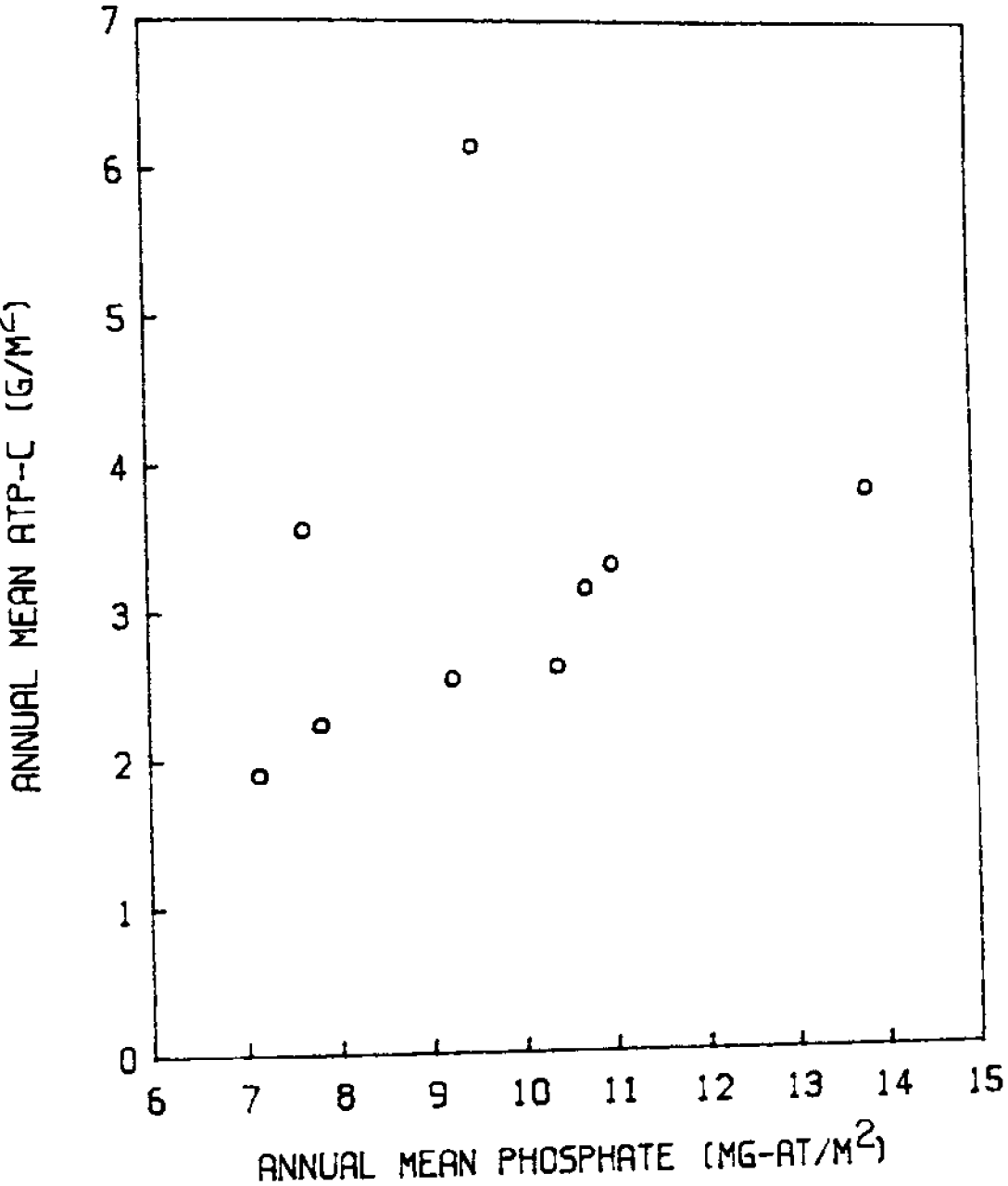

Figure 11. Relationship between annual mean phytoplankton carbon and annual mean phosphate concentrations in lower Narragansett Bay for 1973-1981. Correlation coefficient r = 0.67 (highly significant) excluding aberrant 1979 datum.

expected correlation indicating that annual variations in phosphate input or recycling regulated annual mean phytoplankton and, thereby, zooplankton biomass levels, the potential sources of phosphorus were evaluated. The annual mean phosphate levels were inversely related (statistically significant) to the volume of inflow from the Blackstone River, which accounts for about 50% of the fluvial discharge into Narragansett Bay. (The other nutrients were not correlated with river flow.) Despite this correlation, there was no correlation with the flux of phosphate in this flow, nor was water column phosphate correlated with the benthic flux of phosphate, either solely or when combined with the river flux. This suggested that since the annual mean phosphate levels represent only the residual remaining after uptake, the rates of phosphorus recycling and amounts found in the plankton needed to be considered.

These analyses revealed that the annual mean phosphate concentrations present in the phytoplankton + zooplankton + water column have progressively increased since 1973. That is, a phosphorus build-up has been occurring in lower Narragansett Bay without any evidence for an increase in dissolved phosphate levels (Figure 5). A positive statistically significant correlation ($r = 0.65$) occurs between the annual river flux of phosphorus, from which the annual mean dissolved levels were subtracted (i.e., it represents unused phosphorus), and phytoplankton biomass converted to phosphorus on the basis of the ratio between phosphorus and chlorophyll. Moreover, a highly positive and statistically significant correlation ($r = 0.68$) occurs between the annual mean phosphorus flux from rivers and benthic processes and the annual mean phytoplankton + zooplankton biomass converted to phosphorus.

These correlations implicate riverine influxes of phosphorus as a factor contributing to the apparent increased buildup of plankton biomass in Narragansett Bay over the last decade. However, as emphasized earlier, climatological trends during this period have resulted in lower in situ light levels (Figure 3) and higher temperature (Figure 4). Statistically, temperature and diatom abundance are positively correlated, while in situ irradiance and total phytoplankton abundance are negatively correlated. It must also be emphasized that there is no nutrient evidence for an increasing eutrophication of lower Narragansett Bay, yet increased biomass requires increased nutrient availability, and riverine inputs are implicated. And so we are left with a dilemma. Is there a progressive eutrophication occurring in lower Narragansett Bay which is not detectable through nutrient assessment? Alternatively, have the observed long-term climatologic trends, with which phytoplankton abundance is correlated, simply altered the rates of nutrient recycling and other processes significant to plankton dynamics? If the latter, then the observed annual variations and long-term increase in plankton abundance might simply reflect the natural range of eutrophication (fertility) characteristic of lower Narragansett Bay.

CONCLUSIONS

Variability is an intrinsic characteristic of climate, hydrography and plankton communities. Its documentation here for Narragansett Bay illustrates, therefore, a general property of marine ecosystems and environments rather than a unique situation. The results revealed correlations between climatological variations and plankton processes, and they revealed that the correlative factors were operational on a time scale of a year, 5 years and even 10 years. Moreover, the relative importance of growth factors varied with time. The results even suggested that there may be an ongoing eutrophication process in lower Narragansett Bay hidden within the patterns of normal variability, modified by climatologic trends, and without any obvious signals of the kind sought in monitoring programs.

It is well known among marine ecologists that plankton and environmental surveys are powerless to identify cause and effect; the latter can be identified only by experimentation. Surveys are, after all, mere descriptions of a phenomenon; and biological monitoring is a survey, nothing more. Monitoring as usually applied within a pollution assessment context serves only to give warning or documentation of apparent change, and can indicate neither the causes of such change, nor the relative importance of the various factors and trophic processes contributing to the change. It may be argued that the role of biological monitoring is truly descriptive--to document change. Upon evidence of change, however, a manager must decide whether the change is the result of a pollutant. This requires data interpretation which carries with it the need to sort out changes due to natural variability. But, as pointed out, biological monitoring never deals with the problem of natural variability; the time series surveys are too short. Thus, biological monitoring is inadequate in two key methodological procedures: the survey itself and quantitative procedures.

A process-oriented, in situ study involving rate measurements is needed to help establish causes and effects of oscillations in environmental variables and associated plankton dynamics. It follows, then, that in situ monitoring as traditionally carried out cannot meet its basic objectives; namely, to discover whether change is due to anthropogenic or natural causes, whether it will be only a temporary transient dysfunction or disruption, and to predict ensuing changes.

What revised scientific form should pollution assessment programs take to provide answers to such basic questions? First, one must go back to first principles and incorporate the approach of contemporary marine ecology. The surveying techniques must be quantitative and statistically sound, and the survey must be carried out on the relevant properties over a sufficient time period. Second, key processes must be measured by means of experiments us-

ing suitable experimental design and analytical equipment. Third, meso-microcosm experiments using entrained communities which can be manipulated are needed. In this procedure, the influence of selected perturbants on selected communities, or community components, including their influence on key rates (processes) and routes (transformations), is tracked under controlled conditions. This "hybrid" between the classical monitoring approach, in situ process-oriented studies, and meso-microcosm experiments, I am convinced, is not merely suitable for scientific evaluation of problems of pollution, it is essential. All three activities must complement each other, rather than be carried out piecemeal. An approach short of this represents a flawed conception of how marine systems work, and ignores the functional holism that exists between climate, habitat and biotic structure.

ACKNOWLEDGMENTS

Deborah French assisted with the data processing, developed computer programs and, because of my absence at sea, delivered my paper at the conference on Meaningful Measures of Marine Pollution Effects in April, 1982, at Pensacola Beach, Florida. Dr. Deneb Karentz and Ms. Ellen Deason carried out multivariate statistical analysis on the phytoplankton and zooplankton, respectively. Ms. Blanche Coyne typed the manuscript. This study was supported by National Science Foundation Grants 68-1500, 71-0056, OCE-76-22563, and Department of Commerce (NOAA) Grant No. NA80RA-D00064.

REFERENCES

Hitchcock, G.L. and T.J. Smayda. 1977. Bioassay of lower Narragansett Bay waters during the 1972-1973 winter-spring diatom bloom using the diatom Skeletonema costatum. Limnol. Oceanogr. 22: 132-139.

Lewis, W.M., Jr. 1978. Analysis of succession in a tropical phytoplankton and a new measure of succession rate. Amer. Nat. 112: 401-414.

Longhurst, A., M. Colebrook, J. Gulland, R. LeBrasseur, C. Lorenzen and P. Smith. 1972. The instability of ocean populations. New Sci. Vol. 54: 1-4.

Smayda, T.J. 1974. Bioassay of the growth potential of the surface water of lower Narragansett Bay over an annual cycle using the diatom Thalassiosira pseudonana (oceanic clone 13-1). Limnol. Oceanogr. 19: 889-901.

Smayda, T.J. 1980. Species succession. In: I. Morris (ed.), The Physiological Ecology of Phytoplankton, pp. 493-570. Blackwell Scientific Publ., Oxford, England.

Aquatic Field Monitoring and Meaningful Measures of Stress

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INTRODUCTION

One basic question underlies the effort to understand pollution-induced changes in aquatic systems: what is required to predict the environmental effects of a given toxic or stimulatory substance on a given ecosystem? The basic differences between field and laboratory approaches to this problem are well known and will not be reviewed in detail here. However, over the past two decades, toxicological methodology has evolved to a point where comparative toxicology is a well-established method for evaluating the acute and chronic effects of toxicants on laboratory populations. Unfortunately, field evaluations of such effects on aquatic populations and communities are not as well developed. The reasons for this lack of certainty are well known. Natural systems undergo extreme variation in space and time. A given population responds to multiple stimuli. Thus, the response to anthropogenic effects is superimposed over natural environmental variability in a manner that precludes generalizations from species to species and habitat to habitat. Natural variability is high in many estuarine and coastal areas, where the ecosystem is constantly in flux. The overall response of the system represents the sum total of countless interactions among various populations, each of which is characterized by its own peculiar needs and sensitivities to both natural and anthropogenic forms of stress. In short, field ecology has yet to achieve the level of reproducibility and predictability that has been attained through standardized toxicological techniques.

FIELD APPROACHES AND THE VERIFICATION PROCESS

A concise review of the problems of applying field results to the verification process is given in Figure 1. Field background variability (IA), which includes spatial and temporal components, is the single most important problem in the design of field experiments. Scaling problems (II) are associated with various aspects of variation. How does one take a representative field sample? Alternatively, how do laboratory artifacts limit the applicability of experimental results to field processes? These questions represent obverse sides of the same problem. The definition of the scope of field sampling that is necessary to answer a specific question is the essence of the scaling dilemma. Lack of an adequate definition of the scope of the research effort remains a stumbling block in that microhabitat distribution is difficult to reproduce in the laboratory and is often poorly understood in the field. Essentially, scaling should include those habitat characteristics and biological interactions that define a given environmental process. Depending on the species, the scaling process must consider spatial and temporal aspects of climatological phenomena, microhabitat conditions, water quality factors and specific biological interactions that are effective at the population (III) and community (IV) levels. Reproduction and recruitment of individual species will eventually be reflected at various levels of organization within the context of food web processes and patterns of energy distribution. This problem can be investigated in the field through experimentation under full field and semi-field conditions. However, there is still no consensus among field researchers concerning the relative importance of competition and predation to community structure (Peterson 1979).

Primary productivity, the source of energy for a given system, should be an important part of any community evaluation. Under laboratory conditions, processes such as primary and secondary production are usually difficult to simulate. Natural processes of predation, reproduction and recruitment are not usually a part of a given experimental procedure. Without simultaneous calibration of the laboratory system with background field data, the step between the bioassay and a field situation is indeed a long one. Extrapolation and verification of bioassay information should thus be qualified by natural history information and background (field data). The observational data can provide the basis for field experimentation and manipulation which, in turn, will allow an insight into the ecological processes involved in a given situation.

The problem of realistic simulation in laboratory tests rests on the identification of those aspects of the experimental system that most accurately represent the natural condition. Resolution of this problem can go only one way: from the field to the laboratory. Such an effort rests on baseline and monitoring programs that are not always easily linked to "effects" studies. These problems have

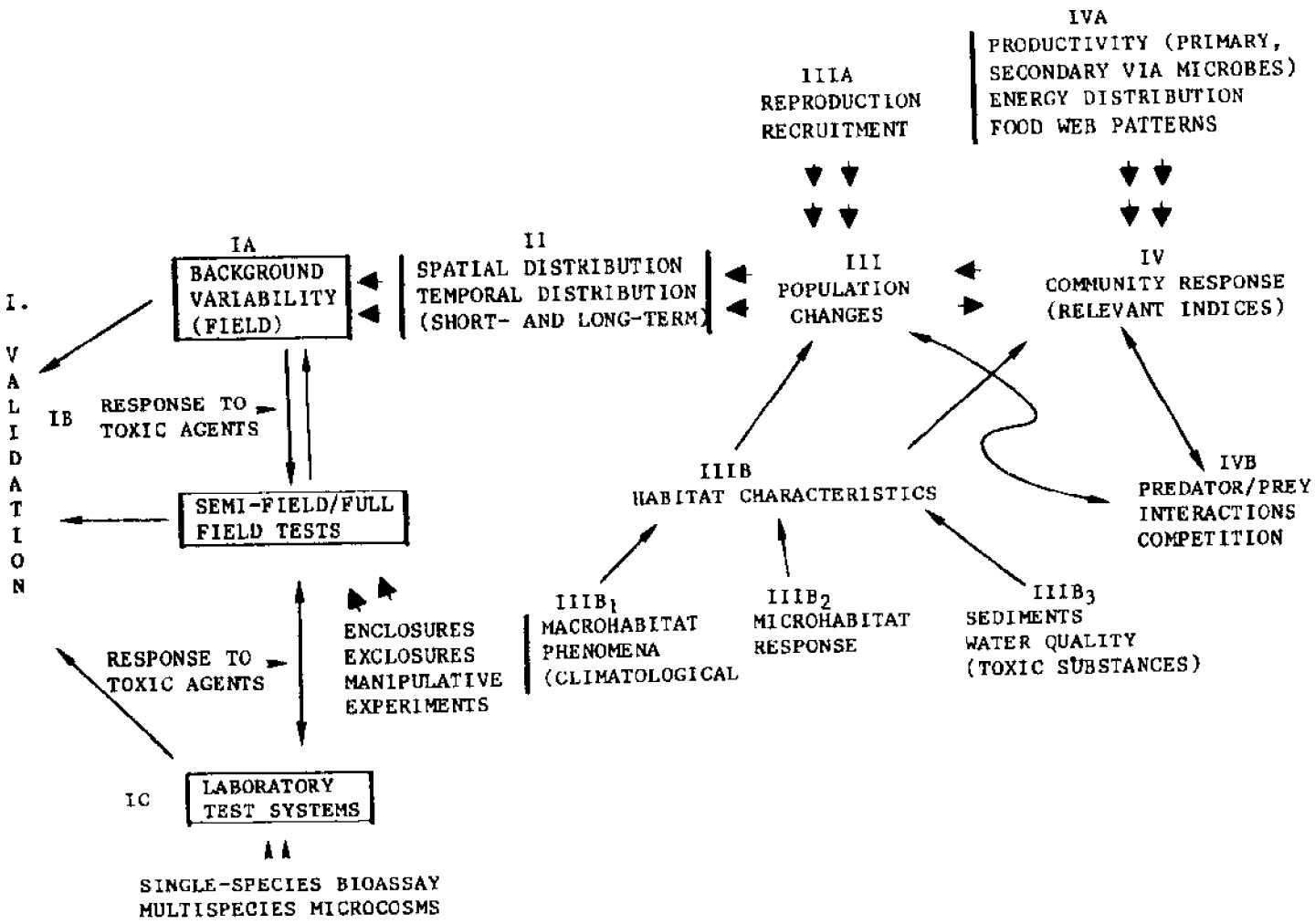

Figure 1. Laboratory/field approach to impact analysis.

been reviewed in a cooperative research report (ICES 1978) in which monitoring data are defined in the broadest possible sense. For example, there should be some attempt to define well-being in morphological, physiological and behavioral terms. A given bioassay test should be qualified by field data from areas with an identifiable potential of risk from pollution. Specific techniques in the laboratory should be field tested for the detection, measurement and evaluation of effects induced at actual environmental levels of field exposure. For example, in cases where there is a definable gradient of a given pollutant (e.g., heavy metal content of sediments), the field response of a given population or community can be defined, and such field results can be compared with bioassay results for the same pollutants. This approach can lead to field transplantation experiments and a coordinated field/laboratory approach whereby both research efforts contribute to an ultimate resolution of the question. In this case, no single field sampling approach is likely to be adequate; what is needed is a suite of procedures that involve various taxonomic and functional levels. Fate and effects monitoring should thus be used as complementary (not mutually exclusive) approaches to the bioassay procedure. Experimental approaches should ideally extend from laboratory bioassay (single species, multispecies) through semi-field testing (enclosures, exclosures, manipulative experiments) to full-field conditions.

CASE STUDY: NORTHEASTERN GULF OF MEXICO

My Florida State University research group has been involved in continuous studies of two bay systems (Figure 2) along the Florida Gulf Coast since 1971. A multidisciplinary approach (Figure 3) has been used to define research problems in a continuous process of hypothesis testing between the field and laboratory. Continuous field data have been taken since 1971-72 concerning the interaction of various physicochemical factors and leading biological components. The original research questions were related to the impact of kraft pulp mill effluents on grassbed areas in Apalachee Bay and the impact of pesticides and upland forestry operations on the Apalachicola estuary. These studies are not completed and have been expanded into a comprehensive analysis of the spatial and temporal variability of system function, population and community response to habitat gradients (temperature, salinity, pH, dissolved oxygen, pollutants, etc.), sources and direction of energy flow, trophic interrelationships and the influence of feeding habits of key populations on community structure. There have been associated efforts to develop an integrated computer system for analysis of extensive multidisciplinary data sets. In addition to various key physicochemical functions, the field monitoring data include

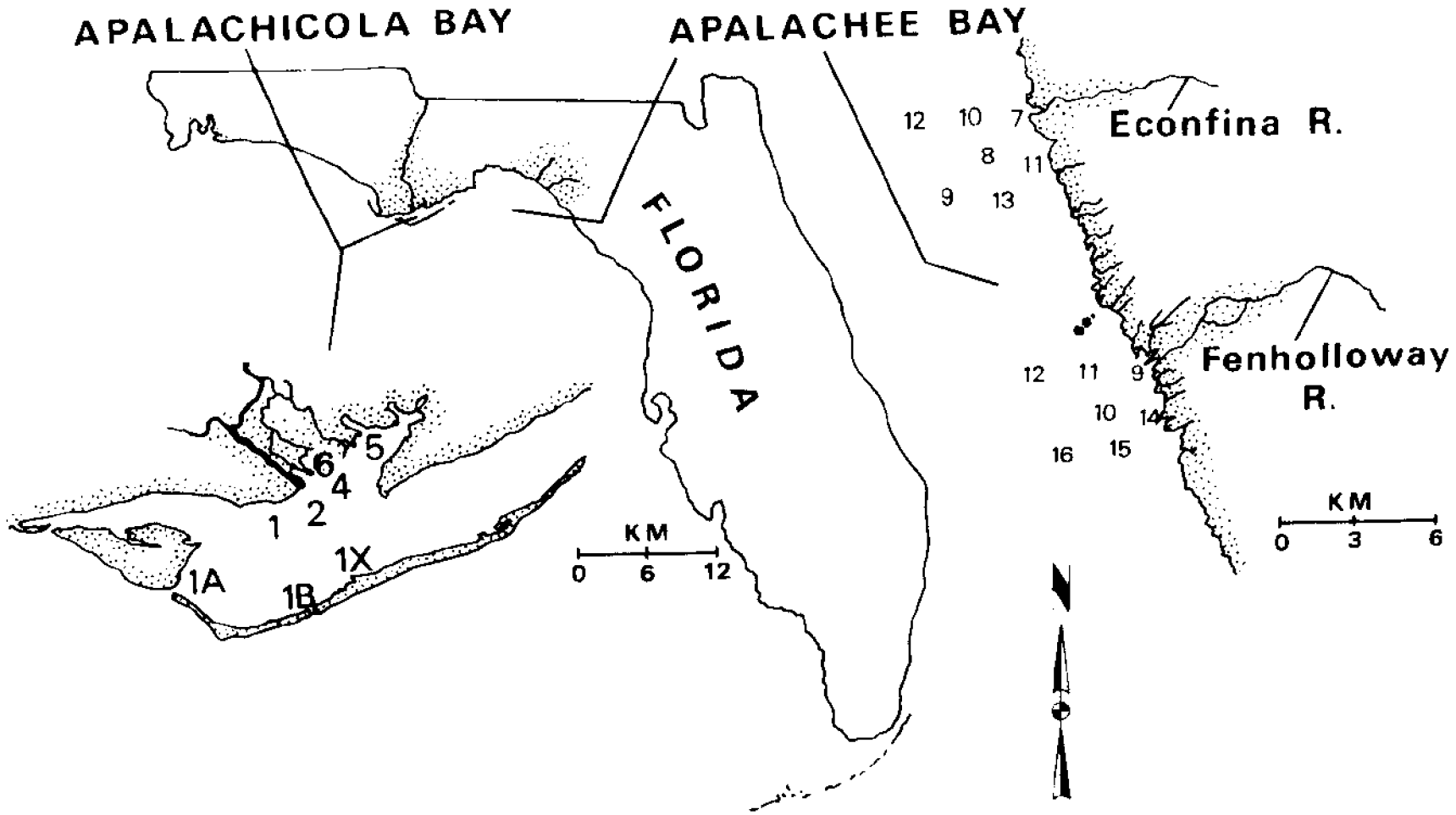

Figure 2. Area of study of the Florida State University Aquatic Studies Group. Numbers represent permanent sampling stations.

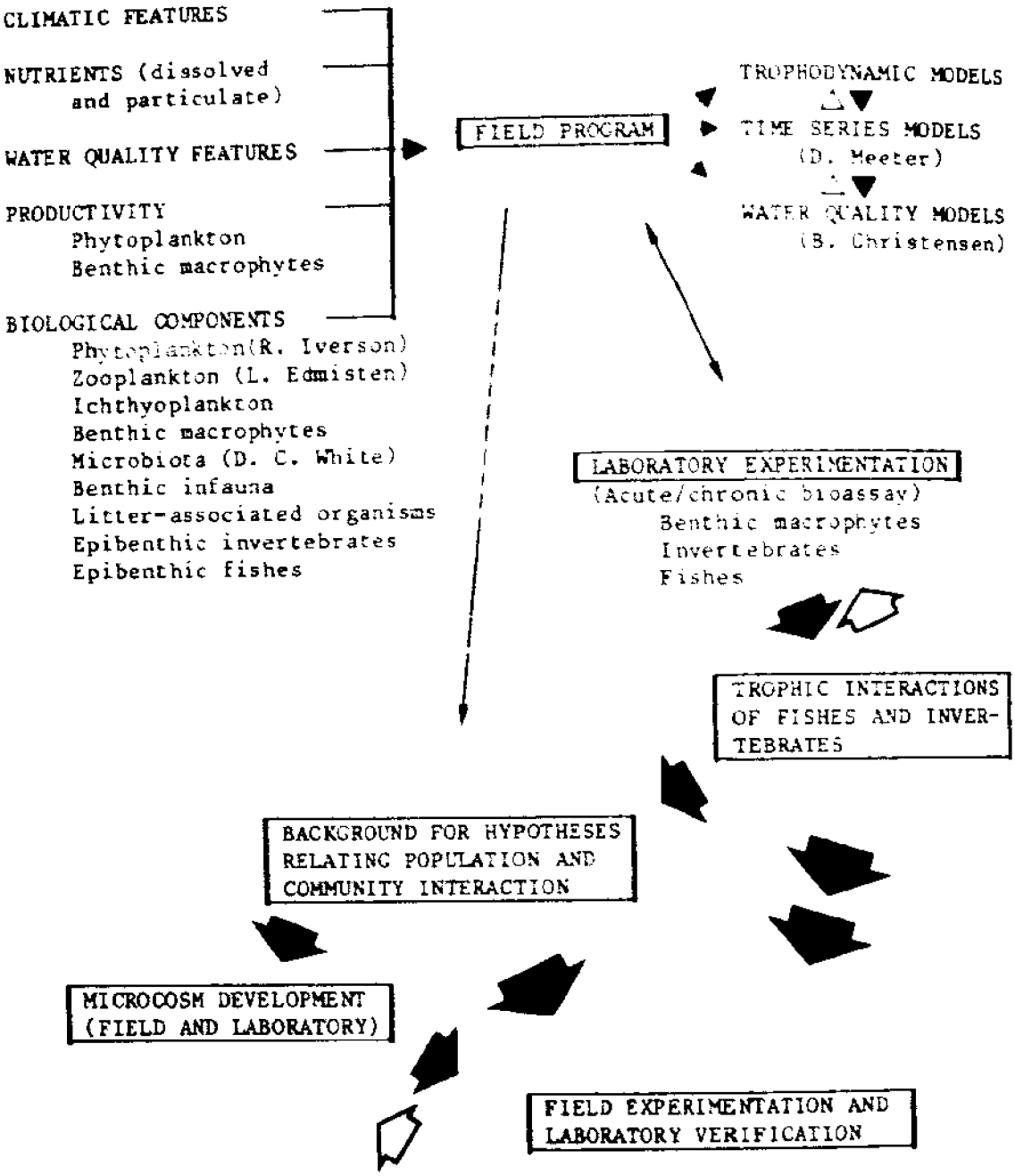

Figure 3. Comparison of a shallow macrophyte-dominated bay system (Apalachee Bay) with a river-estuary characterized by high phytoplankton productivity and input from allochthonous detritus (Apalachicola Bay).

detritus-associated organisms, benthic macrophytes (seagrass and algae), benthic infauna and benthic epifauna (fishes and invertebrates). Cooperative research with other investigators in the primary study areas includes analysis of microbiota, phytoplankton and zooplankton. The entire data base has been utilized to develop models of projected population/community distribution and phased biological response to key physical factors and predator-prey interactions. These data are currently being prepared for publication. Associated laboratory studies include plant and animal bioassays, behavioral studies and the development of multispecies microcosms. Such laboratory efforts are directed at specific questions related to findings in the field program.

The overall research program has been divided into several components: (1) Laboratory/analytical: Experiments have been conducted at the Florida State Marine Laboratory concerning the influence of color and kraft pulp mill effluents on the productivity of dominant grass species such as Thalassia testudinum. In cooperation with Dr. David C. White of the Department of Biological Science, microcosms have been developed for determination of energy flow in laboratory simulations of field-tested food web associations. Associated studies have been conducted concerning impact on ATP/ADP/AMP functions and activity patterns of estuarine fishes. Other analytical determinations have been made concerning dissolved and particulate contributions (carbon/nitrogen relationships) to the energy budgets and biological assemblages of the subject bay system. (2) Field: Continuous physicochemical and biological monitoring of coastal assemblages has been conducted to determine basic mechanisms of short- and long-term variation. A major effort is now under way to analyze long-term (7-year) changes of feeding habits of fishes in formerly polluted areas of Apalachee Bay and to relate such changes to the succession of recovering grassbeds which at one time were polluted. Such data are being compared with data from an unpolluted estuary. Ultimately, there will be a comparison of long-term changes in the two study areas relating the turbid, river-dominated Apalachicola estuary characterized by high phytoplankton productivity and rich allochthonous detritus to the adjacent Apalachee Bay, which is distinguished by extensive benthic macrophyte development. (3) Statistical/computational: Together with Dr. Duane A. Meeter of the FSU Statistics Department, we have analyzed over 50 years of data concerning river flow fluctuations, temperature and rainfall patterns to determine the importance of long-term cycles of meteorological phenomena to biological response in associated coastal systems. This analysis includes a uniform statistical analysis of the field results (time-series analysis) and the development of models of long-term biological variation. This effort will be integrated with engineering models (developed by Dr. Bendt Christensen of the University of Florida) concerning salinity distribution and

water quality parameters in the areas of study. Also associated with this work are statistical treatments of LANDSAT data relating changes in upland configurations (clearcutting and draining, related forestry operations) to observed changes in water quality and biological functions in receiving portions of the drainage system. The computerized analysis combines a comprehensive, interactive method for data manipulation with an extensive, multidimensional data base to answer questions concerning complex changes and biological variability in these two bay systems. A recently completed study of the impact of pulp mill effluents on three river systems in South Carolina, Mississippi and Alabama will be added to the comparative analysis.

Increasingly, this research effort has been directed toward trophodynamic interactions including organic carbon/nitrogen relationships, fluctuations of particulate organic matter (POM), nutrient distribution and the relationship of fluxes of organic matter in wetlands systems (U.S. Geological Survey) with estuarine biological organization. The energetics of physiological (ATP/ADP/ AMP) and behavioral responses of fishes have been studied. In addition, the trophic relationships of blue crabs (Callinectes sapidus) and various other crustaceans have been analyzed. Microhabitat influences on the key components of the benthic inverteberate fauna are being detailed, while assessment of the trophodynamic significance of prey availability to the feeding habits and distribution of coastal fishes is now in the final stages of completion. Day/ night feeding habits of fishes and activity of invertebrates have been analyzed. The emphasis on field experimentation and microhabitat diversity is complementary to the ongoing, long-term field work. Thus, after 11 years of careful collection of field and laboratory data, we are now using these data to design a new program with an emphasis on field experimentation and analysis of the validation process.

SOME OBSERVATIONS AND CONCLUSIONS

Our central theme involves delineation of "meaningful measures of marine pollution effects." The term "meaningful" is difficult to interpret without full qualification of underlying assumptions in the experimental process. Unless a given research question is scaled in an orderly way, controversy regarding generalization of results is almost inevitable. From our recent long-term effort, certain tentative observations and conclusions can be made concerning the development of a research effort to answer specific impact questions.

1. The bioassay approach can be extremely useful in an assessment of the boundaries of a given problem, but it is not necessarily predictive of field response. For example, Laughlin et al. (1978a)

found that blue crabs (Callinectes sapidus) in the laboratory showed a marked avoidance reaction to acidic runoff from clear-cut timber areas above a north Florida estuary. Such effects were also noted with test water characterized by experimentally reduced pH. An analysis of blue crab distribution within similar gradients in the field gave entirely divergent results. Indeed, small crabs were more abundant during periods of high runoff (and low pH) than at any other time during the long-term sampling program. While such results are obviously due to an extremely complex population response to various factors in the field, a direct extrapolation of the bioassay results to field conditions was not feasible because of "the limitations imposed by laboratory artifacts and unavoidable assumptions" (Laughlin et al. 1978b). Another demonstration of this problem is given by Koenig et al. (1976). Blue crabs were noted in a north Florida marsh area that was contaminated with DDT and its metabolities. Total DDT-R residues equaling 39.0 ppm and 1.43 ppm were noted in the hepatopancreas and swimmeret muscle tissue, respectively. Despite bioassay data showing that blue crabs are quite sensitive to DDT, no field mortality was noted during summer/fall months relative to other (uncontaminated) control areas. Only as winter cold fronts moved through the area was there a widespread kill noted among the DDT-exposed blue crab populations. Thus, exposure to DDT together with reduced water temperature produced mortality in the field, while direct extrapolation of the laboratory results was not applicable unless the environmental modifying factor (i.e., low temperature) was taken into account. Once again, field response to multiple factors cannot be predicted from laboratory bioassays alone.

An integrated field and laboratory approach can thus be very worthwhile. Recent experiments with soft-sediment microcosms indicated that, under laboratory conditions (i.e., without natural predators), such assemblages change in a way that is more compatible with changes in areas where predators have been excluded than with those in natural unvegetated, soft-sediment areas. Thus, the laboratory microcosm is an artifact of the separation of the subject organisms from natural conditions (i.e., predation). Field data can provide the basis for experimental questions and hypotheses that can then be tested in an organized fashion and under controlled conditions. Field manipulation experiments, based on life history information and descriptive data, can be useful for analysis of dynamic processes such as the influence of predation and competition on community structure. Habitat alteration can influence such relationships, leading to subtle yet important structural changes in aquatic assemblages. Such factors should be taken into consideration in the experimental design of laboratory bioassays.

2. The inherent spatial and temporal variability of a given aquatic system should be evaluated when a specific method of impact evaluation is developed. We have recently shown that, while

the response to predation by macroinvertebrate assemblages in coastal areas characterized by generally high salinity (25-35 ppt) is pronounced (Livingston unpublished data), such response has not been noted in estuarine areas characterized by low salinity and high productivity (Mahoney and Livingston 1982). Thus, the qualification of a given factor (in this case, predation) must be made with respect to the specific ecological relationships of the system in question. The research question should be qualified by a rigorous scaling effort to fit the question to a particular set of habitat conditions. Too often, one small portion of the environment is studied as if it existed in isolation from other parts of the system. For instance, areas in Florida along the northeastern Gulf coast are physically dominated by runoff, which has its origin not in Florida but in Georgia and Alabama, since the primary source of freshwater runoff for the Apalachicola River occurs in upland areas of the Piedmont. Salinity and productivity in the estuary are thus determined in areas hundreds of miles away from the primary study site. Such factors may be intimately related to laboratory artifacts associated with assemblages in the associated estuarine areas. Ultimately, there is no easy way to scale a particular research problem since working from too few data will eventually complicate the hypothesis-testing process.

3. Generalization from one level of biological organization to the next is not recommended, and such generalization can lead to serious mistakes in interpretation. Response time, sensitivity to limiting factors and pollutants, and interspecific relationships vary from species to species. The elimination of a seagrass bed in Apalachee Bay as a result of a pulp mill discharge was found to be dependent on seemingly slight changes in the physical environment that, in themselves, would not have affected a wide range of animals (Livingston, unpublished data). However, elimination of the grassbeds almost certainly led to altered habitat relationships and food web patterns with the resulting loss of productivity and changes in community structure. Without detailed field information concerning interspecific linkages and a precise laboratory demonstration of macrophyte response to altered water quality, such causative relationships would have been difficult to demonstrate.

4. In addition to qualification of underlying assumptions when a given research program is designed, the experimental approach (whether field or laboratory oriented) should be carried out using appropriate statistical models. While such a basis has been, in principle, agreed upon by most researchers, there is often a tendency to rely on tried and true statistical approaches, which may not be adequate for the intended purpose. Meeter and Livingston (1978), working with the application of standard statistical applications (e.g., ANOVA, multiple regression, factor analysis, canonical correlation) to long-term field data, found that such methods were

not always satisfactory because of the abrogation of assumptions inherent in the statistical models and the natural variability of estuarine systems. In any event, it is advisable that a quantitative approach, based on appropriate statistical methodology, be used in the design of a given experimental approach to impact analysis.

The environmental sciences represent a relatively new discipline, compared with other areas such as physiology and anatomy. However, there is already a premature fragmentation of ecologists and pollution biologists into various subdisciplines based on parochial lines. Such ideological fracturing pits the basic or theoretical researchers against the applied, the laboratory against the field, the experimental against the descriptive, the holist against the reductionist. In part, this situation is due to the complexity of the environmental processes that we study. However, such artificial limitations do not advance our knowledge of how aquatic systems respond to natural and anthropogenic stress. Cairns et al. (1981) summarized the different kinds of information that are necessary to evaluate the potential hazard of toxic agents to natural systems. In a given situation, one should not be limited to any single discipline or area of specialization. The essential problem is to fit the research question to the underlying assumptions and restrict overgeneralization by relating results to a well-defined scaling of the approach. If the question involves a system response, there is no easy substitute for an integrated multidisciplinary effort with careful attention to the design of experimental procedures based on background information concerning the organisms of the area in question. In an integrated discipline, there is little room for a parochial or reductionist approach to the solution of system-level problems.

REFERENCES

Cairns, J., Jr., M. Alexander, K.W. Cummins, W.T. Edmondson, C.R. Goldman, J. Harte, R. Hartung, A.R. Isensee, R. Levins, J.F. McCormick, T.J. Petcrle and J.H. Zar. 1981. Testing for effects of chemicals on ecosystems: A report by the Committee to Review Methods for Ecotoxicology, Commission on Natural Resources, National Research Council. National Academy Press, Washington, D.C.

Hammons, A.S., J.M. Giddings, G.W. Suter, II and L.W. Barnthouse. 1981. Methods for ecological toxicology: A critical review of laboratory multispecies tests. ORNL-5708, EPA-560/11-80-026, U.S. Environmental Protection Agency, Washington, D.C.

ICES (International Council for the Exploration of the Sea.) 1978. On the feasibility of effects monitoring. Cooperative Research Rep. No. 75, Copenhagen, Denmark.

Koenig, C.C., R.J. Livingston and C.R. Cripe. 1976. Blue crab mortality: Interaction of temperature and DDT residues. Arch Environ. Contam. Toxicol. 4: 119-128.

Laughlin, R.A., C.R. Cripe and R.J. Livingston. 1978a. Field and laboratory avoidance reactions by blue crabs (Callinectes sapidus) to storm water runoff. Trans. Am. Fish. Soc. 107: 78-86.

Laughlin, R.A., R.J. Livingston and C.R. Cripe. 1978b. Comments: Reactions of blue crabs to low pH. Trans Am. Fish. Soc. 107: 870-871.

Mahoney, B.M.S. and R.J. Livingston. 1982. Seasonal fluctuations of benthic macrofauna in the Apalachicola estuary, Florida: The role of predation. Mar. Biol. 69: 207-213.

Meeter, D.A. and R.J. Livingston. 1978. Statistical methods applied to a four-year multivariate study of a Florida estuarine system. In: J. Cairns, Jr., K. Dickson and R.J. Livingston (eds.), Biological Data in Water Pollution Assessment: Quantitative and Statistical Analyses, pp. 53-68. ASTM STP 652, American Society for Testing and Materials, Philadelphia, Pa.

Peterson, C.H. 1979. Predation, competitive exclusion, and diversity in the soft-sediment benthic communities or estuaries and lagoons. In: R.J. Livingston (ed.), Ecological Processes in Coastal and Marine Systems, pp. 233-264. Plenum Press, New York.

Biological Effects Versus Pollutant Inputs: The Scale of Things

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INTRODUCTION

"The acquisition of data is a means to an end" (Oakley 1979).

Environmental decision-makers need to know something about the "significance," or importance, of measured effects of marine pollution. Too often, however, evaluation of significance is not offered or, if it is, it is in a form so alien that it is frequently overlooked.

There always will be justifiable debate about the meaning of any scientific measurement. However, there is one measure of marine pollution that at least lends itself to constructive debate and decision-making. That is the scale of damage caused by polluting activity. The questions are these: Are we not able to measure the areal extent of biological effects and, further, are we able to predict how the extent of the damage will change as a result of various remedial or disposal actions?

We believe the answer to both questions is a qualified yes. There is now a large reserve of field data showing the extent to which pollutant inputs of various magnitudes have and have not contaminated or damaged marine and coastal ecosystems. The data reserve includes numerous in situ field "experiments" that show the extent to which changes in inputs have changed the scale of impacts. And there is a growing theoretical base for making projections of impacts in physically complex coastal and oceanic

ecosystems. In short, new opportunities are at hand for evaluating and projecting the magnitude of some biological impacts of marine waste disposal activities.

The goal of this report is to provide, by way of example, some suggestions for evaluating the importance of measures of pollutant effects in terms of the amount of organisms and size of habitat affected. Our objectives are to (1) identify the kinds of scales of contamination and damage that have been associated with certain disposal activities; (2) derive correlations that appear to fit the relationships between the magnitude of certain kinds of biological effects and the waste inputs associated with them; and (3) suggest what we can do to improve our ability to document, predict and convey to managers the scale of biological effects of various waste disposal strategies. Ultimately, we hope the approach outlined here might be used to help identify and compare impacts of alternative remedial and waste disposal activities, and perhaps help identify those which are least disruptive to marine ecosystems.

Background

Klapow and Lewis (1979) viewed "scale" as an essential target of marine pollution assessment and monitoring. We presume Teal et al. (1979) likewise were referring to scale when they asked, "How large a pollutant input and how long a chronic pollutant input is required for the (biological) community to leave its normal set of configurations entirely? How much larger a push will prevent its return?" These questions reflect an assumption that the scale of impact increases with waste input and, further, that there may be an input threshold above which impacts increase markedly.

In the 1970s scientists at the Southern California Coastal Water Research Project (SCCWRP) attempted to relate biological impacts to waste emissions from a suite of municipal marine sewage outfalls. During the course of the work it became obvious that the overall magnitude of benthic impacts was related to the amount of sewage discharged and that mass emission rates were better predictors of effects than were effluent concentrations (Mearns and Young 1978). Moreover, data presented by Reish (1980) suggested that 150 million liters (40 million gallons) per day represented a "threshold" flow rate below which adverse benthic community impacts did not occur in this coastal region.

Later, analysis of additional survey data identified exponential relationships between solids mass emission rates (metric tons per year) and size of bottom areas with "changed" or "degraded" benthic communities (Mearns and Word 1982). Because these relationships were exponential, the existence of a threshold was conceivable. Moreover, the relationships formed the basis for predicting the size of degraded and changed areas of the sea floor that might occur as a consequence of several alternative sewage treatment strategies (SCCWRP 1978; Mearns 1981; and at least five PL 92-

500, Section 301h Applications for Waivers of Secondary Treatment).

These observations suggested that (1) spatial scale was both a useful and a meaningful measure of effects, and (2) mass emission rates of effluent constituents were more meaningful measures of inputs than were concentrations alone.

Definitions

This experience led to a search for similar relations elsewhere and to qualification of our title "Biological Effects vs. Pollutant Inputs: The Scale of Things." For the present purpose we define scale as the spatial (horizontal) distribution of biological effects caused by a waste disposal or pollution event. We equate biological effects to biological damage (such as direct mortality, disease or unnatural changes in community structure) or to any response that renders useless a productive marine resource (such as chemical or microbial contamination that exceeds unacceptable limits for harvesting or consumption). By "scale" we mean the size, extent or relative proportion of a population or habitat that has, in fact, been measurably damaged, is unusually contaminated or has been rendered useless. For habitats such as beaches, the sea floor or the water column, the units of measurement are length of coastline (one dimension), area of sea floor or sea surface (two dimensions), or volume of sea water (three dimensions). By "contaminant inputs" we mean the amount of contaminant expressed in units that relate to the scale of effects in a useful way. To a decision-maker this immediately brings to mind the total amount of material in terms of mass (e.g., metric tons (mt)) or volume (e.g., m^3), and to one concerned with a chronic situation, this may mean mass emission rate (e.g., metric tons per year ($mt\ y^{-1}$), cubic meters per day ($m^3\ d^{-1}$) or million gallons per day ($mg\ d^{-1}$)).

We used these definitions to search the literature for data that would allow quantification of relationships between the spatial scale of biological impacts and corresponding mass emission rates of pollutants or wastes. Below is a sample of what we discovered, together with some comments on the meaning of the relationships.

EXAMPLES OF SCALES AND THEIR RELATIONS TO INPUTS

Many reports we examined identified, usually through maps, something about the spatial scale of pollutant impacts on the sea floor or sea surface. A number of scales are noted by Gerlach (1981). However, few relate the amount of impacted habitat, population or community to some corresponding waste input or pollution event. The number of examples increases somewhat when literature is searched first for the "effects" reports and later for the "inputs" data.

A suite of examples is summarized in Tables 1 through 3 and Figures 1 through 10. The impacts include (a) DDE contamination of mussels along the Southern California coast (Risebrough et al. 1980), Figures 1-3; (b) lengths of coastline contaminated by various oil spills (references as cited in Table 1, data plotted in Figure 4); (c) areas of surface and deep water in Puget Sound, Washington, which contained material from pulp mills in concentrations sufficient to induce abnormalities in developing oyster larvae (Cardwell et al. 1977), Table 2 and Figures 5 and 6; and (d) area of the sea floor occupied by changed and degraded benthic communities, and by unusually high benthic biomass, all resulting from known inputs of sewage from Southern California ocean outfalls (Mearns and Word 1982), Table 3 and Figures 7-9.

Overall, the impacts are represented by alongshore (linear) scales that range from less than 1 km to at least 300 km and scales of areal impact that range from less than 1 km^2 to nearly 100 km^2. Ranges of corresponding inputs are (a) 1000 to 21,000 kg y^{-1} of DDT responsible for the contamination of mussels; (b) 7 to nearly 65,000 mt of oil responsible for the beach contamination; (c) about 50,000 to nearly 400,000 kg d^{-1} (18,250 to 146,000 mt y^{-1}, dry weight) of BOD from pulp mills; and (d) 15 to 140,000 mt y^{-1} (dry weight) of suspended solids from sewage outfalls. These ranges cover several orders of magnitude and raise the specter of plotting data on two-, three-, and sometimes four-cycle log paper. Below is a more detailed account of the relationships identified from these data sets.

DDE Contamination of Intertidal Ecosystems

During the last decade, and probably for many years before, the pesticide DDE contaminated marine life throughout the Southern California Bight and along more than 290 km of the adjacent coastal zone (Figure 1). The major source of the DDE was DDT (mostly DDE) discharged from the 60-m deep Los Angeles County sewer outfall system on the Palos Verdes Peninsula (Young et al. 1978). When the source was identified in 1971, total sewage DDT emissions were 21,600 kg y^{-1}. Control (of the offending manufacturer) in 1971 resulted in a rapid (3-4 year) decline to total emission = 1000 kg y^{-1} (Figure 2a). During this period (1971-78) several researchers documented commensurate declines of DDE contamination in mussels at up to 16 stations along 290 km of the adjacent coastline (summarized in Risebrough et al. 1980).

These decreases were replotted in terms of the lengths of coastline (km) occupied by mussels bearing various concentrations of DDE (Figure 2b). Comparison of these plots with data in Figure 2a suggests that the mussels responded to the decreased emissions within several years.

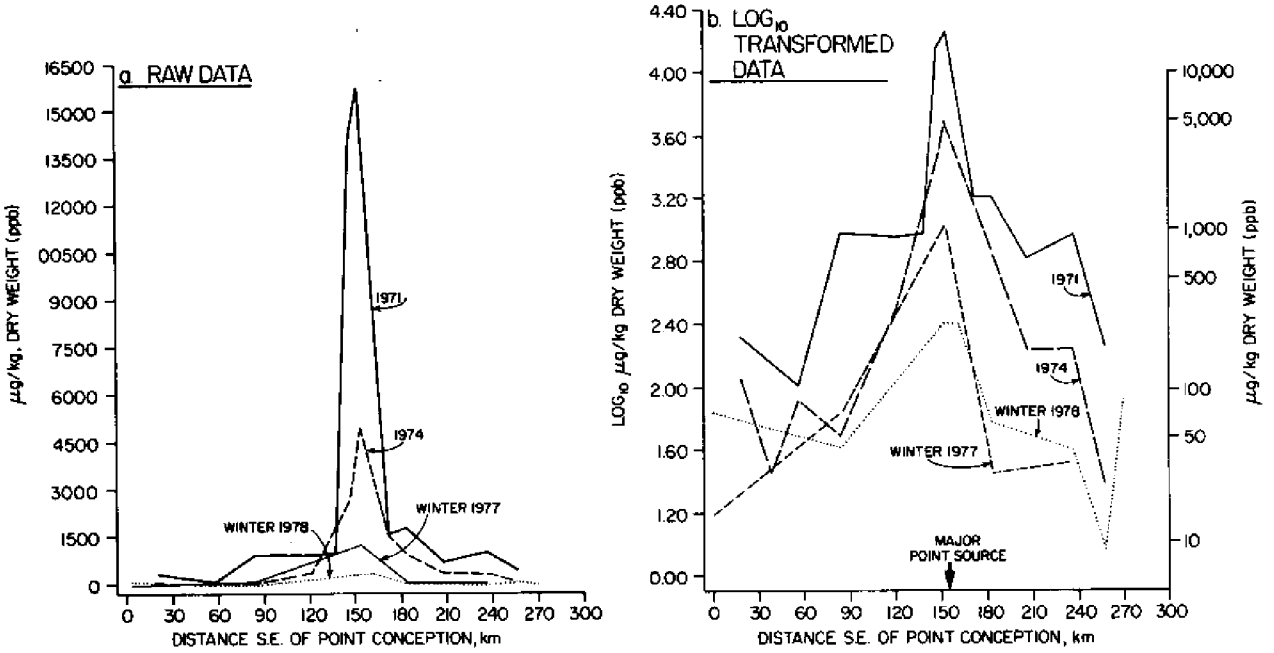

Figure 1. Decline of DDE contamination in intertidal mussels (Mytilus spp.) along 300 km of the Southern California coast, 1971 through 1978. Plots are (a) raw data, and (b) $\log_{10}$-transformed data. The major source was Montrose Chemical Company, discharging through the Los Angeles County outfalls, approximately 150 km southeast of Point Conception (km 0). Data are from the California Mussel Watch program (Risebrough et al. 1980). The 1971 and 1974 data are from collections reported by Young and Szpila (1975).

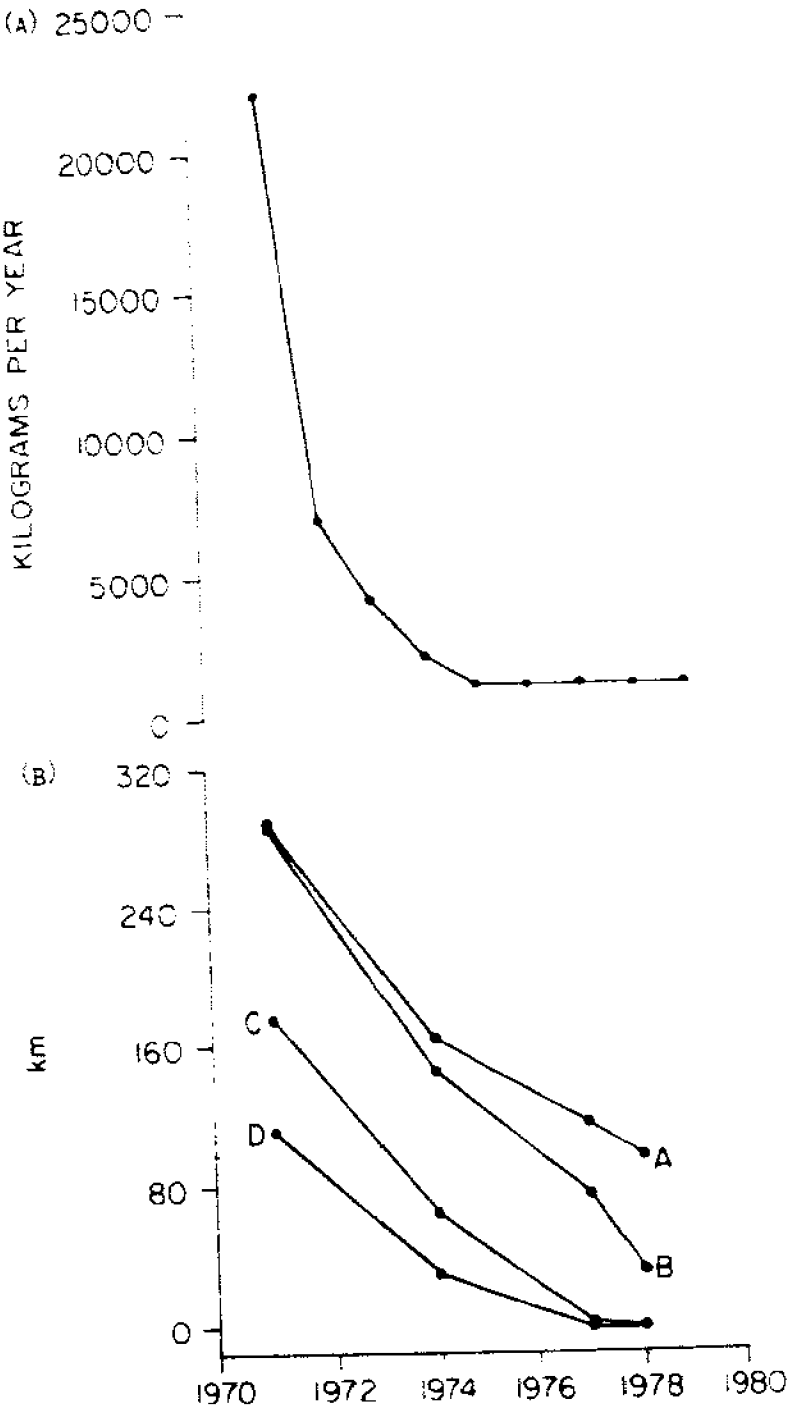

Figure 2. (a) Annual change in total DDT mass emission rate from major Southern California ocean outfalls, 1971 through 1979. Decline was due principally to source control initiated in late 1970 by Los Angeles County Sanitation Districts. Data are from compilation by the Southern California Coastal Water Research Project (see Schafer 1980). (b) Changes in length of coastline bearing mussels contaminated by DDE, 1971 through 1978. Four levels of contamination, in μg/kg dry weight (ppb) are (A) $\geq$50, (B) $\geq$100, (C) $>$500, and (D)$>$1000. Coastline lengths were interpolated from data in Figure 1 (b).

First-order relations between the mass emission rates of DDT and the lengths of coastline bearing mussels containing ≥ 0.1 ppm DDE and > 0.05 ppm DDE are shown in Figure 3. In both cases, DDT emissions were $\log_{10}$ transformed to help normalize the curves. In the case of ≥ 0.1 ppm DDE (Figure 3a), the relationship is nearly significant ($r = 0.939$, p is slightly greater than 0.05 and $r^2 = 82.3$); in the case of DDE ≥ 0.05 ppm (Figure 3b), the relationship is significant ($r = 0.964$, $0.05 > p < 0.01$, $r^2 = 89.1$).

Obviously, there are too few data points ($n = 4$, Figures 3a and 3b) to justify developing a predictive tool. If they exist, data from 1972 or 1973 would be useful in providing data points for intermediate values. Likewise, surveys following any future reductions in DDT emission would help. Nevertheless, the approach used here suggests how "mussel watch" surveys might be designed and used as management tools. For example, rough predictions could be made for effluent DDT emissions and concentrations required to reduce the scale of DDE contamination below some specified level. Such "feedback" estimates have not heretofore been offered.

Oil Spills: Lengths of Contaminated Coastlines

Scales of habitat contamination similar to those for DDT result from oil spills. An analysis of data from a few oil spill reports (Table 1) suggests some possibly useful relationships for identifying the potential magnitude of onshore impacts. The 223,000 T Amoco Cadiz spill in March 1978 off Brittany, France, resulted in 60,000 to 65,000 T of beached oil that impacted up to 300 km of shoreline (Hayes et al. 1979). The 1977 Urquiola incident on the Spanish coast spilled 110,000 T of beached oil covering up to 215 km of coastline (Gundlach et al. 1978). The Metula spilled 50,000 T of oil in the Strait of Magellan, Chile, in August 1974, resulting in 21,000 T of beached oil that covered an estimated 140 km of shoreline (Gunnerson and Peter 1976). In contrast, 150-200 T of oil from the 250,000 T Burmah Agate spill near Galveston, Texas, in November 1979, impacted only about 20 km of shoreline; another 71 T impacted 10.5 km of shoreline, while a third input of 5 T impacted 15 km (Thebeau and Kana 1981).

Taken together, these data offer an interesting relationship between the amount of oil beached and the length of shoreline contaminated ($r = 0.84$, $p \leq 0.01$, Figure 4a). The Arrow data, in Chedabucton Bay, Canada (Vandermuelen 1977), deviate markedly from an otherwise smooth relationship. Not unexpectedly, replotting the data on double log paper helps to resolve the scatter among the four smallest episodes and to reduce the relative variation among the largest (Figure 4b). Likewise, double-$\log_{10}$ transformaton improves the correlation slightly ($r = 0.867$, $p \leq 0.01$). The equation for this line is:

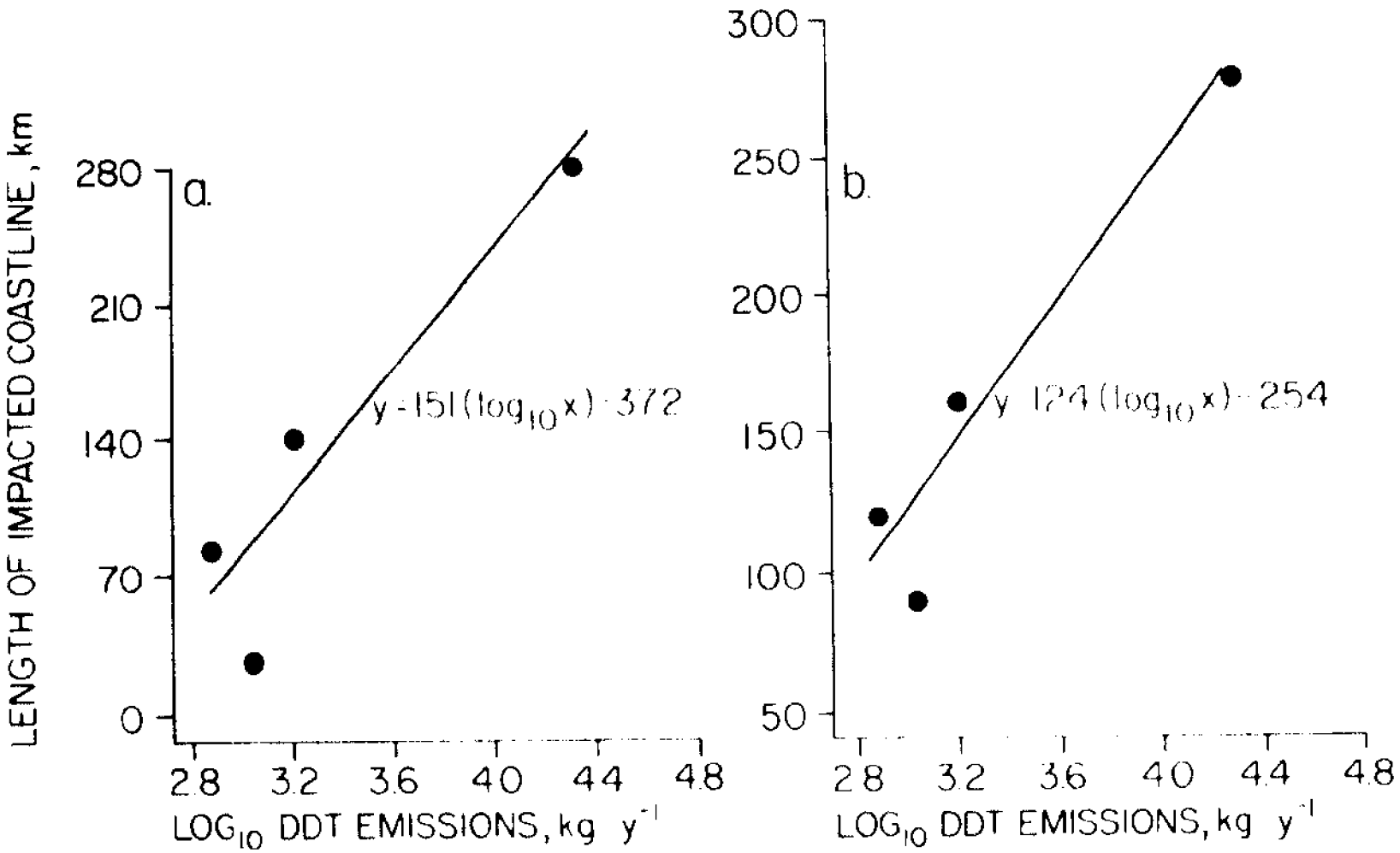

Figure 3. Relation between lengths of Southern California coastline bearing mussels contaminated by DDT and corresponding $\log_{10}$-transformed sewage-borne mass emission rates for the same year. The two concentrations, in $\mu g\, kg^{-1}$ dry weight, are (a) ≥ 100, and (b) ≥ 50. Data are from Figure 2.

Table 1. Eight cases of beached oil and corresponding estimates of lengths of coastline containing beached oil.

Source	Location	Date	Estimated Oil Beached (mt)	Length of Coast Oiled (km)	REFERENCE
Burmah Agate	Texas	Nov. 1979	5	15	Thebeau and Kana 1981
Burmah Agate	Texas	Nov. 1979	71	11	Thebeau and Kana 1981
Burmah Agate	Texas	Nov. 1979	175	20	Thebeau and Kana 1981
Tsesis	Sweden	Oct. 1977	1000	18	Kineman et al. 1980
Arrow	Canada	Feb. 1970	1875	200	Vandermeulen 1977
Metula	Chile	Aug. 1974	21000	140	Gunnerson and Peter 1976
Urquiola	Spain	May 1978	25000-30000	215	Gundlach et al. 1978
Amoco Cadiz	France	Mar. 1978	60000-65000	300	Hayes et al. 1979

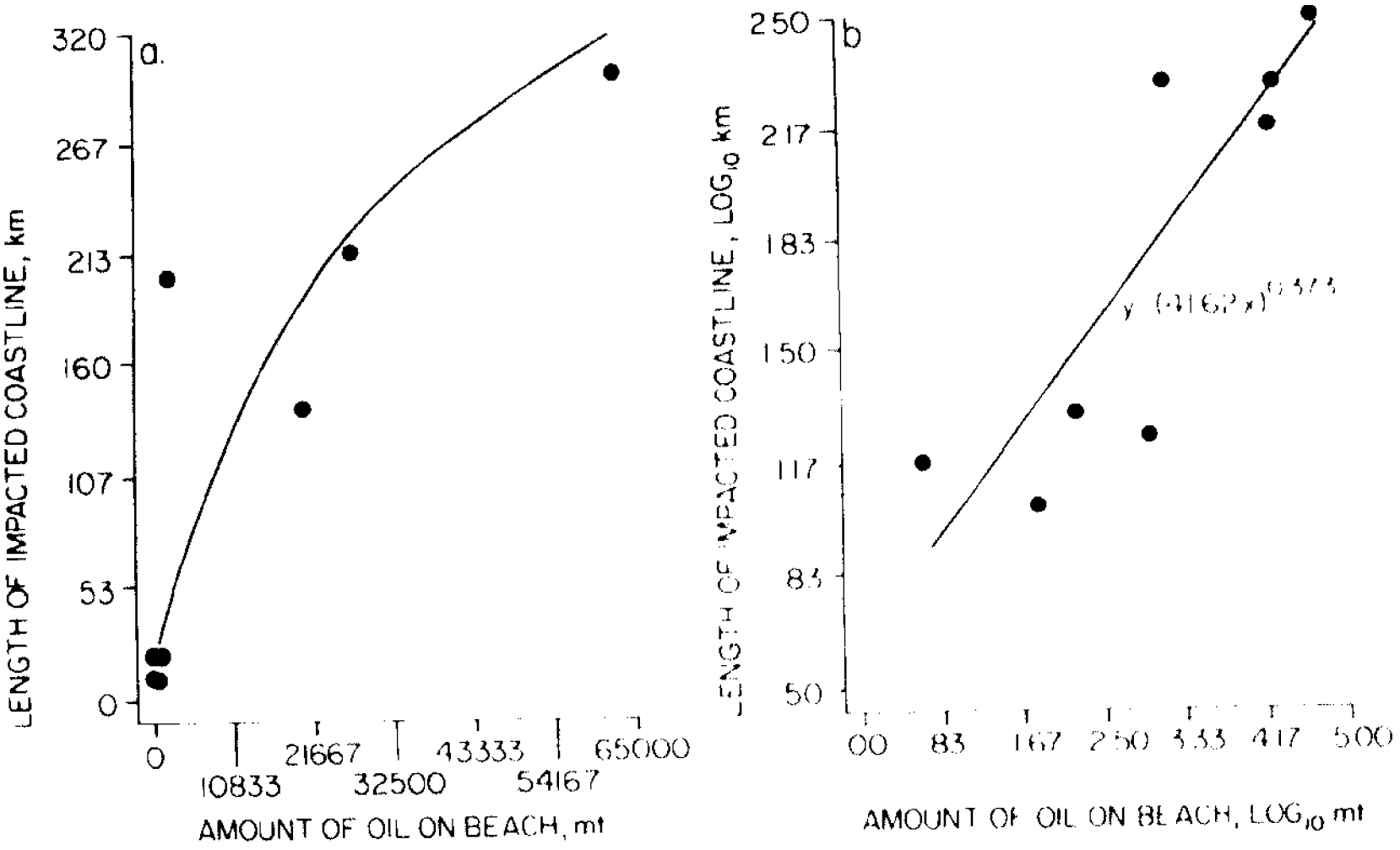

Figure 4. Relation between length of coastline bearing beached oil from spills and the amount of oil beached. Plots are (a) raw and (b) corresponding $\log_{10}$-transformed data from sources identified in Table 1.

$$\log_{10} Y = 0.737 (\log_{10} X) + 0.604 \quad (1)$$

where

Y = length of oiled coastline in km
X = amount of oil beached in mt

Solving for Y produces the exponential equation:

$$Y = (41.6\ X)^{0.373} \quad (2)$$

This means that as the amount of beached oil doubles, the length of coastline oiled does not double but increases by about one-third. This relation may have intuitive, if not predictive, importance. For example, although 10,000 mt of beached oil can impact 122 km of sea coast, it does not follow that 10 times that amount--or 100,000 mt--will impact 1220 km but rather something on the order of 300 km (284 km is predicted). Again, the Arrow spill is an important deviation from this trend.

Presumably, increased volumes of oil on beaches imply increased thicknesses of oil more than increased lateral dimension. We are not prepared to speculate about the properties of the sea coasts or the oil that result in such a relationship. However, the example generally suggests that there may be some inherent limit to the extent to which beaching oil can impact shoreline ecosystems. Inclusion of this example, according to our definitions, implies the assumption that there is corresponding biological damage resulting from this contamination. We are not prepared to challenge or support this assumption.

Size of Toxic and Eutrophic Water Masses

Several studies developed data from which it is possible to infer relationships between waste mass emissions and the areal extent of impacts on organisms of the water column.

Cardwell et al. (1977) found that developmental abnormalities were induced in oyster larvae from various areas within grids of surface and deep water samples taken offshore of several Puget Sound pulp mills (Table 2). As shown in Figure 5, the area, in km^2, of incidence of abnormalities $\geq$ 50% of the exposed oysters decreased in a curvilinear fashion with decreasing BOD emissions as several pulp mills improved treatment and reduced toxicity. The curve at the left in Figure 5 indicates responses commensurate with reduction of pulp mill BOD inputs into surface (<37 m) waters of the Everett--Port Susan region. The middle curve indicates responses commensurate with reduction of pulp mill BOD inputs into deep (100 m) waters of the same region where BOD inputs were two to three times greater than in surface waters. The points to the right represent sea surface areas sustaining $\geq$50% abnormalities coincident with reductions in even larger BOD inputs from a pulp mill discharging at Port Angeles in the Strait of Juan de Fuca.

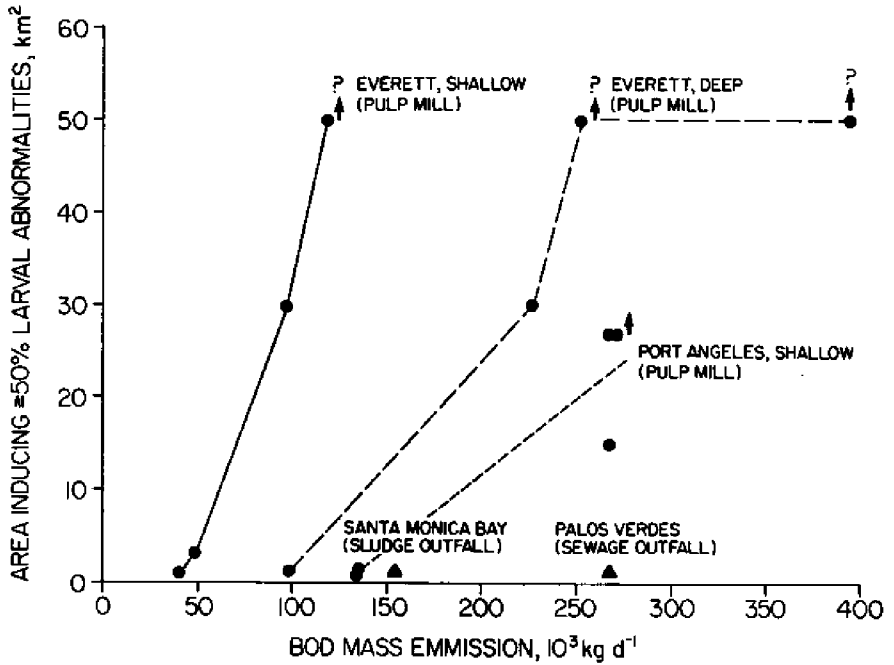

Figure 5. Relations between areal distribution (km^2) of receiving-water toxicity to developing invertebrate embryos and mass emission rates of wastes from adjacent outfalls as indicated by BOD_5. Toxic response is ≥50% incidence of developmental abnormalities in 48-hour-old embryos of oysters (dots) and sea urchins (triangles). Data for Everett and Port Angeles, Washington, are from a survey of pulp mill effects reported by Cardwell et al. 1977 (see Table 2); data from Santa Monica Bay and Palos Verdes, California, are from Oshida et al. 1982.

Table 2. Location and depth of Puget Sound, Washington, pulp mill BOD emissions and area of surface or deep water inducing ≥50% developmental abnormalities in 48 h oyster embryos.*

Year	Location	Ecosystem	Depth	Total BOD ($kg\ d^{-1}$)	Toxicity ≥ 50% Area (km^2)
1972	Everett	Enclosed embayment	Shallow (<37m)	117,232	> 50
1973	Everett	Enclosed embayment	Shallow (<37m)	97,032	30
1974	Everett	Enclosed embayment	Shallow (<37m)	49,420	3
1975	Everett	Enclosed embayment	Shallow (<37m)	41,300	1
1972	Everett	Enclosed embayment	Deep (37-75m)	252,000	> 50
1973	Everett	Enclosed embayment	Deep (37-75m)	395,000	> 50
1974	Everett	Enclosed embayment	Deep (37-75m)	227,000	30
1975	Everett	Enclosed embayment	Deep (37-75m)	98,000	1
1972	Port Angeles	Open Straits	Shallow (<37m)	268,000	15
1973	Port Angeles	Open Straits	Shallow (<37m)	268,000	> 27
1974	Port Angeles	Open Straits	Shallow (<37m)	268,000	> 27
1975	Port Angeles	Open Straits	Shallow (<37m)	134,000	0.7

* Cardwell et al. 1977 .

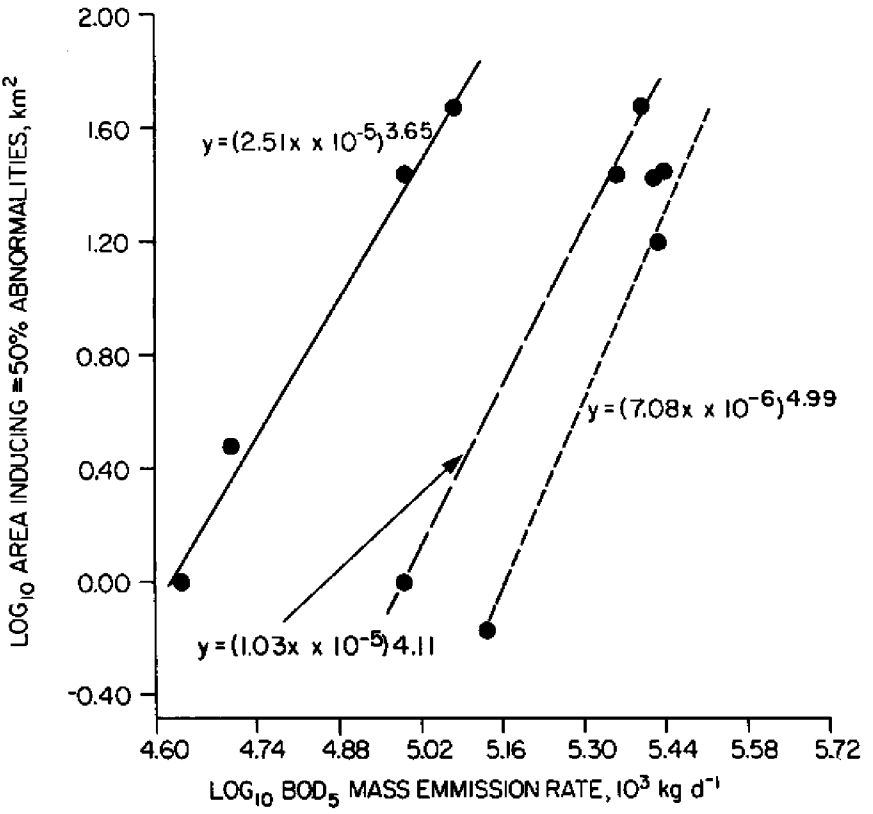

Figure 6. Possible exponential relationships between areal distribution of receiving water toxicity to oyster larvae (km^2) and changing waste mass emission rates from three pulp mill effluents; based on $\log_{10}$-$\log_{10}$ transformation of data in Figure 5 and Table 2 (from Cardwell et al. 1977).

When all data (x and y coordinates) are transformed to $\log_{10}$, all three curves become statistically significant exponential relationships ($p \leq .05$) whose exponents (slopes) range from 3.65 to 4.99 (Figure 6). That is, as the emissions double, the area of toxic water increases approximately 12.5- to 32-fold (or to 1/12.5 or 1/32 of the original impact if they are halved). These are dramatic changes and represent the first example of direct biological damage.

Also shown in Figure 5 are two points confirming limited induction of abnormalities in sea urchin embryos (Oshida et al. 1981) exposed to surface and subsurface waters near the mixing zones of a sewage sludge outfall and a major sewage effluent outfall on the open coast of Southern California (from Oshida et al. 1982). Their data indicated no effect at BOD_5 emissions comparable with those in Puget Sound. While there are obvious uncertainties in comparing oysters and sea urchins, additional uncertainties in comparing pulp mill and sewage outfalls, and serious doubt that BOD_5 is in fact the agent causing the responses, the comparison offers some intriguing speculation. For example, the curves and points might represent a "family of curves" in which conditions on the left of Figures 5 and 6 suggest considerably less tolerance to the emissions than those to the right. Is it possible that the curves represent, from left to right, increasing amounts of water available for dilution (from a shallow impounded lens of inshore water out to the more vigorous Straits of Juan de Fuca and the open coast of Southern California)?

In a study of eutrophication and phytoplankton stimulation, Malone (1982) estimated that the area of the New York Bight required to assimilate the dissolved nitrogen input from Hudson River runoff and sewage varied seasonally from 670 to 1350 km^2 in concert with changes in river flow and stratification. Since phytoplankton production in this area is nitrogen-limited, Garside et al. (1976) concluded that changes in nitrogen input would change the size (or scale) of the area in which primary production is affected but would not alter the production rate itself. Thus, it is the spatial dimension rather than the intensity of production within a eutrophic water mass that varies with the nutrient input.

Benthic Impacts from Outfalls

Scales of impacts on relatively immobile intertidal and subtidal benthic communities have been documented and related to changes or differences in waste inputs. Figure 7 shows the spatial distribution of several measures of benthic infaunal impacts around three Southern California outfalls. The figures are arranged from top to bottom in order of decreasing flows and suspended solids mass emission rates. All discharge at the same depth (60 m) and all are subject to similar current regimes (Hendricks 1977). The figures on the left show isopleths indicating two major discontinuities in the structure of the communities as measured using an index developed by Word (1979).

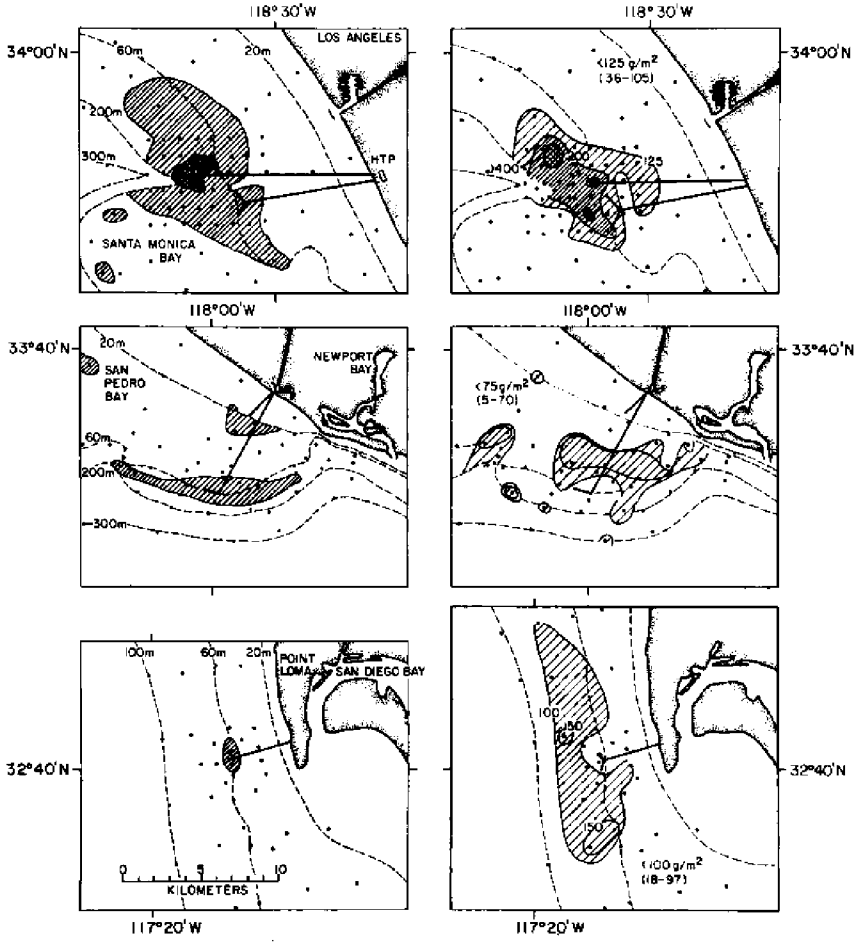

Figure 7. Areal responses of benthic infauna to three Southern California sewage outfall systems: (a) Santa Monica Bay receiving 1215 x 10^6L d^{-1} of effluent and 78,800 mt y^{-1} of suspended solids; (b) southern San Pedro Bay receiving 675.2 x 10^6 L d^{-1} of effluent and 33,300 mt y^{-1} of suspended solids; and (c) coast off Point Loma, San Diego, receiving 435 x 10^6 L d^{-1} of effluent and 20,500 mt y^{-1} of suspended solids. Left column of figures shows distribution of "changed" and "degraded" benthic community structure as measured by the Infaunal Trophic Index (Word 1979; Bascom et al. 1979). Right column of figures shows distribution of increased biomass (contour in g m^{-2}). Data are from surveys conducted in 1977 and 1978 (Bascom 1979).

These plots clearly suggest a direct relationship between the scale of alterations in community structure and the magnitude of wastewater emissions. On the right are isopleths defining areas of above-normal biomass (g m^{-2}) of the communities. Here there is no visible relation between the size of area affected (by elevated biomass) and the magnitude of the discharges. However, there are several areas of extremely high biomass (200 to nearly 1000 g m^{-2}) at the largest discharge that do not occur at the smaller ones.

As noted previously, Mearns and Word (1982) identified from these and two other outfall sites several exponential relationships between the size of area occupied by "changed" and "degraded" community structure (as defined by Bascom et al. 1979) and the corresponding mass emission rates of suspended solids. Equations fitting these data are shown in Figure 8a. The exponents for the double-$\log_{10}$-transformed data are 2.16 for the "changed" fauna and 2.55 for the degraded fauna. Thus for each doubling of mass emission rates of solids, the area of sea floor with "changed" community structure increases 4.5-fold and that of "degraded," 5.9-fold.

In contrast, areas occupied by elevated or "excess" biomass increased linearly (exponent 0.96) over the entire range of observations (Figure 8b). However, the actual amount, or tonnage, of the excess biomass increased nearly as the square of the input (exponent=1.75, Figure 8c).

Flow and mass emission rates of most marine outfalls are much lower than those represented by the five large Southern California systems examined by Mearns and Word (1982). To determine if the original relationships were applicable to smaller scales of impact, additional data from several smaller sewage discharges were examined. Estimates of impacts on benthic biomass are tabulated in Table 3, together with the original data used by Mearns and Word (1982). The new equations and exponents (Figure 9) are not substantially different from the original ones, suggesting that the equations may be useful in predicting scales of impacts for a wide range of treatment plants, at least in California. It remains to be determined whether these relationships are applicable to the shallow, semi-enclosed estuaries or embayments of the Atlantic coast. On the other hand, the equation for "changed" fauna appears to predict, at least within a factor of two, the 260 km^2 of benthic community impact described by Boesch (1982) for the New York Bight (as analyzed in Mearns and Word 1982).

Scales of impact of sewage on rocky subtidal communities and their relation to waste inputs have also been documented. Among these is a partial relationship between decreased solids emissions from the Southern California Palos Verdes outfall and the recovery of adjacent large stands of kelp (Macrocystis) forest (Wilson et al. 1980); this relationship has been exploited to predict the size of the beds (Garrison, 1981).

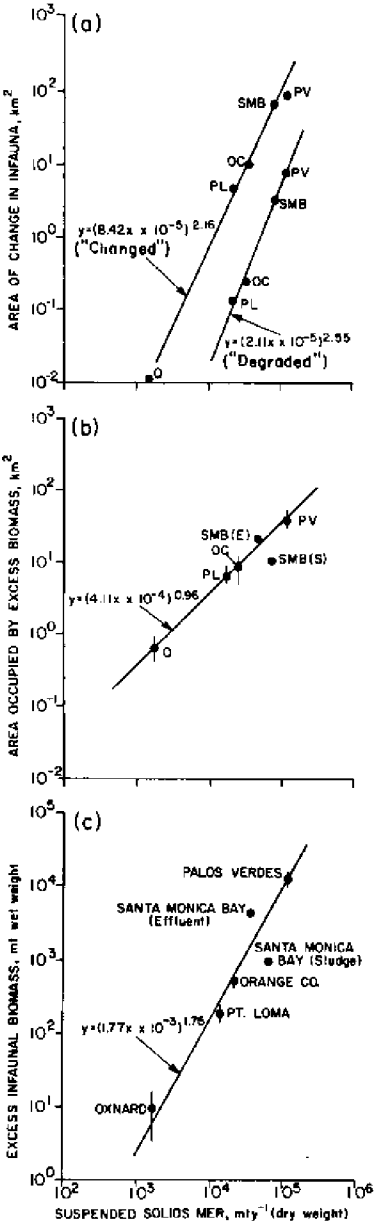

Figure 8. Relationships between the mass emission rates (MER) of suspended solids from sewage outfalls and the magnitude of impacts on (a) area of change in infauna; (b) area occupied by excess biomass; (c) excess infaunal biomass (Mearns and Word 1982).

Table 3. Flow and suspended solids emissions for seven Southern California sewage outfall systems and associated estimates of benthic biomass responses.*

	INPUT				RESPONSE				
	Discharger	Year	Depth (m)	Flow ($L\ d^{-1} \times 10^6$)	Suspended Solids ($mg\ L^{-1}$)	($mt\ y^{-1}$)	Excess Biomass (mt)	(km^2)	Source of Biological Data
1	Montecito	1973	10.7	2.65	15.0	15	N.D.***	<0.01	MBC (1973)
2	San Elijo	1978	41.2	41.75	12.25	644	60	1.7	CSD (1979)
3a	Oxnard	1976	14	43.9	91	1,465	3.4	0.37	EQA (1976)
3b	Oxnard	1975	14	38.2	168	2,340	17.0	0.95	Mearns and Word (1982)
4a	Point Loma	1976-1977	60	393.7	138	15,200	150	5.0	City of San Diego (unpub. data)
4b	Point Loma	1977	60	435.5	128	20,500	2,200	44.0	Bascom (1979)
5a	Orange County	1975	60	647.3	107	25,200	455	5.0	Greene (1976)
5b	Orange County	1977	60	675.2	132	33,300	829	21.8	Bascom (1979)
6a	Hyperion, effluent	1975	60	1,306	85	40,460	4,644	22.5	City of Los Angeles (unpub. data)
6b	Hyperion, sludge	1975	100	16.3	10,300	69,720	>984	>11.1	City of Los Angeles (unpub. data)
6c	Hyperion, combined	1975	60+100		1,322	N/A**	110,800	5,625	>30.0	Mearns and Word (1982)
6d	Hyperion, combined	1977	60+100		1,225	N/A**	78,800	8,087	42.0	Bascom (1979)
7a	L.A. County	1975	60		1,291	276	130,966	11,760	>30.0	L.A. County Sanitation Districts (unpub. data; see Garrison, 1981)
7b	L.A. County	1972-1977	60		1,338	286.5	139,670	13,773	>33.0	
7c	L.A. County	1977	60		1,336	284	138,000	13,011	>62	

* Input data for discharges 3 through 7 from Southern California Coastal Water Research Project (e.g., see Schafer, 1980).
** N/A = Not analyzed
*** N.D. = Not determined

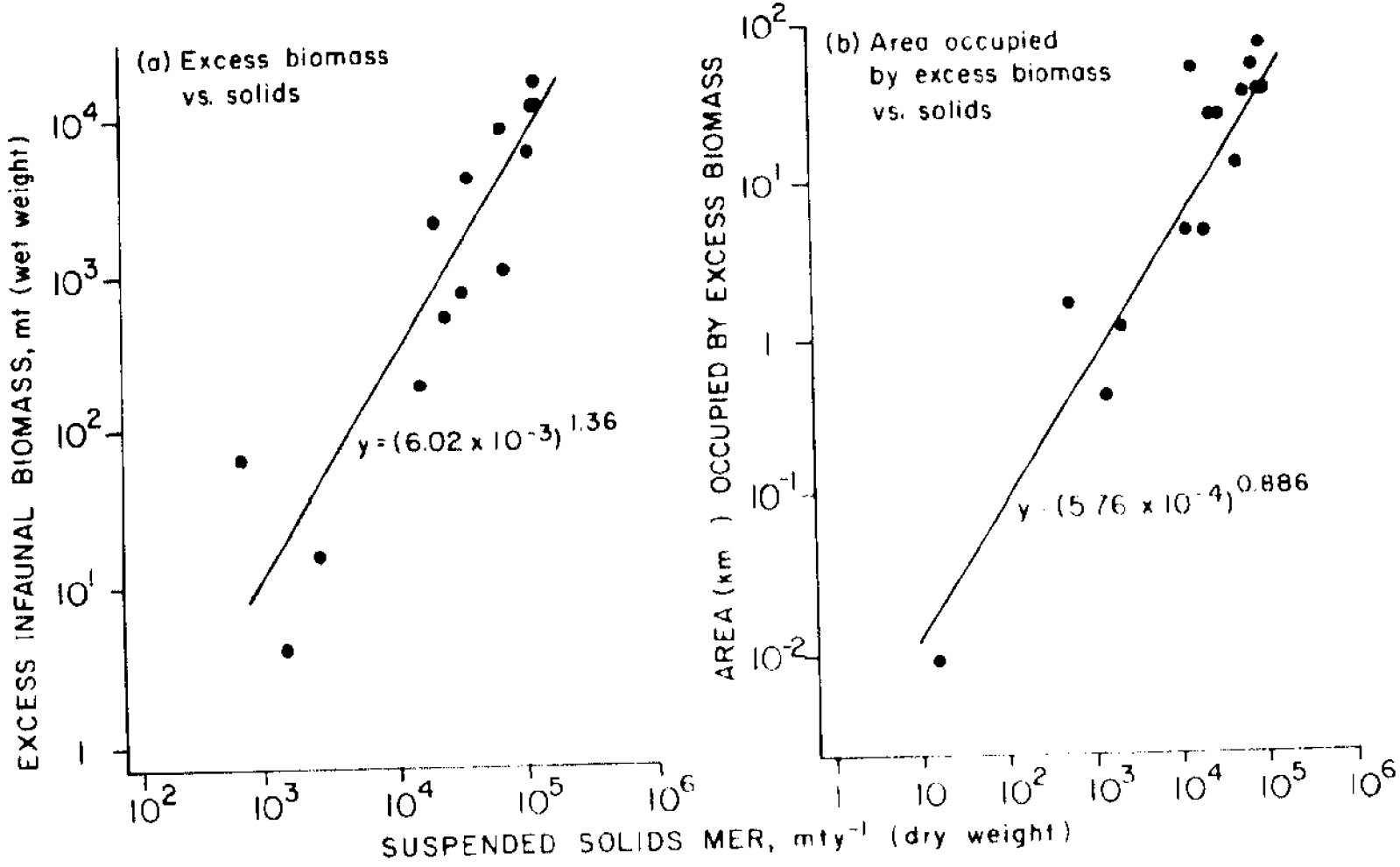

Figure 9. Relationships between the mass emission rates of suspended solids from sewage outfalls and the magnitude of impact on (a) "excess" infaunal biomass and (b) area occupied by the excess biomass. Relationships are based on a wide range of outfalls as described in Table 3.

In a similar study, Dicks and Iball (1981) provided data from which it is possible to deduce relationships between the flow and mass emission rates of oil from a refinery effluent and the fractional area of an adjacent salt marsh damaged by the discharge. The authors speculate on conditions that might represent a threshold below which the refinery effluent does not impact this very shallow "benthic" ecosystem.

DISCUSSION AND IMPLICATIONS

Our aim has been to suggest ways to evaluate measures of pollution effects. We postulated that spatial scale of response was itself a meaningful measure of waste inputs and thus sought to answer three questions, rephrased as follows:

1. What are the scales of observed pollutant effects in nature?
2. How do changes in these scales relate to changes in waste inputs?
3. What can we do to improve our ability to document, predict and convey to managers the scale of effects of various waste disposal strategies?

The answer to the first question is simple. Scales of impacts attributable to known pollutant or waste inputs can be detected over several hundred kilometers of coastline and many tens of kilometers of sea surface or sea floor. However, some observations, such as those relating to contamination of intertidal resources by DDT and oil, are not sufficient to determine the extent of actual biological damage. Some biological indices are needed and a more detailed review might reveal data that could be used to identify and examine such relationships. In any case, some spatial scales of damage can be documented by using simple and well-tested techniques such as the larval bioassays (Cardwell et al. 1977; Oshida et al. 1981) and measures of community structure (Boesch 1977; Word 1979).

The answer to the second question is both simple and complex. There is indeed a reasonable correspondence between the amount or rate of pollutant or waste input and the spatial scale of contamination or biological effects. The complexity arises when we attempt to determine the nature of the relationships: some are linear, as in Case 1, Figure 10; others are exponential, as in Cases 2 and 3, Figure 10. For example, it appears that the increase in area of sea floor occupied by excess biomass of infauna near sewage outfalls is linearly related to suspended solids inputs; the exponent, 0.96, approximates 1.0 and thus satisfies Case 1 in Figure 10. In other words, a doubling of the input doubles the area occupied by excess biomass.

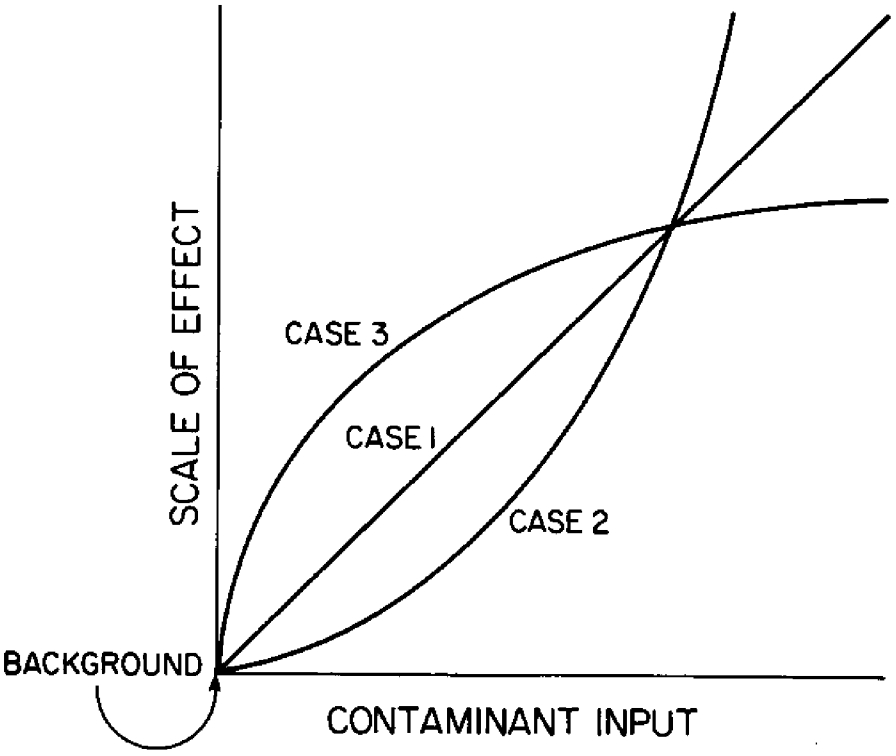

Figure 10. Three of several possible relationships between the mass emission rate (input) of a contaminant and the scale of magnitude of a biological impact or contamination. Case 1 is linear; cases 2 and 3 are exponential, with exponent > 1 for case 2 and < 1 for case 3.

On the other hand, for tanker spills the relation between length of oil-contaminated coastline and amount of oil beached is represented by an exponent much lower than 1 (i.e., 0.365); as the amount of beached oil doubles, length of coastline increases only by one third. Finally, most of the relationships fall into the Case 3 category shown in Figure 10. The exponents we found ranged from 1.75 for the relation between tonnage of excess biomass near sewage outfalls vs. suspended solids mass emission rates, to 4.99 for the relation between sea surface area near a Port Angeles pulp mill inducing $\geq$ 50% developmental abnormalities in oyster larvae and the pulp mill BOD input. Such relationships mean that as inputs double, the area or amount of impact much more than doubles, increased by factors ranging from 4 to 32. Alternatively, such relationships also mean that a halving of pollutant input will result in considerably more than a halving of the scales of effects and may, from an operational point of view, result in the "appearance" of thresholds—the final "push" suggested by Teal et al. (1979).

What is needed to improve our ability to document, predict and convey something about the significance of scales of effects? Documenting spatial scales means "mapping." Klapow and Lewis (1979) recognized the need for spatial mapping in monitoring surveys, but we are not convinced that mapping of effects has been an explicit objective of most surveys and monitoring studies. Rather, replicate sampling, sufficient to demonstrate statistically significant differences between two sampling sites, is an objective of many current monitoring programs. Given limited resources, this approach is not conducive to mapping. Since the confidence required for mapping gradients could be somewhat lower than those required to make sure two sites are not slightly different, the same limited resources could be devoted to this end. Field studies should stipulate explicitly whether mapping is an objective; we urge that more "effects" studies be directed toward "mapping."

We also note that reports of many field studies fail to include quantitative information about the inputs presumably causing the alleged impacts. It would be most helpful if future reports included such information.

Much remains to be initiated to improve our ability to predict scales of effects of various waste inputs. There is a definite need to find out how scales of biological effects relate to scales of sea floor contamination projected on the basis of physical models. Physical models, for instance, would never yield the Case 3 (Figure 10) correlations between inputs and scales of contaminant concentration. The correlations we have found are between waste inputs and scales of biological response. They incorporate but do not separately identify correlations between contaminant concentration and biological response. Some of these projections cover larger scales than those we have considered such as the 10,000 km^2 of sediment PCB contamination expected to result from ocean dump-

ing of sewage sludge over the continental slope (Swanson et al. 1982; O'Connor et al. 1982) and the many tens of thousands of km^2 of abyssal sea floor expected to be disturbed by deep ocean mining (OOME 1981; Jumars 1981).

Teal et al. (1979) suggested experimental manipulation of a coastal ecosystem as a way of developing predictive relationships about impacts of contaminants. We suggest that there are many other experimental opportunities (e.g., existing discharges) awaiting analysis. Regardless of the approach, scale should be among the measures. There also ought to be some possibilities for linking mesocosm experiments such as MERL (Donaghay et al. this volume) with field operations such as monitoring and mapping surveys around outfalls. Both kinds of studies could be designed to converge on predictions of scales of biological effects.

Scale inherently conveys something of significance or importance to managers and decision-makers. That importance can be magnified by offering information in the manager's terms. For example, one could offer at least rough forecasts of the scale of effects that are possible as a result of increasing or decreasing inputs or, conversely, of identifying the inputs below which a given scale of effect would not be expected to occur. If we wished to limit "degraded" infauna around a Southern California outfall to less than 0.01 km^2 (the size of a small zone of initial dilution), then solids mass emission rate could be as high as 31,900 mt y^{-1} or 176,800 lb d^{-1}. The emission rate could be converted to a maximum allowable average suspended solid concentration in an effluent, given the average sewage flow rate (i.e., in millions of liters per day). In other words, it could be used as a tool to help design the treatment system.

In this report we are not prepared to discuss why the different wastes and different effects correlate so differently. A number of factors must be considered, including the chemical stability and physical chemistry of the wastes in sea water; the dispersing characteristics of associated water masses; and the rates at which marine plant and animal communities die, recruit, migrate and metabolize waste constituents. Physical boundaries, such as the sea floor and coastline, serve to place limits on the scales over which pollutants might otherwise disperse. The presence or absence of these limitations might explain some of the dramatic exponential differences between water mass impacts and shoreline/sea floor impacts. However, even with the open sea there are invisible boundaries to scales of vertical and horizontal density gradients which involve both physical and biological processes. The identification by Iles and Sinclair (1982) of physical, predictable herring "larval retention areas" over the continental shelf offers both an example of limitation to scale and a caution that the sea and its biota are not well-mixed everywhere.

In this paper, the definition of scale has been limited to "spatial distribution." Scales in time have not been considered except inadvertently in examples of recovery of polluted ecosystems. The persistence or reversibility of impacts is of obvious concern and should be dealt with directly.

Likewise, we have not quite returned to the question of significance or importance. In a sense that humans are worried over impacts, is 10 km^2 of degraded benthic communities significant for its own sake or only if damage over that scale has consequence to valued fisheries? We cannot answer this. However, we can suggest that the question is more clearly debatable when the scale of the problem has been measured or predicted and the results conveyed in a useful form to decision-makers.

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REFERENCES

Bascom, W. 1979. Life in the bottom: San Pedro and Santa Monica Bays, Palos Verdes and Point Loma Peninsulas. In: Coastal Water Research Project Annual Report 1978, pp. 57-80. Southern California Coastal Research Project, El Segundo, Calif. (PB 299830/AS, National Technical Information Service, Springfield, Va.)

Bascom, W., J.Q. Word and A.J. Mearns. 1979. Establishing boundaries for normal, changed and degraded bottom conditions. In: Coastal Water Research Project Annual Report 1978, pp. 81-94. Southern California Coastal Water Research Project, El Segundo, Calif. (PB 299830/AS, National Technical Information Service, Springfield, Va.)

Boesch, D.F. 1977. Application of numerical classification in ecological investigations of water pollution. EPA-600/3-77-033, Ecological Research Series Rep., U.S. EPA, Office of Research and Development, Corvalis, Ore.

Boesch, D.F. 1982. Ecosystem consequences of alteration of benthic community structure and function in the New York Bight region. In: G.S. Mayer (ed.), Ecological Stress and the New Bight: Science and Management, pp. 543-568. Estuarine Research Federation, Columbia, S.C.

Cardwell, R.D., C.E. Woelke, M.I. Carr and E.W. Sanborn. 1977. Evaluation of the efficiency of sulfite pulp mill pollution abatement using oyster larvae. In: F.L. Mayer and J.L. Hamelink (eds.), Aquatic, Toxicology and Hazard Evaluation, pp. 201-295. ASTM STP 634, American Society for Testing and Materials, Philadelphia, Pa.

CSD (County of San Diego). 1979. Application for Modification of Waiver for Secondary Treatment for the Elijo Lagoon Outfall. San Diego, Calif.

Dicks, B. and K. Iball. 1981. Ten years of salt marsh monitoring--The case history of a Southampton water salt marsh and a changing refinery effluent discharge. In: Proceedings 1981 Oil Spill Conference, March 2-5, 1983, Atlanta, Georgia, pp. 361-374. American Petroleum Inst., Washington, D.C.

EQA (Environmental Quality Analysts). 1976. City of Oxnard: Receiving Water Monitoring, First Quarter Progress Rpt., Env. Qual. Anal., Costa Mesa, Calif.

Garrison, W.E. 1981. Ocean monitoring and research, annual report 1980-1981. Los Angeles County Sanitation Districts, Monitoring Section Technol. Services Dept., Whittier, Calif.

Garside, C., T.C. Malone, O.A. Roels and B.A. Sharfstein. 1976. An evaluation of sewage-derived nutrients and their influence on the Hudson River Estuary and New York Bight. Estuarine Coastal Mar. Sci. 4: 281-289.

Gerlach, S.A. 1981. Marine pollution: Diagnosis and Therapy. Springer-Verlag, New York.

Greene, C.S. 1979. Responses of benthic infauna to the initiation and termination of sewage discharge. Task Report to the U.S. Environmental Protection Agency, Grant R801152, Corvallis, Ore.

Gundlach, E.R., C.H. Ruby, M.O. Hayes and A.E. Blount. 1978. The Urquiola oil spill, La Coruna, Spain: Impact and reaction on beaches and rocky coasts. Environ. Geol. 2(3): 131-143.

Gunnerson, C.G. and G. Peter. 1976. The Metula oil spill. NOAA Special Rep., NOAA, Environmental Research Laboratories, Boulder, Colo.

Hayes, M.O., E.R. Gundlach and L. D'Ozouville. 1979. Role of dynamic coastal processes in the impact and Dispersal of the Amoco Cadiz oil spill (1978), Brittany, France. In: Proceedings 1979 Oil Spill Conference, March 19-22, 1979, Los Angeles, Ca., pp. 193-198. American Petroleum Inst., Washington, D.C.

Hendricks, T.J. 1977. Coastal currents. In: Coastal Water Research Project Annual Report 1977, pp. 53-62. Southern California Coastal Research Project, El Segundo, Calif. (PB 299830/AS, National Technical Information Service, Springfield Va.).

Iles, T.D. and M. Sinclair. 1982. Atlantic herring: Stock discreteness and abundance. Science 215: 627-33.

Jumars, P.A. 1981. Limits in predicting and detecting benthic community response to maganese nodule mining. Mar. Min. 3: 213-229.

Kineman, J.J., R. Elmgren and S. Hansson (eds.). 1980. The Tsesis oil spill: Report of the first yeart scientific study. NOAA, Office of Marine Pollution Assessment, Boulder, Colo.

Klapow, L.A. and R.H. Lewis. 1979. Receiving water studies for preliminary design. In: S.H. Jenkins (ed.), Marine Municipal and Industrial Waste Water Disposal. Progress in Water Technology, Vol. 4, pp. 77-86. Pergamon Press, New York.

Malone, T.C. 1982. Factors influencing the fate of sewage derived nutrients in the lower Hudson estuary and New York Bight. In: G.S. Mayer (ed.), Ecological Stress in the New York Bight: Science and Management, pp. 389-400. Estuarine Research Federation, Charlotte, N.C.

MBC (Marine Biological Constultants, Inc.). 1973. Montecito Sanitary District: Biological survey of receiving waters, Mar. Biol. Consult., Inc., Costa Mesa, Calif.

Mearns, A.J. 1981. Effects of municipal discharges on open coastal ecosystems. In: R.A. Geyer (ed.), Marine Environmental Pollution, Vol. 2, Dumping and Mining, pp. 25-66. Elsevier, Amsterdam, Netherlands.

Mearns, A.J. and J.Q. Word. 1982. Forecasting the effects of sewage solids on marine benthic communities. In: G. Mayer (ed.), Ecological Stress in the New York Bight: Science and Management, pp. 495-512. Estuarine Research Federation, Charlotte, N.C.

Mearns, A.J. and D.R. Young. 1978. Impact of nearshore development on open coastal resources. In: J.N. Baskin, M.D. Dailey, S.N. Murray and E. Segal (eds.), The Urban Harbor Environment, Proceedings of the First Symposium, pp. 23-47. Southern California Ocean Studies Consortium, Harbor Plaza, Long Beach, Calif.

Oakley, H.R. 1979. Application of field data to system design. In: S.H. Jenkins (ed.), Marine Municipal and Industrial Waste Water Disposal, Progress in Water Technology, Vol. 4, pp. 167-178. Pergamon Press, New York.

O'Connor, T.P., Akira Okubo, M.A. Champ and P.K. Park. 1983. Deep ocean dumping of sewage sludge. Can. J. Aquat. Fish Sci.

OOME (Office of Ocean Minerals and Energy). 1981. Deep sea bed mining: Final programmatic environmetal impact statement, Vol. I. NOAA, Washington, D.C.

Oshida, P.S., T.K. Goochey and A.J. Mearns. 1981. Effects of municipal wastewater on fertilization, survival and development of the sea urchin, Strongylocentrotus purpuratus. In: Vernberg, F.J., A. Calabrese, F.P. Thurbert and W.B. Vernberg (eds.). 1981. Biological Minitoring of Marine Pollutants, pp. 389-402. Academic Press, New York.

Oshida, P.S., S. Bay, A. Haeckl, K. Goochey and D. Greenstein. 1982. Seawater and wastewater toxicity experiments. In: Coastal Water Research Project Biennial Report 1981-1982. Southern California Coastal Water Research Project, Long Beach, Calif.

Reish, D.J. 1980. Effects of domestic wastes on the benthic marine communities of southern California. Helgol. Meeresunters. 33: 377-383.

Risebrough, R.W., B.W. DeLappe, E.F. Letterman, J.L. Lam, M. Firestone-Gillis, A.M. Springer and W. Walker, II. 1980. California Mussel Watch 1977-78: Vol. III. Organic pollutants in mussels, Mytilus californianus and M. edulis, along the California coast. Water Quality Monitoring Rep., State Water Resources Control Board, Sacramento, Calif.

SCCWRP (Southern California Coastal Water Research Project). 1978. Report to LA/OMA on Task Order No. 3: Forecast effects. In: Modification of Secondary Treatment Requirements for Discharges into Marine Waters: Hearings Before the Subcommittee on Water Resources of the Committee on Public Works and Transportation, House of Representatives, Ninety-Fifth Congress, Second Session, May 24 and 25, 1978, pp. 133-224. El Segundo, Calif. (U.S. Government Printing Office, Washington, D.C.)

Schafer, H.A. 1980. Characteristics of municipal wastewater. In: Coastal Water Research Project Biennial Report 1979-80, pp. 235-240. Southern California Coastal Water Research Project, Long Beach, Calif.

Swanson, R.L., M.A. Champ, T. O'Connor, P.K. Park, J. O'Connor, G. Mayer, H. Stanford, E. Erdheim and J.L. Verber. 1982. Sewage sludge dumping in the New York Bight Apex: A comparison with other proposed ocean dumpsites. Draft manuscript, NOAA, Office of Marine Pollution Assessment, Rockville, MD.

Teal, J., B. McCain, P. Jumars, J. Stegeman and R. Howarth. 1979. Biological effects--A generic view. In: E.D. Goldberg (ed.), Proceedings of a Workshop on Scientific Problems Relating to Ocean Pollution, Estes Park, Colo., July 10-14, 1978, pp. 204-219. NOAA, Environmental Research Laboratories, Boulder, Colo.

Thebeau, L.C. and W.T. Kana. 1981. Onshore impacts and clean-up during the Burmah Agate oil spill--November 1979. In: Proceedings 1981 Oil Spill Conference, March, 2-5, 1981, Atlanta, Ga., pp. 139-146. American Petroleum Inst., Washington, D.C.

Vandermeulen, J.H. 1977. The Chedabucto Bay spill--Arrow, 1970. Oceanus, 20: 31-39.

Young, D.R. and I.S. Szpila. 1975. Decreases of DDT and PCB in mussels. In: Coastal Water Research Project Annual Report, 1975, pp. 123-126. Southern California Coastal Water Research Project, El Segundo, Calif. (PB 274467/AS, National Technical information Service, Sprinfield, Va.)

Young, D.R., T.-K. Jan and T.C. Heesen. 1978. Cycling of trace metals and chlorinated hydrocarbon wastes in the Southern California Bight. In: M.L. Wiley (ed.), Estuarine Interactions, pp. 481-496. Academic Press, New York.

Chapter 9. Summary and Synthesis

Summary and Synthesis

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INTRODUCTION

This chapter attempts to summarize and synthesize the weaknesses and strengths of environmental assessments that have been pointed out in this book. It is an overview of some of the important problems and questions, as well as the opportunities, we now face in developing better and more meaningful measures of marine pollution effects. Wherever possible, the chapter presents the ideas and opinions that were generally expressed at the workshop where these papers were first presented. However, in order to highlight the weaknesses of presently available means for measuring such effects and to suggest possible improvements, it has been neither possible nor desirable merely to restate views expressed in earlier chapters. Instead, this chapter presents the opinions of its authors on the lessons from past experiences and the directions that should be taken to improve pollution assessments.

DEVELOPMENT OF ENVIRONMENTAL ASSESSMENT

The papers in this volume provide a survey of the techniques and methods presently used to assess man's pollution impact on the marine environment. At the workshop where the papers were presented, the participants generally agreed that assessment

capabilities have been improved over the past decade. However, many participants expressed some dissatisfaction with the direction and rate of that development.

Such dissatisfaction can be traced back to the relatively recent and somewhat chaotic emergence of strong public concern with marine pollution. During the 1960s and 1970s, justified public outcries set an environmental protection process in motion before governments or, for that matter, scientists were adequately prepared to recommend appropriate actions. For example, with few exceptions, the first courses dealing with the science of pollution effects were introduced at American universities at approximately the same time that the U.S. Environmental Protection Agency (EPA) was established. Yet the newly formed agency was mandated to base its programs of environmental protection on this still nascent area of applied science.

Even today, few universities offer training in hazard assessment, that multi-disciplinary procedure that combines information on pollutant loadings, fates, ecosystem effects, human health risks, and economics in order to evaluate the probable consequences of pollution. However, it is now clear that such a holistic approach must be used to provide a satisfactory basis for rational decision making. Further, the public must be informed of the need for such an approach and thereby develop realistic expectations for pollution control effects. At present neither the public nor their legislative representatives have a clear grasp of the tremendous complexity of this assignment.

Some technical mistakes of the past--such as those pointed out by Smith and by Patton and Couch (this volume), where nonspecific responses were measured in the field without valid controls--need never be repeated. The lessons learned from such past errors must, however, be brought to the classroom, so that the next generation of environmental scientists is trained to recognize and follow good experimental design. Further, such training must break away from the narrow disciplinary approach that has dominated many environmental science curricula in the past. Successful environmental assessments have amply demonstrated the need for interactive teams, not just superficial exchanges of information among specialists. We need to train people who are comfortable in such teams and who can understand, operate in, and interact with the array of traditional disciplines that are required to conduct complete hazard assessments of marine environmental problems.

A further difficulty that has plagued the development and application of environmental science is the high frequency of poor communication among research managers, regulators and scientists. This situation may improve as students trained in the holistic hazard assessment approach move into environmental research and management positions. Nevertheless, environmental regulators must make decisions, whether or not these are based on scientific-

ally defensible assessments. Too often at present regulators fail to express their needs and sense of inadequacy so that research scientists can provide advice with the necessary scope and focus. On the other hand, it is not unusual for scientists to build their entire careers around narrow specialties, with the result that they become myopic in their approach to environmental problems and so advocate underdeveloped or even inappropriate methods for the solution of complex problems.

These are not the only stumbling blocks, of course. Lawmakers are subjected to intense lobbying from all sides and must depend on the advice they receive, whether good or bad. Adequate funding is a perennial problem. Furthermore, funding agencies by the very nature of the legislative process operate largely on one-year budget cycles, thus making it difficult to maintain support for vital long-term studies. Public and Congressional priorities shift rapidly, creating an ephemeral atmosphere in which directed investigations move from one crisis to the next and never expend the concerted effort needed to reach long-term solutions.

CRITICAL OVERVIEW OF THE STATUS OF POLLUTION ASSESSMENT STRATEGIES AND METHODS

Need to Apply the Scientific Method

Too frequently environmental studies, ostensibly conducted to determine pollution effects, fail to advance beyond the preliminary descriptive phase of the scientific method. Several papers in this volume, including those by Leffler, Heck and Horwitz, Carpenter and Huggett, and Green, point out the need for field studies to move to the hypothesis and testing phases of the scientific method. Heck and Horwitz contend that most field impact studies are observational rather than experimental, and therefore cannot establish causation. Carpenter and Huggett state that while "emphasis on rigorous testing can be overdone when the hypotheses are trivial, data collection without clear hypothesis formulation is really the cause of the prevention of environmental science developing as a science." They observe that several recent expensive government programs have collected descriptive data for purposes that have not been clearly defined. If such programs are to help in solving pollution problems, they must be directed at quantifying processes connecting loadings, fates, ecological effects, human health risks, and economic impacts related to the specific problems under consideration. The scientific method should be adhered to rigorously and vigorously. Hypotheses should be established with respect to quantification of each process, and the requisite data should be gathered as fully and precisely as required to test these hypotheses.

Choice of Meaningful End Points

One of the most pressing, but too often ignored, demands in any environmental assessment is to define meaningful assessment end points, i.e., measures of impact whose importance is widely accepted. Both Duke and Green urge scientists to state clearly the references against which the significance of detected impacts have been judged. Not surprisingly, researchers' diverse expertise, environmentalists' particular values, and regulators' specific mandates often color what are viewed as meaningful end points. Grice considers impacts on long-lived species to be especially important. Malins et al. suggest that measurements of the uptake and effects of contaminants on organisms can be very useful in conjunction with measurements on the sediment distribution of those contaminants. Both Jeffries and Livingston believe that critical end points are those reflecting ecosystem-wide responses, and Jeffries goes further to say that meaningful emergent properties at the ecosystem level can be defined in a straightforward manner. Other end points suggested include mortality, fecundity, growth rate, age distribution, population stress indices, and community change indices (Hansen, Gentile and Schimmel, Hinton and Couch, Singleton et al., Azam et al., Grassle and Grassle, Smith, and Saila et al., inter alia). Obviously there is little agreement on what these measures should be. Two generalizations can be made, however: (1) measures that can be easily judged by human values (e.g., effects on commercially important fish stocks) are usually more meaningful than more esoteric ones and (2) impact end points related to higher levels of biological organization (i.e., communities and populations) are more meaningful than those related to lower levels (i.e., individual organisms or their organs, tissues, and cells).

Proper Use of Statistics

Many papers in this volume raise questions about application of statistics to environmental data and the use of statistics in analysis and interpretation. Taub indicates that microcosm data have many of the same problems that characterize data from natural ecosystems: time series data are not statistically independent, replicate systems are often out of phase with each other, and many of the data sets are noisy. Heck and Horwitz caution that analysis of variance is merely an exploratory technique that should be used to identify consistency of association rather than causality. Further, they contend that in the rush to appear quantitative the potential analytical rigor of statistics has been diluted through widespread misapplication. Carpenter and Huggett emphasize that water-column and sediment concentrations of chemical contaminants and their derivative species are highly variable in both time and space. Hence, when field exposure concentrations are measured, uncertainty estimates (variance and standard error) should be determined and stated. Indeed, uncertainty and probability state-

ments may be a common ground between science and the societal judgements underlying legislation and litigation. If lawmakers, regulators and judges are apprised of the reliability of scientific results, then they have a basis for weighing scientific determinations against other societal judgements.

Inadequacy of Diversity Indices

Several authors in this volume warn that diversity indices are clearly inadequate as the sole measures of ecological impacts of contaminants. Smith indicates that, while effects of pullutants can be reflected by changes in community composition, diversity indices are often too insensitive to be good indicators of such changes. However, Saila et al. report on a situation where informed use of such techniques leads to detection of significant differences between a relatively disturbed and a relatively undisturbed environment. They believe that more discriminative application of diversity formulae can at least help to guide more definitive studies. Heck and Horwitz on the other hand contend that diversity measures are too simplistic to reflect the complexities of ecosytems. Finally, Green cautions that diversity indices may be merely artificial mathematical expressions that only marginally address the larger questions of ecological significance.

Need for Verification

Several chapters in this book express concern that predictions of chemical fates, bioaccumulation, and dose responses which are based solely on laboratory results for single species bear little relation to actual events in the field. Field verifications for such predictions have not often been conducted, but when they have been, the predictions have usually proven insufficient to describe events in the real world (Pritchard and Bourquin; Cairns and Buikema). Hansen points out that even simple toxicity tests are rarely conducted under a sufficient variety of conditions to reflect the large range in potential situations found in the field. Gentile and Schimmel show that sometimes the predictions are improved substantially when the results from a number of different tests are considered simultaneously. Hartung, as well as Gentile and Schimmel, makes an important distinction between predictive and retrospective assessments and suggests that the latter can be used to verify the former. Bender et al. discuss the Kepone situation as an excellent example of the need to demonstrate field accuracy of laboratory predictions.

Recently several innovative approaches have been emphasized to bridge the gap between laboratory results and actual field conditions. These include experiments conducted in elaborate but controlled laboratory regimes that simulate many aspects of natural regimes; studies that take advantage of accidental spills and discharges; and projects that bound portions of nature and artificially

contaminate them. These more natural test regimes have been used to compare and calibrate simple laboratory tests. Whatever the approach, however, field verifications of laboratory-based predictions are urgently needed, because unverified predictions--albeit often the only feasible approach at present--are never completely definitive.

Appropriate Role for Toxicity Testing

In the past, laboratory testing to determine the toxicity of specific hazardous substances to specific biological species has played a predominant role in providing the basis for regulation of the marine environment. Hansen, Gentile and Schimmel, Cairns and Buikema, Pritchard and Bourquin, and Hinton and Couch raise basic questions as to what role such tests should play in the future, which tests are most meaningful, and, more specifically, what weight should be accorded the results (e.g., LD50's) of such testing.

Lately there has been a de-emphasis on toxicity tests that determine mortality and more emphasis on those that measure sublethal responses. However, sublethal effects assume significance only when they can be placed in a larger context of what they actually mean at the level of individual organisms, populations or communities. Sublethal effects should be related quantitatively to mortality, growth and/or reproduction; taken alone and out of context, such effects do not constitute an appropriate basis for regulation.

Further, both lethal and sublethal tests merit close examination to eliminate artificial construction and to delineate their most meaningful aspects. For example, it is necessary to decide which of the many quantifiable properties of dose-response curves should be used. It is important to ask which properties best describe effects that have impacts on other components of the ecosystem. The slope of the dose-response curve may be more closely related to substantial ecosystem effects than is the commonly used 50% effect level. As a first approach, the significance of various test responses can be analyzed using one of the field verification techniques cited above. Such an examination or crude validation of presently employed "quick-fix" tests will provide guidance to channel resources into the more productive science efforts as well as identify the value of toxicity testing in support of legislation and litigation.

In general, it can be concluded that past programs of environmental regulation have tended to rely too heavily on toxicity testing, probably because there have been few practical alternatives. However, the techniques of hazard assessment have now advanced to the state where primary reliance on such testing should no longer occur. This does not imply, however, that toxicity testing should be abandoned. On the contrary such testing will be required for the foreseeable future, but only as an initial part of the com-

plex process of hazard assessment. Gentile and Schimmel, Cairns and Buikema, and Pritchard and Bourquin express the clear need for such tests as a first step to guide the design of definitive field studies as well as to confirm, evaluate and establish the significance of these studies.

Measurement of Significant Chemical Parameters

Several contributors discuss the critical distinction that should be made between analytical chemistry results and the biological significance of those results. Carpenter and Huggett emphasize the large difference that often exists in the oceans between the total concentration of a metal and the amounts of that metal that are present as bioavailable toxic species. Malins et al. warn that when a compound disappears from the analytical window, a more toxic degradation product which is not being measured, or even possibly cannot be detected with the techniques presently available, may have been formed. They also agree with Carpenter and Huggett that the bioavailability and toxicity of the species of toxic materials found in an environment, not just the total concentrations present, are the parameters of significance. Hence, there is a general call to avoid environmental surveys of chemical contaminants unless other measurements that allow one to calculate and interpret the significance of such data are included.

Interpretation of Bioaccumulation Measurements

Several authors underline the need for information on the relationship between bioaccumulation and effects. Peddicord, in fact, points out that, if we fail to discover the connection between tissue burdens and effects, then the only utility of bioaccumulation measurements is to determine whether seafood concentrations exceed regulatory action limits for human consumption. The influences of biological and physical-chemical properties on the accumulation-effects relationship should be quantified for important contaminants. For example, Carpenter and Huggett remark that the bioavailability of a contaminant may depend less on its total content in the sediment than on the organic content of the sediment and the chemical form in which the contaminant occurs. As a first step toward studying effects of tissue burdens, Jenkins and Brown describe studies on detoxification mechanisms which effectively sequester bioaccumulated contaminants. Detoxification processes are one of the reasons that there is often poor correlation between body burdens and effects.

As discussed by Peddicord and by Young, food chain biomagnification occurs with far fewer contaminants than once thought. A systematic approach is needed to discover which chemicals biomagnify and how the physical-chemical environment and the taxonomic composition of the food chain affect this process. Young elaborates on the difficulties in determining biomagnification

potential and links these difficulties to our inability to assign organisms to trophic levels.

Utility of Micro- and Mesocosms

Several papers emphasize the potential utility of micro- and mesocosms in environmental assessments. Donaghay points out that some systems of this type already stand ready to assist in fates and effects forecasting and in data gathering for management purposes. Santschi et al., Pilson, and Oviatt all discuss promising directions for use of mesocosms, particularly as models for determining an environment's recovery potential and recovery rate once contamination has ceased; for predicting fates and effects of new chemicals as well as those that have been used for many years; and for determining contaminant effects on the highest levels of biological organization. Grice states that micro- and mesocosms can be more sensitive than conventional toxicity tests, at least in detecting pollutant effects on plankton. Taub suggests that microcosms may aid in establishing the laboratory-to-field link by identifying those ecological components and processes (e.g., nutrient cycling) that are most likely to be disturbed by a given stress. With such identification the response of these components and processes to contaminants can be examined individually in controlled experiments. Grice identifies an important limitation of micro- and mesocosms: as presently designed, they cannot accommodate most commercial species, although juveniles and, in a few cases, even small adults of these species can sometimes be included. Considering the wide experimental applications and unique advantages of micro- and mesocosms, it behooves knowledgeable scientists to explain the properties of these relatively new techniques to the regulatory community and to recommend applications to meet specific regulatory needs.

Requirements for Long-Term Studies

A call for long-term studies is made by a number of contributors, including Oviatt, Green, Livingston, Malins et al., and Smayda. Although no explanation of "long-term" is offered, such studies can be viewed as involving sufficient data-gathering efforts in time and space so that natural variability can be statistically separated from variability due to anthropogenic activity. Such data bases may take years to decades to acquire. Smayda illustrates the folly of drawing conclusions about impacts from a relatively short time series of highly variable data. On the other hand, Mearns and O'Connor show what can be learned from a data base that is sufficient to establish quantitative relationships between contaminant loadings and spatial extent of impacts. Of course, the ecological significance must still be substantiated, but meanwhile their study demonstrates the advantages of a sufficiently dense data base. Several authors, including Malins et al., Green, and Liv-

ingston, note that long-term projects require large amounts of stable funding. To assure maximum return from funding of pollution research and monitoring, sponsoring agencies should develop means to make commitments to long-term studies when these are called for.

RECOMMENDATION—THE DEVELOPMENT OF HOLISTIC STRATEGIES

In this volume, Green emphasizes that a single index cannot usually reflect the environmental health or well-being of an area, but rather a proper "mix" of measures is necessary. Duke expresses the need for a "balance" between laboratory and field studies in order to provide meaningful assessments, while Smayda calls for an approach that is a "hybrid" of conventional monitoring, process-oriented studies, and mesocosm experiments. Certainly assessments should be carried out by combining various individual techniques, however, this must be done in an integrated and coordinated manner. In the past environmental impact assessments have often included scores of individual studies, on the justification that an "interdisciplinary approach" was thereby achieved. Unfortunately, such assessments routinely failed to stipulate how their parts fit together, how conflicting indicators were to be combined, and how the results obtained by one investigator in the "mix" related to those of the others. The interaction of the pieces to form a whole has rarely been provided for. Unless we take steps to assure that environmental assessments follow a holistic approach, these assessments will continue to fail in meeting even limited goals.

Under special circumstances certain aspects of a holistic assessment may be shortened or even eliminated. In general, however, the following broad elements are required:

1. Determination of contaminant loading. How much enters the marine environment, from which sources, and in what geochemical forms?

2. Determination of physical transport and biogeochemical fate. Where and at what rate does the contaminant move? At what rate does it enter various sinks? How quickly does it degrade? How much exists in a bioavailable form and in which environmental compartments?

3. Determination of ecological effects. How are primary and secondary productivity and nutrient cycles affected? How do food chain dynamics shift? What are the most sensitive ecosystem compartments? At what rate is the contaminant taken up by various organisms from various sources? At what rate is the contaminant bio-

transformed and eliminated? What is the effect of body residues and biotransformation products on growth, survival, and mortality of various organisms and populations throughout their life cycles? How do these population changes affect other compartments of the ecosystem?

4. Determination of human risks. What are the effects of the above phenomena on human health, fisheries, and other human activities? What are the economic consequences of the contaminant? What are the relative values placed by society on these effects?

The holistic approach clearly calls for mathematical models of the behavior of contaminants and the systems they enter. In retrospect, it is unfortunate that a chapter on assessment modeling is not included in this volume to evaluate the present status of fates, hazards, and risk assessment models and their adequacy or inadequacy to meet assessment needs. As a first step, however, assessments should be required to develop holistic conceptual models of the systems being studied. These should then serve to catalyze the construction of functional models and to integrate the various components of the assessments.

A piecemeal approach to environmental assessment does not often work. Non-interacting investigators measuring different processes with incompatible methods and at different times and sites usually cannot a posteriori reconstruct a holistic picture linking contaminant loading to important impacts. Rather, interacting investigators must study the same contaminant problem at the same site, synergistically building a coherent and unified model. It is not the complexity of the holistic approach that stands in the way, so much as the common failure so far to organize interactive teams at the start of an assessment and to assure that these teams consider all the elements necessary to analyze contaminant problems of concern.

We challenge the environmental science community to consider the following issues seriously:

- What general scientific problems are encountered in the construction and verification of a unified, quantitative model tracking a particular contaminant from its initial loading to its ultimate effects on ecosystems and humankind? What legislative and political problems are encountered? How are these various difficulties best overcome?

- Which contaminants and environments lend themselves best to the holistic approach? Should these necessarily be the initial focus of attention?

- How best does one solicit, support, and manage a team of specialists working on a holistic approach, especially if they must be drawn from geographically separated institutions?

- How long does a holistic assessment require for some typical contaminant problems? What should be the products of such a program? Who will use the products, and what limitations will the users encounter?

- Which suite of governmental, academic, private, and other institutions is best suited to guide debate on the above issues? Which is best suited actually to develop specific holistic assessments? How can public education and public participation facilitate these endeavors?

Although these questions have been directly treated by few authors to this time, they point the way to the challenging and vital problems that must be resolved if we are to develop truly meaningful environmental assessments.

Index