Natural Toxins and Human Pathogens in the Marine Environment

Rita R. Colwell
Editor

NATURAL TOXINS AND HUMAN PATHOGENS IN THE MARINE ENVIRONMENT: PROBLEMS AND RESEARCH NEEDS

Report of a Sea Grant-Sponsored Workshop

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Rita R. Colwell, Workshop Leader

INTRODUCTION

The National Sea Grant College Program is charged with promoting the development and wise use of our nation's marine resources through the utilization of the talents of American universities. Currently our nation's ability to harvest its abundant shellfish and tropical finfish resources is limited to a great extent by public health problems. These public health problems are associated both with the presence of pathogenic bacteria and viruses in the marine environment and with outbreaks of paralytic shellfish poisoning and ciguatera caused by naturally occurring toxins. Significant increases in shellfish and tropical finfish utilization will require that these public health threats be better controlled and alleviated.

In November 1982, a workshop was held to identify how university research supported by the National Sea Grant College Program can best address these issues. Knowledgeable workshop participants were invited from the university community, from state and federal agencies with regulatory responsibilities, and from the seafood industry. During the workshop, the participants addressed the following:

1) What barriers exist, with respect to pathogens and natural toxins, to the greater utilization of the shellfish and tropical finfish resources of the U.S.?

2) What information do managers, regulators, and processors need to overcome these barriers?

3) Which needs can be best addressed by university based research efforts?

The workshop was structured around four working groups, 1) ciguatera, 2) paralytic shellfish poisoning, 3) viruses and 4) bacteria and newly recognized pathogens. Plenary sessions were held, facilitating interchange and cross-fertilization of ideas among working groups.

The goal of the workshop was to produce, for each area, a guidance paper identifying barriers to increased utilization and identifying the appropriate research needed to overcome barriers that were identified. Wherever possible, the participants placed priorities on the research needs. The guidance papers presented in this report are structured to identify succinctly the priority research areas formulated by the workshop participants.

One of the main purposes of the workshop was to bring members from several disciplines together to discuss the direction of future research endeavors related to disease agents, particularly with regard to Sea Grant supported activities. The discussions focused primarily on priority research areas for Sea Grant to assist program directors and the national office of Sea Grant in guiding the substantial ongoing Sea Grant

research program directed to natural toxins and pathogens in the marine environment. It is hoped that this report will also be helpful to other agencies and groups involved in this field of research.

EXECUTIVE SUMMARY

Chapter 1. Ciguatera Seafood Poisoning: A Circumtropical Fisheries Problem

Ciguatera is a serious human illness resulting from the consumption of coral reef-associated fish that accumulate ciguatoxin through their diet. Thousands of inhabitants and tourists in tropical areas of the world are affected annually and the resulting publicity has impacted the sale of seafoods in tropical and subtropical regions. A variety of commercially important reef fish may contain the currently undetectable, heat-stable toxin, thereby presenting a real threat to development of selected tropical fisheries. Research needs include the development of market-place detection methods for ciguatoxin in suspect fish, a thorough description of the molecular structure of the toxin, delineation and culturing of ciguatoxin-producing dinoflagellates, a comprehension of the mechanism by which the toxin is introduced into the ecosystem, and an understanding of the toxin's mode of action through the utilization of animal models. An inherent problem in conducting these proposed chemical and biological studies is the lack of sufficient quantities of ciguatoxin. Laboratory cultures of dinoflagellates have not yielded the expected amount of ciguatoxin-like material. Because other toxic components are produced which may play a role in the disease process and culture conditions optimum for ciguatoxin production have not been determined, large-scale extraction of suspect fish remains the only means of acquiring a reasonable supply of ciguatoxin.

The priority areas of needed research are:

Laboratory Production of Ciguatoxin

* Determine the culture conditions for production of toxin(s) in the laboratory.
* Develop culture system(s) for toxin production.

Chemical Research

* Determine the molecular structure of ciguatoxin.
* Identify the chemical composition of other toxins often found in association with ciguatoxic fish and determine their relationship, if any, to ciguatoxin.

Dinoflagellate Research

* Determine the growth phases and environmental conditions which support the biosynthesis of ciguatoxin in unialgal and axenic cultures.
* Isolate and culture species of dinoflagellates which occur in regions known to harbor ciguatoxic fish and test for toxin(s) production.

* Chemically and biologically define toxins produced by dinoflagellates that exhibit properties similar to ciguatoxin.
* Determine the mechanism by which dinoflagellates introduce toxin(s) into the ecosystem.

Ciguatoxin Detection and Analysis

* Develop sensitive immunological methods for the detection of ciguatoxin and associated toxins.

Toxicology Studies

* Elucidate the molecular events involved in ciguatera poisoning.

Chapter 2. Human Viruses in the Marine Environment: The Problem and Research Needs

Different types of fecal waste discharges, including those from conventional treatment systems, package plants, lagoons, septic systems, boats and marinas and nonpoint sources such as run-off from community and animal feed lots and sludge dumping operations result in the entry of varying amounts of more than one hundred virus types into coastal waters used for recreational purposes and containing shellfish and other seafood sources. Some of these viruses, notably hepatitis A and the Norwalk-type gastroenteritis viruses, have been responsible for disease outbreaks due to contaminated bathing water and edible molluscan shellfish.

The contamination of coastal environments by human viruses of public health significance underscores the need for virologic surveillance, assessment of public health hazards and development of plans to keep the coastal environments clean and safe. Available data from past work dealt only with more widely studied and easily cultivable viruses such as polio-, coxsackie- and echoviruses. Health effects significance of difficult-to-cultivate gastroenteritis viruses (rotaviruses, Norwalk-type viruses and several other candidate viruses) and hepatitis A virus in coastal environments is yet to be understood. Only when methods for efficient and rapid cultivation or other means for the detection of these viruses are developed, will the full extent of health hazards due to water and shellfish transmission of viruses be known. For accomplishing this goal, research recommendations have been made for enteric viruses in general, but with priority of attention directed toward hepatitis virus A, and gastroenteritis viruses. These include: 1) improved concentration and reconcentration from estuarine water, shellfish and sediments; 2) rapid detection methods with sensitivity and specificity; 3) round-robin or cooperative testing to verify methods acceptability; 4) assessment of the significance of highly polluted and less polluted coastal waters; 5) transport, distribution and survival of viruses in both highly polluted and less polluted coastal waters; 6) bio-accumulation, extent of elimination and low-level persistance of viruses in shellfish under relay and de-

puration conditions, and 7) an appraisal of whether fecal indicator bacteria or easily cultivable enteroviruses can indicate potential health hazards due to hepatitis virus A and gastroenteritis viruses in coastal environments.

The priority areas of needed research are:

Virus Cultivation and Assay

* Develop and/or further improve cultivation and infectivity assay methods for HAV, Norwalk-type viruses, HRV and other difficult-to-cultivate viruses, as well as other enteric viruses.

Methods for Recovering and Detecting Viruses in Coastal Environments

* Evaluate and develop improved methods for recovering all viruses from coastal waters, shellfish, sediments and suspended solids.

Control of Virus Contamination of Shellfish by Relay and Depuration

* Determine the rate and extent of elimination of enteric viruses and fecal indicator bacteria by shellfish under relay and depuration conditions, especially for HAV and Norwalk-type viruses.
* Determine, quantitatively, how environmental factors influence the rates at which shellfish eliminate microbial contaminants.
* Determine the persistence of viruses at low levels in shellfish under relaying and depuration conditions, and the reliability of fecal indicator bacteria and other potential fecal indicators to reflect the virological quality of relayed or depurated shellfish.

Occurrence, Transport, Distribution and Survival of Viruses in Coastal Environments

* Determine the extent to which geographic and temporal differences in the physical, chemical and biological composition of coastal waters and sediments are responsible for differences in virus survival.
* Determine with comparative studies the occurrence, transport, distribution and survival of difficult-to-cultivate and other viruses in coastal environments.
* Determine the relative importance of various environmental factors that may influence the transport and survival of viruses in coastal environments.

Epidemiology and Health Risk Assessment

* Use epidemiological approaches for assessing the health risk from exposure to viruses in coastal environments, including the determination of dose-response relationships and the sanitary quality of potential viral vectors.

Characterization and Control of Sources of Viral Pollution in Coastal Environments

* Determine quantitatively the sources and significance of virus contamination in sensitive coastal environments.
* Develop new and improved treatment systems, waste management strategies and operational controls.

Chapter 3. Paralytic Shellfish Poisoning

A significant barrier to the optimal utilization of many shellfish resources (e.g. clams, mussels, oysters, and other bivalves) stems from the sporadic blooms of a variety of toxic dinoflagellates. These blooms represent serious economic and public health problems because of the resulting accumulation of paralytic, neurotoxic, or diarrhetic shellfish poison (PSP, NSP, DSP) in shellfish tissues in addition to the recently recognized adverse impacts on certain finfish resources. Not only are large expanses of the U.S. coastline currently affected, but there is strong evidence for a general increase in the magnitude, duration, and geographic distribution of the toxic outbreaks in recent years.

The nature of the toxic dinoflagellate problem requires a multidisciplinary approach. Not only is it necessary to study the ecology of the dinoflagellates, shellfish, and finfish involved, but it is also necessary to elucidate the toxin chemistry and the mechanisms whereby the toxin is accumulated and transformed during its movement through trophic levels. The working group felt it necessary to designate general categories, each containing several specific research programs. We feel that the areas listed below should be considered priority research directions warranting concurrent funding. Other less pressing needs (or those that must follow other studies) are included in the text.

Resource/Toxin Interactions

* Determine rates of toxification and detoxification in affected shellfish species of the toxins PSP, NSP, or DSP.
* Develop a rapid, sensitive, simple, and inexpensive assay method for each kind of dinoflagellate toxin.
* Determine the alternative vectors for toxin transfer and the magnitude of the impact. Emphasis should be placed on the effect of toxic dinoflagellates on commercially important finfish, both adult and larval stages.
* Determine the role played by direct ingestion of toxic cysts in toxic outbreaks.

Toxins

* Expand existing knowledge on the structure and distribution of dinoflagellate toxins, in vivo, in vitro, and following their bioconversion in shellfish.

* Study the physiology of the differential binding of shellfish tissues (in one species and between species) for toxins.
* Investigate the use of toxin composition as a chemo-taxonomic tool or "fingerprint" to detect relationships between dinoflagellate populations.
* Establish a source of reference standards.
* Study life cycle and environmentally-induced changes in toxin levels and composition.
* Study depuration and processing methodologies to permit canning or utilization of PSP-contaminated stock.
* Investigate the previously unrecognized diarrhetic shellfish poisoning (DSP) problem to learn its magnitude and distribution. This potentially serious problem has long been misdiagnosed to be of bacterial or viral origin.

The Organisms

* Determine the mechanisms controlling the encystment/excystment process whereby dormant cysts are formed during toxic blooms and motile, vegetative cells are introduced at the beginning.
* Study the vertical and horizontal distribution of dinoflagellate populations through time and under a variety of hydrographic conditions. This work should be conducted on a regional basis since extrapolations from one area of the U.S. to another will be of limited utility.
* Determine the quantitative role of cysts in bloom dynamics, and general cyst distribution patterns (horizontally and vertically) in the sediments.
* Examine the genetic variability between toxic populations (in terms of toxin composition, growth tolerances, etc.) and assess the implications of the observed differences.
* Explore the significance of endocellular bacteria or viruses as possible regulators of toxin production.
* Determine the abundance, feeding rates, and feeding strategies (e.g. preferences, avoidance) of relevant herbivores in areas subject to shellfish toxicity. This information is important both in terms of regulation of dinoflagellate population development and the transfer of toxins through trophic levels.
* Define the response of the vegetative cells to various combinations of light, temperature and nutrients.
* Study the importance of heterotrophic nutrition and exogenous growth factors in dinoflagellate growth.
* Establish and maintain culture archives of important strains and species.

Environments

* Study the factors that lead to localization of toxic blooms in time and space.

* Accumulate "surface truth" data bases and develop techniques to best utilize remote sensing as a tool for long range monitoring and predictions of dinoflagellate distributions.
* Determine the relative magnitude of human impact on the toxic dinoflagellate phenomena. Such factors as dredging, effluent discharges, and shellfish transplants are of concern.

Prediction and Control

* Recognizing that much of the preceding information is needed before realistic efforts can be made towards prediction and/or control, projects are nevertheless needed that examine the sensitivities of the target species to various control methodologies (chemical, biological and physical). Since manipulations are unlikely to be successful in open waters with large water masses and transport systems, initial emphasis should be restricted to confined natural systems or enclosures.

Future Workshop or Meetings

* There was a general consensus that the previous two international conferences on toxic dinoflagellate blooms provided an invaluable forum for exchange of information, techniques and ideas and that there is now a need for a third general meeting. The published proceedings of both conferences have proven to be important basic documents for those working in this field.

Chapter 4. Bacterial and Parasitic Pathogens in the Marine Environment

Bacterial pathogens and an expanding group of "newly recognized" pathogens including amoebae and nematodes pose a significant barrier to greater utilization of the Nation's seafood resources. Shellfish resources are particularly affected. A large percentage of the shellfish areas nationwide are closed to harvesting due to findings of bacterial contamination. In addition, incidences of food poisoning and other infections that are traced to seafood consumption cause xerious damage in consumer acceptance of seafood products.

The following presents the priority research needed to address the problems of bacterial and parasitic contamination in seafood. In addition, the importance and need for a nationally coordinated education and awareness program are presented. The priority areas for concurrent effort are:

Education and Public Awareness

* Develop and conduct standardized consumer and industry education programs on safe seafood handling requirements and methods.

* Identify the major seafood products that show inconsistency in processing and/or quality and determine practical means of improving quality.

Epidemiological/Ecological Studies

* Delineate the habitat (both macro and micro habitats) occupied by pathogens of interest.
* Determine the conservative and non-conservative limiting factors which control pathogen occurrence and survival.
* Delineate how pathogens are incorporated into shellfish or seafood and how the organisms are maintained, concentrated or grow.
* Determine how sewage and waste disposal (as well as other impact activities) influence the distribution, abundance, and pathogenicity of pathogens.

Detection of Potential Pathogens

* Develop diagnostic methods that are reliable, reproducible, economical and simple.
* Conduct taxonomic surveillance of atypical strains to detect unknown human and shellfish disease agents, food spoilage agents, and organisms with indicator potential.
* Use environmental concepts as a basis for the development and modification of enrichment and isolation media.
* Determine repair methods for stressed cells and identify gene products, such as intracellular enzymes, as a tool for rapid taxonomic identification of microorganisms.

Improved Bacterial Standards and Indicators

* Determine indicator-pathogen relationships in estuarine waters.
* Determine the densities of indicator bacteria and pathogens (both autochthonous and allochthonous) in shellfish obtained from growing areas of characteristic indicator densities.
* Determine bacterial concentrations in different shellfish growing area sediment types and determine factors affecting bacterial survival.
* Determine the significance of physiological stress on bacterial recovery in order to evaluate enumeration procedures and numerical standards.
* Determine the relationships between hydrographic characteristics and the distribution and densities of indicator and pathogenic bacteria.
* Develop improved detection and enumeration techniques for the presence of pathogens in seafood products.
* Evaluate the efficacy of the fecal coliform market standard in view of the occurrence of false coliforms and identify and determine the regional extent of false coliforms.

Human Pathogens in Marine Aquaculture

* Develop better methods of recognition of human fecal contamination of estuarine/coastal waters.
* Determine the effects of extensive aquaculture on the ecology of growout areas.
* Determine the survival characteristics of viruses and resistant stages of other microbial pathogens.

Newly Recognized Pathogens

* Develop methods for the isolation and identification of previously unrecognized pathogens.
* Develop better understanding of pathogenicity such as differences in penetration enzymes, host immunologic responses, host mimicry and life cycle stages.
* Determine environmental factors affecting the concentration and distribution of pathogenic parasites.
* Develop treatment methods for water contaminated with amoebae and other water-borne protozoans.
* Determine the immunological mechanisms of marine organisms.
* Develop rapid and reliable methods for detecting endopathogens in imported seafoods.
* Determine the undesirable chemical changes in seafood resulting from the enzymes secreted by parasites.

Seafood Processing Methods

* Develop improved seafood packaging and handling techniques.
* Determine the thermal death times of pathogenic marine bacteria and the effect of storage conditions on these organisms.

Genetics of Marine Bacteria

* Determine the molecular basis of virulence and the expression of virulence factors.
* Isolate genes encoding specific virulence factors.
* Construct probes for the detection of pathogenic isolates in a total population of pathogenic and non-pathogenic bacteria.
* Determine the expression of gene products in marine vibrios.
* Determine the extent and nature of interspecies or intergenic gene transfer or phase transition.
* Determine the interactions between pathogenic marine vibrios, the marine animal host and its environment.

Chapter 1

CIGUATERA SEAFOOD POISONING: A CIRCUMTROPICAL FISHERIES PROBLEM

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Introduction

There are several human illnesses associated with the consumption of particular seafoods that, by virtue of the publicity they have received, have a far-reaching, negative impact on the entire fishing industry. One such illness that seafood consumers in tropical and subtropical areas of the world have long recognized is the very unpleasant and potentially dangerous illness called "ciguatera." This particular poisoning is acquired by the ingestion of fish that accumulate through their diet a heat-stable, currently undetectable toxin named ciguatoxin. The fish may be any one of a wide variety of tropical or subtropical reef fish, including those of commercial and recreational value, such as snapper, grouper, barracuda, and amberjack. In addition to affecting public health directly, ciguatera can have a dramatic impact on the economy of an entire regional fishing industry through the local public's concern and apprehension regarding the safety of all seafoods. And while the illness is usually limited to the warmer climates of the world, ciguatera has been reported with increasing frequency in temperate climates due to the return of tourists afflicted with ciguatera, as well as intensified interstate commerce of tropical reef fish. Only recently have members of the northern U.S. fishing industry and physicians practicing in temperate climates become aware of this illness and of its dramatic impact on tropical fisheries. For more detailed information on ciguatera, the reader should consult the recent review article by Withers (1982).

Impact of Ciguatera on Human Health

Ciguatera is a serious health and socio-economic problem, involving the fisheries in tropical and semitropical areas of the world (Banner, 1976; Halstead, 1978; Helfrich and Banner, 1968). One difficulty in assessing the health impact of ciguatera is the absence of diagnostic means to identify and evaluate clinical cases. Reasonable estimates suggest that each year between 10,000 and 50,000 individuals are afflicted worldwide. Documented annual morbidity statistics for specific geographical areas range from approximately 0.5 cases per 1,000 population in low incidence areas such as Florida (Lawrence, et. al., 1980) to 27 cases per 1,000 population in high incidence areas such as the U.S. Virgin Islands (McMillan, Grande and Hoffman, 1980). Other

areas that experience frequent episodes of ciguatera include Puerto Rico, Fiji, American Samoa, French Polynesia, the Queensland coast of Australia and the British Virgin Islands (Bagnis, 1980; Dawson, 1977; Lewis, 1981). Fortunately, the mortality is less than 1 percent of reported cases (Lewes, 1981; Bagnis, Kuberski and Laugier, 1979). Even though ciguatera is not a reportable disease, the Center for Disease Control (Atlanta, GA) has listed ciguatera as the most frequently reported foodborne illness of chemical origin (Hughes, 1979).

Symptoms of ciguatera involve gastrointestinal, neurological, and cardiovascular systems, leading in extreme cases to death by respiratory failure and/or severe dehydration (Lawrence, et al., 1980; Bagnis, 1973). Diagnosis of ciguatera is based on clinical observation and patient history; there is no confirming laboratory test. An episode of ciguatera intoxication does not appear to confer immunity; in fact, an inhanced reaction to the pharmacological effects of the toxin may occur with successive exposures. The unusual neurological symptoms of paresthesia and temperature reversal are considered diagnostic hallmarks, and these often recur or are exacerbated following physical duress or the consumption of an alcoholic beverage. Unfortunately, there is no "cure" for ciguatera and management of patients is based solely on supportive measures.

Despite the absence of reliable morbidity statistics, it is immediately apparent that the illness is of widespread concern in both the Caribbean and South Pacific areas, where it presents a serious health hazard and limits the utility of fish resources in island regions whose populace depends on reef fish as an important source of protein.

Impact of Ciguatera on the Development, Growth and Stability of the Fishing Industry

Because of the publicity associated with the devastating long-term effects of this illness, ciguatera has had a dramatic impact on the tropical fishing industry and its associated trades. Public apprehension of seafood safety that borders on hysteria was dramatically illustrated by the public's reaction to a ciguatera case reported by the banner headline "Death at the Dinner Table" in the San Juan Star's Sunday Supplement (October 4, 1981). Unfortunately, ciguatera cases are often reported with melodramatic flair, which generates panic and forces health officials to confiscate restaurateurs' entire stock of fish, even when such fish cannot reliably be incriminated in the illness. In addition, many of those afflicted are often associated with the local fishing industry and therefore do not report an incident that could have a serious impact on their livelihood. Reports of ciguatera are almost always followed by an immediate and drastic reduction in the sales of seafoods. Additional economic loss to the seafood industry occurs when afflicted individuals refuse to eat fish due to personal aversion or clinical admonition for many months or years after the intoxication. The greatest constraint to the industry occurs when reports of ciguatera have led to the ban on sale of selected fish species (barracuda in Dade

County and Puerto Rico, large amberjack in Hawaii, etc.). Estimates of losses to the industry have not been adequately determined, but it is reasonable to approximate the annual losses at $10,000,000 to the Florida/Caribbean industry.

Any effort to start a fishing industry, or to expand a marginal operation in the tropics, immediately encounters the problem of ciguatera. And while many islanders often regard ciguatera as an expected nuisance, or the price paid as a consequence of eating local reef fish, the potential threat of ciguatera continues to be a major hurdle in fishieries development, from both a public health perspective and from litigation issues which often follow (Hughes, 1979). Recent court cases of ciguatera, and pending litagation, have forced seafood wholesalers to pay a higher price for liability insurance. As reported by Dammann (1969), the danger of ciguatera poison remains one of the major deterrents to efficient and widespread marketing of most tropical species of shallow-water food fish. Until the health hazards of ciguatera can be resolved, there appears to be little possibility of developing or improving the present inshore fisheries in ciguatera-endemic tropical areas.

Laboratory Production of Ciguatoxin

An inherent difficulty in carrying out laboratory-based research on ciguatera is the ability to acquire quantities of toxin(s) sufficient to conduct the chemical and biological studies needed to advance our understanding of the illness. Although readily capable of causing serious human illness, ciguatoxin fish contain extremely small amounts of the poison. Its isolation and purification was accomplished by Scheuer, et al. (1967) 15 years ago, when 2,000 pounds of liver tissue from toxic moray eels yielded only 1.5 mg, or 0.00006 ounce, of toxin (intestines, gonads and liver were found to harbor fifty to two hundred times more ciguatoxin than muscle). Extraction of ciguatoxin from clinically-incriminated fish flesh yielded ciguatoxin but in such small quantities as to make this source impractical for chemical studies.

Recently, the dinoflagellate Gambierdiscus toxicus has been implicated as the microorganism that synthesizes ciguatoxin and initiates its transmission through the food web (Adachi and Fukuyo, 1979; Yasumoto, 1980; Withers, 1981). This finding raised hopes that laboratory culturing of the dinoflagellate could yield significant quantities of ciguatoxin. Unfortunately, this optimism has been tempered, since the presence of ciguatoxin has not been confirmed in laboratory cultured cells as distinct from wild cells (Helfrich and Banner, 1968). Cultured cells do produce maitotoxin, a water soluble toxin. Both maiotoxin and scaritoxin are secondary toxins which frequently coexist with ciguatoxin in suspect fish; their involvement in ciguatera, however, needs to be resolved. Since wild G. toxicus cells collected from their natural habitat yielded substantial amounts of ciguatoxin-like material (Helfrich and Banner, 1968), it is reasonable to assume that physiochemical parameters may be found that will result in production of significant levels of toxin within the laboratory setting. Hopefully, such studies may ulti-

mately result in the use of cultured cells for obtaining ciguatoxin. Until these culture conditions have been ascertained, however, researchers will have to rely on large-scale extraction of suspect fish as their source of ciguatoxin.

Chemical Research

A complete description of the molecular structure of ciguatoxin, the principal lipid toxin responsible for ciguatera, may be forthcoming. Only recently has ciguatoxin been crystallized; unfortunately, the crystals are too small for structural elucidation by X-ray diffraction (Hokama, Kimura and Miyahara, 1980). One milligram of crystalline toxin constitutes the entire supply worldwide and more is needed to enhance crystal size in order to resolve its structure. Since the parameters for crystal nuclei formation are not known, "trial and error" experiments must be conducted to find optimum conditions for maximum crystal size. With a limited toxin supply, only one or two experiments can be conducted at a time and each experiment takes several weeks. Furthermore, no crystallographer has succeeded to date in determining the molecular structure of any molecule of the size of ciguatoxin (M.W. = 1,112) by direct methods. A crystalline heavy atom derivative of ciguatoxin must therefore be prepared if successful X-ray crystallography is to be realized. Obviously, the possibility of obtaining a molecular structure for ciguatoxin will be much improved once additional quantities of the toxin become available.

In addition to elucidating the structure of ciguatoxin, chemical studies are required in order to identify several other toxins often found in association with ciguatoxic fish. Preliminary evidence suggests that dinoflagellates, in general, and G. toxicus, in particular, produce a variety of toxic material; only by carefully defining these materials on the basis of their chemical composition will their relationship, if any, to ciguatoxin be determined. Furthermore, an accurate chemical definition of the responsible agent is mandatory, if similarities or differences in the illness among geographic regions (i.e., Pacific vs. Caribbean) are to be documented. Unfortunately, an inadequate chemical description of the extracted "toxin(s)" used in many former studies makes it virtually impossible to interrelate data. A thorough knowledge of the toxin's structure is a key element in many other endeavors, including the development of a practical field test for ciguatoxin, the analysis of its toxicological effects, the development of specific antidotal therapy, and the verification of the microbial source(s) of the toxin.

Dinoflagellate Research

From all available evidence, it is almost certain that the toxin(s) responsible for ciguatera have their origin in one or more benthic dinoflagellate species. The range of species involved, and the environmental conditions that favor toxin synthesis, are unknown. Obviously, a thorough knowledge of the incriminated dinoflagellates will greatly enhance

our understanding of the vagaries of the illness, including the mechanism for transmitting the toxin through the food web. In general, research investigations involving dinoflagellates comprise several inter-related studies. (i) Although G. toxicus is known to produce ciguatoxin in its natural habitat, the production of this toxin has not been achieved in laboratory cultures. It is necessary, therefore, to determine growth phases and environmental conditions which support the biosynthesis of ciguatoxin in unialgal and axenic cultures. (ii) Since other dinoflagellates may synthesize ciguatoxin, it is important to isolate and culture other species of dinoflagellates which occur in regions known to harbor ciguatoxic fish and to test for toxin(s) production. (iii) To date, various species of dinoflagellates have been shown to produce toxins, some of which exhibit properties similar to ciguatoxin, maitotoxin, okadaic acid or saxitoxin. However, most of these toxins have not been purified or well defined chemically or biologically, and a great deal of effort must be applied if these results are to have meaning.

Underlying all research endeavors involving the marine dinoflagellates is an attempt to understand the process by which dinoflagellates introduce the toxin(s) into the ecosystem. Once the mechanism is known, it may be possible to predict and/or monitor localized marine areas as reservoirs for ciguatoxic fish on the basis of the prevailing environmental conditions that favor dinoflagellate growth and toxin production.

Ciguatoxin Detection and Analysis

Several options have been considered for minimizing the threat of ciguatera. These options range from simply avoiding those fish often incriminated in the illness, to seeding suspected "ciguatoxic hot spots" with mutant dinoflagellates that are defective in toxin biosynthesis. The comparatively large number of fish species that have been involved with ciguatera obviates a simple avoidance procedure for resolving the problem, and the technology for selecting mutant dinoflagellates and for altering conditions that would permit the mutant clones to overgrow the natural population has not been developed. The option that has received the greatest attention involves the development of a practical "market place" test for the presence of ciguatoxin, with a view to removing suspect fish from the stream of commerce.

Although this latter option appears most practical, the only reliable methods to date for detection of ciguatoxin (or any of the associated toxins) is animal bioassay, for which the mouse has become the favored animal model. Unfortunately, mice are relatively refractory to the effects of ciguatoxin and death, the end point of the bioassay, is not a classical response in humans. Nevertheless, this animal bioassay, although labor intensive, expensive and of questionable sensitivity, is currently the only acceptable basis for determining the biological activity of ciguatoxin preparations.

Without knowledge of the chemical structure of ciguatoxin, the possibility of developing a test to identify the toxin by classical

chemical means is unlikely. Because of the minute amount of toxin in fish flesh, and the lack of sensitivity intrinsic to many of the traditional chemical detection systems, sensitive immunological methods need to be pursued, with a goal of developing a ciguatoxin-specific reagent (anti-ciguatoxin antibodies). Although convincing evidence for the existence of this immunological reagent has not appeared, it is believed that this approach is most likely to provide the basis for future ciguatoxin assays.

Toxicology Studies

Ciguatera is an exceedingly unpleasant illness in man, with serious gastrointestinal and neurological disturbances occasionally culminating in death (Lawrence, et al., 1980). Unfortunately, there is as yet no medical treatment for ciguatera beyond measures designed to relieve some of the early symptoms. If the illness runs its typical course, gastroenteritis develops several hours after ingestion of a ciguatoxic fish and may last for several days, followed by a generalized weakness. Neurological symptoms, particularly paresthesia (a sensation of prickling or tingling of the skin that has no objective cause) may last from two days to several weeks (or longer). In severe cases, death results from respiratory failure associated with paralysis of the respiratory musculature. If the illness is untreated, death may also result from severe dehydration secondary to vomiting and diarrhea.

Unfortunately, the molecular events that cause these symptoms are unknown. Progress towards elucidating the toxin's mode of action has been severely hampered by the lack of biologically and chemically defined toxic material for experimentation. The available information has been gained by utilizing crude toxin preparations from small individual fish samples associated with isolated episodes. The mode of operation has only permitted the most superficial endeavors. Elucidating the molecular events leading to neurological disturbance and/or death is a prerequisite to the development of a rational approach to a specific antidotal therapy. Careful and systematic studies with animal model systems, including in vitro organ transplants, are urgently needed, not only to develop effective treatment regimes but also to resolve some of the existing difficulties (i.e., sensitivity) present in the only ciguatoxin assay currently accepted, the mouse bioassay. In addition, detailed knowledge of the toxin's mode of action will contribute to our pool of biological methods, which can be used to differentiate ciguatoxin from other marine toxins, particularly those toxins which have been associated, directly or indirectly, with "ciguatera."

Summary

Ciguatera is a serious human illness resulting from the consumption of coral reef-associated fish that accumulate ciguatoxin through their diet. Thousands of inhabitants and tourists in tropical areas of the world are affected annually and the resulting publicity has impacted the sale of seafoods in tropical and subtropical regions. A variety of com-

mercially important reef fish may contain the currently undetectable, heat-stable toxin, thereby presenting a real threat to developing selected tropical fisheries. Research needs include the development of market-place detection methods for ciguatoxin in suspect fish, a thorough description of the molecular structure of the toxin, delineation and culturing of ciguatoxin-producing dinoflagellates, a comprehension of the mechanism by which the toxin is introduced into the ecosystem, and an understanding of the toxin's mode of action utilizing animal models.

Chapter 2

HUMAN VIRUSES IN THE MARINE ENVIRONMENT

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Introduction

Fecal contamination of estuarine and coastal marine environments is of growing public health concern as permanent and transient coast populations and associated developments continue to increase. People can be exposed to and become infected with pathogenic microorganisms by ingesting fecally contaminated coastal waters and sediments in the course of primary contact recreation such as bathing, scuba and skin diving and water skiing and in occupational activities such as commercial and military diving operations. People can also ingest and become infected with microbial pathogens by eating raw or partially cooked bivalve mollusks such as oysters and clams that have become fecally contaminated. These mollusks feed by filtering particulate matter, including microorganisms, from large volumes of water that they pump through their bodies (Galtsoff, 1964).

Although most microorganisms in coastal waters, sediments and shellfish are not detrimental to people, enteric pathogens from a variety of fecal waste sources can enter coastal environments. Enteric viral contamination of coastal waters, sediments and shellfish has been documented by both the recovery of viruses from environmental samples (Table 1) (Ellender et al., 1980; Gerba and Goyal, 1978; Gerba et al., 1980; Metcalf and Stiles, 1965; 1968; Sobsey et al., 1980; Vaughn et al., 1979; 1980) and the occurrence of waterborne and shellfish-borne outbreaks of diseases due to hepatitis A virus (HAV) and gastroenteritis viruses (Appleton, 1981; Bryan, 1980; Centers for Disease Control, 1982; Gerba and Goyal, 1978; Goldfield, 1976; Larkin and Hunt, 1982; Murphy et al., 1979).

More than 100 different types of viruses can be shed in the feces of infected people and subsequently contaminate coastal environments (Table 2). Polioviruses have been recovered more frequently than other enteric viruses. This is most likely due to ongoing immunization programs in which young children are fed live, attenuated viruses that replicate in the intestinal tract and are shed for several days to weeks. Feces from such immunized children and from people infected with disease-causing enteric viruses may contain virus levels of 10^3 to 10^6 per gram. Some other enteric viruses, such as the rotaviruses that cause acute gastroenteritis in infants and young children, are shed fecally for as long as 1 week at concentrations of 10^7 to 10^9 per gram (Konno et al., 1977). Such viruses may be present in feces at levels approaching those of coliform bacteria. Enteric virus concentrations in domestic raw

TABLE 1. VIRUS ISOLATES FROM BIVALVE MOLLUSKS

Source	Type of shellfish	Percent of samples positive for virus	Viruses isolated	Reference
Market	Oysters*	10	Coxsackievirus A16, B2, B3, and B4 and Polioviruses 1, 2 and 3	Denis, 1973, 1974
	Oysters	<0.01	Poliovirus 1	FDA 1978#
	Mussels*	20	Coxsackievirus A16	Denis 1973
	Mussels+	#	Echovirus 3, 9 and 13	Bellelli and Leogrande 1967
Samples from approved waters	Oysters	7	Poliovirus 1 and 3 Fugate et al. 1975	Fugate 1972
	Oysters	20	Poliovirus 1	Metcalf et al. 1972
	Oysters	<1	Poliovirus 1 1980	Ellender et al.
	Oysters	20	Echovirus 1 and Poliovirus 1	Goyal et al. 1979
	Oysters	25	Coxsackieviruses and Echoviruses	Vaughn et al. 1979, 1980
	Clams	28.5	Polioviruses and Echoviruses	Vaughn et al. 1979, 1980

*Source: France
+Source: Italy
#Data not provided

#Unpublished data, Virology Branch, Div. of Microbiology, Cincinnati, OH

TABLE 2. POTENTIAL HUMAN VIRUS CONTAMINANTS OF SHELLFISH

Name	No. of Types
Picornaviruses	
Polioviruses	3
Coxsackieviruses A	24
Coxsackieviruses B	6
Echoviruses	34
Enteroviruses	4
Hepatitis A virus	1?
Reoviruses	3
Rotaviruses	?
Human adenoviruses	38
Norwalk-type viruses	<3
Other gastroenteritis viruses	?

sewage may range from less than 10 to greater than 10^5 infectious units per liter and tend to vary both seasonally and geographically.

Although conventional sewage treatment processes usually reduce enteric virus concentrations by 90 to 99%, treated sewage effluents may still contain considerable amounts of enteric viruses (see reviews by Bitton, 1980; Gerba, 1981).

Enteric virus levels in coastal environments are further reduced by dilution and natural inactivation processes. However, exposure to even low levels of viruses in coastal environments is of concern because human infection can be produced by as little as one cell culture infectious unit of virus (Plotkin and Katz, 1965; Westwood and Sattar, 1976; Akin, 1981).

The sanitary quality of recreational waters and edible shellfish and their harvesting waters is based on levels of total and/or fecal coliform bacteria as indicators of fecal contamination and on the identification of sources of fecal contamination through sanitary surveys (national Shellfish Sanitation Program, 1965; Wilt, 1975, National Technical Advisory Committee, 1968; U.S. EPA, 1976). However, recent epidemiological and microbial findings have raised concerns about the reliability and validity of present coliform bacteria standards for recreational waters (Cabelli, 1980) and for shellfish and their harvesting waters (Portnoy et al., 1975; Grohmann et al., 1981). Field studies have shown that enteric viruses can sometimes be found in shellfish obtained from approved harvesting waters and that there is no consistent relationship between levels of enteric viruses and coliform bacteria in shellfish or the waters and sediments from which they are harvested

(Ellender et al., 1980; Fugate et al., 1975; Goyal et al., 1979; Vaughn et al., 1980).

It is the conclusion of this report that the important research needs are for completely and effectively assessing the public health significance of enteric viruses in coastal environments and for developing and evaluating strategies and methods for their control.

Virus Cultivation and Assay

The lack of simple, convenient and reliable methods to cultivate and quantify hepatitis A virus (HAV), Norwalk-type viruses, human rotaviruses (HRV) and other viruses implicated in acute gastroenteritis, hampers much needed research on these viruses relative to their public health significance as contaminants of coastal environments. Recent success in laboratory cultivation of HRV (Banatvala et al., 1975; Esparza et al., 1980; Moosai et al., 1979; Schoub et al., 1979; Sata et al., 1981; Wyatt et al., 1980) and HAV (Daemer et al., 1981; Flehmig, 1980; Froesner et al., 1979; Provost and Hilleman, 1979) and in quantifying HRV in feces, sewage and contaminated aquatic samples (Smith and Gerba, 1982) indicate that creative and persistant research efforts will lead to the development of better and reliable methods for cultivating and assaying all of these important enteric viruses. The availability of such assay methods will then make it possible to perform comparative studies on the behavior of: (i) these less studied and difficult to cultivate viruses, (ii) the more widely studied and easily cultivated viruses (e.g., polioviruses, echoviruses and coxsackieviruses), and (iii) fecal indicator bacteria that are found in coastal environments.

Research is needed to develop and/or further improve cultivation and infectivity assay methods for HAV, Norwalk-type viruses, HRV and other difficult-to-cultivate gastroenteritis viruses, as well as other enteric viruses.

Methods for Recovering and Detecting Viruses in Coastal Environments

Methods for detecting enteric viruses in shellfish, water and sediment from coastal environments have been developed, evaluated and utilized in field studies (see reviews by Cliver et al., 1983; Gerba and Goyal, 1982a; Gerba et al., 1978; Sobsey, 1982-83). However, these methods have been developed and optimized using only a few model viruses that are easy to cultivate and assay. The methods have not been applied to the difficult-to-cultivate viruses such as HAV and Norwalk-type viruses implicated in shellfish and swimming-associated disease outbreaks. Thus, only a small and variable proportion of the total viruses actually present in field samples are being detected using current methods. When samples from coastal environments are found to be virus negative, it cannot be concluded that they indeed contain no viruses. Considering the importance of obtaining reliable field data on all enteric viruses in coastal environments for such purposes as disease outbreak investigations, epidemiological studies, development of rational quality

criteria, and other studies on the nature and extent of virus contamination, the availability of simple, reliable and effective detection methods for all viruses is essential.

With continued development and modification of virus detection methods, it has not been possible to standardize methods and critically evaluate them for precision and accuracy in carefully designed intra- and inter-laboratory tests. Reliable information from collaborative testing of standardized methods for virus detection in shellfish, water and sediment is especially lacking (Larkin and Metcalf, 1980). Research is especially needed in the following areas:

Evaluation of Methods to Recover all Viruses

* Existing methods for concentrating viruses from shellfish, water and sediment from coastal environments must be evaluated for their ability to recover HAV, Norwalk-type viruses, HRV and other difficult-to-cultivate viruses that have been implicated in disease outbreaks. If existing methods are found to be inadequate, then the development of new or modified methods that are effective for these viruses will be needed.
* Development and field testing is needed on infectivity assays for difficult-to-cultivate viruses, such as HAV and Norwalk-type viruses in concentrates obtained from environmental samples.
* Immunochemical methods such as enzyme linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) should be evaluated as adjuncts to infectivity assays for rapid detection and quantification of viruses in concentrates of environmental samples.

Improvements in methods to recover viruses from coastal waters

* Evaluation of different types of adsorbent filters for virus concentration by adsorbent-elution methods.
* Development and evaluation of new or improved eluents and elution methods for recovering viruses adsorbed to filters. Such studies should include comparison of the effectiveness of different proteinaceous and nonproteinaceous eluents.
* Improvements in and comparison of different reconcentration methods such as filter adsorption-elution aluminum hydroxide precipitation, polyethylene glycol hydroextraction and metal oxide adsorption.

Improvements on methods to recover viruses from shellfish, sediments and suspended solids

* Development and evaluation of improved virus extraction protocols including assessment of virus recovery efficiency and field testing.

* Evaluation of existing eluents or development of improved eluents or techniques to extract or desorb viruses from proteins, sediments or other particles.
* Development and evaluation of improved procedures to reduce or eliminate cytotoxicity of sample extracts in cell culture assay systems.
* Development of improved methods for further concentration of viruses in sample extracts down to microliter volumes in order to assay samples for viruses by immunochemical method.

Collaborative and cooperative testing

* Collaborative and cooperative studies involving different laboratories are needed in order to develop and evaluate standardized methods for virus extraction and detection in shellfish, sediments and other coastal environment samples.

Control of Virus Contamination of Shellfish by Relay and Depuration

It is impossible to entirely eliminate fecal contamination from coastal shellfishing areas, and therefore, some degree of virus contamination of edible shellfish, however small, must be expected and accepted. Reducing viral contamination of shellfish to acceptable levels in a reliable manner would not only further assure the quality of shellfish harvested from approved areas, but might also make it possible to increase shellfish resources by reclaiming virally contaminated shellfish from prohibited areas.

Shellfish have the natural ability to purge themselves of viruses and other microbial pathogens in the course of normal filter feeding and respiring when they are placed in uncontaminated water (Hoff and Becker, 1969; Liu, 1970; Liu et al., 1965; 1976; Metcalf and Stiles, 1965b; Metcalf et al., 1980a; Mitchell et al., 1966). When this process occurs under controlled environmental conditions in shore-based commercial facilities it is called depuration; when it is done by transferring contaminated shellfish to uncontaminated natural waters it is called relaying.

Although self-cleansing of shellfish by relaying to approved waters or by depuration under controlled environmental conditions has the potential for reducing viruses and other microbial contaminants in shellfish, these processes have not been adequately evaluated to determine the environmental conditions for optimum viral eliminations (Furfari, 1966; 1976). Relatively little information is available on the rate and extent of virus uptake by shellfish in contaminated waters or on the rate and extent of virus elimination by shellfish placed in uncontaminated waters. Quantitative information is lacking on how environmental factors such as temperature, salinity, pH, and turbidity influence the rate and extent of virus uptake and elimination by shellfish, especially for the difficult-to-cultivate viruses such as HAV, Norwalk-type viruses and HRV.

Research is needed to determine the rate and extent of elimination of enteric viruses and fecal indicator bacteria by shellfish under relay and depuration conditions, especially for HAV, Norwalk-type viruses, other viruses implicated in shellfish-associated disease outbreaks and for potential indicator viruses such as poliovirus. Quantitative information on how environmental factors influence the rates at which shellfish eliminate microbial contaminants must be determined in order to optimize conditions for maximum viral elimination. Especially needed are studies on persistence of viruses at low levels in shellfish under relaying and depuration conditions, and the reliability of fecal indicator bacteria and other potential fecal indicators to reflect the virological quality of relayed or depurated shellfish.

Occurrence, Transport, Distribution and Survival of Viruses in Coastal Environments

Virus occurrence, transport, distribution and survival in coastal environments influences the extent to which they will contaminate shellfish, water and sediment. The ability of viruses to survive for long periods is especially important because such persistence can lead to their accumulation and wide-spread dissemination by environmental transport mechanisms. Viruses have been shown to survive longer in marine waters and sediments than do coliform bacteria (see reviews by Akin et al., 1971; Bitton, 1978; Kapuscinski and Mitchell, 1980; Sattar, 1981), and the ability of coliforms to adequately indicate the virological quality of shellfish and coastal waters has been questioned (Gerba and Goyal, 1978; Gerba et al., 1980; Goyal et al., 1979; Larkin and Hunt, 1982).

The ability of viruses to adsorb to and accumulate in coastal sediments and in the region of the sediment-water interface, has also been demonstrated (Gerba et al., 1977a; LaBelle et al., 1980). Furthermore, the association of viruses with suspended particles and sediments tends to prolong their survival (LaBelle and Gerba, 1979; 1980; Smith et al., 1978). These persistent viruses may then be transported and become widely distributed by such mechanisms as current, tides, storms, boat wakes and dredging.

A number of environmental factors that influence virus survival in coastal environments have been identified, such as temperature, sunlight, antiviral microbial activity, and antiviral chemicals (see reviews by Bitton, 1978; Kapuscinski and Mitchell, 1980; Sattar, 1981). However, there are unexplained and unresolved discrepancies about which of these specific factors has the greatest influence on virus survival. The extent to which geographic and temporal differences in the physical, chemical and biological composition of coastal waters and sediments are responsible for the reported differences in virus survival has not been determined.

There are over 100 different human enteric viruses that can contaminate coastal environments. Although these viruses are generally similar in structure and chemical composition, they differ in their ability

to survive and be transported in coastal environments (Gerba et al., 1980). A few representative enteric viruses that are easy to cultivate have been studied, to some extent, with respect to their occurrence, survival and movement under different coastal environmental conditions; however, many enteric viruses, including HAV, Norwalk-type viruses and HRV, have not been studied at all with respect to these phenomena. The fact that HAV and Norwalk-type viruses have been responsible for shellfish- and swimming-associated disease outbreaks suggests that these viruses may be more resistant to coastal environmental conditions, and/or more plentiful than the easily cultivated viruses that have been studied so far.

There is a need for further research on the occurrence, transport, distribution and survival of viruses in coastal environments. Information is especially needed for those difficult-to-cultivate viruses (e.g., HAV, Norwalk-type viruses and HRV) that are known or strongly suspected to be responsible for disease outbreaks due to the ingestion of shellfish and coastal water. With recent successes in the cultivation and assay of some of these viruses, it is now possible to initiate these much needed studies.

Comparative studies are needed on the occurrence, transport, distribution and survival of: (i) these difficult-to-cultivate viruses, (ii) the more easily cultivated viruses, and (iii) fecal indicator bacteria such as coliforms and fecal streptococci. Such studies will make it possible to determine if fecal indicator bacteria or the easily cultivated enteric viruses are reliable and practical indicators for the difficult-to-cultivate enteric viruses.

There is a need to determine the relative importance of various environmental factors that may influence the transport and survival of viruses in coastal environments. Seasonal studies and coordinated or joint research efforts between workers in different geographic locations are needed. Such studies will make it possible to determine if seasonal and geographic differences in physical, chemical, and biological factors are responsible for the reported differences in the transport and viability of viruses in the marine environment.

Epidemiology and Health Risk Assessment

Epidemiological approaches are needed to assess health risks from human exposure to viruses in coastal environments. This approach is essential for developing dose-response information on the relationship between virus exposure via ingestion of shellfish or coastal water and the occurrence of virus infection and illness. Such dose-response information will make it possible to establish rational criteria and standards for the sanitary quality of edible shellfish, bathing waters and other potential vehicles of human exposure to viruses in coastal environments. Determination of the sanitary quality of vehicles of possible virus transmission (e.g., shellfish, bathing water, etc.) by measurement of levels of both viruses and fecal indicator bacteria is an integral part of such studies.

Recently, the promise and utility of such epidemiological studies was indicated by the findings of Cabelli and colleagues on the relationships between risks of gastrointestinal illness and bacteriological quality of bathing waters (Cabelli et al., 1979; Cabelli, 1980; Cabelli et al., 1982). Using illness assessment by an individual's responses to personal interview query and measurement of fecal indicator bacteria in bathing water, the investigators discovered a mathematical relationship between the rate of swimming-associated gastrointestinal illness and the levels of enterococci bacteria in the water. Although a viral etiology was hypothesized for much of the observed gastrointestinal illness, no data on the virological quality of the bathing waters were obtained in these studies.

Carefully designed epidemiological studies are needed on the risks of infection and illness from exposure to shellfish, water and sediment of coastal environments of differing sanitary quality. Such studies should be designed to develop rational standards for the sanitary quality of shellfish, water and sediments which humans ingest or otherwise contact.

Characterization and Control of Sources of Viral Pollution in Coastal Environments

Conventional sewage treatment systems were not designed specifically to eliminate viruses or other microbial pathogens. Although the virological quality of effluents from conventional sewage treatment systems has been studied to some extent (see reviews by Bitton, 1980; Gerba, 1981; Malina, 1976), information is needed on the levels and significance of viruses in wastes discharged into coastal environments by widely-used small community treatment systems (e.g., package plants, lagoons, etc.); by septic waste treatment systems; by waste discharges from boats and marinas; by non-point sources, such as runoff from municipal areas and agricultural waste sources; and by sludge dumping operations. Small community systems deserve special attention because they are often poorly designed and operated and, consequently, prone to failure. Septic systems are of particular concern because they often leach into coarse soils and shallow water tables characteristic of many coastal areas, thus resulting in poor removal of viruses and other pathogens (Gerba et al., 1977a; Goyal et al., 1978; Sobsey and Scandura, 1981). Waste discharges from boats, marinas and non-point sources may contribute intermittent and transient viral contamination that is difficult to detect, quantify, or control.

Research is needed to quantitatively determine the sources and significance of virus contamination in sensitive coastal environments, such as shellfish areas and bathing waters. Evaluation of waste treatment facilities such as conventional treatment systems, widely used small systems such as package plants and lagoons, and septic waste treatment systems are needed; as is an assessment of the extent of marine contamination from boats and marinas, non-point sources such as community and animal feedlot runoff and sludge dumping operations.

Research is needed to develop new and improved treatment systems, waste management strategies and operational controls to further reduce discharges of viruses and other pathogens to coastal environments from ever increasing waste sources.

Chapter 3

PARALYTIC SHELLFISH POISONING

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Introduction

A significant barrier to the optimal utilization of many shellfish resources stems from the sporadic blooms of a variety of toxic dinoflagellate species. Not only are large expanses of the U.S. coastline currently affected by paralytic, neurotoxic, or diarrhetic shellfish poisoning (PSP, NSP, DSP), but there is strong evidence for a worldwide increase in the impacts. There is no doubt that many previously unaffected areas are now subject to recurrent PSP episodes, for example, in New England and Washington state (Anderson et al., 1982; Saunders et al., 1982), while other regions have experienced significant increases in the magnitude and duration of outbreaks (White, 1982). The recent discovery of toxic strains of Gonyaulax tamarensis in the rich shellfish regions of Long Island and Connecticut support our view that no regions are currently immune from the potential incursion of PSP--that the causative organisms can thrive in a variety of environments and thus pose a significant socioeconomic and public health threat to recreational and commercial shellfish resouces throughout coastal waters of the U.S.

It has only recently become evident that toxic dinoflagellates may be linked to many cases of the most common health problem associated with shellfish consumption—acute transitory gastrointestinal reactions. A large number of consumers have experienced this problem after eating shellfish, with the symptoms often attributed to allergies or bacterial contamination. Recently one cause of this unpleasant GI disorder was shown to be the dinoflagellate Dinophysis fortii. Yasumoto et al. (1978) first isolated this toxin, (named diarrhetic shellfish toxin) in Japan, but it is now believed to occur throughout the world. These authors further stated that most of the gastroenteritis outbreaks attributed to Vibrio in Japan in the past were probably due to the Dinophysis toxin. This is clearly a high priority research topic that was totally unanticipated a few years ago.

Another relatively new and unexpected impact from the toxic dinoflagellates relates to their consumption by a variety of planktonic herbivores. Recent data now document the ingestion of Gonyaulax species by zooplankton which then pass the toxin along to fish. Thus the toxin dinoflagellate has been linked to large fish kills (herring, menhaden), with even greater potential impact expected on the more sensitive larval stages (White 1977; 1979; 1981; 1982). Insufficient data are available to assess the magnitude of this problem, but the potential effects may well be substantial with respect to the recruitment success of large numbers of commercially important species.

The magnitude of the economic losses associated with PSP needs little emphasis, ranging from catastrophes, such as the one in southern New England in 1972 which caused $4-6 million worth of damages (Jensen, 1975), to the recurrent costs associated with preventative shellfish monitoring in each state with a potential for PSP, or with the loss of revenue from resources not utilized. If one adds the losses associated with the decrease in demand for "safe" seafoods during toxic outbreaks, the net result is a significant economic impact.

Despite the apparent widespread nature of the DSP problem, it is not possible to estimate economic losses for this toxin since outbreaks have in all likelihood been attributed to bacterial or viral causes. Economic impacts caused by NSP and shellfish bed closures in Florida are also significant; however, more income and revenue are lost from direct and indirect industries associated with tourism (e.g. hotels, motels, beach resorts, charter fishing boats and shore/beach activities) due to widespread news accounts of "red tides" and fish kills.

Years ago it seemed that the PSP problem could be envisioned as the relatively simple production of one toxin by dinoflagellates and the accumulation of that toxin by shellfish. Now it is evident that the situation is far more complex, with 12 different toxin structures identified from Gonyaulax alone (Fix Wichmann et al., 1981b; Boyer, et al., 1978; Shimizu, et al., 1978b), each with different specific toxicity and potential for biotransformation to more or less toxic forms within shellfish (Shimizu and Yoshioka, 1981). Implications of these results are profound, ranging from the validity and safety of assay methodologies to the validity of the use of the federal standard for enacting harvesting closures.

Funding for research on the toxic dinoflagellates has been as sporadic as the outbreaks themselves. Progress has been made in certain areas, but there is a clear need for: a) long-term continuity of funding, and b) development of integrated regional programs that recognize the uniqueness of the organisms and environments in each area. The following discussion outlines the areas where research funds would be the most productive. The fact that the list is so extensive demonstrates that a narrow, focused national program is not sufficient for significant progress. Neither would a large one-time infusion of funds be appropriate. The interactions between toxin source (the organisms), toxin accumulators (shellfish, finfish, zooplankton, larvae) and the dynamic environment of the coastal waters are truly complex. This is not a problem that will simply disappear on its own in the near future. In fact, evidence suggests the opposite. The consensus was reached that there is great potential for prediction and possibly management of the shellfish toxicity phenomenon. Such efforts remain premature, however, and must be placed in the correct sequence following the fundamental studies outlined below, most of which can yield direct economic, public health, and recreational benefits.

We have found it necessary to place specific research topics into several general categories. Any numerical listings are purely organizational and are not to be interpreted as a ranking of priorities. We feel

that all of these areas warrant concurrent funding. Low priority topics have been omitted.

Resource Analysis

Regional economic data are needed for the assessment of actual or marine resource impacts due to PSP, NSP and DSP. Data needed for economic evaluations include: 1) commercial and recreational harvest of shellfish (e.g. clams, mussels, oysters) by area and species and projected retail value; 2) total economic losses at the retail level due to closures of shellfish beds and, 3) actual and potential loss of leaseable water column and bottom habitat acreage for mariculture ventures (mollusks, crustaceans, finfish) and the accompanying loss in value of the saleable product. Currently, only commercial landing statistics are available to assess losses due to closures without any projections for losses due to further restrictions in resource utilizations. Likewise no comprehensive estimates are available to assess the magnitude of the impact on recreational shellfishing closures.

We should stress that we see a need for this information, but feel that it is within the jurisdiction of several federal and state agencies to collect and compile it. The role of Sea Grant is not to fund such resource analyses but perhaps to facilitate the collection and dissemination of relevant data from other agencies.

Resource/Toxin Interactions

It is important to recognize that toxic dinoflagellate blooms are a problem because they serve as a food source for shellfish. We must therefore learn more about the ways in which the toxin is delivered to the shellfish, accumulated and depurated. We consider the following topics to be of high (and approximately equal) priority.

Rates of Toxification and Detoxification. Given the large geographic areas subject to toxic dinoflagellate outbreaks, we must study the mechanisms of toxin uptake, retention and depuration for numerous important shellfish species. Not only will this lead to more flexible regulations and guidelines (with species-specific closures for example), but it should also aid in the development of depuration methodologies and in the optimal economic design of state monitoring programs. It is noteworthy that nothing whatsoever is known about the uptake and depuration of DSP in the United States. Japan has already established quarantine levels for this toxin while we remain dangerously ignorant of the nature and extent of our similar problem. Other benefits from this research would lie in the increased utilization of formerly unused portions of shellfish (the viscera of scallops, for example), as well as the potential resolution of the long standing enigma that many shellfish remain toxic in times and places where motile cells are not or have not been present.

Assay method. There is a need for a rapid, sensitive, simple and inexpensive assay method for dinoflagellate toxins. This could be a bioassay (such as the fly assay now under development), which could replace the mouse bioassay for monitoring of total toxicity in shellfish for regulator purposes. Other methods could include rapid enzyme immunoassay systems. One or both would reduce the costs incurred by states in monitoring shellfish for toxicity and would allow more efficient and detailed monitoring coverage. An example of the cost/benefit aspects of rigorous monitoring should be emphasized. John Hurst, Maine Division of Marine Resources, used fine-scale monitoring in the Casco Bay region during 1979. By keeping closed only the portions of the clam flats that exceeded quarantine, 0.5 million dollars of shellfish were landed, with "value added" estimates of 2.7 million dollars. Clearly one obstacle to widespread use of this type of program is the time and expense of the present assay system.

Antidotes. No antidote is available for PSP. The mode of action of the toxins is to inactivate the fast sodium channels in excitable cells, with the primary site of action being the nervous system. In severe poisoning, the muscles of respiration are affected, but generally if the victim is able to receive sustained artificial respiration, chances of recovery are good. Since the onset of clinical PSP symptoms following a meal is rapid and medical assistance is often unavailable or inadequate to correctly diagnose the problem, there is an obvious need for an effective antidote that can be administered prior to hospitalization and treatment. The large number of toxins and their different chemical properties suggest that this could be a difficult problem to solve.

Effects on Finfish/Larvae. In recent years it has become apparent that the effects of toxic gonyaulacoid dinoflagellates extend beyond the well known shellfish toxicity problems and include negative impacts on finfish resources as well (White, 1977, 1979, 1981, 1982). We feel it is important to examine alternative vectors for the toxin and to assess the magnitude of this previously unforeseen problem area. It has already been established that several commercially important finfish species are sensitive to Gonyaulax toxins, but nothing is known of the effects of the dinoflagellates on the more sensitive larval stages. Likewise little is known of the magnitude of the adult fish kills beyond the clear demonstration that they occur. Research in this area may provide data on larvae and adult mortality from PSP that add greatly to the understanding of fluctuations in recruitment and overall fish stocks from year to year.

This area of research logically falls under the National Marine Fisheries Service. Although this should not preclude Sea Grant sponsorship of such projects every effort should be made to attract NMFS funding so that Sea Grant dollars can be used for the other topics listed above.

Cyst Ingestion. In addition to the motile, vegetative phase of a toxic dinoflagellate life cycle, it is important to recognize that non-motile cyst stages (Anderson and Wall, 1978; Dale, 1977), differing in morphology and often not recognized in the past, may be significant as a source of toxicity (Dale et al., 1978), particularly to benthic organisms in deeper waters (Bourne, 1965). It is of particular significance that cysts may be subject to hydrodynamic mechanisms of transport that classify and focus sedimentary material and may therefore concentrate cysts.

Cysts are of their nature refractory, however, and it remains to be demonstrated that the toxin they contain is available to consuming organisms.

The Toxins

Of the syndromes due to natural toxins in the fisheries products, diarrhetic shellfish poisoning (DSP) and neurological shellfish poisoning (NSP) are due to substances that have some similarities, although their structures are not yet fully known. In contrast, paralytic shellfish poisoning (PSP) is caused by substances related to saxitoxin (STX). Despite the differences in chemistry, we will treat as a single group these three and related syndromes not yet named. We have identified five subject areas with required emphasis:

Toxin Structures. Although the structures of the 12 neurotoxins extractable from Gonyaulax are now known (Boyer et al., 1978; Fix Wichmann et al., 1981a; Fix Wichmann et al., 1981b; Schantz et al., 1975; Shimizu et al., 1978b; and Boyer et al., 1978), it is recognized that bioconversions occur within shellfish (Shimizu and Yoshioka, 1981) which may cause structural modifications not yet known. Substances apparently related to STX but differing from the 12 Gonyaulax neurotoxins have been isolated from several species of shellfish (Hsu et al., 1979; Fix Wichmann et al., 1981a; Konosu et al., 1969; Kotaki et al., 1981; Koyama et al., 1981).

Fragmentary information is available on the structures of the DSP toxins and the structures of some NSP toxins have been determined. Based on the success of studies of the Gonyaulax neurotoxins (Hall et al., 1980), it is likely that progress in both areas will result from systematic efforts to mass culture the source organisms, with careful attention to optimizing growth conditions for toxin production. With respect to Gonyaulax, it should be remembered that attention has been focused on STX and its derivatives which are responsible for the acute toxicity of the genus. There are some indications that Gonyaulax cells contain in addition other toxins of lower potency and entirely different nature which are still a significant threat to public health. A clear understanding of toxin structures and the toxin composition of dinoflagellates and shellfish is essential for the development of more efficient assay methods.

Toxin Supplies. It is necessary to insure that reference standards of the toxins are produced for supply to research groups requiring them. In most cases, they can best be produced by culture of the appropriate dinoflagellate strain. It would be of great value to produce, by similar means, radio-labelled toxins for studies of toxin binding and pharmacology (Henderson et al., 1973).

Chemotaxonomy. While morphological taxonomy of Protogonyaulax has proven complex and is at present in a state of uncertainty (Taylor, 1975), various chemical markers may be of use in constructing "fingerprint" profiles of dinoflagellate strains that will be useful in mapping the distribution of populations (Hall et al., 1980). Of the possible markers, toxin composition is of the most immediate interest and has been shown to be a conservative property of Protogonyaulax strains, while great differences are seen among strains. Given the substantial differences in the properties of the various saxitoxin derivatives, it is of great importance to map the distribution of each compositional type.

Binding and Metabolism. Toxins that have been accumulated by an animal may be conserved, converted, or lost. It is well known that bivalves differ in the levels of toxicity they attain (Neal, 1967; DuPuy, 1968; Sribhibhadh, 1963) and the rates at which they lose toxicity, and it has been shown that the saxitoxin derivatives can be altered by shellfish metabolism (Shimizu and Yoshioka, 1981).

It is likely that the accumulated toxin load undergoes change due both to interconversion by metabolism, eventually to non-toxic products, and to selective retention of the more strongly bound toxins. The relative significance of these processes is not known. It is important to establish whether high toxicity and low rate of toxin loss are due to strong retention or slow metabolism. Elucidating the mechanisms of binding and metabolism will facilitate the development of methods for depuration of contaminated product.

Variations in Toxin Content and Composition. Existing data show that the gross toxicity of cultured Gonyaulax varies greatly, partly in response to differing growth conditions (White, 1978; White and Maranda, 1978). It is important in all cases to define the variation of toxin content and composition with differing environmental conditions, with life cycle stage, and stage in vegetative growth. It must be recognized that culture conditions which are expedient may induce serious artifacts and must be carefully evaluated. Cultured organisms may become quite different from those found in wild populations. Among the differences, ploidy and the accompanying bacterial flora may both have significant effects on toxicity.

Depuration and Processing: Simple detoxification methods for shellfish with marginal toxicity should be investigated. There are indications that some toxins are chemically unstable. We should study this further and devise practical methods to reduce the toxicity to the

safe regulatory level for canning, etc. There is an associated need for depuration studies since the aquaculture industry has indicated that PSP depuration must be accomplished in 48 hours or less if it is to be economically viable.

A sixth area, biogenesis of the toxins, is of the greatest importance in understanding the nature and significance of the toxins, but of secondary importance in a practical sense. Studies of toxin biogenesis, however, would likely aid in the prediction of other organisms likely to be sources of toxicity.

The Organisms

It is of paramount importance to recognize that there is a diverse assemblage of organisms responsible for PSP, DSP and NSP. Although some studies will be broadly applicable, in most cases it will be necessary to fund research that addresses similar questions in several regional laboratories simultaneously. The general categories of high priority research are as follows:

Encystment and Excystment Mechanisms. Dormant cyst stages have recently been described for several important toxic dinoflagellates (Anderson and Wall, 1979; Walker and Steidinger, 1979). Although benthic stages of other important NSP and DSP producers (i.e. Ptychodiscus, Prorocentrum and Dinophysis) have not been described, it is quite possible that these species reproduce sexually to produce benthic resting stages; Ptychodiscus brevis for example does produce gametes and a planozygote (Walker, 1982). There is thus a significant impetus to study the mechanisms underlying the encystment/excystment process. This information is of fundamental importance to our understanding of the population dynamics of dinoflagellates and is of obvious importance in prediction and management efforts. This work should include both laboratory and field studies so that the relevant forcing functions can be pinpointed.

Cyst and Motile Cell Distributions. The shellfish toxicity problems we are concerned with occur in the dynamic coastal environment. We thus need much more information on the vertical and horizontal distribution of the dinoflagellate population under a variety of hydrographic conditions. Likewise it is necessary to study cyst distributions and their link to bloom initiation and decline. This should include: i) quantitative studies of excystment/excystment fluxes during the early and late stages of bloom development; ii) distributional baseline surveys to help in an assessment of apparent spreading events; and iii) examination of the role and fate of cysts buried below the sediment surface.

Genetics. This general category encompasses a variety of important topics. As previously stated, it is acknowledged that the diversity of the organisms is responsible for shellfish toxicity problems. These differences are at both the genus and the species level, with strong indi-

cations that even small areas may be affected by genetically different strains of the same species. Every effort should be made to recognize and describe the population variability (in terms of toxin composition, growth tolerances, etc.) and to assess the implications of such differences. In a similar vein, we must recognize the important role of taxonomy and systematics in these problems. The multitude of closely-related species and strains of a given species necessitate continual scrutiny of the organisms being studied in the laboratory and the field.

Bacteriophages. Expression/repression of toxin production in some microorganisms is regulated by the presence of bacteriophages. Therefore, since bacteria have been documented as endocellular (and even endonuclear) in dinoflagellates (Dodge, 1972; Silva, 1978), this possible regulatory relationship should be explored.

Grazing Impacts. Despite the direct link between herbivore activity and phytoplankton mortality, remarkably little is known of the magnitude of grazing impacts on toxic dinoflagellate blooms throughout the country. In fact, recent studies on non-toxic dinoflagellates indicate that some zooplankters may actively avoid the dinoflagellate accumulations (Huntly, 1982), providing a substantial competitive advantage for population development. Thus we must stress the need for studies on the abundance and feeding rates of relevant herbivores in areas subject to shellfish toxicity. We also recognize the potential that fecal pellets from Gonyaulax grazers may be toxic and thus represent a new vector for toxin transport to deeper waters or to the benthos.

Metabolism and Growth Factors. The dinoflagellates responsible for PSP are all photosynthetic. Other cellular properties are also important, however. There is clear evidence that an external supply of vitamin B_{12} is essential, and strong suggestive evidence that the marine bacteria tightly associated with dinoflagellate cells are essential in the successful growth pattern. Furthermore some cells exhibit heterotrophic metabolism in that they can actively accumulate and assimilate amino acids from their environment. These three parameters need to be examined critically in view of the increasing urbanization of the coastlines of the entire world with resultant increased runoff which supplies nutrient wastes, vitamin B_{12}, and growth hormones. Such enrichment can increase dinoflagellate populations in confined areas and support these blooms for longer periods of time, thus lengthening impacts.

Culture Archives. Several laboratories now have extensive cultures of toxic dinoflagellates that vary by geographic origins as well as toxin or growth characteristics. The maintenance of these valuable "archives" is an absolute necessity for continuity and standardization of research results. The unusual culture media often required for the maintenance of many highly sensitive dinoflagellate species and the special precautions necessary to prevent the accidental release of new species to the local environment suggest that this culture collection be separate

from those currently used for other phytoplankton species. We recognize there are costs associated with this operation that should become budget items with long-term funding commitments.

Environments

Localization in Time and Space. Just as we acknowledge the variability between toxic dinoflagellate species in different geographic regions, we also must stress that differences in environments necessitate regional research programs.

Since there is evidence for both geographic localization and temporal patchiness of toxic dinoflagellate populations in certain areas (Anderson et al., 1982; Watras et al., 1982), there is great potential in studies directed at elucidation of the factors that dictate such distributions. Thus studies contrasting locations and times where the toxin producers are or are not present are encouraged. These might include investigations into such factors as cyst concentrations, temperature tolerances, chemical constraints (e.g. trace metals) or hydrodynamics.

As outlined in the previous section, it is critically important to conduct field surveys that define the time and space (vertical and horizontal) variability in the dinoflagellate populations for each region. This must then be coupled with hydrodynamic studies to pinpoint the forcing functions or physical processes that determine population distributions (Pingree et al., 1977; Holligan, 1979; Tyler and Seliger, 1978).

Remote Sensing. Well-developed coastal and large estuarine frontal systems are detectable through aerial or satellite imagery depending on the sensors and resolution. Such frontal systems are characterized by surface thermal gradients and/or high chlorophyll levels. Since these fronts and associated boundary layers are often suitable areas for developing dinoflagellate populations (where growth exceeds loss through advection and predation), remote sensing has been used to detect blooms and follow their movement. This technology could also be used to predict blooms in certain areas if hydrographic features such as fronts are associated with the initial stages of bloom development (Holligan, 1979; Steidinger and Haddad, 1981). We should stress, however, that good remote sensing applications are only expected in those regions with adequate baseline information on the distribution of dinoflagellate populations and shellfish toxicity (Yentsch and Yentsch, 1982). Premature application of this technology would not lead to realistic assessment of its utility.

Human Impact. In addition to the supply of nutrients and growth factors in sewage effluents with their currently unknown effects on dinoflagellate populations, there are other aspects of human impact that call for evaluation and possible control.

Dredging and the construction of new harbors are examples. Dredging can totally transform the ecosystems of an area by destroying natural fauna and flora, by changing the natural nutrient cycling, and by

altering bathymetric features that could affect dinoflagellate cyst "seed beds." In the special case of building new harbors or marinas, there is often a change in the flow pattern of the water such that flushing decreases, thus creating a favorable low advection environment for dinoflagellate accumulation.

Relaying or transplanting of shellfish stock from a polluted or perhaps poor growth area to a "clean" area can also transfer toxic cysts, thus providing a potential seed bed for future water column blooms in a previously unaffected area. Research is clearly needed to study the viability of ingested cysts and the magnitude of this hypothesized spreading mechanism. This may lead to regulations similar to those directed at movement of diseased or contamined stock from state to state.

Prediction and Control

Prediction of PSP, NSP, and DSP events by area would be a valuable management tool because it would allow for concentrated monitoring efforts and possibly provide time to initiate alternative strategies, such as transfer of mariculture stocks or depuration of shellfish. However, predictions will only come with a knowledge of the variables and mechanisms that interact to produce dangerous concentrations of motile cells. It must thus be kept in correct sequence behind higher priority research directed at these mechanisms.

Dinoflagellates are sensitive to a variety of chemical and physical parameters. In confined systems such as laboratory culture, their growth can be limited by temperature, salinity, nutrients, chemicals such as flocculents or lysates, and even predators such as parasites or competitors. Therefore, in confined natural systems with little mixing and advection, it may be possible to control growth providing that treatment: 1) does not substantially alter other biological components of the system, 2) is cost productive, and 3) does not require constant monitoring. It, of course, would be more effective to control benthic seed beds than motile populations. Such control is unlikely in open waters with large water masses and transport systems. As with prediction, we would stress that control methodologies cannot be adequately evaluated until a sound understanding of many of the mechanisms described in previous sections is obtained. Small scale research programs aimed at characterizing the sensitivity of the target species to various control methodologies should be encouraged.

Chapter 4

BACTERIAL AND PARASITIC PATHOGENS IN THE MARINE ENVIRONMENT

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Introduction

Bacterial pathogens and an expanding group of "newly recognized" pathogens including amoebae and nematodes pose a significant barrier to greater utilization of the Nation's seafood resources. Shellfish resources are particularly affected. A large percentage of the shellfish areas nationwide are closed to harvesting due to findings of bacterial contamination. In addition, incidences of food poisoning and other infections that are traced to seafood consumption cause serious damage in consumer acceptance of seafood products.

The following presents the priority research needed to address the problems of bacterial and parasitic contamination in seafood. In addition, the importance and need for a nationally coordinated education and awareness program are presented. The priority areas for concurrent effort are:

* Education and Public Awareness
* Epidemiological/Ecological Studies
* Detection of Potential Pathogens
* Improved Bacterial Standards
* Human Pathogens in Marine Aquaculture
* Newly Recognized Pathogens
* Seafood Processing Methods
* Genetics of Marine Bacteria

Education and Public Awareness

The safety of seafood products is neither worse nor better than other food products. Information from the Center for Disease Control (CDC), for 1979, shows that of confirmed bacterial food-borne outbreaks, seafood products were responsible for 6 of 119 or 5% (U.S. Department of Health, Education and Welfare, 1981). Adjusted to a per capita consumption basis, this incidence would be similar to other food products. Another report indicated that the top five causes of these food-borne outbreaks were: (i) improper cooling, (ii) lapse of one or more days before serving, (iii) infected persons in contact with cooked food, (iv) inadequate thermal processing, canning or cooking, and (v) improper hot storage (National Research Council, 1982). This indicates, in most cases, that the food is the vehicle and not the cause. Consumer education is the only practical means of correcting this situation.

Incidents such as these and the more recent involving V. vulnificus (Louisiana Department of Health and Human Resources, 1982) are conveniently grouped into "emerging pathogens." This is somewhat of a misnomer, as these organisms have been in our environment for many centuries. Refinements in laboratory procedures; increased awareness on the part of physicians, scientists and hospital laboratories; plus changes in, and the increased sophistication of, taxonomic systems have brought these "newly recognized" pathogens to the forefront.

While it is crucial that we continue to conduct basic research on the isolation and identification of potential pathogens, the problems they might create, from a public health point of view, could be circumvented through consumer education. The overall result is confidence in and continued growth of the U.S. seafood industries.

Examples of how mishandling of seafood by the consumer has led to food-borne illnesses unique to seafood products are exemplified in outbreaks involving species of the genus Vibrio. In 1972, an estimated 600 persons became ill with acute gastroenteritis after attending a shrimp boil (U.S. Department of Health, Education and Welfare, 1972). Of all the food items served, only shrimp could be related to the incidence of the disease. Vibrio parahaemolyticus was subsequently isolated from frozen samples of the shrimp and the clinical, epidemiologic, and laboratory features were compatible with V. parahaemolyticus gastroenteritis. It was speculated that inadequate cooking allowed survival of the small number of V. parahaemolyticus present on the product and that subsequent storage for 4-5 hrs at ambient temperature resulted in rapid multiplication of the organism. After a 58 year-old woman was hospitalized for diarrheal illness and Vibrio cholerae isolated from her stool, it was determined that the vehicle was crabs that had been boiled and then held 6 hrs without refrigeration (U.S. Department of Health, Education and Welfare, 1978). Five of 9 other persons who had eaten the crabs at the same time also developed diarrheal illness. Studies conducted at the time showed that V. cholerae artificially infected into crabs survived up to 8 minutes of boiling, but not 10 minutes.

Information on the handling of seafood products has been published by most Sea Grant Advisory Services in one form or another. In addition, researchers in Sea Grant Seafood Technology programs have developed information on times and temperatures related to storage or destruction of pathogens during cooking. A national effort to combine appropriate food scientists and microbiologists for the purpose of collecting and reviewing all available information would accomplish several goals. First, it would insure that the information being disseminated was relevant and standard. Secondly, it would identify the gaps that exist in research related to the incidence and temperature sensitivity of various microorganisms of public health concern in different seafood products. The final phase of the program would be the incorporation of this information into educational programs through Marine Advisory Services, National Fisheries Institute, National Marine Fisheries Service, Food Marketing Institute, National Restaurant Association, and state and regional marketing programs under the various development founda-

tions. The end product is useful, standardized information on the safety of handling seafood products that goes much further than "flakes easily with a fork."

Seafood Processing

Quality in seafood products usually refers to its state of freshness or decomposition. Since most of the undesirable changes associated with a loss of freshness are caused by bacterial action, bacteriology can be a very useful tool in monitoring and improving seafood quality. Attempts to monitor or regulate seafood quality at the retail level have failed and will continue to fail. It is very seldom that a sample at retail reflects the quality of a product as it left the plant whose label it bears and who also must assume ultimate responsibility. Another reason for this failure is that the levels of acceptability are based on some arbitrary percentage of samples taken during retail surveys. The question of whether these samples came from plants operating under good quality control practices or not must be answered. It is important that the consumer receive a good quality product at retail. The development or refinement of processes that insure good bacteriological quality, coupled with proper handling during transportation and retail will achieve this goal.

Because of the diversity of seafood products produced and processed in the U.S., it is logical that this be a nationally coordinated effort. As with the safety problem, much information on the bacteriology of seafood products during processing does exist. Unfortunately, because of changes in laboratory techniques, the purpose of the original work, the type of sampling (blend vs. swab), different incubation temperatures, etc., the information is useful only in the planning of the program and not its implementation.

A task force of industry, government and academic food scientists and bacteriologists should identify the major seafood products that show inconsistency in processing and/or quality. After identifying standard sampling and testing procedures, various researchers would be selected on the basis of product geography. Working with the plants, the advisory specialist or agent, in cooperation with the National Marine Fisheries Service, National Fisheries Institute, National Food Processors Associations, and the Food and Drug Administration, would conduct a series of bacteriological tests. Results would be compared to processing plant conditions and processing to determine the most practical means of improving quality without deterring from productivity. The data would reflect what was achievable under good processing conditions and methods and could be used by industry through Sea Grant Marine Advisory Services to improve quality voluntarily.

Epidemiological/Ecological Studies

The marine environment constitutes a reservoir for the transmission of potential human pathogens via direct contact (Blake et al., 1980b) and/or seafood consumption (Blake et al., 1980a; Tacket et al.,

1982). The pathogens may represent part of the indigenous flora (Blake et al., 1982) or result from human influences. The abundance and distribution of the organisms, as well as their pathogenicity, appear to be regulated by environmental parameters which tend to be unique to a given goegraphical location. Correlative evidence from field studies for a few gross conservative factors, such as temperature and salinity, exist only for well-studied pathogens, such as V. cholerae (Colwell et al., 1977; Lee et al., 1982; Roberts et al., 1983; Hood et al., 1983) and V. parahaemolyticus (Kaneko and Colwell, 1978). However, the effect of various components of salinity are not understood, even for these organisms. Further, conservative parameters have not been delineated for newly recognized pathogens (i.e., Capylobacter spp., Yersenia spp., V. fluvialis, and other less well defined Vibrio spp.) and many classical pathogens (Salmonella and Shigella). Even less information is available for a thorough understanding of the relationship between non-conservative environmental factors and the abundance, distribution and pathogenicity of microorganisms of public health significance which occur in the marine environment. These non-conservative factors include, but are not limited to, trace elements, organics, particulate load, and biological interactions.

These research recommendations concern the behavior and ecology of potential pathogens in the natural environment and should be focused in given geographical areas.

* Delineate the habitat (both macro and micro habitats) occupied by the pathogen of interest.
* Determine the conservative and non-conservative limiting factors which control its occurrence and survival.
* Delineate how the pathogen is incorporated into shellfish or seafood and how the organisms are maintained, concentrated or grow.
* Determine how sewage and waste disposal (as well as other impact activities) influence the distribution, abundance, and pathogenicity of these microorganisms.

Detection of Potential Pathogens

The concept of indicator organisms originated over 100 years ago. It was based upon the premise that feces should not be present in any product for human consumption. Detection of microflora that is normally present in the gastrointestinal tract of the entire population is quantitatively easier than efforts to isolate enteric pathogens which are ordinarily present only in the feces of infected individuals and/or healthy carriers. Escherichia coli (Bacterium coli) was recognized as the indicator organism of choice (Frobisher, 1944). Methods for detection of E. coli were developed based upon gas production as the end product of the fermentation of lactose at either 35° C (Hobbie, et al., 1977 and Mallman and Darby, 1941) or 44.5° C (Hajna, 1938).

Application of these methods revealed that other bacterial species would also be recovered. Many of these organisms are normal

inhabitants of water and soil. However, their presence in potable waters and milk supplies were considered indicative of poor handling practices and provided a safety factor. Thus, the "coliform group" came to be accepted as the indicators of choice, although there is no direct relationship between allochthonous indicators and autochthonous pathogens (Colwell and Kaper, 1977).

Quantitation of numbers of coliform organisms was considered necessary in order to indicate the degree of pollution. The most probable number (MPN) method is based upon a statistical consideration of positive results from a series of three tenfold dilutions consisting of either three or five tubes per dilution (American Public Health Association, 1980). To reach a valid endpoint, additional dilutions must often be added to the minimum three dilution series. Although this increased the "precision" of the MPN methods (Woodard, 1957), a considerable workload and consequent expense is added as well.

Detection of human pathogens in seafoods, waters (American Public Health Association, 1970; Bacteriological Analytical Manual, 1978) and the aquatic environment (Kaper et al., 1979; Litsky, 1979; Roberts and Seidler, 1982) utilize media developed by and for the clinical laboratories in a MPN procedure. Time for analysis by MPN procedures may extend for several days to several weeks. Poor recoverability of "standard methods" have been shown by environmental microbiologists (Daley and Hobbie, 1975; Hobbie et al., 1977). Recent work (Xu et al., in press) has shown that both allochthonous E. coli and autochthonous Vibrio cholerae survive in the environment in a nonrecoverable, but viable state. Although the role of stress upon recoverability of microorganisms has been studied extensively, the pathogenic potential and/or virulence of stressed pathogens is now being documented. The following are priority research needs with respect to the detection of potential pathogens:

Rapid diagnostic methods that are reliable, reproducible, economic and simple are perceived as high priority research goals:

* Direct microscopic methods using immunological probes, i.e. immunofluorescence and stains for viable cells.
* Development of culture media and methodologies that will yield maximum recovery and enumeration of pathogens and indicator organisms, both allochthonous and autochthonous, from estuarine and marine seafood growing waters.
* In concert with these techniques, develop immunological probes that identify specific cell associated antigens, virulence factors, membrane proteins, etc.
* Rapid screening methods suitable for testing large numbers of isolates using gene probes for virulence factors utilizing nonradioactive labeled reagents.

* Accurate identification of isolates using a minimum number of physiological and serological parameters. This may require a reassessment of current accepted taxonomic definitions, particularly as used in definition of indicator organisms and newly recognized pathogens.
* Rapid detection of bacterial groups that produce toxins.

Taxonomic surveillance of atypical strains to detect unknown human and shellfish disease agents, food spoilage agents, and organisms with indicator potential.

Use environmental concepts as a basis for the development and modification of enrichment and isolation media for the recovery of human pathogens from the aquatic environment: Environmental concepts include consideration of stressed organisms. The same consideration should be given when attempting to isolate these same pathogens from seafoods, sewage, and other sources.

Basic research:

* Investigation of repair methods for stressed cells.
* Detection of gene products, such as intracellular enzymes, as a tool for rapid taxonomic identification of microorganisms.

Improved Bacterial Standards and Indicators

Any bacterial indicator is necessarily a compromise approach for protection of the public health. Aside from limitations inherent in an indirect or surrogate measurement, compromise results from the application of a given indicator to situations which may be dissimilar in both temporal and qualitative respects. As knowledge of environmental factors affecting microbial growth and survival increases, questions invariably arise concerning the universal applicability of a given indicator in view of local and regional habitat differences which can uniquely influence indicator and pathogen occurrence, survival and detectability.

A second consideration is that any indicator and standard, including those for shellfish growing waters or shellfish meats, should ideally exist in a state of regular and sponsored review. In this regard an indicator standard is a developmental concept subject to modification through advances in research and epidemiological investigations. Information gained through such activities should be disseminated regularly to appropriate industry, regulatory, advisory and scientific interests. The oft-repeated adage that a particular standard should not be changed because "it works" (despite the absence of supportive empirical data), does not constitute sufficient justification for complacency regarding the soundness of a standard's derivation and argues for continuous evaluation.

A final remark relates to the inadequacy of a bacterial indicator, even assuming perfect correspondence between indicator and all pathogens, to protect the public health with regard to chemical agents. Man's utilization of estuarine waters has produced significant changes in water chemistry and biology. An aspect of these changes are the industrial and domestic pollutants available for concentration by shellfish (Neff, 1979) and whose presence (and public health risk) would not necessarily correlate with enteric indicator levels.

Shellfish Growing Area Standards. Total coliform and fecal coliform bacterial standards now in use to clarify shellfish growing waters were developed in response to epidemics of typhoid and partyphoid associated with the consumption of contaminated shellfish (Furfari, 1968; Hunt, 1977; Kehr et al., 1941). The numerical value of the indicator derived was not based on epidemiological data qualitatively relating indicator to pathogen densities, but rather evolved from assumptions regarding the volume of receiving waters required to dilute enteric bacteria in sewage to a given density. Basic assumptions were made concerning the minimum number of infective agents required to produce disease symptoms and the indicator to pathogen ratio. Furthermore, bacteria were considered as essentially conservative elements, an assumption that is certainly inconsistent with current thinking (Mitchell and Chamberlin, 1975).

A significant body of literature now exists concerning the advantages and disadvantages of this coliform indicator (Hoadley and Dutka, 1977; Pipes, 1982). Our understanding of the ecology and microbiology of marine receiving waters has changed considerably since its derivation. Evidence now exists which questions the predictive value of the bacterial indicator with regard to viral pathogens (LaBelle et al., 1980; Wood, 1979). Using new techniques and methods, basic assumptions are being questioned regarding correlation of indicator densities with classical pathogens, such as Salmonella (Kaper et al., 1977), as well as "newly emerging" (Yersinia enterocolitica, Campylobacter jejuni) pathogens. In addition, pathogens native or autochthonous to estuarine environments have been identified as a significant disease causing agent capable of being transmitted through consumption of raw or contaminated shellfish and other seafoods. These pathogens include Vibrio parahaemolyticus, Vibrio vulnificus, and Vibrio cholerae (Blake et al., 1979; Carpenter, 1979; Colwell et al., 1981; Kelly and Avery, 1980). As these pathogens are not derived from sewage, the classical fecal indicator appears of little value in predicting their incidence. The lack of predictive value of the classical fecal indicator for both allochthonous and autochthonous pathogens certainly demonstrates a need for basic research describing indicator-pathogen relationships in estuarine waters.

In addition to recognition of the lack of correspondence between the indicator and certain pathogens in the water column, there are observations which suggest bacterial densities in the water column may not reliably correlate with indicator and pathogen concentrations in shellfish (Wood, 1979). This has resulted in recommendations that repre-

sentative sampling of the shellfish themselves, rather than the growing waters, be used to assess product quality (Wood, 1979). This is a reasonable suggestion and basic research should be performed to measure the densities of indicator bacteria and pathogens (both autochthonous and allochthonous) in shellfish obtained from growing areas of characteristic indicator densities.

Other investigators (Erkenbrecher, 1981; Goyal et al., 1977) have noted significantly higher concentrations of indicator bacteria and pathogens in estuarine sediments, compared to the water column. As shellfish are located at the benthic boundary layer, where sediment particles may be quite mobile during tidal excursions, the suggestion has been made (Erkenbrecher, 1981) that shellfish growing area standards be based on concentrations of indicator or pathogens in sediments. However, to evaluate this concept, basic information is required describing bacterial concentrations in different growing area sediment types, as well as an understanding of factors affecting bacterial survival.

If there are justifiable reasons to question the validity of the current bacterial indicator, then even more questionable is the quantitative derivation of the growing area standard and the public health significance of its assigned numerical value. Certainly, an increase in the level of indicator bacteria tolerated would result in increases in marketable product. The absence of quantitative data providing means to assess the epidemiological consequences of tolerating even small increases in indicator levels would seem to provide sufficient need for statistical comparison of pathogen burdens in shellfish and growing waters characterized by small differences in indicator concentrations.

Another question regarding the absolute numerical value of the bacterial indicator standard is related to viable enumeration procedures used for detection of indicator or pathogen in all sample types. The literature reveals that enumeration procedures for recovery of these organisms are affected by their history of exposure, which is a function of source, physical-chemical conditions, biological processes and physiological stress (Hackney et al., 1979; Kaper et al., 1977; Rhodes and Kator, 1982; Rhodes et al., 1982) and that the basic assumption that indicator and pathogen exhibit at least parity in die-off may not hold for all seasons and physical-chemical regimes. The significance of physiological stress on bacterial recovery must be determined if enumeration procedures (and numerical standards) are to be of value.

A final remark about the correspondence of indicator bacteria to pathogen concerns the classical idea that municipal sewage comprises the most significant source of these pathogens. In many estuaries nonpoint source runoff has been identified as frequently responsible for closure of shellfish beds after significant rain events. Although some data exist on the levels of indicator bacteria and pathogens in storm runoff (Olivieri et al., 1978), there is a basic lack of quantitative data on pathogen occurrence in estuarine receiving waters following runoff events. There is a need for this type of information by regulatory personnel who manage these resources, especially in view of a recent outbreak of shellfish associated gastroenteritis attributed to storm runoff (Centers for Disease Control, 1982).

Two additional topics associated with the indicator concept require further research. The first of these is the regulatory use of so-called "buffer" zones contiguous with potential or known sources of fecal pollution. Shellfish harvesting is prohibited within these zones on yearly or seasonal bases. One concern is the blanket application of this concept by coastal states toward marinas and the arbitrary criteria used to construct these zones in the absence of quantitative microbial or hydrographic data (Young, 1981). A data base must be developed relating hydrographic characteristics, indicator and perhaps pathogen densities to buffer zone dimensions. This information is needed to judge if reductions of potential shellfish growing area acreage are based on justifiable criteria. A preliminary marina study (Kator et al., 1982) suggests each marine-buffer zone combination must be individually evaluated.

The other problem related to the shellfish growing area standard concerns relaying oysters from restricted areas to approved waters for the purpose of depurating pathogens. Considering depuration (in situ or in controlled environments) as a process which can facilitate greater utilization of shellfish resources, then efforts to increase the efficiency of this process are reasonable goals. Theoretically, a depurated product would be "safer" (and therefore of enhanced desirability) if it can be shown free of not only pathogens, but also chemical pollutants or toxicants which may be present in shellfish from approved areas. Intervals required for depuration of relayed shellfish may be longer than necessary. Basic research should be conducted on the depuration process in the field for related oysters, focusing on factors controlling densities of indicator bacteria, pathogens (both autochthonous and allochthonous) and chemical pollutants. The information obtained could result in a shorter required depuration interval and reveal whether determinations of indicator or pathogen densities in the meats should be required for a measure of product safety.

Product Standards. Bacterial standards for seafood products should be developed considering these products as unique foodstuffs. Therefore, one important question is whether the fecal coliform shellfish market standard and the aerobic plate count (APC) are adequate criteria by which to judge the public health aspects of seafoods. Although the FC standard and the APC may be valid indicators of fecal contamination or food spoilage, correlation of these indicators with the presence of native estuarine pathogens should not be anticipated. Recognition of this fact has become important following observations that autochthonous estuarine pathogens previously mentioned (V. parahaemolyticus, V. vulnificus, V. cholerae) are disease-causing agents responsible for acute gastroenteritis, septicemia and even death (Blake et al., 1979; Carpenter, 1979; Colwell et al., 1981; Kelly and Avery, 1980). These pathogens, as normal constituents of the estuarine microbiota, may be initially present in or on the food, or introduced as contaminants during processing, and can grow to appreciable numbers at ambient temperatures. The initiation of research to develop methods for the detection and enumeration of these pathogens in shellfish and blue crabs is recom-

mended, with the immediate objective of providing data on which to develop a new standard based on these pathogens. Regional influences on the occurrence of these pathogens should also be evaluated.

Finally, differences in geographic habitats may affect the interpretation of fecal coliform enumeration tests. Aside from the recognized significance of sublethal stress on enumeration efficiency, personnel in the Gulf of Mexico (Hackney, C.R., personal communication) have reported extremely large numbers of fecal coliforms in oyster meats obtained from approved growing waters. Such oyster meats are not marketable and represent significant economic losses to watermen. There is some evidence that these elevated counts are due to indigenous marine bacteria, which can transiently ferment lactose and produce gas in EC medium and which may grow in the oyster after harvesting during the period prior to shucking. Research is necessary to evaluate the efficacy of the fecal coliform market standard in view of these false fecal coliforms, to identify them, and to determine the regional extent of their occurrence.

Human Pathogens in Marine Aquaculture

Public health problems may develop from fecal contamination of open water growing areas in extensive aquaculture, or from microbial contaminants not killed before use of human or other mammalian or avian sewage as a nutrient source for aquaculture. A secondary problem (at present) is increase of heterotrophic organisms in areas enriched by aquaculture production--some of these organisms may be toxic to humans or to the cultured animals.

The seafood industry is somewhat unique in that any hint of a public health problem with one product has immediate repercussions on the rest of the industry. The aquaculture industry, struggling for survival in this country, is especially vulnerable to adverse actions.

Multiple use of estuarine/coastal waters is a fact of life in industrialized countries. There are already examples, in Japan and the United States, of loss of extensive aquaculture growout areas (particularly for shellfish) as a consequence of gradual or rapid increases in industrial pollution. There are also examples of outbreaks of human disease directly attributable to consumption of contaminated shellfish. These outbreaks occur despite national and state efforts at control of harvest, transport, and use.

Additionally, there are attempts--some of them pilot stage or operational--to use treated sewage as a nutrient source for aquaculture production. The Far East has long used human feces as a source of fertilizer. Questions persist about survival of viruses and certain bacterial and protozoan spores after various treatments.

Populations of vibrios and other heterotrophs may be enhanced by organic enrichment--whether by aquaculture or by human organic additions. Infection pressure on stressed aquaculture populations is thereby increased, and the potential for human infection from consumption of inadequately processed seafood is also enhanced. Priority research needs are:

* Continued development of better methods of recognition of human fecal contamination of estuarine/coastal waters, beyond the usual coliform tests. New and rapid methods should be incorporated quickly into regional and national monitoring programs, concentrated in aquaculture areas.
* Expand research on the effects of extensive aquaculture on the ecology of the growout areas--emphasizing the critical role of vibrio and other microbial populations and the significance of eutrophication.
* Further studies of survival of viruses and resistant stages of other microbial pathogens in the marine environment should be conducted, emphasizing possible protective roles of particulates and alternative inactivation techniques.
* Coastal Zone planning in industrialized countries must consider, in any multiple use program, the primacy of aquaculture areas, and must develop regulatory frameworks to protect such areas from actions which would increase fecal pollution.

Newly Recognized Pathogens

Although a number of marine organisms are considered below as "newly recognized" pathogens transmissible to humans via seafoods, in some instances their occurrence has been known for decades. However, their exact identification, clinical symptoms and diagnosis of the diseases caused by them, and their prevalence, especially in the United States, remain essentially unknown and as a result, their impact has not been fully appreciated.

Among these "newly recognized" pathogens may be listed the bacteria Campylobacter spp. and Yersinia spp., amoebae of the Hartmannella-Naegleria-Acanthamoeba complex, certain species of nematodes of the family Anisakidae, and trematodes of the family Heterophyidae. Also, although a specific example is at present limited to the hard clam, Mercenaria mercenaria, parasitized by metacercariae of the trematode Himasthla spp., undesirable biochemical alterations in seafoods resulting from degradation due to enzymes of parasite origin can cause widespread clinical syndromes, e.g., short-term but, nevertheless, severe gastroenteritis, resulting from the ingestion of toxic, short-chain fatty acids (Cheng, 1965; Cheng, 1973).

It is noted that both Campylobacter and Yersinia are human pathogens possibly transmissible through seafoods (Toma, 1973; Rabson et al., 1975; Munger et al., unpubl.).

The pathogenicity of the Hartmannella-Naegleria-Acanthamoeba complex of amoebae, first reported by Wang and Feldman (1961, 1967), has been reviewed by Chang (1971). In brief, these microorganisms are usually free-living in soil; however, they are capable of facultative parasitism and are pathogenic, causing a commonly lethal syndrome known as amoebic meningoencephalitis. The fact that this group of amoebae can be passed from soil into sewage and from thence to shell-

fish beds (Cheng, 1970a, Chang, 1971) would appear to be of sufficient importance to warrant attention from individuals interested in pathogens transmissible by shellfish. However, the finding by Cheng (1970b) that Hartmannella does indeed invade oysters under environmental stress signals a potentially explosive situation from the standpoint of public health. It is noted that at least 50 cases of human meningoencephalitis have been documented (Callicot et al., 1968; Duma et al., 1969; and others).

The occurrence of anisakiasis transmitted from marine fish to humans was first reported in Holland by Van Thiel et al., (1960) and has since become recognized as a global problem (Cheng, 1973; Jackson, 1975). The etiologic agents are larval nematodes belonging to the genera Anisakis, Belanisakis, Phocanema, Porrocaecum, Paradujardinia, Pseudoterranova, Cloeoascaris, Phocascaris, and Contracaecum (Myers, 1975; Cheng, 1982). They are ingested in raw or inadequately cooked (including smoked) fish fillet and they cause the development of commonly lethal gastric or intestinal granulomatous lesions. Globally, at least 300 human cases of anisakiasis have been documented (see Jackson, 1975, for review). In the United States, at least a dozen cases have been reported.

Those species of heterophyid trematodes, which can be transmitted from marine fish to humans have been reviewed by Cheng (1973). They are members of the following genera: Heterophyes, Metagonimus, Centrocestus, Cryptocotyle, and Stellantchasmus. Although human infections occur most commonly in the Orient, these intestinal parasites occur in Hawaii and Cryptocotyle occurs along the Atlantic coast of the United States. Parasitization by these parasites usually only result in mild intestinal discomfort; however, as the worms mature and commence depositing eggs, these microscopic bodies are commonly carried to various tissues in the body in circulation, including the heart. According to Kean and Breslau (1964), 14.6% of cardiac failures in the Philippines result from inflammation of cardiac muscles due to heterophyid eggs. Priority research needs are:

* As stated, one of the major reasons the "newly recognized" pathogens have not been readily recognized is because of difficulties in isolation and identification. Therefore, research involving modifications of such modern techniques as direct and indirect ELISA, immunofluorescence, etc. for rapid, inexpensive, simple, and reliable identification should be encouraged.
* There are some isolated pieces of evidence which suggest that not all strains of the listed pathogens are equally as pathogenic, at least to mammals. Therefore, studies directed toward the understanding of pathogenicity (e.g., differences in penetration enzymes, host immunologic responses, host mimicry, life cycle stages, etc.) should be conducted.

* Although the prevalence of certain groups of parasites has been ascertained to some degree in Scandanavia, Great Britain, Holland, and Japan (e.g., the anisakid nematodes), as well as the environmental factors favoring their concentration and shifts from one location to another, such studies have not been conducted off the coast of North America. Such should be encouraged in order to gain a better understanding of the epidemiology of the resulting human diseases. Such information could lead to the development of preventive measures through correction of fishing methodologies.
* It needs to be pointed out that the prevention of water-borne protozoan diseases differ from that of bacterial diseases in one major aspect. Specifically, most pathogenic bacteria are anaerobic and water treatment as currently practiced contributes to the reduction of bacterial growth. On the other hand, amoebae and other water-borne protozoans are aerobic and treatment of contaminated water requires alternative methodologies. Studies along this line should be encouraged.
* In studying the ecology of pathogenic eukaryotes, the concept of facultative parasitism of immunologically compromised hosts should be remembered, which holds true for invertebrates (crustaceans and mollusks), as well as vertebrates (fishes). We need to gain considerable additional basic information relative to the immunology of these marine animals, keeping in mind that pathogens harbored by them have, at least in certain cases, been demonstrated to be zoonotically transmissible to humans.
* In view of the fact that fresh and prepared seafood products are being introduced into North America from areas where some of these "newly recognized" pathogens are endemic, e.g., echinocephaliasis (caused by larvae of the nematode Echinocephalus crassostreai in Crassostrea gigas) (Cheng, 1975) from Hong Kong, research leading to rapid and reliable detection of endopathogens in imported seafoods should be developed.
* Increased emphasis should be placed on determining undesirable chemical changes in fresh seafoods, especially mollusks and crustaceans, resulting from degradation by enzymes secreted by parasites.

It is a well established ecologic principle that animals maintained under stress conditions, e.g. crowding, elevated levels of excreted materials, lowered pO_2, etc., are more susceptible to facultative parasitism and metabolic diseases. In view of the active promotion of mariculture, emphasis should be placed on research directed at the identification of pathogens, the importances of which are magnified under culture conditions. Along this line, it should be pointed out that biological control agents (viruses, bacteria, protozoans) currently in use or being developed for agriculture in coastal areas may comprise a portion of potential facultative pathogens of estuarine animals.

Another area of increasing concern involves the emerging biomedical problems associated with direct human contact with polluted offshore waters, for example, increased gastrointestinal disturbances, and eye, ear, and throat infections on divers and swimmers. Many of these appear to be caused by bacteria and protozoans while others may be of viral etiology.

Seafood Processing Methods

The possible presence of human pathogens has an effect on new process or product development. For example, the possible presence of Clostridium botulinum type E may influence the use of flexible pouches for pasteurized crabmeat. Currently, pasteurized crabmeat is packaged in one pound cans and heat is applied until the cold point reaches 85° C for one minute (Cockey and Tatro, 1974). It often takes upwards of 90 minutes to achieve pasteurization. Considerable accumulated lethality is derived from this process. Pasteurization in one pound cans has a number of disadvantages. The long processing time is expensive because of high energy costs. The one pound can is often too large for the average American family, which is getting smaller. In addition, cost of the one pound can is considerable, often exceeding $6.00. The long processing time sometimes causes the crabmeat, farthest from the cold point, to become overheated and to turn blue. Finally, the one pound can does not freeze well and often shows rust. Thus, it is apparent that current packaging for crabmeat needs to be changed. The flexible retort pouch may provide the answer (Heintz, 1980). The pouch could be made small enough for single servings, i.e., 4-6 oz. Its small size would allow quick heat penetration and less energy usage. Also, the pouches will freeze easily and could be placed into printed paperboard boxes for an attractive display. In addition, restaurant managers should find the single serving pouch appealing because of better portion control. However, microbiological considerations are important. Whenever container size is changed, the heating process must be re-evaluated. Pasteurization time for crabmeat in retort pouches would be reduced and the accumulated lethality would be less. Studies will be needed to assure that C. botulinum type E would not be able to survive pasteurization.

Products which may be pasteurized in retort pouches in the future include shrimp and crawfish tails. It is important to note that the heat resistance of microorganisms will vary with different foods. Therefore, time and temperatures used to eliminate C. botulinum type E in crabmeat may not be adequate to assure safety for pasteurized shrimp.

Other research needs concerning human pathogens and seafood processing include determination of thermal death times of pathogenic marine bacteria in various seafoods and the effect of storage conditions on these organisms. Pathogenic marine bacteria of concern include Vibrio cholerae, Vibrio parahaemolyticus and Vibrio vulnificus. These microorganisms are relatively heat sensitive (Sakazaki, 1979). However, many seafoods, such as oysters, are consumed raw or after only a minimal heat treatment. Oysters have been responsible for food-borne

outbreaks of cholera and V. vulnificus and V. parahaemolyticus food poisoning (Sakazaki, 1979; Spite et al., 1978). Processing procedures are needed which will eliminate these microorganisms from oysters, without changing the organoleptic characteristics of these mullusks. Since these pathogenic marine bacteria are heat sensitive, it is possible that a relatively mild heat treatment, such as that used in heat shocking, may destroy these bacteria. Heat shocking is a process where shell stock oysters are immersed in hot water to facilitate shucking (Hackney et al., 1980). At present, no effort is made to insure uniform heat distribution, but this process will greatly reduce the number of V. parahaemolyticus and coliforms in oysters (Hackney et al., 1980). It might be possible to refine this process, to reduce the levels of pathogenic marine bacteria. Thermal death time studies on pathogenic marine bacteria are needed before a study of this nature could be undertaken. In addition, thermal death time studies are needed to evaluate cooking procedures for fish and shellfish.

Another important research area concerns the storage of seafood products before and after processing. It is important to know the fate of indicators, pathogenic marine bacteria and enteric bacteria, such as Campylobacter jejuni in shell stock oysters during storage. Limited studies with V. parahaemolyticus indicate that their number should not increase when stored at 25° C (Thompson and Vanderzant, 1976). However, oysters harvested along the Gulf coast are often held at temperatures in excess of 32° C for 8-10 hours. Coliform and fecal coliform levels in these oysters often reach counts of 100-1000 per gram. Research is needed on the fate of human pathogens during storage at these high temperatures.

Finfish are often stored in modified controlled atmospheres (Lanelongue et al., 1982). This process will greatly extend the shelf life of the product by inhibiting psychotrophic Gram-negative aerobic bacteria. Psychotrophic pathogenic bacteria, such as Yersinis enterocolitica may present problems. In addition, studies on the effect of possible consumer abuse to these packages are needed. A list of recommendations follows.

* Determine the pasteurization parameters for new packaging approaches, in particular the flexible pouch.
* Determine thermal death times of pathogenic marine bacteria in seafoods and the effect of storage conditions on these organisms.
* Determine the fate of indicator bacteria, pathogenic marine bacteria and enteric bacteria in shellfish during storage.
* Determine the bacterial quality of seafoods stored in modified controlled atmospheres.

Genetics of Marine Bacteria

In recent years there has been substantial progress in our understanding of the ecology, taxonomy and epidemiology of pathogenic marine vibrios. However, a lack of basic information is now seriously

impairing further progress towards the control of human diseases transmitted via seafood.

Studies of the distributional ecology of marine vibrios have been stymied by the inability to detect specific pathogenic strains of marine vibrios. One crucial point is that it is important to distinguish pathogenic from non-pathogenic strains of organisms if we hope to develop realistic policies impacting upon the transmission of human diseases.

Studies of the molecular basis of virulence and expression of virulence factors in marine vibrios will not only provide a means for the development of rapid tests for their detection, but will also eliminate the barriers preventing the complete utilization of marine resources.

Currently there are only a few, but exciting, areas of research concerning virulence factors in pathogenic marine vibrios. The virulence mechanisms include: a plasmid-mediated iron transport system in the fish pathogen Vibrio anguillarum (Crosa, 1979; Crosa, 1980; Crosa and Falkow, 1981; Crosa and Hodges, 1981; Crosa et al., 1980; Crosa et al., 1977) and a similar mechanism in the human marine pathogen, Vibrio vulnificus; chemotaxis, motility and attachment in Vibrio cholerae, Vibrio mimicus and perhaps the other pathogenic vibrios (Freter et al., 1979; Guentzel and Berry, 1975); and evidence for a new enterotoxin distinct from cholera toxin produced by non-01 Vibrio cholerae and Vibrio fluvialis isolates from humans (Nishibuchi and Seidler, 1983).

The wave of developments in genetic engineering and molecular genetics is upon us. The major initiative made by Sea Grant in this area is welcome. Recognition of the potential applied and basic sciences benefits will reduce the barriers to seafood utilization currently caused by bacterial pathogens. Genetic engineering offers the opportunity to utilize recombinant DNA technology for detecting specific virulence genes present in food-borne pathogens or causing epizootics in important aquaculture species. Consequently, in order to efficiently apply this technology the bacterial and host factors that affect virulence must be known.

The following research topics are of high priority for defining the basis of pathogenicity by marine vibrios.

* It is recommended that recent advances in recombinant DNA technology be used to study the molecular basis of virulence.

    1. Identification of virulence factors using a variety of animal and tissue culture bioassays.
    2. Isolation of genes encoding the specific virulence factors. This will be accomplished by cloning onto specific plasmid and bacteriophage vectors and selecting for clones expressing the putative virulence factors.
    3. Construction of probes with the isolated genes to be used for the detection of pathogenic isolates in a total population of pathogenic and non-pathogenic bacteria. This methodology will be, of course, very rapid, reliable, economical and amenable to development of commercially produced diagnostic kits.

* It is recommended that expression of gene products in marine vibrios should also be investigated in depth. The following are suggested approaches:

    1. Immunological probes for products of virulence genes should be obtained by using both monoclonal and classical antibody approaches. These probes will be a reflection of the expression of the virulence genes.
    2. Influence of nutritional and environmental conditions in the expression and control of genes in marine vibrios. Some unusual genetic regulatory mechanisms in the marine vibrios have been uncovered (Crosa, 1979; Crosa, 1980; Crosa and Falkow, 1981; Crosa and Hodges, 1981; Crosa et al., 1980; Crosa et al., 1977; Greenberg et al., 1979; Ulitzur, 1974). These mechanisms are not yet well defined but should be important to an adequate understanding of virulence. For instance, there is a need for intensive investigations in the mechanisms of control of motor behavior, specifically chemotaxis and flagellar type variation (Shinoda et al., 1974; Ulitzur, 1974); export of enterotoxins; cell size; and the effect of environmental stress, for instance, iron limitation (Crosa, 1979; Crosa, 1980) upon the physiological state of the pathogen.

* Research on genetic events which occur in marine vibrios is needed. Any investigation of interspecies or intergenic gene transfer or phase transition would be of the utmost importance to the understanding of the molecular genetics of pathogenicity and to the development of new measures for the control of those pathogens.
* Research concerning the interactions between pathogenic marine vibrios, the marine animal host and its environment should be a research priority.

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