Chesapeake Bay Environmental Effects Studies

Toxics Research Program

Chesapeake Bay Program

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1994 Workshop Report

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CHESAPEAKE BAY ENVIRONMENTAL EFFECTS STUDIES
TOXICS RESEARCH PROGRAM

1994 WORKSHOP REPORT

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INTRODUCTION

BACKGROUND

In order to restore the productivity and ecological health of the Chesapeake Bay, the federal/state Chesapeake Bay restoration program set as a goal the reduction of nutrients and toxic substances into the estuary. While the desire to reduce anthropogenic influence on the Bay was recognized, there was a need to establish how these inputs affected the ecological processes of the Chesapeake Bay.

Since 1985, Congress has appropriated funds for the National Oceanographic and Atmospheric Administration (NOAA) to support environmental effects research in the Chesapeake Bay. The Chesapeake Bay Environmental Effects Committee (CBEEC) was established in 1987 by NOAA to oversee this program. CBEEC includes representation from Virginia, Maryland, Pennsylvania, and the federal government. Research funds are awarded via a competitive peer-review process, and the funds are administered by the Maryland and Virginia Sea Grant College Programs.

Beginning September 1985, a major effort was initiated to address the effects of low dissolved oxygen in Chesapeake Bay. The hypoxia program was conceived as an ecologically oriented study focusing on system-level effects. This research has greatly refined our understanding of the complex relationships between nutrients, production of organic material, carbon cycling, and the development of hypoxia. In addition, the impact of low dissolved oxygen on functioning and productivity of key species and communities was found to be significant. *Oxygen Dynamics in the Chesapeake Bay*[1], summarizes the current level of understanding of the interaction of physical, chemical and biological processes that create hypoxic conditions.

In 1990 the Environmental Protection Agency's (EPA) Chesapeake Bay Office joined the Environmental Effects Research Program. In addition, members of the Chesapeake Bay Toxics Subcommittee were added to the CBEEC, and the focus of the research program was redirected from hypoxia to studies of toxic contaminants in Chesapeake Bay, an area where considerable information is needed to support management actions.

In conceiving the Toxics Research Program (TRP), CBEEC felt that it was important to maintain an ecological focus on system-level environmental effects, similar to the hypoxia program. Thus, the general goals of the TRP are:

- to understand how Chesapeake Bay ecosystem processes influence the transport, fate and effect of toxicants; and

- to understand the effects that representative toxicants have upon ecological processes, including trophic dynamics, in Chesapeake Bay.

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[1] David E. Smith, Merrill Leffler, and Gail Mackiernan, Eds. *Oxygen Dynamics in the Chesapeake Bay.* (Maryland Sea Grant, 1992) UM-SG-TS-92-01. VSG-92-01.

Long-term objectives of the TRP are based on the Chesapeake Bay Program Research Planning Committee's "Toxics Research Prioritizations" document, and focus on increasing the understanding of the source, transport, fate, and effects of toxicants in support of development of ecological risk assessments for the Chesapeake Bay. A conceptual diagram of the interaction between ecosystem processes and contaminants in Chesapeake Bay is presented in Figure 1. The CBEEC prepared a Request For Proposals based on these needs, emphasizing an ecosystem approach to the issue of toxics in Chesapeake Bay. This request has been updated annually to reflect progress of the TRP and the needs of water quality managers in the Bay community.

Funding for TRP projects commenced in September 1990. At the time of this workshop, twenty-one research projects have been completed, four are nearing completion, and six projects continue through 1995. A list of project titles and principal investigators is provided in Appendix 1; addresses of principal investigators are listed in Appendix 2.

1994 WORKSHOP

This document is a summary of the third Toxics Research Program workshop, which was held at the Virginia Institute of Marine Science in Gloucester Point, Virginia on 6-7 December 1994. The workshop agenda and a list of attendees are provided in Appendices 3 and 4, respectively. Sponsored by the Chesapeake Bay Environmental Effects Committee and the Scientific and Technical Advisory Committee (STAC) of the Chesapeake Bay Program, the purpose of the workshop was to provide a forum for:

- reviewing recent findings of TRP research, highlighting project integration and relationship to goals of the Chesapeake Bay Program;
- discussion among researchers and resource management representatives regarding TRP research priorities and communication among various constituencies;
- consideration of the role of TRP research in fulfilling objectives of "Directed Toxics Assessments" of the *Chesapeake Bay Basinwide Toxics Reduction Strategy;*
- discussion of the role of STAC in implementation of a "management/scientific framework" as directed in the *Chesapeake Bay Basinwide Toxics Reduction Strategy.*

The prime objective of the workshop was to promote better cooperation and dialogue among groups that have responsibility for addressing the problem of toxic contaminants in Chesapeake Bay.

The first half day of the workshop was devoted to an overview of toxics research in Chesapeake Bay. Dr. Joel Baker led off by addressing the question, "Are toxics a problem in Chesapeake Bay?" followed by summary presentations of findings from Toxics Research Program projects (see Agenda, Appendix 3). Highlights of these presentations begin on p. 6. Research findings from each project begin on page 31.

Following an overview of the *Chesapeake Bay Basinwide Toxics Reduction Strategy* by Rich Batiuk, most of the afternoon of the first day was spent in two breakout discussion groups for resource managers and researchers to discuss the role of the toxics research program in relation to management of Chesapeake Bay. The discussion was continued among the whole group on the second day of the workshop. These discussion sessions are summarized on pages 21-28.

Dr. Ray Alden led discussion of the "management-scientific framework" as called for in the *Chesapeake Bay Basinwide Toxics Reduction Strategy*. The main points of this discussion are summarized on page 27-28.

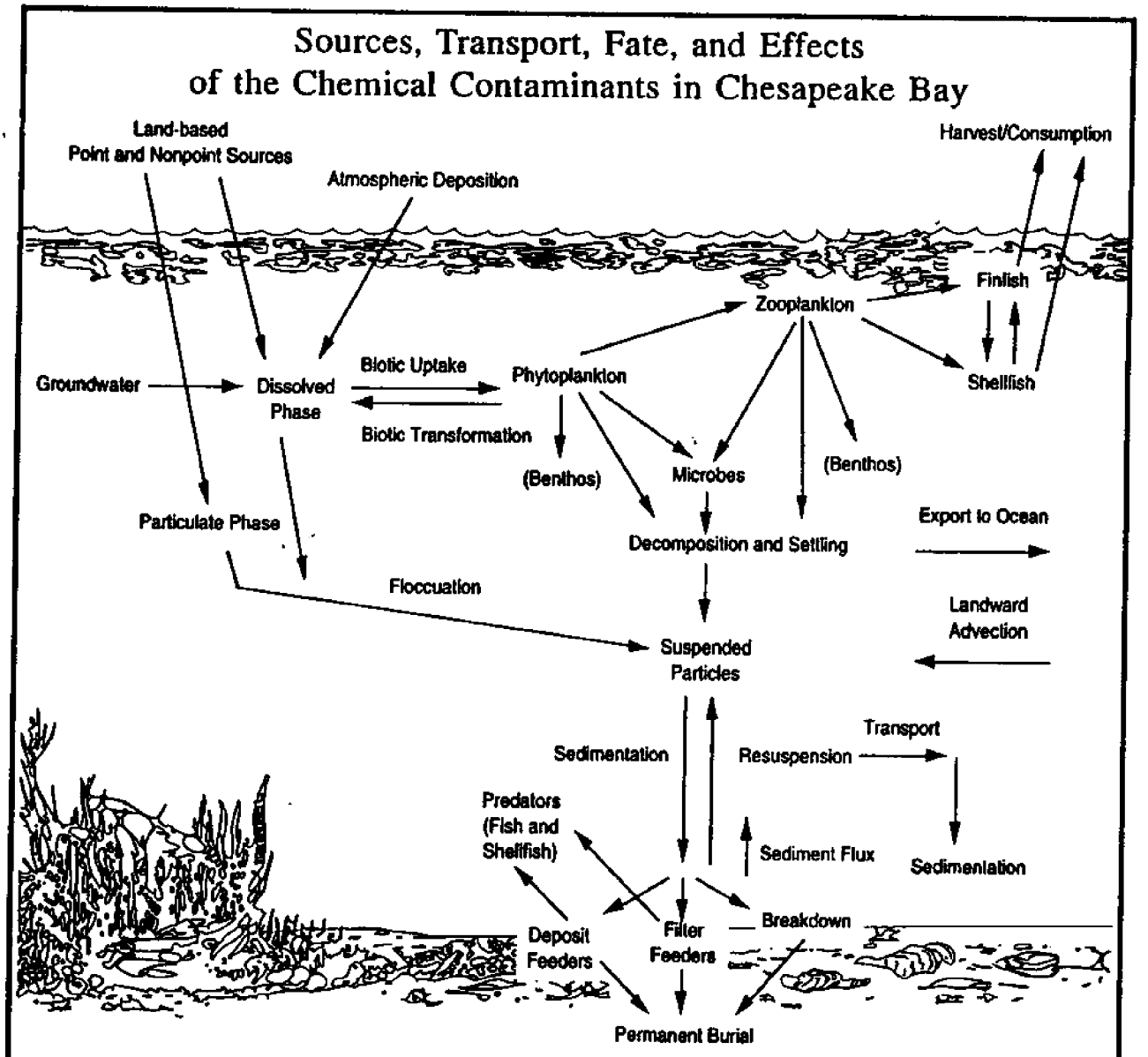

Fig. 1. Conceptual model of the sources, transport, fate, and effects of chemical contaminants on Chesapeake Bay trophic dynamics and ecosystem processes. Adapted from *Chesapeake Bay Basinwide Toxics Reduction Strategy Reevaluation Report.* (Chesapeake Bay Program, 1994) p.47.

ARE TOXICS A PROBLEM IN THE CHESAPEAKE BAY?

THE THIRTEEN QUESTION METHOD

Joel E. Baker
Associate Professor of Environmental Chemistry
Chesapeake Biological Laboratory
Center for Environmental and Estuarine Studies
University of Maryland

I say the thirteen question method is the one to use.
The thirteen question method is the one to use if you want to have some fun,
The thirteen question method is the one to use.

C. Berry

QUESTION 1. Are toxics present in the Chesapeake Bay?

'Toxic' chemicals, generally understood to be anthropogenic organic chemicals or anthropogenically-mobilized elements, have been detected throughout the Chesapeake Bay (Helz and Huggett, 1987; Hale *et al.*, 1990; Leister and Baker, 1994; Ko and Baker, 1995). This is not surprising, as these chemicals are found broadly distributed throughout the world's aquatic environments, even in remote regions of the Arctic and Antarctic. Skilled chemists using modern analytical methods can detect as little as one part per quadrillion (picogram/liter, or one millionth of a part per million) of an organic pollutant in water. Previous and, unfortunately, some ongoing monitoring programs continue to use antiquated methods (often dictated by regulatory agencies) with exceptionally poor sensitivities. The best available analytical techniques are often 1000 to 10,000 times more sensitive that 'EPA-certified' methods, yet cost essentially the same. One would only go hunting with a blind dog if you didn't want to find anything.

While we can debate the significance of low levels of contaminants in the Chesapeake Bay, there is no scientific justification for the argument that toxics simply are not present.

QUESTION 2. Where do toxics come from?

While there is a long list of possible sources of toxic chemicals to the Chesapeake Bay, recent work suggests that on a bay-wide basis, riverine discharges, urban storm water runoff, atmospheric deposition, and, perhaps, agricultural runoff, dominate the loadings (Chesapeake Bay Program, 1994). Riverine discharges are large simply because river flows dominate the hydrologic budget of the bay. Much of the chemical load borne by the rivers may result from industrial and municipal discharges to the rivers near and above their fall lines and, therefore, the riverine loads may be controllable. (A majority of industrial discharges are located on tributaries rather than on the bay proper). While there is a great deal of

uncertainty about the magnitude of urban storm water runoff, the loadings of trace elements and organic chemicals (petroleum-derived hydrocarbons) are likely large. Urban storm water has not been adequately characterized since the National Urban Runoff Project in the early '80's, and likely represents one of the most readily controllable sources of toxics to the Chesapeake Bay. Combustion of fossil fuels and incineration drive the loadings of toxics from the atmosphere to the Chesapeake Bay, and reducing emissions on a regional scale will be required to lessen these loadings. Runoff from farm fields may deliver large but (likely) short-lived loadings of current-use agrichemicals to the bay and its tributaries. While implementation of best management practices (e.g., no-till) may reduce runoff, application rates of agrichemicals may increase. It seems unlikely that transport of contaminants through groundwater is a significant route of toxics loadings to the Chesapeake Bay.

QUESTION 3. What is the relative importance of these sources?

Recent attempts to place the relative magnitude of toxics loadings from various sources to the Chesapeake Bay have been partially successful, but limited by large gaps in the data and by inconsistencies in the methods used to measure or estimate loadings. If one takes the fall lines as the boundary of the Chesapeake Bay waters (as distinct from the tributaries and watershed), then riverine loadings and internal recycling (i.e., reentrainment of contaminants from sediments) likely are the dominant sources of toxics to the water column bay-wide. Needless to say, this generalization does not apply to those waters adjacent to extensively contaminated areas such as Baltimore Harbor, the Anacostia River, and the Elizabeth River. In addition to historical loadings stored in their sediments, these systems also receive considerable urban storm water runoff.

QUESTION 4. Do all sources have the same potency?

Current toxics loading estimates are at best statements of the <u>total</u> contaminant entering the Chesapeake Bay, regardless of its form. Toxic contaminants exist not only dissolved in the water, but also associated with a wide variety of aquatic and eroded terrestrial particles. The toxic 'potency' depends not only upon the specific characteristics of the chemical and its total concentration, but also upon its physicochemical form in the water. Dissolved contaminants are readily available for uptake by aquatic organisms across gill and membrane surfaces, while particle-associated toxics may be ingested by filter-feeding organisms. Speciation also controls the spatial distribution and water column lifetimes of contaminants, as particle-associated chemicals settle from the water column into the sediments near their discharge point. Some contaminants, such as high molecular weight polycyclic aromatic hydrocarbons, are discharged into the environment tightly bound to relatively inert soot particles. Others, such as many trace elements, also occur on particles but may be easily leached once discharged to surface waters, supporting dissolved inventories.

Not all forms of toxics loadings are equal. Point source discharges of particle-reactive chemicals likely impact localized areas. In contrast, diffuse loadings, such as atmospheric deposition, delivers chemicals directly to surface waters throughout the bay. A kilogram of phenanthrene discharged from a storm drain in Baltimore's Inner Harbor will likely have a

much different impact than a kilogram of gaseous phenanthrene absorbed into the surface waters of the bay during a plankton bloom.

QUESTION 5. Are toxics evenly distributed spatially?

A defining characteristic of an estuary is the sharp spatial gradient in habitat, geochemistry, physics, and living resources. It would be surprising, therefore, to find toxics evenly distributed through the bay's waters. The Chesapeake Bay has recognized 'hot spot' areas where (virtually!) everyone agrees toxic contaminants are present in undesirable levels. Much less is known, however, about how far these areas exert an influence and about spatial gradients of chemicals in the waters and sediments of the mainstem bay. In general, levels of particle-reactive chemicals in mainstem bay sediments decrease sharply from north to south (Helz and Huggett, 1987; Eskin *et al.*, 1994). This has been attributed to higher loadings to the northern bay (perhaps from atmospheric deposition; Helz and Huggett, 1987), but may also simply reflect the enhanced deposition of fine-grain sediments above and within the turbidity maximum. Interestingly, Eskin *et al.* (1994) reported elevated concentrations of trace elements and some organic contaminants in the sediments of the northern-most tributaries of the Chesapeake Bay (e.g., Sassafras River) far above the Baltimore metropolitan area. In the absence of local sources, this enrichment of sedimentary toxics may result from efficient 'capture' of fine-grain riverine sediments from the Susquehanna River.

Recently, we have measured large gradients in polycyclic aromatic hydrocarbons and agrichemicals southward from the Susquehanna River (Nelson *et al.*, 1995; M^{c}Connell, 1994). Inventories of particle-associated PAHs are highest below the Coniwingo Dam, and decrease sharply along a transect above the turbidity maximum, presumably due to efficient particle settling. In contrast, concentrations of dissolved PAHs are nearly uniform in the upper bay, and monotomically decrease downstream of the turbidity maximum (Nelson *et al.*, 1995). Elevated concentrations of pesticides (e.g., chloropyrifos) in the Susquehanna River during spring flow decreased sharply as the water mass moved downstream, perhaps due to degradation or to volatilization (M^{c}Connell, 1994). In general, concentrations of toxics in the water column and in the sediments are quite low in the southern Chesapeake Bay, with the obvious exception of the Elizabeth River region.

QUESTION 6. Are toxics evenly distributed throughout the water column?

On long time scales (i.e., years), toxics enter the Chesapeake Bay's surface waters and are transported both down the bay and into the sediments. On seasonal and daily scales, vertical concentration gradients of toxic chemicals are only likely when physical mixing is minimal. In an intensive study of organic contaminant cycling in the mesohaline Chesapeake throughout the year, Ko and Baker (1995) found that dissolved contaminant concentrations were relatively constant both with depth and among seasons, but that particle-associated contaminant inventories changed by orders of magnitude in response to internal recycling. Storm- and tidal-induced resuspension of surficial sediments maintained a particle-rich nepheloid layer within 1-2 meters of the sediment-water interface, resulting in higher contaminant inventories compared to the relatively particle-poor surface waters.

QUESTION 7. Are toxics evenly distributed in time?

Temporal changes in water column inventories of toxics may result either from fluctuations in loadings or from changes in removal processes. Current use agrichemicals with relatively short environmental half-lives are enriched in the air and waters of the Chesapeake Bay during their application to regional fields (Glotfelty *et al.*, 1990). Inputs from urban storm water runoff are also likely to be highly variable, and depend upon both the amount of precipitation and the antecedent moisture conditions. Although catastrophic spills could potentially result in short-term pulses of toxics in the bay, such accidents are (to date) a minor part of the overall toxics loading inventory (Chesapeake Bay Program, 1994). Internal recycling of toxics resulting from release from the sediments alters water column levels. For example, a major storm in October 1990 resuspended significant amounts of sediments and their associated organic contaminants in the mesohaline bay (Ko and Baker, 1995; Ko *et al.*, 1995), increasing inventories of PAHs and PCBs in the water two to ten times higher than during calm periods. Trace elements may be released from sediments as well, particularly when bottom waters are anoxic.

QUESTION 8. Have levels of toxics changed over time?

There is no question that environmental inventories of many anthropogenic chemicals have decreased during the past three decades in response to production bans (e.g., organochlorine pesticides; alkylated lead additives) and decreased emissions (e.g., combustion products). Documenting such changes for a specific chemical at a given location is often difficult, as only in few cases have consistent analytical methods been used for a long enough duration to show statistically significant trends. One example is the excellent record of Kepone contamination in James River fishes. Once external loadings of this organochlorine insecticide were stopped, levels in striped bass decreased exponentially with a characteristic first order rate constant of 0.3 $year^{-1}$. Other regional monitoring programs which apparently show temporal trends in bioaccumulative chemicals are hindered by changing analytical procedures and inconsistent sampling programs.

Longer term records of contaminant trends are preserved in sediments (Owens and Cornwell, 1995; Baker, unpublished data) and wetlands (Khan and Brush, 1995). Applying radiochemical dating tools to cores from relatively undisturbed environments, these investigators demonstrate that the Chesapeake Bay has responded in a favorable way to decreased toxics loadings over decade time scales, with current trace metal deposition rates lower than the historical maximums observed twenty to forty years ago. To date, geochronological studies have been limited primarily to unimpacted regions of the bay, although it is conceivable that undisturbed sediments could be located in highly contaminated harbors and rivers as well.

QUESTION 9. Does Chesapeake Bay have a toxics 'memory'?

It has been argued that most if not all of the toxics in the Chesapeake Bay are the result of past activities, and that current regulatory programs have decreased contemporary loading to an insignificant level. There is little doubt that contaminant levels bay-wide are less now than

during the past several decades. The jury is out, however, whether current toxics levels reflect a 'new equilibrium' with present-day loadings, or if the levels are continuing to decline. There are clearly locations where internal recycling of toxics from contaminated sediments is likely to dominate loadings to the water column. However, our understanding of the relative importance of 'new' *versus* 'recycled' toxics inputs is insufficient to conclude whether further reductions of controllable sources, especially urban runoff, would result in further declines in toxic levels.

QUESTION 10. Do toxics bioaccumulate in the Chesapeake Bay food web?

Persistent hydrophobic chemicals and trace metals are enriched in higher trophic levels in the Chesapeake Bay food web, just as they are in other aquatic systems. Fish consumption advisories are in effect in several locations throughout the bay. The complex and interwoven food web in this estuary, the dominance of migratory species, and the rapidly changing and wildly fluctuating stocks of finfish and shellfish prevent a simple description of the trophic transfer of toxics. Ironically, eutrophication of the bay may 'protect' higher trophic levels from bioaccumulative pollutants as the contaminants are diluted by large amounts of plankton biomass which is relatively inefficiently incorporated into higher trophic levels. Evidence from the Great Lakes region and the Baltic Sea suggests persistent contaminants accumulate to a greater degree as systems become more oligotrophic. One might speculate that the reduction in nutrient loads to the Chesapeake Bay may increase toxic exposure!

QUESTION 11. Are Chesapeake Bay waters toxic?

Using single species bioassays, Hall and co-workers have partially mapped the ambient toxicity of Chesapeake Bay surface waters. While they have found acute and chronic toxicity at a variety of locations at different times of the year, understanding the consequences of these findings are limited by three factors. First, ambient toxicity is not observed consistently with time at any given location, with toxicity occasionally observed at 'control' sites and no toxicity found in highly contaminated areas. Second, when toxicity is observed it cannot be clearly ascribed to the presence of a single chemical or to a mixture. Finally, it is unclear how to extrapolate toxicity to a single species to community or population-level effects. One could argue that toxicity simply displaces sensitive plankton species with more robust organisms, with little effect on gross measures of ecosystem function (e.g., productivity). Several areas of the bay contain sediments which are clearly and consistently toxic to infauna. In at least one case in the Patapsco River, observed toxicity in the sediments was linked to the presence of high levels of chromium, lead and zinc (Hall *et al.*, 1992). Further mapping of ambient and chronic toxicity is needed at a much higher spatial and temporal resolution.

QUESTION 12. Are other bad things other than toxicity occurring?

The presence of potentially toxic chemicals in the waters and sediments of the Chesapeake Bay may cause three types of problems. First, most obviously, and least likely, high levels of chemicals may kill the most sensitive species. Second, persistent chemicals may bioaccumulate to unacceptable levels in finfish and shellfish consumed by either humans or

piscivorous raptors and mammals. Third, chemical exposure at levels insufficient to kill the organism outright may impair their physiological functioning. Examples of these 'sub-lethal' effects include reduced fecundity and increased susceptibility to disease. Anderson and co-workers (see Anderson, et al., p. 150.) demonstrated that oysters exposed to levels of metals commonly found in contaminated regions of the Chesapeake Bay loose their ability to fight disease. Interestingly, they observe a dose-response relationship between the metal exposure level and the extent of suppression of the oyster's immune system, establishing a strong link between toxics and this sub-lethal effect.

QUESTION 13. Is there a toxics problem in the Chesapeake Bay?

Are toxics causing people to drop dead in the streets? Of course not. Are toxics causing a widespread, ecosystem-level change to the Chesapeake Bay comparable to that which resulted from excess nutrient loadings? Probably not, although some synergisms are conceivable. Are there regions of the bay where levels are toxics are high enough to cause toxic effects on (i.e., kill) the native organisms? Absolutely. Are we exposing ourselves to harmful levels of toxics? Probably depends upon who you are, where you live, and what you eat. Relative to smoking and driving, the risks of Chesapeake Bay toxics to a healthy adult are likely small. Whether the same is true for a child breathing contaminated, ozone-rich air in a city and getting a large fraction of their dietary protein from catfish and crabs caught from beneath an interstate overpass is much less clear. Who is the regulatory system trying to protect?

If you are waiting for a definitive 'Yes' or 'No' answer to the above question, you are going to be disappointed. This, like many other issues, comes down to a value judgement and gut instinct. More knowledge, such as that generated by the CBEEC program, helps us to sharpen that judgement and to more intelligently weigh the costs and benefits involved. No research study, however, (and certainly not the risk analysis paradigm currently in vogue) will alleviate our responsibility to make tough calls. When in doubt, I believe that we should err on the side of the non-renewable resources rather than to favor the short term gain.

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KEY RESEARCH FINDINGS: SUMMARY OF PRESENTATIONS

AIR-WATER PARTITIONING AND TRANSFERS

Dr. Rebecca Dickhut discussed the role of gas exchange across the air-water interface in facilitating/impeding the flux of organic contaminants into Chesapeake Bay. She emphasized:

- Flux across the sea surface can be in both directions, into and out of the surface layer. Flux rates are significant relative to other sources.

- Fluxes are extremely variable (orders of magnitude) both in time and space. Temperature appears to regulate the direction of flux for at least one organic compound (phenanthrene). Wind speed is also correlated to flux rates.

- The surface microlayer contains extremely high concentrations of contaminants relative to the rest of the Bay. In this layer, photodegradation of some compounds can occur. In addition, concentrations may be high enough to cause impacts to organisms that dwell in this layer.

SEDIMENT ASSOCIATED RESUSPENSION AND TRANSPORT

Dr. Donald Swift presented current knowledge of seabed processes and summarized findings from work done to date:

- Sediment surface "roughness" greatly affects the amount of energy needed to resuspend sediments. Temporal variation in roughness is a factor of seasonal changes in wind regime and bioturbation from benthic biota.

- Seabed sediments are readily resuspended by wind-induced and tidal currents.

- Surface sediments and the contaminants contained within them, therefore, can move in any direction within the Bay, often ending up in a location distant from their original site of deposition.

- Over time, sediments are more permanently buried below the zone of resuspension. However, bioturbation and the dynamics of organisms can cause contaminants that were buried to resurface.

SEDIMENT FLUX PROCESSES

Dr. Gerhardt Riedel summarized research involving the flux and transformation of contaminants within sediments:

- Organisms have a significant effect on toxic fluxes and transformations. In benthic environments, organisms substantially increase flux rates (both into and out of sediment) of organic and metal contaminants and can transform and degrade some

contaminants. This activity results in small-scale spatial heterogeneity in concentrations of contaminants in sediments.

- Environmental conditions also affect fluxes. Anoxia kills benthic organisms responsible for fluxes and transformations and also alters metal flux.

- The behavior of some functional groups of contaminants is predictable. For example, reducing metals (As, Mn, Fe) are mobilized from sediment while non-reducing metals (Zn, Cd, Ni, Pb) are fixed in sediment by anoxia. Organic compounds with low log K_{ow} will move deeper into sediments through the activities of benthic organisms than will compounds with higher log K_{ow}.

Speciation and Trophic Transfer of Contaminants

Dr. James Sanders presented findings concerning how the chemical form of contaminants can affect uptake by organisms and eventual trophic transfer:

- Trace metals are largely found in complexed forms. The most biologically available form of the metal, the free ion, is present in small amounts only. Both inorganic and organic complexes are important. Organic complexation may also reduce the availability of some organic contaminants to biota, as well.

- Partitioning between dissolved, colloidal, and particulate phases also greatly affects contaminant bioavailability. Both suspended particles and living microbial cells (bacteria, phytoplankton) play important roles in determining partitioning ratios, which vary seasonally and with different types of contaminants.

- Biological packaging of contaminants not only affects uptake by higher trophic levels, but can also determine the rate of burial, with dead organisms and fecal pellets sedimenting rapidly.

- Once contaminants are taken up by higher trophic levels, metabolic processes within the organism can lead to substantial transformation/degradation of compounds, to new compounds which may be more or less toxic. We do not yet know how large these contaminant pools may be, or how quickly they can turn over within the organism and its surrounding environment.

Chemical Contaminant Exposure

Dr. David Wright presented preliminary findings of two current studies examining the impacts of two compounds, mercury and dimilin.

- Extremely sensitive chemical analysis have been developed to measure ambient concentrations of these contaminants in organisms.

- The insecticide dimilin is extremely toxic to estuarine crustaceans such as copepods and amphipods. Salinity does not effect the toxicity of dimilin.

- Mercury is also highly toxic to estuarine organisms. Trophic transfer and bioaccumulation of mercury occurs readily from the water column, with uptake by phytoplankton and transfer to copepods being the key transfer mechanism. The toxicity of mercury decreases with increasing salinity.

CONTAMINANT-ORGANISM RESPONSE INTERACTIONS

Dr. Robert Anderson reviewed the current status of a diverse group of projects that are studying the responses of organisms to contaminants:

- Low concentrations of trace metals can induce a variety of responses in organisms, such as the induction of metallothionein in microorganisms, or the inhibition of metamorphosis and set in oyster larvae.

- Oysters exposed to contaminants have increased susceptibility to the disease *Perkinsus marinus*. Other estuarine organisms exposed to pollutants may also show decreased resistance to environmental stressors.

- Use of cell cultures, rather than whole organisms, may present a cost effective mechanism for examining contaminant toxicity.

MODELING FRAMEWORK

Dr. John Kucklick discussed the modeling efforts done to date within the program:

- The Chesapeake Bay mesohaline ecosystem model has been utilized as a structure for modeling exercises assessing the resiliency of planktonic communities to contaminant stress.

- A trophic transfer model representing the mesohaline portion of the Bay has been constructed and calibrated with PCB data collected from field projects.

- A simulation model of exposure and bioaccumulation is being constructed to represent one of the Bay's regions of concern—Baltimore Harbor.

DISCUSSION SUMMARIES

MANAGEMENT DISCUSSION SESSION

Resource managers participated in a discussion session led by Ron Klauda, Maryland Department of Natural Resources. To begin the dialogue, Dr. Klauda asked managers to identify what types of information they would like from the Toxics Research Program over the next five years.

Managers stated that their primary concern is knowing what adverse effects ambient chemical contaminants have on living resources. The management community needs information regarding what human activities should be regulated to minimize environmental impacts. They responded emphatically that this information is what would be the most useful. Managers feel they know very little about the link between contaminant loadings and their ambient effects. To better understand this link, participants recommended that the program strive for a balance between process-orientation and chemical-contaminant related adverse effects. Although managers recognized the importance of loadings information, their main concern is how that information translates into community effects. In the future, managers would like to develop a regionally-based assessment protocol to measure community level adverse effects.

The focus of the Toxics Research Program seems to be determined by the *Chesapeake Bay Basinwide Toxics Prevention and Reduction Strategy*. Participants addressed concerns regarding the geographical focus outlined in the *Strategy*. Managers recommended that while some research should be focused within the Regions of Concerns, the majority of projects should be located outside of these highly contaminated areas. While research in those areas is important, they represent worst case scenarios. The management community is interested in preventing other areas from reaching such extreme contamination levels. Research in these areas does not further management's main concern — effects of low-level contaminants on living resources. Participants also felt that, as much as possible, research sites should be balanced between urbanized and agriculturally-dominated watersheds. Managers would like to see the TRP expand into the tributaries, so that the TRP does not solely focus on the mainstem.

Managers agreed that modeling efforts should address the link between loadings and fate mechanisms to bioconcentration and bioaccumulation. Participants recommended that future research should examine synergistic effects. For example, how does speciation affect contaminant bioavailability? Does anoxia affect the bioavailability of chemical contaminants? Managers also questioned the impact of synergistic effects at low-level concentrations. Past research has shown organisms in highly contaminated areas, like the Elizabeth River, to be more susceptible to disease. Participants would like to gain a better understanding of the prevalence of immunosuppression outside of those areas. Managers commented that this area, in particular, provides an appropriate role for modelers. They felt that modeling efforts must attempt to link loadings via transport and fate mechanisms to bioconcentration and direct adverse effects.

The group recommended that the TRP place more emphasis on metals listed as Toxics of Concern. Metals are an immediate regulatory concern. Recent evidence suggests that metals should be of particular concern, even at what were thought to be low ambient concentrations. Managers would like to see future research address the impact of metals on the Bay's living resources.

Participants noted the need for consistent monitoring, however, they would also like to see the program generate more "big picture" syntheses. This type of information, which explains the results of research, also needs to be effectively communicated to the public. Currently, the general public is largely unaware of toxics-related issues and the value of continued toxics research. If the eventual goal of the program is to establish/enhance toxics regulations, then public education is needed. An informed public is less likely to strongly resist regulation, especially if the link is made between toxics loadings and impacts to the Bay's living resources.

The group posed the following specific questions and recommendations concerning toxics research in Chesapeake Bay in support of management:

- Can we design a regionally-based assessment protocol to measure community level adverse effects?
- Can we design the sampling regime necessary to fully characterize the nature and extent of chemical contaminant related effects within a defined geographic region?
- Are there better measures of adverse ambient effects beyond those within the existing Regions of Concern identification protocol?
- How do we better define, or gather evidence for, the presence/absence of a causal relationship between measured ambient chemical contaminant concentrations and observed adverse effects?
- How might we direct research in support of a watershed approach?
- We need to develop a set of meaningful/relevant bioindicators of adverse effects that are responsive to low level contaminant concentrations at the community level.
- How do we measure synergistic effects relevant at low concentration levels?
- How do complexation and speciation effect the bioavailability of Toxics of Concern?
- Can we determine tributary/region specific permanent sediment burial rates?
- What are the results of anoxia and changes in nutrient enrichment on the bioavailability of chemical contaminants?
- Can we measure immunosuppression outside of the highly contaminated regions?

- What are the best environmentally sensitive ways to dispose of highly contaminated sediments?

- Need more research focused on metals on the Toxics of Concern List.

- The role for modelling must be to link loadings via transport and fate mechanisms to exposure, uptake, and adverse effects.

- Place emphasis on tributaries as well as mainstem of the Bay.

- Continue to focus on exposure and adverse effects of toxics on biota.

RESEARCH DISCUSSION SESSION

Dr. James Sanders, The Academy of Natural Sciences, led discussion in the breakout group for researchers. The goal of this session was to conceptualize a framework that would guide toxics research over the next five years. Dr. Sanders posed several questions, along two major themes, to help focus the discussion:

1. Communication

   - How can we more effectively communicate the importance of the scientific findings to the management community and the general public?

   - How can managers provide input to the research community regarding their need for information?

2. Program Direction

   - What processes are important in understanding the transport, fate and effects of toxics in Chesapeake Bay?

   - What processes do we still need to study?

   - What should the scope of the program be? Can the program's focus be narrowed?

   - Where should we be five years from now?

Initial discussions raised the question: "What is the magnitude of the problem to be solved?" Several participants felt that the Bay was already showing some signs of improvement, and the lack of support for the research program from management may be related to the perception that toxics in the Bay are not a problem. Showing cause and effect is difficult even in "hot spot" areas, and may be very difficult to do outside of these areas. It was noted that some managers fear that research on low-level toxics might uncover a problem that they are unprepared to address. There was not a consensus on what was meant by "low-level" contaminants, even though the *Chesapeake Bay Basinwide Toxics Prevention*

and Reduction Strategy directs that the effects of low-level contaminants be evaluated. It was suggested that low-level effects be defined as any effects less than acute toxicity.

The group agreed that an ecological process-oriented approach to understanding toxics problems in Chesapeake Bay was the correct approach, but questioned whether most water quality managers agreed with the approach. The point was made that there seems to be a difference in temporal perspective; managers are faced with day-to-day compliance issues, whereas the researchers address complex long-term questions of toxic contaminants in the environment. The researchers agreed that for the most part, they have not addressed well the effects of multiple compounds in the environment.

The group felt that current funding constraints limit scientific progress toward understanding the interaction between toxics and ecological processes in the Bay, and it was suggested that alternative funding sources should be investigated. Several participants agreed that tapping into parallel sources of information both from other national and international environmental programs could expand our current understanding of toxic process without serious funding increases. Chemical surveys throughout the Bay should be continued because they are important for developing future management strategies. Also, such "trends" data prompt relevant research questions.

The group leader presented a historical perspective: To date, NOAA (through CBEEC) is the major funding agency for toxics research in Chesapeake Bay. Up to $600,000 annually has been spent on studies that have primarily focused on transport and fate of toxics within the Bay system. Focus of the Toxics Research Program seems to be shifting toward the effects of toxics on organisms.

Several participants agreed that effects should be investigated more thoroughly, but emphasized that in order to make rational decisions about toxic inputs, important links must first be drawn between loading, transport, and fate of toxins which are harmful to Bay organisms. In order to eventually "pinpoint" the origin of a toxin and to "predict"' the severity of a toxic effect on a biological organism, many more pieces of the puzzle need to be put together. Current data sets are not complete enough to provide sufficient information for creating a risk assessment model. Since funding constraints are the reason for the lack of data, it was suggested that one of several commercially important species be selected for continued research. Other members felt, however, that the big picture might become distorted by focusing on a few groups of organisms. Several participants pointed out that managers may not understand the necessity for continued process-oriented research and that emphasis should be placed on educating managers on the utility of this approach.

The argument was made that water quality managers have done a poor job conveying their information needs to the research community. The researchers were then asked "What information do you think the managers need?" Answers included: more basic data which allows ability to make correlations; more monitoring type data; standardized assessments; rates of atmospheric transport and deposition of pesticides and other contaminants. One participant commented that the Bay Program, and the Toxics Research Program in particular, should not have to change course each year with each new "hot topic." Researchers agreed

that their job was to supply the basic information of how the Chesapeake Bay system responds to toxic contaminants, and that ultimately, what the managers need are the tools to conduct comparative ecological risk assessments.

Opinions differed about the definition of low level toxins. The *Chesapeake Bay Basinwide Toxics Reduction Strategy Reevaluation Report*[2] has a list of regions of concern, which could be potential study sites with lower levels of toxins, in contrast to the known "hotspots" (i.e., Baltimore Harbor, Back River, Anacostia River, and Elizabeth River). Several scientists agreed that downstream of these highly contaminated areas might be logical areas for continued studies. Others questioned the need for lowering toxins beyond certain "safe" levels which have yet to be determined. Following trends of decline in certain toxins (i.e., those already banned) could also be a good way of determining if additional management strategies for these toxins are necessary. Examining trends would possibly help identify future toxics of concern. Participants also felt the non-point sources should be the major targets for toxic reductions, since many of the point sources have already been strictly regulated.

In general, scientists regarded the current research program as a relatively "young" program which has much work to do to provide managers with the tools to conduct ecological risk assessments across the range of contaminants, organisms and habitats. With limited funding, however, there is a need to bring the program into a more narrow focus. Suggestions included focusing by geographical area (regions of concern or specific watersheds), target organism (commercially important organisms, certain trophic levels, levels of cellular organization, etc.), and contaminant type. Final consensus on the best way to focus the program was not reached. Other suggestions included: studying synergistic effects of toxins on organisms; collecting better data sets (chemical and biological), better integrating modeling into the research program, and better information exchange between the research program and managers.

The research group developed the following list of goals for the Toxics Research Program over the next five years:

- Continue process-oriented research that produces information which will lead to an enhanced ability to conduct comparative ecological risk assessments.
- Reduce the overall scope of the research program.
- Better utilize existing data sets (loadings, ambient toxicity) from past and ongoing monitoring studies of toxic contaminants in the Bay.
- Come to agreement on interpretation of "low-level concentrations" and "effects" of toxics in the Bay.

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[2] *Chesapeake Bay Basinwide Toxics Reduction Strategy Reevaluation Report.* (Chesapeake Bay Program, 1994) CBP/TRS 117/94.

- Better integrate modelling efforts with the fundamental research.

- Synthesize findings from the Toxics Research Program and communicate these findings to a broader audience than the research community (i.e., management and the public).

- Have a better dialogue with managers so that important findings of the program are readily conveyed to managers and the information needs of managers are readily conveyed to the researchers (via CBEEC).

FULL GROUP DISCUSSION: FINDING COMMON GROUND

Frank Dukes (Institute for Environmental Negotiation, UVA) and Rich Batiuk (EPA Chesapeake Bay Office) led the discussion among all of the workshop participants on the role and direction of the Toxics Research Program with regard to supporting management of Chesapeake Bay resources.

Rich Batiuk and Jim Sanders presented their summaries of the management and research breakout group discussions, respectively, held the previous day. Many of the ideas expressed during the breakout groups were discussed among the full group, but are not repeated here.

Modeling

The opening discussion concerned the role of modeling in research and management of toxics in Chesapeake Bay. The model currently being developed under TRP funding is a process model specific for organic contaminants; it is not applicable to metals and does not contain previously generated information on transport and effects. It will have value as interpretive tool — integrating data for Baltimore Harbor Region of Concern.

We have a pretty good understanding of the movement of organic contaminants in the Bay system (i.e., a success story for TRP), but we have not done a good job of putting the story together in an understandable way. This information would be a good candidate for a conceptual model and a process model, incorporating some rates leading to exposure and effects.

What do we expect from the modelling effort? There are a variety of types (and hybrids) of models that can be developed to answer specific questions. What are the questions that managers wish to have answered? The ultimate goal is risk assessment.

The modelling effort is perceived by some as expensive and not able to produce desired results. Are we asking too much of the modelling effort? No, it is critical to linking together loadings, transport, exposure and effects information. We know conceptualization and some rates now. We should move toward mathematical models.

TRP Research Priorities

There was a consensus that the effects of low-level contaminants on living resources in Chesapeake Bay should be investigated, and that the linkage between contaminant loadings and their effects on biota must be understood because it is the loadings that can be managed. Thus, there was agreement on the need for process-oriented research on toxics in Chesapeake Bay in addition to research on the effects of those contaminants on living resources. It was noted, however, that if there is no effect of low-level contaminants on living resources, the "process" of exposure may be of little significance to management decisions.

If an ecosystem process changes as a result of toxic contaminants, then it should be considered an effect, and there is no reason to distinguish "process-oriented research" from "effects research."

Is TRP research useful to managers? Clearly, different managers have different information needs. In general, the TRP research was considered useful by managers, but only when put in terms that are useful. Some managers recognize that not all findings will be immediately useful, i.e., research is a long-term investment. Researchers noted that if the ultimate goal is risk assessment of toxics in Chesapeake Bay, that goal will not be reached for some time. The objective of comparative ecological risk assessment should be more clearly stated as a goal of the TRP.

Risk assessment is an important goal. It was noted that managers have contaminant reduction goals to meet, but don't know what benefits will be achieved (i.e., impacts on living resources) by reaching that goal. Ranking contaminants by "risk" would be helpful for managers, as an intermediate goal toward the ability to assess risk of toxics loadings in the Bay.

Communication

Participants agreed that better communication among researchers, managers, and the public, with interests in Chesapeake Bay contaminants, is necessary. Greater emphasis should be placed on the outreach component — reporting the findings in a form that is useful to various audiences. It was felt that existing resources (university, agency, *Bay Journal*) would be appropriate. Most important is the need to synthesize information generated by TRP research into a format that is relevant to resource managers and to the educated public. The CBEEC should make this a high priority.

It is also important that managers convey to researchers what their information needs are. The "management - scientific framework" (see below) should also help in the transfer of information between managers and researchers.

MANAGEMENT - SCIENTIFIC FRAMEWORK

The Chesapeake Bay Basinwide Toxics Reduction and Prevention Strategy (Oct 1994) calls upon the Scientific and Technical Advisory Committee (STAC) to:

By January 1995, establish and support a management\scientific framework that provides for comprehensive peer review of technical reports, research proposals, budget initiatives, and strategy implementation products; identifies management issues requiring technical information building on the findings from the reevaluation; prioritizes, plans, and implements research strategies to address the identified management issues; monitors and evaluates the directed research efforts; and synthesizes and communicates the management implications of research findings to the Chesapeake Bay community.

As a member of the Bay Program's Scientific and Technical Advisory Committee and the Toxics Subcommittee, Dr. Ray Alden of Old Dominion University introduced the concept of the "framework" and led discussion of its merits. It was envisioned that STAC would lead the effort in coordination with the Toxics Subcommittee and the CBEEC. Most participants agreed that the concept had merit, but that there were many details to be worked out prior to implementation. It was noted that such a "framework," if successful, could serve as an example for other Bay Program subcommittees. This forum could also act as liaison to other subcommittees with an interest in toxics issues.

Project Reports

AIR/WATER PARTITIONING AND MASS TRANSFER PROPERTIES OF TOXIC ORGANIC CHEMICALS [R/CBT-1]

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INTRODUCTION

Chemical transfer across the air/water interface is a dominant process controlling concentrations and residence times of toxic organic chemicals in aquatic ecosystems. In the Chesapeake Bay watershed, researchers conducting the Chesapeake Bay Atmospheric Deposition (CBAD) study have determined the wet and dry depositional fluxes of selected hydrophobic organic chemicals (HOCS) and trace elements to Chesapeake Bay (Leister and Baker, 1994; Scudlark *et al.*, 1994; Dickhut and Gustafson, 1995). Furthermore, gas exchange is estimated to contribute significantly to the air/water transfer of organic contaminants (Dickhut and Gustafson, 1995). Through our CBEEC research, we measured the air/water partitioning and kinetic mass transfer properties of HOCs necessary for modeling the volatilization/absorption (vapor exchange) of organic contaminants in Chesapeake Bay.

RATIONALE/APPROACH

The rate of gas transfer between air and water reservoirs, across stagnant films at the interface, is assumed to be governed by molecular diffusion and driven by the concentration (or fugacity) gradient between the equilibrium concentrations at the interface and bulk reservoirs. The volatile flux (F_{vol}) is:

$$F_{vol} = k_{ol}(C_{f,w} - C_{v,atm}RT/H) \quad [1]$$

where

$$1/k_{ol} = 1/k_w + RT/Hk_a. \quad [2]$$

and k_{ol}, k_w, k_a are the overall, water, and air mass transfer coefficients, respectively, $C_{f,w}$ is the freely dissolved concentration of a HOC in surface water, $C_{v,atm}$ is the vapor phase concentration of a chemical in the atmosphere, R is the gas constant, T is temperature (K), and H is the compound specific Henry's law constant.

Determinations of the air/water partitioning (H) and kinetic mass transfer coefficients (k_w and k_a) for organic chemicals under various environmental conditions are required to evaluate the passive/diffusive flux of HOCs to Chesapeake Bay. These coefficients can be determined from the basic physical/chemical properties: saturation vapor pressure (p_{sat}), aqueous solubility (x), air and water molecular diffusivities (D_a and D_w); of the HOCS.

OBJECTIVES

To develop and calibrate accurate techniques for measuring and estimating the physical-chemical properties p_{sat}, x, D_a and D_w for predicting the air/water partitioning properties (H) and kinetic mass transfer coefficients (k) for semivolatile organic contaminants.

FINDINGS TO DATE

Generator systems for measurement of p_{sat} for both liquid and solid organic chemicals were developed. Vapor pressures were measured for benzene and a series of chlorinated benzenes at five temperatures between -15° C and 40°C. Phenanthrene vapor pressure was also measured at three temperatures (0, 25, 40°C) and the effects of humidity on p_{sat} evaluated. The vapor pressure measurements on benzene and phenanthrene were utilized to evaluate our methods and we found that our experimental techniques compare well (within 10-20%) to those of others. Results indicate that p_{sat} of HOCs is strongly dependent on temperature, increasing as temperature increases. In addition, we observed no effects of humidity on the vapor pressure of phenanthrene.

Relationships between the enthalpies of vaporization and chlorine number were used to develop a predictive equation for chlorinated benzene vapor pressure. This correlation predicts p_{sat} of chlorinated benzenes within a factor of three. Alternatively, vapor pressures of liquid chlorinated benzenes are accurately ($\pm$20%) estimated using the modified Watson correlation, an equation based on the boiling point temperature of the compound. However, at present, the modified Watson correlation does not accurately predict p_{sat} values for solid aromatic chemicals with very low vapor pressures. These findings are reported in Liu and Dickhut (1994).

Our solubility research has primarily included studies of the effects of dissolved organic substances on the solubility of HOCS. Organic substances in water are of potential importance in air/water transfer research as they can influence solubility of HOCs in the surface microlayer of an aquatic ecosystem. Solubility at three temperatures (10, 25, 40°C) has been measured for three PAHs (naphthalene, phenanthrene, and acenaphthene) in three organic solvent/water mixtures (methanol/water, ethanol/water, 1-propanol/water) ranging from 0-100% organic solvent. We are examining thermodynamic and molecular models to describe organic contaminant-solvent interactions in solution, and are preparing a manuscript for publication.

Systems for measuring gas-phase and aqueous molecular diffusivities of organic contaminants were developed and calibrated in our laboratory (Gustafson, 1993). We

measured molecular diffusion coefficients in air (-5°C to 40°C) and water (4°C to 40°C) for several organic chemicals. Molecular diffusivities in both air and water were found to decrease with molecular size and increase with temperature. The effects of salinity on aqueous molecular diffusivity of HOCs were determined to be insignificant; salinity effects were not larger than the uncertainty in the experimental measurements.

Property estimation techniques for diffusivity in air are based on the Chapman hard sphere model and in water are based on the Stokes-Einstein equation for fluid flow around spherical particles. We observed that diffusivities for PAHs predicted using recommended techniques (Fuller *et al.*, 1966; Hayduk *et al.*, 1974) deviate exponentially from measured values with increasing molecular size. Consequently, predictive equations have been modified to accurately predict HOC molecular diffusivities (Gustafson, 1993; Gustafson and Dickhut, 1994a&b).

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DETERMINATION OF THE VOLATILE/ABSORPTIVE EXCHANGE OF HYDROPHOBIC ORGANIC CONTAMINANTS ACROSS THE AIR/WATER INTERFACE OF LOWER CHESAPEAKE BAY [R/CBT-18]

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RATIONALE

To accurately assess atmospheric inputs of hydrophobic organic contaminants (HOCS) to surface waters such as Chesapeake Bay, it is necessary to consider all of the major air/water exchange processes (Mackay et al., 1986). Preliminary assessments show that vapor transfer fluxes of selected HOCs are of the same order of magnitude as depositional fluxes in the southern Chesapeake Bay region and may be directed either into (absorption) or out of (volatilization) the Bay surface waters (Dickhut and Gustafson, 1995). This gaseous transfer of toxic organic chemicals between the atmosphere and an aquatic system is driven by concentration or fugacity gradients between the compartments, the physical-chemical properties of the contaminants, and environmental conditions such as wind speed and temperature (see CBT-1).

In this study, we are assessing the volatile/absorptive exchange of HOCs across the air/water interface of lower Chesapeake Bay by measuring the fugacities, and the spatial and temporal variability of HOC fugacities, for selected HOCs in the atmosphere and surface waters of the southern Chesapeake Bay region. We are also evaluating the influence of the surface microlayer on the potential for diffusive flux of HOCS. With the data collected in this study and those we have been assembling as part of the Chesapeake Bay Atmospheric Deposition (CBAD) study, we propose to examine models for determining the net flux of HOCs between the atmosphere and lower Chesapeake Bay.

OBJECTIVES

To quantify the volatile/absorptive exchange of HOCs across the air/water interface of lower Chesapeake Bay, by:

[1] Measuring the fugacity gradients (air/water, air/surface microlayer, surface microlayer/water) for selected HOCS, evaluating the spatial and temporal variability of HOC fugacities, and assessing the influence of the surface microlayer on the air/water diffusive exchange potential; and

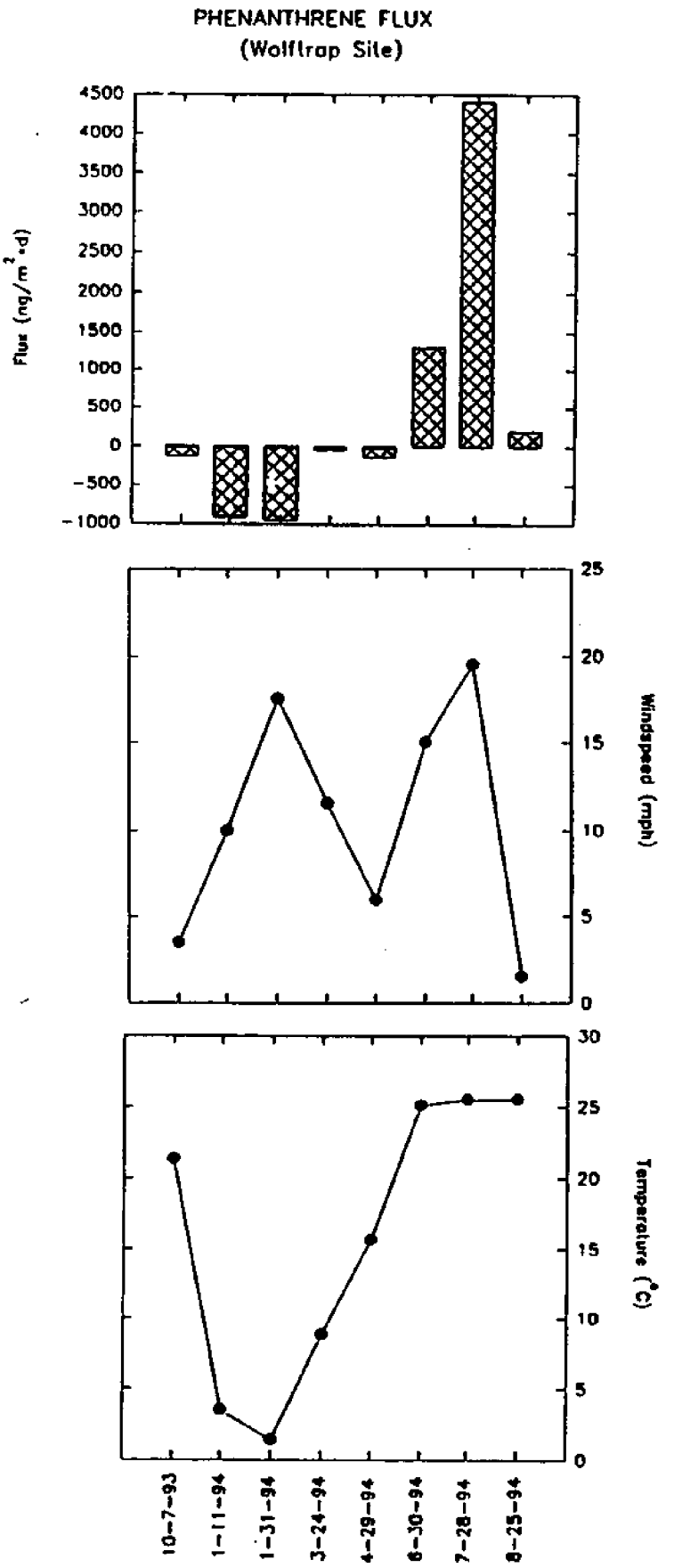
[2] Experimentally determining HOC mass transfer coefficients, particle uptake and photodegradation rates by surface microlayer material, to assess whether this region acts as an inert layer of resistance to HOC gaseous exchange or a separate, active, potentially controlling phase in HOC vapor transfer at the air/water interface.

APPROACH AND FINDINGS

To determine the air/water gas exchange fluxes of organic chemicals in Chesapeake Bay, we have measured HOC fugacities in air and surface waters at various sites in the lower Bay, throughout one year. We have evaluated several methods for measuring the freely dissolved surface water concentrations (i.e. fugacity) of HOCs including a floating sparger apparatus (Sproule *et al.*, 1991), semipermeable membrane devices (Huckins *et al.*, 1990), and filtration followed by extraction of dissolved HOCs with Amberlite® XAD-2 resin (Dickhut and Gustafson, 1995). Our comparison revealed that the sparger technique suffers from artifacts due to particle ejection from the water, and that the artifact increases as the volatility of the HOC decreases. Semipermeable membrane devices (SPMDs) were found to be useful for measuring the freely dissolved concentrations of HOCs in estuarine surface water, however, continued development of the analytical methodology with SPMD lipids is required. Further, XAD-2 isolated concentrations of HOCs compare well to SPMD measured fractions, indicating no "oversampling" with XAD-2 fractionation due to potential collection of dissolved organic carbon (DOC) or colloidally bound HOCS. In addition, DOC measurements before and after XAD-2 extraction were not observed to be significantly different. Consequently, filtration with XAD-2 isolation of dissolved HOCs has been used to measure surface water concentrations of HOCs in this study. Nonetheless, the SPMD technique is under further development in our lab.

A preliminary assessment of the volatile/absorptive fluxes of HOCs in the lower Chesapeake Bay region is as follows. Gas exchange fluxes determined thus far range from 2-200 times that of atmospheric deposition and operate both as a source and loss mechanism for HOCs from Chesapeake Bay (see figure 1). Moreover, wind speed and temperature are important meteorological variables in determining the overall air/water vapor flux (see figure 2). Atmospheric fugacities of HOCs vary spatially up to a factor of ~ 100 and temporally tip to a factor of ~ 10, whereas surface water fugacities vary spatially up to a factor of ~ 10 and temporally by only a factor of ~ 2. Due to the large variation in fugacities and meteorological conditions, gas exchange fluxes are expected to vary greatly both spatially and temporally.

We have also collected surface microlayer samples at two sites in lower Chesapeake Bay, throughout one year, using a rotating drum apparatus. Total suspended particulates, particulate organic carbon, and particle-associated HOC concentrations in the surface microlayer were observed to be routinely higher than for surface water (collected at 1 m depth). Moreover, the uptake rate of HOCs by surface microlayer material has been determined to be faster than that of water and photodegradation of polycyclic aromatic hydrocarbons has been determined to occur within hours in the surface microlayer (see figure 3). Using open tubular column liquid chromatography (Gustafson and Dickhut, 1994) diffusivities of HOCs in collected microlayer material were also measured and observed not


PHENANTHRENE FLUX
(Wolftrap Site)
Flux (ng/m^2·d)
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4000
3500
3000
2500
2000
1500
1000
500
0
-500
-1000
Windspeed (mph)
25
20
15
10
5
0
Temperature (°C)
30
25
20
15
10
5
0
10-7-93
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3-24-94
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7-28-94
8-25-94

Figure 1.

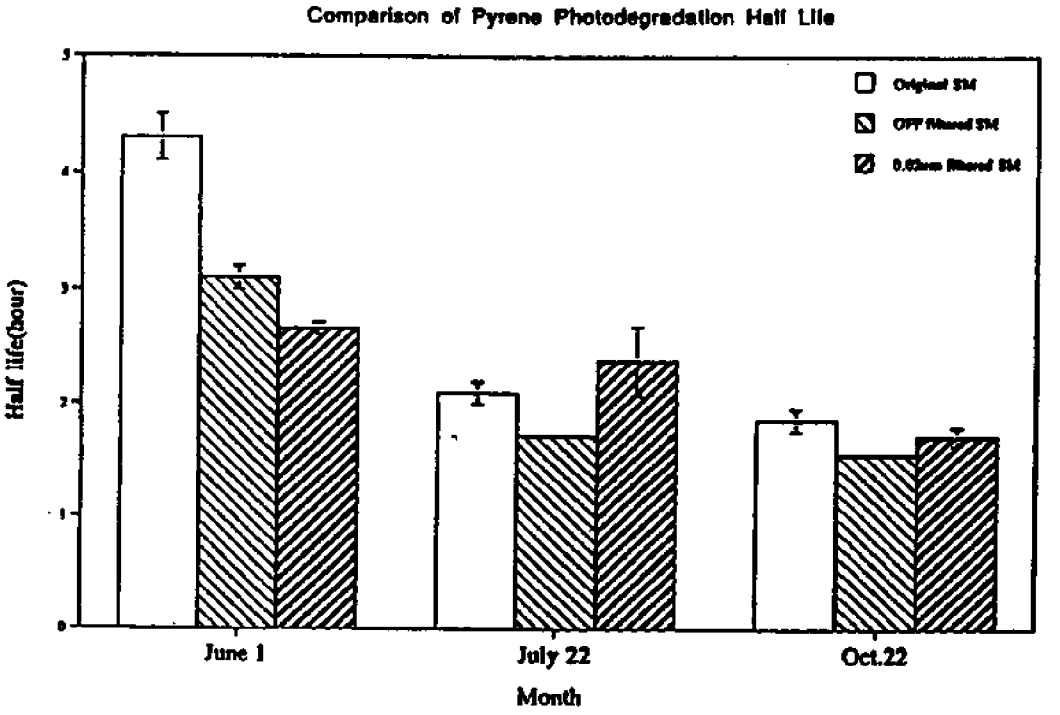

Figure 2.

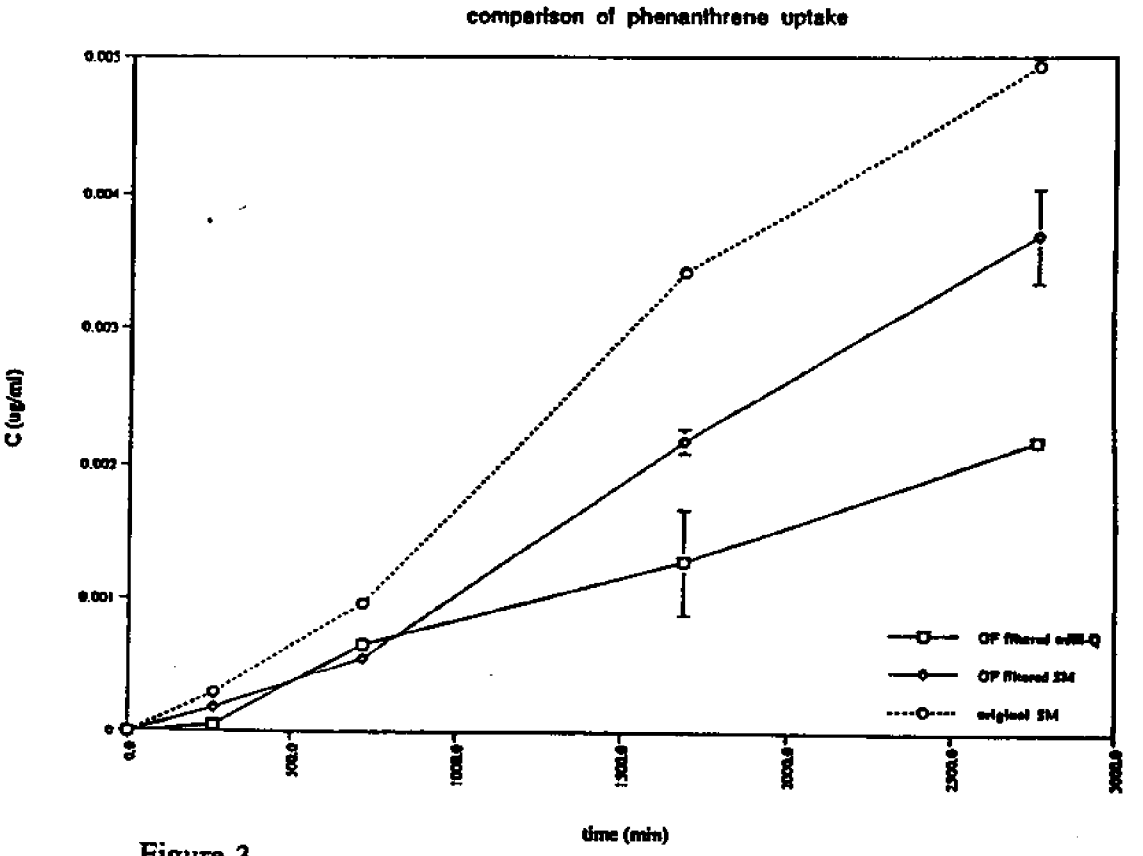

Figure 3.

to be significantly different from those measured in water at the same temperature and salinity.

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PARTICLE-REACTIVE RADIONUCLIDES AS ANALOGUES OF PARTICLE-REACTIVE POLLUTANTS IN THE CHESAPEAKE BAY [R/CBT-2]

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OBJECTIVES

Many of the toxic pollutants that make their way into the Chesapeake Bay, for example, metals such as zinc, copper and lead, and organic compounds such as DDT, Kepone, hydrocarbons and PCBS, undergo extensive interactions between the dissolved and particulate phases in the aquatic environment and associate preferentially with the particulate phase. They are classified as the "particle-reactive" pollutants. The overall objective of this study is to understand and quantify the geochemical dynamics that govern the fate of these pollutants in the Bay. Specifically, this study attempts to determine:

1. in the sediments, the relative effectiveness of the various sedimentary sub-environments of the Bay as sinks for particle-reactive pollutants.
2. in the water column, the partition of particle-reactive pollutants between the dissolved and the particulate phases, the residence times of these pollutants in the water column and the effects of environmental conditions on the geochemical behavior of these pollutants.

APPROACH

Studying the geochemical behavior of all particle-reactive pollutants individually is often prohibitively time-consuming, costly and thus impractical. Furthermore, it is also often difficult, if not impossible, to extract information involving time, such as reaction rates and residence times, by studying the distributions of these pollutants directly. Several naturally occurring radionuclides, namely, ^{7}Be, ^{228}Th, ^{210}Pb and ^{210}Po, are known to be particle-reactive. The source terms of these particle-reactive radionuclides are well defined and can be determined exactly. Their half-lives, which range from weeks to decades, allow them to act as unique geochemical clocks for studying the geochemical dynamics of the particle-reactive pollutants since many of the processes that may govern the geochemical fate of these pollutants also occur within this range of time scales. Thus, in this study, an "ANALOGUE" approach, utilizing ^{7}Be, ^{228}Th, ^{210}Pb and ^{210}Po as the analogues, is used to study the geochemical dynamics which govern the phase association and fate of particle-reactive pollutants in the Chesapeake Bay.

FINDINGS

The inventory of ^{210}Pb in sediment cores has been used as an indicator of the potential for the accumulation of particle-reactive pollutants in the sediments in the Lower Chesapeake Bay. The distribution of ^{210}Pb in 20 cores collected from the Lower Chesapeake Bay suggests that while the Lower Bay as a whole may be relatively depleted in excess ^{210}Pb and is thus relatively free of particle-reactive pollutants, there is still a marked focusing effect (i.e., preferential burial site) among the various sedimentary sub-environments. The most important burial sites in the Lower Bay are the main channel north of the Rappahannock River and the fringe embayments, Mobjack Bay and Pocomoke Sound. Although there may be considerable accumulation of sediments in the Bay south of the mouth of the York River, little particle-reactive pollutants are buried in this area. Apparently, the materials accumulated there are primarily coarse grained particles that are almost free of excess ^{210}Pb, and thus, particle-reactive pollutants. The inventory of excess ^{210}Pb in the channel along the eastern side of the Lower Bay is also low even though the depth of this channel is about the same as that of the main channel further north. Thus, four types of sedimentary sub-environments in the Lower Bay may be defined: non-accumulating areas, rapidly accumulating areas (depo-centers) that are also preferential depositional sites of particle-reactive pollutants, accumulating areas relatively free of particle-reactive pollutants, and, moderately accumulating areas with moderate loadings of particle-reactive pollutants.

Based on the distribution of particle-reactive radionuclides in the Lower Bay between the Summer of 1991 and the Fall of 1992, the scavenging residence times of particle-reactive pollutants can be estimated to be mostly in the order of less than a day to couple weeks. The residence times are longer in the northern Lower Bay, between the mouth of the Rappahannock River and the Patuxent River, than in the southern Lower Bay, south of the mouth of the Rappahannock River. The residence times in the southern Lower Bay may be slightly longer in the Winter. However, the seasonal trend is not dramatic.

We have tested the possibility of using radionuclides as an analogue to study the changes in the speciation of particle-reactive pollutants in the Chesapeake Bay. We started the study with uranium since its concentration is higher, its inorganic speciation in seawater and its source term to the Bay is relatively well known. We have developed an analytical scheme for the determination of inorganic and organic uranium in marine waters and measured the concentration of these two forms of uranium in Atlantic Ocean water and Chesapeake Bay water. Our data suggest that the Chesapeake Bay may act as a geochemical reactor that can change the speciation of uranium significantly. The source of uranium to the Bay is primarily through the intrusion of seawater from the Atlantic Ocean. Uranium exists exclusively as inorganic uranium in this incoming water. However, within the residence time of the water in the Bay, a significant fraction, up to 45% of total dissolved uranium may be converted to the organic form. A significant amount of colloidal uranium was also found in the Bay. Since uranium is geochemically relatively unreactive, the speciation of the more reactive elements may be even more extensively affected.

Ra-226 and Ra-228 were measured in this study. As a first approximation, the relationship between the concentrations of Ra-228 and Ra-226 falls along a linear band. This

suggests that although these two isotopes belong to different decay series and thus may have different source terms, their distributions in an estuary such as the Chesapeake Bay are governed more by their similarities in their geochemistries than by their differences in their source terms. The relationship between Ra-226 or Ra-228 and salinity may vary with season. A well defined convex curvature was observed on a number of occasions suggesting that Ra-226 and Ra-228 are produced in the Chesapeake Bay. This non-conservative behavior is probably caused by the desorption of the Ra isotopes from particulate material in the estuary.

RESIDENCE TIME OF PARTICLE REACTIVE POLLUTANTS IN THE COASTAL SEA BED: CONTROL BY RESUSPENSION AND SEA BED MIXING PROCESSES [R/CBT-3]

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LONG TERM GOAL

The goal of our study is to develop methods and algorithms that will allow us to predict the residence time of particle-reactive pollutants in the coastal seabed.

SCIENTIFIC OBJECTIVES

We propose to develop methods for computing the frequency and intensity of resuspension of bottom sediments by flow events, methods for computing the diffusion coefficient for biological mixing of the sea bed directly from the species composition and population density of the benthic infauna, and methods for establishing the rate of sedimentation by comparison of profiles of radioisotopes of differing half lives.

BACKGROUND

Toxic pollutants entering estuarine and coastal waters are known to preferentially associate with the particulate phase, and their dispersal is therefore controlled by the natural cycle of fine sediment transport. A key aspect of this cycle is the exchange of sediment particles between the water column and the sea floor. As contaminated particles accumulate on the sea floor, they pass downward through a zone subject to biogenic mixing, and to resuspension by storm and tidal currents, with secondary release of the pollutant into the water column. Eventually, however, the sediment is so deeply buried that it is no longer accessible to these processes, and has entered the zone of permanent burial. By measuring the rates of these competing processes (accumulation, mixing, resuspension), we will be able to predict hypothetical contaminant budgets under a variety of conditions.

APPROACH

In order to determine potential patterns of contaminant dispersal on the floor of southern Chesapeake Bay, we are undertaking fluid dynamical, radiogeochemical and biological investigations. We have developed a numerical model of boundary layer physics that will

allow us to compute our first critical rate, namely the frequency and intensity of particle resuspension, from wind, wave, and tidal current data. We have also collected cores in order to measure radionuclide profiles. The radionuclides ^{234}Th, ^{137}Cs, and ^{210}Pb serve as proxies for the behavior of particle reactive pollutants. In addition, measurements of vertical concentration gradients of radionuclides with differing half lives have allowed us to estimate the other two critical rates, namely accumulation and mixing. We are also therefore analyzing the composition of the benthic infaunal community as a function of depth. By applying random walk theory, we are generating independent estimates of the biodiffusive mixing. Finally, we are developing computational schemes that will allow us to predict scenarios of contaminant release.

PROGRESS TO DATE

Box cores and vibracores from the Baystem Plain of Southern Chesapeake Bay consist of a sandy clayey silt, containing a Polychaete-molluscan community that extends, with vertically decreasing population density, to 30 cm depth. Preliminary calculations suggest the depth-averaged biodiffusion coefficient for the upper 10 cm may be on the order of 1×10^{6} cm^2yr^{-1}. Radiographs indicate that the silt is well mixed in the summertime when winds are mild and the fauna is most active; in the winter, however, the top several centimeters are frequently current stratified. Accumulation rates on the Baystem Plain range from 0 mm yr^{-1} near areas of tidal scour to 7 mm yr^{-1} in protected marginal bays. Computations suggest that the annual resuspension depth due to wave-tide interaction ranges from 0.4 to 1.6 cm in the shallower portions of the Baystem Plain (Fig. 1), and may be several times greater when wind-driven currents coincide with these interactions.

Burial histories for hypothetical contaminant layers vary from "rapid entombment" in marginal coves where accumulation is rapid relative to mixing, to "slow release" scenarios near the Bay mouth, (Fig. 2). As burial proceeds, the particle-reactive contamination leaks away through upward diffusion of the contaminant, coupled with repeated storm flushing of the surface layer. Eventually the contaminant layer is deeper than burrowers can reach, and the seabed contamination stabilizes.

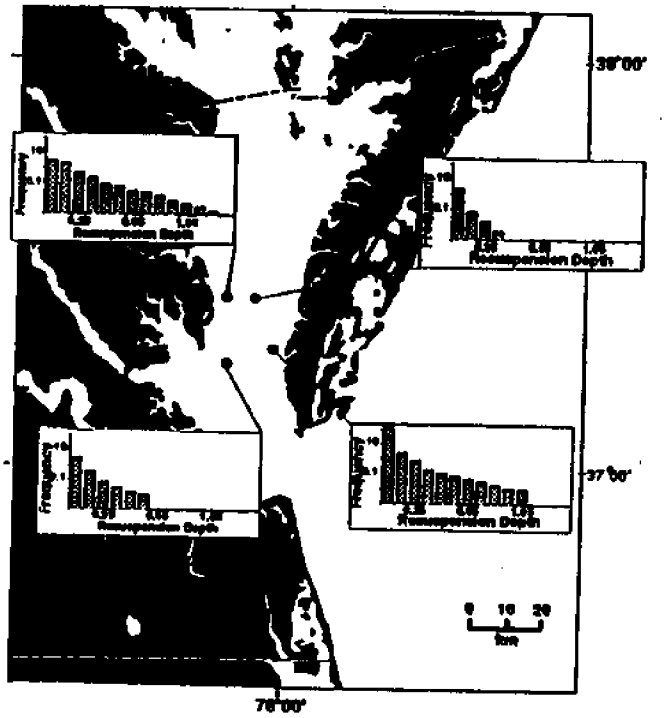

Figure 1. Resuspension frequency (cycles per day) as a function of depth in the sea bed for 4 sites in southern Chesapeake Bay.

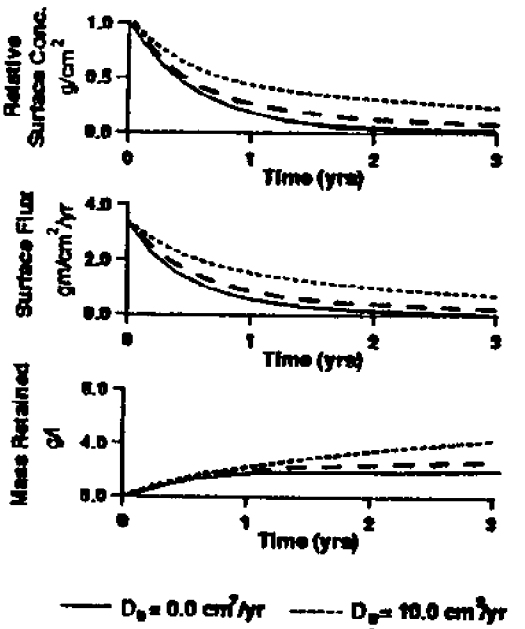

Fig. 2. Simulated burial history for a contaminant spill as determined by the rate of sediment accumulation, the frequency and intensity of resuspension. and the rate of biogenic mixing. For moderate to high values of the biodiffusion coefficient, surface concentration surface flux and total mass retained approach limiting values with time.

DYNAMICS OF SEDIMENT RESUSPENSION: BAY-STEM PLAINS OF THE LOWER CHESAPEAKE BAY [R/CBT-4]

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OBJECTIVES AND RATIONALE

The bay stem plain is the most aerially extensive benthic environment of the lower Chesapeake Bay. It has considerable economic importance as a region of previous and projected dredged material disposal and supports ecologically-valuable benthic communities. The bay-stem plains region of Chesapeake Bay also provides a useful model for other coastal or estuarine habitats which are characterized by fine sediments and relatively high rates of bioturbation (e.g., numerous areas of Chesapeake Bay main-stem and tributaries that are not impacted by critically low dissolved oxygen; much of Long Island Sound; coastal regions of Gulf of Mexico).

This study was designed to characterize sediment resuspension and transport processes as they potentially influence contaminant transport and fate in the lower bay. Specific objectives were to: (1) determine hydraulic roughness and bed stress; (2) determine sediment resuspension potential; (3) determine the roles of physical vs. biological resuspension processes; and (4) assess the appropriateness of existing models for predicting sediment resuspension.

APPROACH

Field measurements were made in all seasons to assess seasonal variability. Instrumented boundary layer tetrapods supporting electromagnetic current meters, turbidity sensors, a pressure sensor and a sonar altimeter were used to record time-varying bed stress and suspended sediment concentrations. Box cores and sediment profile camera imagery provided data on benthic biology. An annular seabed flume was used to obtain *in situ* estimates of threshold shear stress at which sediment is resuspended. Stress and roughness estimates were made by applying the 'law of the wall,' the Komogorov spectrum (inertial dissipation) method and the Grant and Madsen wave-current boundary layer model.

RESULTS

Seasonally-variable biological modifications of bed roughness, substrate tenacity and critical shear stress played key roles in controlling the responsiveness of sediment to physical

disturbance. The floor of the bay-stem appears to approximate a quasi-equilibrium surface with normally-recurrent physical bed stresses, resulting from the combined action of tides and waves, remaining very near to threshold values for resuspension. Minor alterations in parameters such as sediment fabric, elevation of biogenic bed forms (e.g., fecal mounds), surface sediment binding and the construction of roughness features such as tubes by organisms residing on or within the sediments greatly alter sediment resuspension potential. The bed itself remains highly active because active benthic biota mix the sediment column, provide bed roughness elements, and mediate the responsiveness of the bed to physical processes. Recent radionuclide tracer studies (S. Kuehl and others, personal communication) support the idea that the upper layers of the sediment bed are highly mobile from the combined activities of biological and physical processes. In contrast, resuspension of particulate material into the water column is considerably less than what is predicted by existing models. Recent observations by Schaffner and others (in progress) indicate that the binding of fine particles by microbes or into the fecal pellets of benthic or pelagic organisms may be major factors influencing the availability of fine particles for resuspension.

CONCLUSIONS

1) Hydraulic roughness is biologically dominated, but varies seasonally and annually in response to changes in the bioturbation rate, changes in benthic community composition and seasonal variation in biological-physical interactions (e.g., changes in the wave climate in winter vs. summer).
2) The roughness height, k_s (= 30 z_o) can range from less than 0.5 cm to greater than 2.0 cm. Roughness tends to be lowest in winter.
3) Waves, including long period swell, enhance hydraulic roughness and shear stress.
4) Benthic biota have major impacts on fluxes across the sediment-water interface and resuspension potential because they mix the sediment column, provide bed roughness elements that influence bed stress, and mediate the responsiveness of the bed to physical processes.
5) Currents alone do not play a major role in sediment resuspension in this environment.
6) The critical shear stress necessary for predicted sediment entrainment is only exceeded when strong currents interact with moderate waves.
7) Sediment resuspension into the water column is far less than predicted by existing models based on first order assumptions. Microbial binding and pelletization through the feeding activities of benthic and pelagic organisms are believed to have a significant impact on the availability of fine particles for resuspension.
8) The floor of the lower Chesapeake Bay is a highly dynamic environment. Contaminants associated with fine grained or organic-rich particles are unlikely to accumulate through burial processes. These same particles will, however, have a high potential to recycle repeatedly through a 'biologically-active compartment' at or near the sediment-water interface. This increased recycling will enhance the potential for transformations that may alter the fate and effects of the compounds. Deleterious effects on living resources may be greater than predicted based on contaminant inventories in bulk sediments.

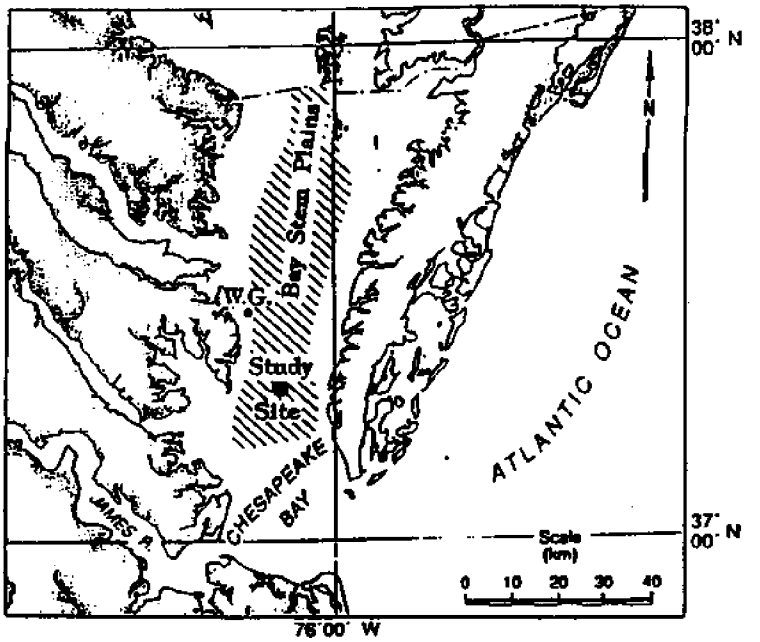

Figure 1. Study site location in lower Chesapeake Bay

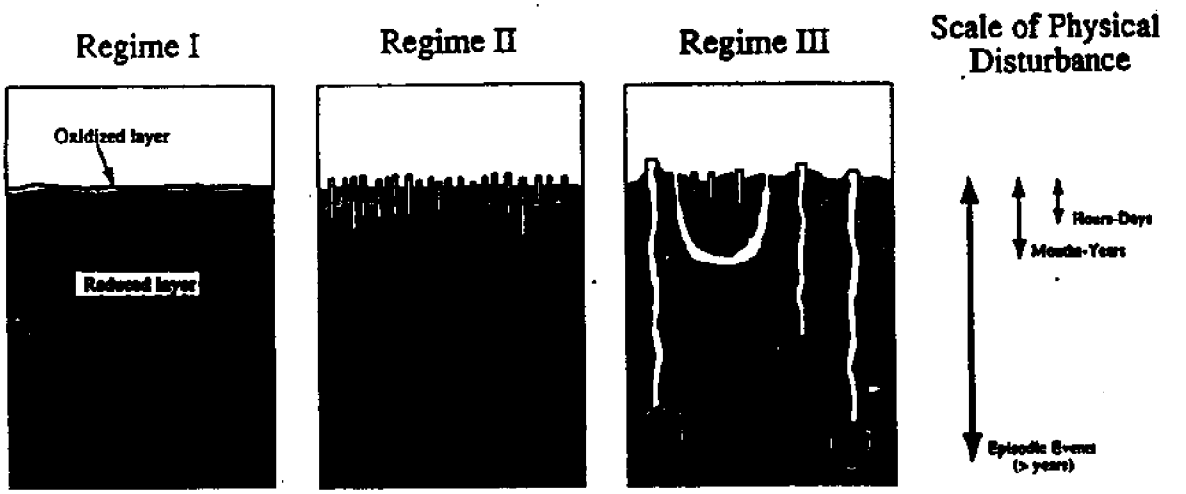

Figure 2. Conceptual model of 3 community disturbance regimes. Regime I typifies areas exposed to high levels of environmental stress. This regime may lack macrofauna altogether. Regime II communities are characterized by high densities of opportunistic macrofauna. These animals are small and live very near to the sediment surface. Bioturbation is limited to the shallow sediment surface layers. This type of community is often observed in moderately stressed environments such as areas impacted by pollutants, low water column dissolved oxygen or low or variable salinity regimes. The Wolf Trap study site is representative of Regime III, areas that have relatively healthy communities with diverse benthic assemblage that include large, relatively long-lived macrofauna. These areas are characterized by high levels of bioturbation (lower estuary, shallow coastal regions).

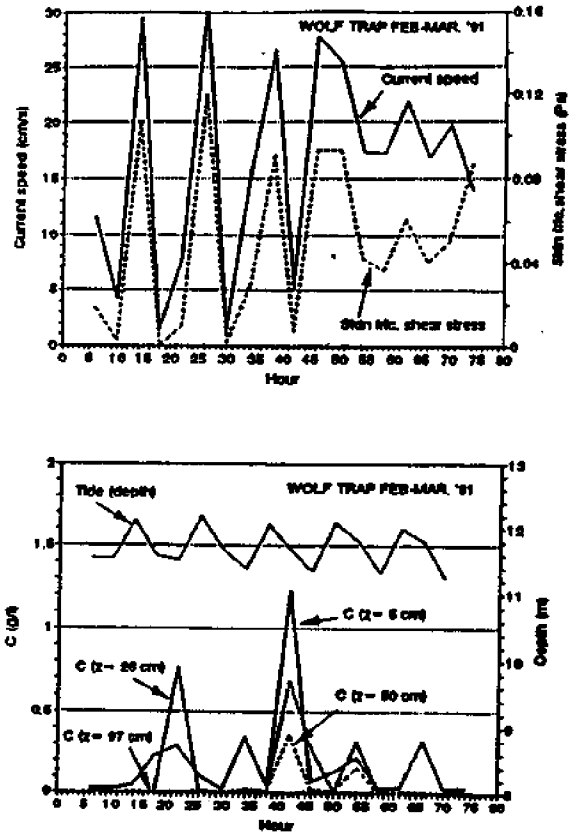

Figure 3. Representative records of current speed (cm/s), skin friction shear stress (Pa) based on the Grant and Madsen model, tidal height and suspended sediment concentration (g/l) at the Wolf Trap study site. Note that sediment resuspension events are rare, except very close to the bed. Peak suspended loads are observed out of phase with periods of maximum bed stress suggesting the possibility of advection from a non-local source.

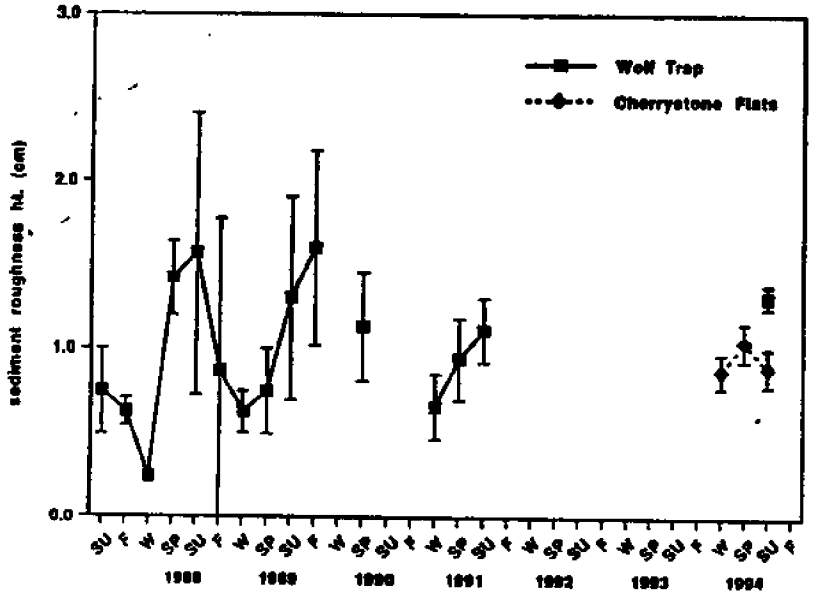

Figure 4. Height of bioturbation-induced sediment roughness due to features such as fecal mounds and pits at the Wolf Trap site. These roughness heights were determined by measuring photographic images that recorded the maximum and minimum depth of penetration into the sediment of a sediment profiling camera. Seasonal and interannual variations in average roughness height (± 1 SD) are apparent. These variations can best be explained by changes in the community composition (interannual), rates of bioturbation (seasonal) and changes in the wave climate (seasonal). Comparable data are shown for a nearby site during 1994 (Cherrystone Flats).

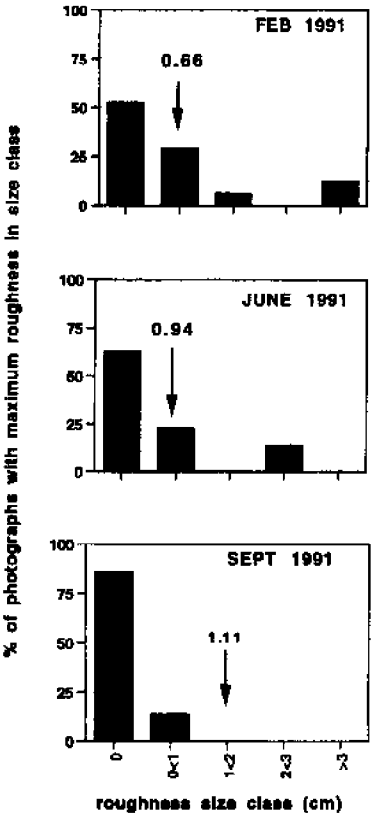

Figure 5. Comparison of biological roughness heights due to projections of organisms and their tubes above the sediment-water interface at the Wolf Trap study site. These figures show that the organisms themselves can dominate biogenic roughness at the sediment-water interface. For comparison, the mean sediment roughness heights due to mounds and pits are indicated with the arrows on each panel.

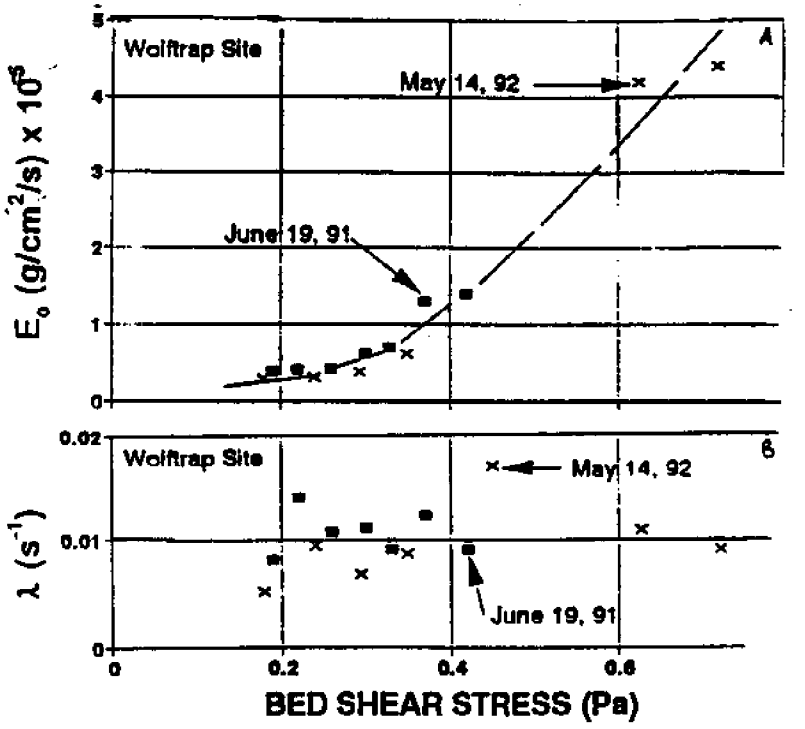

Figure 6a. Sediment erosion rate versus bed stress for the spring of 2 years at the Wolf Trap site. Critical shear stresses observed to date for the bay stem plains of lower Chesapeake Bay have ranged between 0.1 and 0.2 Pa.

Figure 6b. The rate coefficient remains near 0.01.

RESUSPENSION AND TRANSPORT OF SEDIMENT ASSOCIATED TOXICS IN THE NORTHERN CHESAPEAKE BAY [R/CBT-9]

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OBJECTIVES

The objectives of this study were to:

1) Investigate the resuspension and transport of fine sediments in the northern Chesapeake Bay, characterizing temporal and spatial (vertical) variability in resuspension processes through a sequence of combined moored and *in situ* observations.

2) Investigate the influence of resuspension on the flux of toxics across the sediment-water interface, and to examine how resuspension affects partitioning of toxics between continuously suspended particulates, tidally resuspended particulates, bottom sediments, and dissolution.

3) Relate resuspension and transport of sediment associated toxics to more easily measurable or predictable sediment and physical forcing characteristics, in order to facilitate the incorporation of our results in improved toxics transport models.

This project was the physical component of an integrated group of three projects that addressed physical, geochemical, and biological processes controlling transport, fate, and bioavailability of suspended and dissolved toxic compounds in Chesapeake Bay waters. Our project concentrated on physical forcing, sedimentary particulate response, and trace metal contaminants. A second project under the direction of J. Baker, R. Harvey, and R. Dawson concentrated on hydrophobic organic contaminants (HOCS) in different particle size classes and organic carbon pools. A third project under the direction of G. McManus and M. Roman examined the contribution of zooplankton fecal pellet production toward the overall settling flux of HOCS.

RATIONALE

Sediments are the major reservoir of heavy metals and hydrophobic organic contaminants (HOCs) in aqueous environments. Transport into and out of the sedimentary reservoir is controlled primarily by the transport of fine, organic carbon-rich suspended sediments, to which both metals and HOCs preferentially adsorb. Resuspension of bottom sediments is a

major contributor to the pool of fine suspended sediments in shallow, muddy environments like the northern Chesapeake Bay. Thus, the processes that control sediment resuspension in the northern Bay should also affect fluxes of toxics into and out of northern Bay sediments.

RESULTS

The results of our investigations of sediment resuspension processes have shown that:

1) Sediment resuspension in the mid-Bay occurs on a tidal basis, but a critical erosion velocity that in our observations is very close to typical tidal velocities causes a marked asymmetry in the magnitude of tidal resuspension. The response is much larger for slightly higher and more prolonged velocities than average, and the response much lower for slightly lower than average velocities.

2) Wind/storm generated resuspension occurs less frequently than tidal resuspension, but is much larger than tidal resuspension.

3) Temperature/salinity stratification in the water column can play an important role in resuspension by limiting the height to which eroded sediments can be resuspended.

4) Resuspended bottom sediments settle quite rapidly out of suspension in the mid-Bay, resulting in relatively short-lived periods of high suspended sediment concentration. There is a seasonally variable population of much less rapidly settling particulates that is a slowly varying background for the rapidly varying resuspended population.

5) The depth of erosion that results from tidal resuspension in the mid-Bay is on the order of 0.1 to 1 mm thick. These estimated erosion depths are very similar to the thickness of the "floc layer" observed at the very surface of bottom sediments from mid-Bay sites, when sediment samples are obtained by careful box coring.

Combining the results of these investigations with our collaborators' results in the context of the theoretical model begins to reveal a coherent picture of the influence of resuspension on sediment-water pollutant fluxes. The model predicts that resuspension will delay burial of newly settled particles by a time proportional to the ratio of resuspension to sedimentation. Using estimates of total resuspended sediment mass from the anchor station cruises and estimates of sedimentation rate from ^{210}Pb dating performed by J. Cornwell, we estimate a burial delay time of several days to weeks. Measurements of the organic carbon content of the "floc layer" at the sediment surface show it to be substantially degraded relative to fresh material in the water column, at a level consistent with decay rates measured by H.R. Harvey and decay times similar to the burial delay time. The floc layer material is also very similar chemically to the material collected in near bottom sediment traps. Finally, PCB concentrations in the floc layer material are substantially less than in the newer suspended material, and decrease even more into the surface sediments just below the floc layer. Thus, it appears that an important role of resuspension is to short circuit incorporation of organic pollutants into the sediment bed and increase water column recycling.

A summary view of the suggested overall scenario is thus as follows. Toxics in Bay waters are bioaccumulated in the plankton. At most times and throughout most of the water column, this high organic content material is the main component of the suspended particulate pool. It ultimately settles toward the Bay bottom through incorporation into larger, more rapidly settling particles by zooplankton fecal pellet production, agglomeration of planktonic detritus, and other processes that promote coagulation. However, short-lived resuspension events deliver a much larger mass of rapidly settling resuspended particulate material to the water column. The vertical extent of resuspension is determined by the intensity and duration of erosion, density stratification, and the ratio of settling velocity to mixing in the water column. The likelihood of sampling the resuspended particle pool by conventional shipboard sampling techniques is small, since the conditions under which massive resuspension occurs are inhospitable and the material settles back out of suspension quickly, but resuspended particles dominate sediment trap collections. Newly settled material thus constitutes only a small part of the regularly resuspended sediment surface layer, and it takes a period of several days to weeks before it is actually buried below the surface layer and incorporated into the sediment bed. During this time the relatively fresh organic carbon decays and associated contaminants are recycled into the water column, such that little of the original contaminant load remains by the time burial finally occurs.

Finally, our metals analyses have been completed and we have begun examining relationships between metals variability, resuspension, and advection of subpycnocline water. Several conclusions can be drawn from the metals data in correlation with the physical data. These are:

1) The sulfur to carbon (S/C) ratio can be used as an indicator of resuspension. A simple mixing model can be constructed employing the S/C ratio in the flocculent layer and the S/C ratio at the 12m altitude as end members. Using this model the fraction of the flocculent layer resuspended can be calculated;

2) Physically, from the transmissometer and total suspended solids measurements three categories of suspended loads where functionally defined - background, resuspended, and mixed. The primarily background levels and mixed categories are clearly distinguished from the resuspended with the cut off being at 20% resuspended matter based on the S/C determined fraction;

3) All of the metals enrich as the fraction of resuspended matter decreases. The enrichment factors are calculated by making a ratio of the metal of interest to Fe and normalizing the ratio to the ratio found in the flocculent layer, as follows - Enrichment Factor (X) = $[(X/Fe)_{profile}/(X/Fe)_{floc}]$

4) Variations in the metal levels, suspended and dissolved, can be attributed to tidal action and flow direction. However, the interpretation of the data is complex because the variations are influenced not only from to resuspension by tidal action and may be due to influx of other water masses, and;

5) The anoxic event of the summer sampling period was of greater significance in metal mobilization, for some metals as compared to the tidal action of the well mixed, oxygenated periods of the study. The metals strongly influenced by anoxia were Mn, Zn, Ni, and Pb. Cu and Cr appeared unaffected.

THE ROLE OF BENTHIC INFAUNA AND FLUCTUATING OXYGEN CONCENTRATIONS IN THE FLUX OF TOXIC TRACE ELEMENTS FROM CHESAPEAKE BAY SEDIMENTS [R/CBT-7]

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RATIONALE

The purpose of this research program was to investigate the processes by which trace elements are transported into and out of sediments, how benthic organisms or their activities regulate the transport of such elements between the sediments and the remainder of the ecosystem, and how these processes are altered by periodic changes in the oxygen concentrations of bottom waters.

In order to address this problem, we developed three immediate objectives:

1. Investigate the importance of seasonal anoxia and resulting recolonization cycles on redox reactions of representative, important trace elements (arsenic, copper, iron and manganese) within the sediment, and subsequent changes in both the chemical form of the substance and its flux rate;
2. Determine the extent to which sediment biota, especially the macrobenthos, facilitate or impede the flux of representative trace substances across the sediment/water interface under varying oxygen concentrations; and
3. Extend our study of the effects of organisms with different feeding and burrowing habits on trace element flux to a more realistic habitat.

We initially focused our efforts on two toxic trace elements (As, Cu) as representative, model elements because they exhibit quite different geochemical properties and allow us to examine a wide variety of trace element behaviors under varying oxygen conditions. They are also elements which have been found in estuaries in high levels, and for which environmental concern has been expressed. In the second year, upon requests from reviewers and local interested managers, we added Cd and Ni as elements of interest.

METHODS

1991 Season - Experiments with Manipulated Benthic Communities

Sediment samples were collected from the Baltimore Harbor in July 1991 using a Van Veen grab. The sediment was screened through a 500 mm screen to remove debris and larger biota, homogenized and distributed to twenty-one 20L aquaria, which received approximately 10 cm of sediment. The tanks were filled with filtered water from the Patuxent River, sealed

and bubbled with N_2 for 7 days to induce anoxia and kill the remaining biota. After this the tanks were bubbled with air to restore O_2 saturation, and received filtered Patuxent River water at a turnover rate of 50% per day for two weeks, prior to the start of experimentation by the addition of animals. ^{234}Th was added to the top of the sediment layer as a tracer for bioturbation.

Treatments consisted of 3 oxygen levels x 3 organism conditions x 3 replicates, with no treatments with both anoxia and organisms (n=21). The oxygen levels used were anoxic, nearly depleted (5-10% saturation), and saturated. Oxygen levels were maintained by bubbling gas mixtures through the sealed tanks. The organism conditions were control (no organisms), *Macoma balthica*, and *Nereis succinea*. No organisms were added to anoxic tanks. *Macoma* was added to the tanks approximately two weeks after gas and water flows were started to the tanks during continuous flow operation, while *Nereis* was added two weeks later, at the start of a one week stopped flow experiment.

The microcosms were operated for a period of approximately 6 weeks. With the exception of two one week stopped flow experiments, a 50% turnover of water was maintained with a continuous flow. DO, temperature, and salinity were monitored daily. Water samples for trace metal analyses were taken twice weekly during continuous flow experiments, and daily (except weekends) during stopped flow diffusion experiments. At the end of the experiment, organisms were collected for mortality estimates. Core samples were taken to determine metal concentrations with depth in both sediment and interstitial water.

1992 Season - Experiments with Intact Sediments and Fluctuating Oxygen Conditions

Sediments were collected from Baltimore Harbor at the same site as the previous year using a plastic box corer. Sediments were taken from a depth of approximately 20 ft. Cores were placed in PVC trays and brought back to the laboratory. One core was sectioned immediately for trace elements in the sediment and interstitial water, while three cores were sieved to 250 mm for later determination of the organisms. The remaining cores were placed in filtered aerated seawater in the laboratory. The cores were placed in 9 5-gallon aquaria (2 per tank). The sediments received a trickle flow of unfiltered water to provide food for the resident fauna and the tanks were allowed to stabilize for 6 weeks.

Treatments consisted of 3 oxygen regimes with triplicate tanks for each treatment (n=9). The oxygen regimes are as shown:

1. No anoxia;
2. Intermittent anoxia (anoxic 1 week, oxic remainder);
3. Seasonal anoxia (anoxic 2 months, oxic remainder).

Intermittent anoxia is intended to mimic a single brief intrusion of anoxic water into oxic waters. Seasonal anoxia is intended to mimic anoxia seen throughout the summer months in deeper areas of the Chesapeake Bay. Periods of anoxia were created by bubbling N_2 gas through the microcosms. The tanks were allowed to recolonize with infusions of unfiltered water following anoxia.

The microcosms operated for 4 months, during which 4 stopped- flow experiments were carried out. For the first 2 months, a daily 50% turnover was maintained with filtered water during continuous flow periods. After 2 months, all tanks were changed to unfiltered water.

DO, temperature, and salinity were monitored daily. Water samples were collected regularly for trace metal analysis as in the previous season's study. At the end of the experiment, one sediment core from each tank was sectioned to determine metal concentrations with depth in the interstitial water and sediment, and the other core sieved for determination of the benthic community

RESULTS

1991 Season

Arsenic fluxes out of sediment were large, and relatively constant in the anoxic treatment, about 8 ng/cm^2/day. Arsenic fluxes were not measurable from the "no organism" treatments for both the low oxygen and saturated oxygen, and there was some indication that in the case of saturated oxygen, there may have been net flux from the water column to the sediment. Both *Nereis* and *Macoma* in the low oxygen treatment caused fluxes of arsenic similar to those of the anoxic treatment in the absence of organisms. In both cases the fluxes were high immediately after the introduction of animals, and declined relatively rapidly thereafter, suggesting that fresh burrowing was responsible for much of the increase in flux.

Copper behaved in a manner very different from arsenic. In saturated oxygen treatments without organisms, we saw a significant flux of copper from the sediment from the water column, about 3 ng/cm^2/day. When organisms were added to the sediments with saturated oxygen overtop, the flux was reversed, with a net flux out of the water of about 1-4 ng/cm^2/day. A similar net flux of copper into the sediment was observed in the low oxygen treatments with organisms, although no net flux was measurable in the absence of organisms. In the anoxic treatment, a flux from the water of about 1 ng/cm^2/day was also observed.

Manganese and iron flux were also examined, to provide indications of the redox chemistry in the treatments. Manganese showed a pattern very similar to arsenic, except with much greater (100-1000X higher) fluxes. Iron fluxes appear to also have been extremely high in the anoxic treatment, but were largely found as particles, and not measured in the dissolved fraction. We have also examined the interstitial water, and total sediment concentrations of the same elements to compare the fluxes with the inventories of trace elements present in each form.

1992 Season

Flux measurements observed in this experiment were generally comparable to those measured in the first year's experiments. In the second year, both short-term and long-term anoxia caused significant fluxes of arsenic (4-6 ng/cm^2/day) and manganese (1-7 mg/cm^2/day) out of the sediment, which were rapidly stopped by the switch back to oxic water over top. Copper flux was again the reverse of arsenic and manganese, with fluxes from sediment to water under oxic circumstances (ranging from 8-11 ng/cm^2/day in the stopped flow experiments), and from water to sediment during anoxic periods (1-4 ng/cm^2/day). Switching

from anoxic to oxic conditions rapidly restored the outward flux pattern. Cadmium flux was similar in pattern to copper, with apparent flux into the sediment during anoxia (< 0.1 ng/cm^2/day), flux out under oxic conditions (0.4-0.8 ng/cm^2/day), and a large pulse during the reoxidation of the long-term anoxic treatment (1.4 ng/cm^2/day). Manganese flux was again very high under the anoxic treatments (1-7 mg/cm^2/day), and rapidly declined after the introduction of oxygen. For nickel, little trend is discernable, but there is some suggestion it may follow a pattern similar to copper and cadmium. In the final stopped-flow experiment, persistent differences in the flux of the different treatments are seen for both arsenic and copper, suggesting either differences due to the changed benthic communities, or persistent differences in the sediment chemistry.

Examination of the biological samples immediately after the anoxic treatments showed that the oxygen treatments had the expected effects, essentially no surviving fauna in the anoxic treatments, and only a few in the short-term anoxia treatment. After two months of recruitment from raw water however, a significant benthic fauna consisting of small *Macoma* and worms was found in the intermittent anoxia treatment, while the recruitment in the long term anoxia treatment, which was delayed until later in the season, was minimal.

This project has addressed the question of the significance of the threat to the Chesapeake Bay by the relatively large inventories of toxic trace elements found in the sediments of some contaminated areas. Specifically, we addressed the rates, and some of the biological and chemical processes affecting the rates, at which trace elements may leave the sediments, and enter the water column, and thus affect a wider suite of organisms. To briefly summarize, depending on the chemical and biological conditions in the sediment, the sediment may either be a net source or a net sink of toxic trace elements to the overlying water column. However, when considering a water column of the average depth and residence time of Baltimore Harbor, or Chesapeake Bay, the highest fluxes found, while sufficient to play an important role in the concentrations and distributions of the metals studied, are unlikely to lead to situations of acute trace element toxicity. The flux rates are also sufficiently small that toxic trace elements resident in the sediments are not likely to significantly decrease within reasonable time scales. These findings have important implications for managers. One is that sediment sources will continue to put metals into the system for the foreseeable future, whether or not other sources of metal are restricted or stopped. Unless managers are willing to either cover or remove contaminated sediments, there is a level of contamination which is inevitable in the system. Moreover, we have examined some factors which influence these fluxes, and can predict the effect of certain environmental changes on these fluxes. For example, a change from an anoxic to oxic regimes over the sediment, as might occur when organic inputs to a system are lessened, will cause a change from a positive flux of arsenic and loss of copper and cadmium to a system which sequesters arsenic and releases copper and cadmium. Changes in biological characteristics of such a system will have lesser, but potentially significant changes. Increased benthic activity will tend to increase the flux of some materials (e.g., arsenic), but may increase loss to sediment by other materials (e.g., Cu).

MICROBIAL DEGRADATION OF CHLORINATED HYDROCARBONS UNDER ALTERING REDOX CONDITIONS IN CHESAPEAKE BAY SEDIMENTS [R/CBT-11]

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RATIONALE

While chlorinated hydrocarbons are far more resistant to aerobic microbial degradation than their non-chlorinated analogs, the recent recognition of the ability of anaerobic bacteria to reductively dehalogenate a range of such contaminants suggests that environments which naturally undergo temporal oscillation from anoxic to oxic conditions, such as mid-Bay and tributary sediments, may allow for more complete degradation of such compounds. For a relatively large number of contaminant organics including chlorinated aromatics, a previous history of exposure often results in enhanced degradative potential by the microbial flora. Information on the natural capacity of the bacterial flora of these sediments to dechlorinate and mineralize haloaromatics will be useful in the development of remediation strategies.

Hypotheses

1. Exposure of chlorinated aromatic hydrocarbons to reducing conditions in surficial sediments increases their potential for complete oxidation under subsequent oxic conditions.

2. Sustained exposure to chlorinated hydrocarbons and seasonally varying anoxia increases the capacity of surficial sediments for degradation and mineralization of these compounds.

PROJECT RESULTS

2,4-DCP Experiments

Mid-Chesapeake Bay Sediments. Results from our first 2,4-DCP experiment with mid-Bay sediments, while not providing direct support for our "alternating redox" hypothesis, provided evidence for the capacity of the indigenous microbial population for degradation and complete mineralization of 2,4-DCP. After eight months of incubations with ^{14}C-labeled 2,4-DCP (11 μM) these sediments mineralized 2,4 DCP (as measured by $^{14}CO_2$ production) under both anoxic and oxic conditions, after a lag period of c. 30 days. The largest amount and greatest rate of $^{14}CO_2$ production were observed in a treatment held anoxic for 2 months, followed by oxic incubation. The remaining live treatments, initially held anoxic 0. 5, 1, and

4 months before turning oxic, showed essentially similar rates of $^{14}CO_2$ production, due to high variability among replicates. No remarkable change in rates of mineralization was observed following a change in redox conditions and timing of increased CO_2 production in each treatment could not be definitely correlated to length of time each treatment was held anoxic before exposure to oxic conditions. Several problems with experimental design did crop up during the first experiment which may have contributed to the observed variance among replicates. Concern over possible oxygen contamination in some of the anoxic treatments which may be responsible for the observed results, prompted closer direct monitoring for and protection against O_2 contamination of anoxic treatments in subsequent experiments. Chemical analysis of anaerobic batch slurries dosed with 2,4-DCP revealed that 2,4-DCP was completely removed and dechlorinated to 4-chlorophenol (4-CP) within four months. The metabolite, 4-CP, was first detected at 2 months and persisted for at least 2 additional months before being degraded. The potential dechlorinated metabolite, phenol, was not detected at the time points sampled. No chlorophenols were detected in the unamended control sediments.

The experiments with ^{14}C-phenol dosed subsamples showed no significant differences in aerobic mineralization rates between the 2,4-DCP amended and control batch slurries. These rates changed very little between Day 0 and Month 4 subsamples, and in all samples mineralization took place without a lag period.

In related experiments conducted in the summer of 1992 by a REU student in our lab, we also investigated the effects of sediment storage (with respect to methane production, sulfate and carbon depletion) on rates of disappearance of 2,4-DCP in anoxic sediments previously stored either 3 months or freshly collected. 2,4-DCP completely disappeared in aged, sulfate-depleted R-64 sediment in less than one month. Production of 4-CP was evident after one day of incubation. The addition of acetate enhanced degradation rates. Complete disappearance of 2,4-DCP also occurred in aged sediments to which sulfate was added back, though theoretically, sulfate could have been again depleted within the one month time course of the experiment. No significant degradation of 2,4-DCP was observed, over in the same time period with freshly collected, sulfate replete, R-64 sediment. Of great interest was the observation that in aged, sulfate-depleted sediments, where methanogenesis dominated, that inhibition of sulfate reduction by molybdate resulted in no degradation of 2,4-DCP. This suggests that sulfate respirers may use Cl-organics as an alternate electron acceptor in the absence of their normal substrate, sulfate. Results from these preliminary studies are encouraging. We have proposed more in-depth investigations of environmental variables affecting anaerobic degradation of chlorinated compounds, and the microbial communities involved, in Chesapeake Bay.

Baltimore Harbor Sediments. In the second experiment, with Baltimore Harbor sediment dosed with ^{14}C labeled DCP at a higher concentration (100 μm), we have obtained evidence in support of our hypothesis that anoxic incubation will enhance degradation under subsequent oxic conditions. Baltimore Harbor sediment slurries were dosed with ^{14}C-labeled 2,4-DCP and incubated anaerobically for either 1, 2, or 4 months followed by aerobic incubation. Live and killed controls were held either strictly aerobic or anaerobic for this 245 day experiment.

Mineralization, as measured by evolved $^{14}CO_2$, occurred in all live treatments after a lag period of c. 30-50 days, regardless of the redox conditions employed. Treatments initially incubated under anoxic conditions, then later switched to oxic conditions showed biphasic mineralization patterns with a distinct increase in rate upon aeration. The fastest rates of mineralization were observed in treatments held anaerobically I or 2 months followed by aerobic conditions. However, these increased rates were not significantly different ($p = 0.05$) from a control treatment held entirely oxic. Chemical analysis of anaerobic batch sediment slurries revealed that degradation of 2,4-DCP began within one month with rapid loss (84%) occurring within 2 months. Substantial concentrations of 4-CP were first detected at 2 months, and persisted at this level for at least 2 additional months. At the time points sampled, 4-CP production was detected in less than stoichiometric proportion to the amount of 2,4-DCP degraded. Phenol, as the potential dechlorinated daughter product of 4-CP, if present, could not be detected in the sample matrix. It is not clear whether 4-CP is degraded via an initial reductive dechlorination step prior to mineralization. No chlorophenols were detected in the unamended BH sediment slurry controls.

Results from the ^{14} C-phenol experiments with Baltimore Harbor sediments were similar to those observed in Mid Bay sediments. Apart from Day 0 subsamples, there were no appreciable differences in rates of aerobic mineralization between dosed and control batch slurries. Rates of mineralization from both slurries increased in subsamples from the fourth month. Mineralization again was observed without a lag in all samples. The lack of difference between mineralization rates in the dosed and control batch slurries in both the BH and Mid Bay sediments are not surprising in that we never detected any appreciable concentration of phenol in our chemical analyses of amended samples from any time point. What these experiments do show is the sustained rapid facility for aerobic mineralization of these aromatics by microbes incubated several months under anoxic conditions.

For both our uncontaminated Mid Bay station and the contaminated Baltimore Harbor site, degradation and complete mineralization of 2,4-DCP appears to proceed under either aerobic or anaerobic conditions and to be catalyzed by anaerobes and facultative aerobes in the sediments examined. This therefore suggests that the indigenous microbial flora may be adapted to, and capable of responding relatively rapidly to conditions of variable redox. Anaerobic microbial populations at both sites initiated reductive dechlorination from the *ortho* position of 2,4-DCP. The observed onset of reductive dechlorination appeared to be more a function of biogeochemical parameters (as yet clearly defined) of the systems involved rather than a history of previous exposure to chlorinated aromatic hydrocarbons. In both systems the 4CP metabolite, which is more toxic than 2,4-DCP, persisted for a period considerably longer than that of the parent compound.

PCB Experiments

The Baltimore Harbor sediments dosed with a mixture of 10 polychlorinated biphenyl (PCB) congeners did not provide support for either of our hypotheses over the experimental duration. Unlike our chlorinated phenol experiments, we did not observe any significant reductive dechlorination occurring in our anaerobic batch slurries after 4 months of incubation. We also did not observe any significant mineralization of either the ^{14}C-labeled

tetra- or hexa-chlorobiphenyl after 9+ months of incubation under anaerobic, aerobic or variable redox conditions. Chemical analysis for PCBs in the unamended batch slurries revealed that PCBs were present in these sediments at levels of c. 300 ng/g dry wt for total PCB congeners, (tPCB). This background level in BH sediments is an order of magnitude higher than levels of tPCBs found in Mid Bay sediments. The elevated levels in BH sediments were thought to have provided an environment to test whether sustained exposure to PCBs promoted degradation and mineralization of these compounds. However, the BH TPCB levels are in the median range of concentrations observed in sediments of the NOAA National Status and Trends Program and much lower than ambient concentrations in other sediment systems where *in situ* PCB dechlorination or degradation has occurred. This suggests that the ambient PCB levels in sediments from our site in Baltimore Harbor were not sufficiently high to enrich for a microbial population capable of dechlorinating these particularly recalcitrant compounds. These initial findings also point to a substrate specificity for the reductive dechlorination reaction observed in the same sediments with our chlorophenol experiments. It is apparent that an enzyme for PCB dechlorination is not constitutive or active in the microflora of these sediments, though a more lengthy induction period than we observed may be operative.

Mineralization results from ^{14}C-labeled non-chlorinated analogue (biphenyl) dosing of batch slurry subsamples and respective controls yielded results somewhat similar to those in our phenol experiments. Day 0 subsamples from dosed and control slurries exhibited essentially identical rates of mineralization. Subsamples from Month I and 2 showed similar trends in the kinetics of mineralization between dosed and control slurries. Initial rates of biphenyl mineralization in control slurries were faster than in dosed slurries. However, these rates leveled off quite rapidly relative to the dosed slurries. There was an observed lag of 34 weeks in the dosed slurries before significant biphenyl mineralization occurred. Subsequent mineralization rates exceeded those of the control slurries. There may have been some adaptation to biphenyl mineralization from exposure to high levels of PCBs over time in the dosed sediments, even though no significant dechlorination of the dosed congeners occurred within the same time frames.

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DIRECT MEASUREMENTS AND BIOGEOCHEMICAL CONTROLS OF SEDIMENT-WATER FLUX OF TRACE METALS FROM ESTUARINE SEDIMENTS [R/CBT-12]

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RATIONALE

This research program provided, for the first time, a realistic measure of sediment fluxes of trace metals, specifically Cu, Zn, Mn,and Fe in a broad range of sediment types, salinities and degrees of contamination. The trace elements we have chosen to study here have been selected for several reasons. Our interest in Fe and Mn lies in the adsorptive properties of oxyhydroxides. The other toxic elements were chosen for study here (Cu, Zn, Pb) in part because they are toxic substances of interest in Chesapeake Bay and other estuarine environments, and in part because their contrasting geochemical behavior makes them good representatives of classes of toxic elements. As such, an understanding of their behavior in these sediments should allow an extrapolation to other similar toxic elements.

In a more general sense, this program provided a comprehensive investigation of the role of sediment metabolic processes on trace metal fluxes in estuarine sediments. Trace metal diagenesis and fluxes are controlled by the production of 1) complexing ligands (especially sulfides) and 2) the surface area of adsorbing Fe and/or Mn oxides (Mn, Fe reduction or precipitation of FeS): continued descriptive work on estuarine sediments that does not account for such biogeochemical processes will have little predictive value for the combined physical/chemical/biological models that will ultimately be used for decision-making by environmental managers.

PROJECT RESULTS

Iron and Manganese Reduction

Iron and manganese reduction rates have been measured a number of times at the

three main LMER sites located in the northern, middle and southern Chesapeake Bay regions. As expected, the highest rates tend to occur in near surface sediments where the potential release of associated trace metals is most problematic. The results of these studies, combined with rates of sulfate reduction and ΣCO_2 provide the best estimates of the importance of metal reduction to sediment diagenesis in any estuarine system.

Dissolved Trace Metal Pore Water Chemistry and Sediment-Water Fluxes

Considerable effort has gone into developing the chelation ion chromatograph into a useful process instrument for dissolved trace metals. Mike Owens, a Faculty Research Assistant in Cornwell's laboratory, has shown the instrument can provide high precision analyses of dissolved transition metals. As with any new instrument system, we invested much time into making the instrument perform in the mode necessary for our research.We feel that we have been very successful in this regard.

We have collected cores from numerous bay cruises for the measurement of pore water metal concentrations. Our data are consistent with literature concentrations of pore water Fe, Mn, Co, Ni, Zn and Cu and we appear to have no significant contamination problems. Seasonal patterns of pore water chemistry will benefit from the simultaneous measurement of other pore water parameters from the LMER project.

The measurement of benthic fluxes is still very experimental and we continue to modify our techniques. The batch mode of reaction, in which cores are incubated with no water exchange, have not provided reliable rates, partially because of the rapid depletion of dissolved oxygen. We have acquired new chambers and a high precision pump to run our fluxes in a chemostat mode, so that we continuously add new oxygen and remove the products of sediment respiration. Experiments with this new approach are underway.

Metal Geochronology

In cooperation with Cornwell's component of the NSF-sponsored LMER project, we have collected cores from almost 20 sites in the Chesapeake Bay for the determination of trace metal concentrations. The age of each sediment layer has been determined using the ^{210}Pb dating technique, typically analyzing 8-15 sections per core. In contrast to previous efforts, we have emphasized the lateral distribution of sedimentation rates at several bay sites. Overall, this effort provides the first small spatial scale consideration of sedimentation in Chesapeake Bay. The most significant result of this work is that the metals Pb, Cu and Zn are highly enriched in bay sediments, with a concentration maximum corresponding to sediment horizons from the early 1970's. Figure I shows the results from one mid-bay core.

The sharp decline in Pb concentration is consistent with the national trend of decreasing Pb inputs, largely because of point source controls and the elimination of leaded gasoline. The lead inputs above that found in the late 1800's have decreased by 50% from their maximum. The trace metal profiles measured in the project indicate that the environmental controls of metal inputs to the mid-bay region have been effective. We are cooperating in a study of organic contaminant inputs with Dr. Joel Baker of the Chesapeake Biological Laboratory and have supplied him with sediment from the two mid-bay cores with the best geochronology.

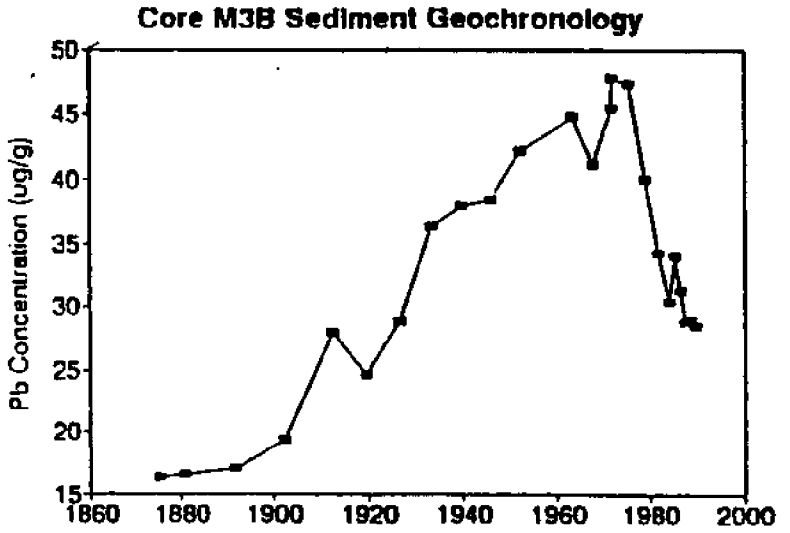

Figure 1. Concentration of acid-extractable lead plotted as a function of year. The geochronology was obtained using ^{210}Pb dating. The sampling site is the main mid-bay LMER site which experiences seasonal anoxia and thus has a minimum bioturbation. Similar peaks were found for Cu, Zn, with similar chronologies of input found in other mid-bay cores. The lower parts of the core are consistent with Helz' 1978 efforts to determine trends of metal input; the major decline in trace metals was barely evident at that time.

Sites which experience bioturbation, typically found in shallow water, also have peaks in trace metals, though bioturbation makes establishing sediment ages more difficult. The trace metal peaks may be a boon to sedimentation studies since we know the peak was about 20 years prior to today. Using stable lead as an indicator of sediment age is analogous to using ^{137}Cs, a product of thermonuclear testing in the 1950's and 1960's, as an event marker.

Seasonal Changes in Metal Concentration

Using surficial sediments from our mid-bay site, we examined solid phase chemistry of the sediment. We observed no seasonal change in the overall concentrations of Cu, Zn and Pb, despite large temporal changes in iron sulfide mineral chemistry and overall redox conditions. In fact, only Mn showed any seasonal pattern, with higher concentrations fall, winter and early spring, with loss of Mn oxides in mid-spring.

PROJECT USES AND BENEFITS

While many of the results of this project are awaiting more data for final interpretation, we have been trying to assist the USEPA in their toxics reevaluation efforts. Our CBEEC-funded time course of metals input to bay sediments will be presented in the basinwide Toxics Reduction Strategy Revaluation Report which is being coordinated with Richard Batiuk of the Chesapeake Bay Program Office. We have also discussed the use of these data with Dr. David Velinsky of the Interstate Commission on the Potomac River Basin.

ROLE OF BENTHIC COMMUNITIES IN SEDIMENT-ASSOCIATED TOXIC ORGANIC CHEMICAL FATE AND TRANSPORT IN LOWER CHESAPEAKE BAY [R/CBT-15]

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OBJECTIVES AND RATIONALE

The benthic subsystem and component processes are important in the transport and ultimate fate of contaminants in coastal systems such as Chesapeake Bay. Benthic organisms have the potential to play a major role in these processes because they influence the movement and mixing of sediments, alter biogeochemical processes and act as links to higher trophic levels. Previous studies have shown that bioturbation dominates sediment reworking throughout a significant portion of Chesapeake Bay subtidal region (above the pycnocline in upper bay, much of lower bay, high mesohaline and polyhaline tributaries). This means that benthic organisms are likely to have major impacts on the transport, flux and burial of contaminants throughout much of the system. Bioturbation may also be important in the short term in areas where physical reworking or sediment accumulation processes dominate the ultimate fate of particle-associated contaminants. Similarly, short periods of high macrofaunal activity in the spring could alter contaminant fate even in areas that experience summer hypoxia or anoxia. Especially important are processes that enhance flux, thereby increasing contaminant residence time within the Chesapeake Bay ecosystem. Flux is enhanced via activities such as particle bioturbation, burrow irrigation and trophic transfer.

Our three major objectives were: 1) to identify and quantify the role of macrobenthic organisms in sediment-associated organic contaminant (PAH, PCB) transport and fate for a representative community of lower Chesapeake Bay; (2) to evaluate seasonal variation in the mechanisms and rates of contaminant transport; (3) to relate contaminant physical-chemical properties to cycling processes in benthic systems.

APPROACH

We conducted a series of laboratory experiments to evaluate macrofaunal effects, via bioturbation, bioaccumulation and biotransformation, on organic contaminant (PAH, PCB) transport and fate. Microcosms with intact benthic communities were dosed at the sediment-water interface with contaminants sorbed to sediment. The contaminants varied in hydrophobicity (e.g., log K_{ow}) and other chemical characteristics. The rates and pathways of contaminant incorporation into microcosm sediments were then followed for up to 2 months.

All major compartments (e.g., organisms by species, biogenic structures such as tubes and burrows, sediments and pore waters) were sampled. Thus we can identify total community effects as well as the effects of individual species as a function of chemical characteristics.

RESULTS

In two 'summer' experiments we found that macrofauna enhanced the loss of compounds from the sediment relative to control microcosms (with macrofauna removed). Macrofaunal effects were similar for all compounds regardless of K_{ow} suggesting that mechanical movement of particles with associated contaminants is a major process governing contaminant flux at the sediment-water interface. During these experiments, we observed significant resuspension of particles into the water column by surface feeding macrofauna. In the estuarine environment, many of these resuspended particles and associated contaminants eventually resettle to the bottom, but this increased recycling leads to greater potential for transformations or detrimental environmental effects. Defaunated microcosms also lost contaminants, but total loss in these systems varied as a function of contaminant physical chemistry (e.g., log K_{ow}) indicating the importance of desorption and microbial degradation processes. In an experiment conducted during the winter of 1994, macrofauna capable of significant resuspension were absent and the net effect of the macrofaunal community was to slightly enhance the retention of contaminants relative to defaunated controls.

Our high resolution sampling of biogenic structures such as feeding pits, burrow walls and fecal mounds allows us to demonstrate the importance of bioadvective processes associated with the feeding activities of large infauna for contaminant burial. Contaminants introduced at the sediment-water interface were rapidly buried under fecal mounds or coils of infauna. Particle-associated contaminants were subducted in the funnels of funnel feeders such as the hemichordate *Balanoglossus balanoglossus*. We also found high contaminant concentrations in the burrow walls of this species. This is the major pathway we documented for the advection of contaminants to deep subsurface sediments (10+ cm) over the time frame for our observations (days to weeks). Profiles from subcores (7.5 cm diameter) used to assess spatially-averaged burial processes indicate that the initial processes that govern contaminant burial into the bed are independent of log K_{ow} suggesting that burial is regulated by physical movement or burial of particles. But the depth of contaminant burial in microcosms with and without macrofauna can be predicted on the basis of physical chemistry, indicating a possible role for diffusive movement of some fraction of each contaminant away from burrow walls and into the adjacent sediments over time. Ultimately, in our experiments buried contaminants did not accumulate in the sediment bed, indicating that contaminant fluxes must be in both directions.

We collected more than 25 species of macrobenthos during our experiments and analyzed their tissues for parent contaminant compounds and daughter products. We found that both surface and subsurface deposit-feeding organisms and predators exhibit rapid uptake of compounds deposited at the sediment-water interface. This indicates that direct ingestion of sediments is not the only mechanism of exposure. We also found that there is high variability in bioaccumulation and biotransformation both within and among major taxa. The production of metabolites by benthic macrofauna is a potentially important biogeochemical route of

contaminant cycling. Recent studies in other labs indicate that these compounds, which often are more carcinogenic than the parent compounds, can be transferred to predators. Ongoing studies are focussing on the production and trophic transfer of metabolites from benthic prey to fish and crab predators.

CONCLUSIONS

Benthic macrofauna, through their feeding, burrowing and irrigation activities, have major impacts on the transport, flux and fate of organic contaminants. Organic contaminants reaching the sediment-water interface are ingested or absorbed by most benthic macrofauna within just a few hours. Active bioturbation and, in particular, bioresuspension and bioirrigation, lead to increased contaminant residence time near the sediment-water interface. This will increase the potential for mixing back into the water column, for microbial degradation in oxygen sensitive processes and for transfer to higher trophic levels.

Another important finding of our laboratory studies is that contaminants are rapidly subducted down burrow and tube structures where the compounds become highly concentrated. This is significant because these structures are known to be important sites of activity within the sediment bed. Benthic organisms, ranging from microbes to small macrofauna, are found in increased densities around these biogenic structures. Oxygenated tubes and burrows are also important sites for critical estuarine processes such as nitrification-denitrification. Importantly, bulk sediment concentration profiles do not reflect the enhanced fluxes and elevated contaminant concentration levels we have observed in association with biogenic structures. Thus, bulk sampling may provide an unrealistic view of organism exposures to organic contaminants in the environment.

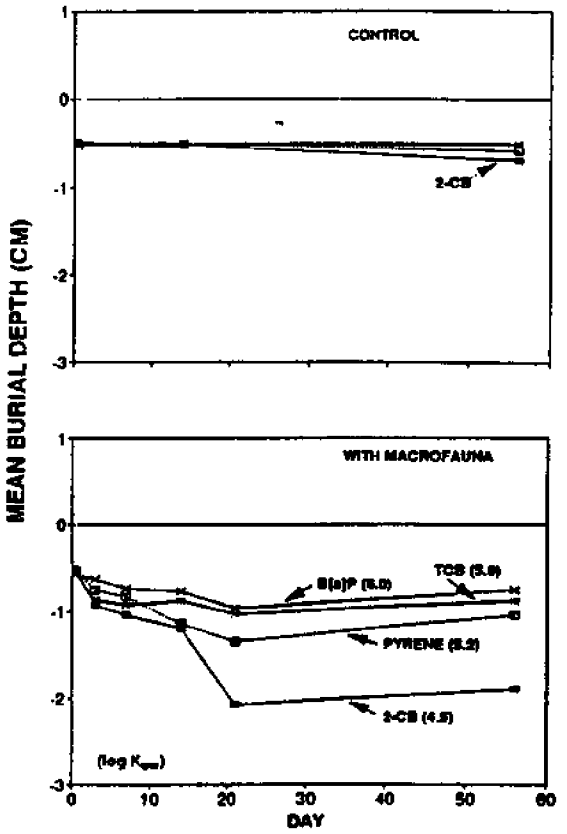

Figure 1. Results from a 'summer' experiment showing contaminant burial depth versus time in microcosms with and without macrofauna (controls). The significant effects of macrofauna and contaminant physical chemistry are apparent.

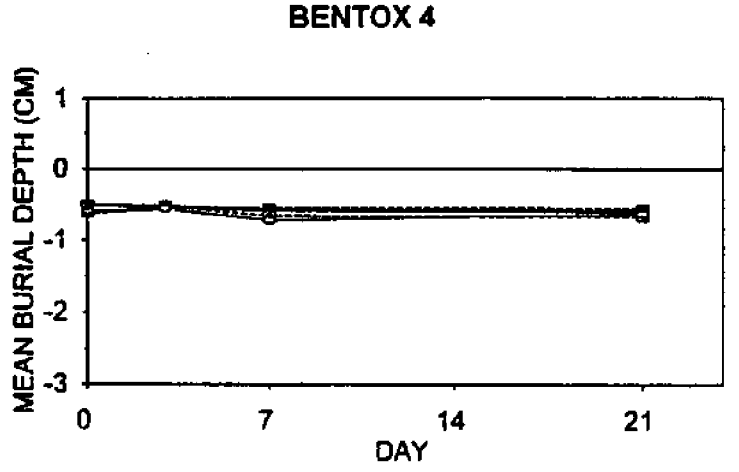

Figure 2. Results from a 'winter' experiment showing contaminant burial depth versus time in microcosms with and without macrofauna. In this figure all treatments are plotted together, indicating the lack of significant effects due to macrofauna or contaminant physical chemistry.

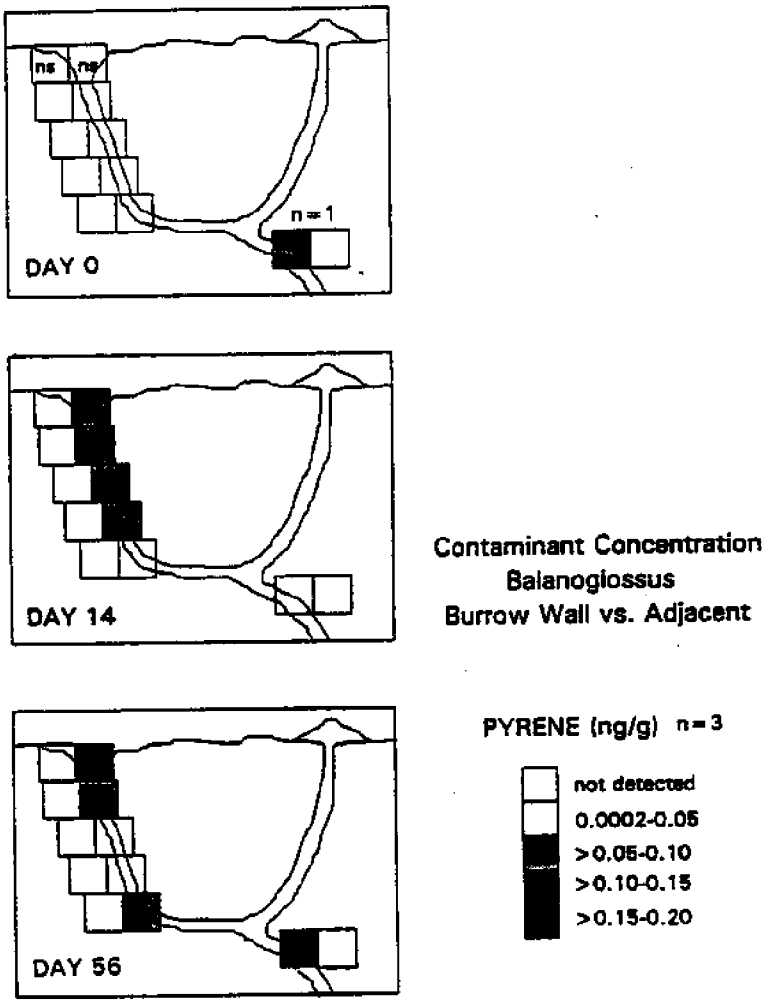

Figure 3. Pyrene concentration in the burrow walls of the hemichordate *Balanoglossus balanoglossus*. The contaminant was introduced at the sediment surface on Day 0. The burrow wall and adjacent sediments away from the burrow wall were sampled at 2 cm depth intervals.

THE SPECIATION OF DISSOLVED COPPER AND CADMIUM IN CHESAPEAKE BAY [R/CBT-14]

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INTRODUCTION

Copper and cadmium are among a group of metals designated as "Toxics of Concern" in Chesapeake Bay. Identification and quantification of the potentially harmful effects of metals such as Cu and Cd in estuaries receiving anthropogenic inputs (e.g., Chesapeake Bay) is difficult because of the multitude of pollutants potentially present and the uncertain relationship between metal concentrations and metal toxicity and bioavailability. Much of the uncertainty about this relationship results from lack of definitive knowledge of the chemical forms (species) of metals in natural waters. Information on the speciation of Cu and Cd is critically important for understanding their toxicity, bioavailability, and geochemical reactivity because the different forms of these metals can have very different behaviors.

The data presented here represent the first comprehensive information on the horizontal, vertical, and temporal variability of the speciation of dissolved copper and cadmium in Chesapeake Bay.

OBJECTIVES

The objectives of this study were to determine the concentrations of the following constituents, and investigate their horizontal, vertical, and temporal variability, in the Chesapeake Bay:

- Total dissolved Cu and Cd;
- Cu- and Cd-complexing organic ligands; and
- Free ionic, inorganic and organically-complexed fractions of dissolved Cu and Cd.

METHODS

Surface water samples were collected from five stations along the main stem of Chesapeake Bay (Figure 1) in August and September, 1992. Water samples from three depths were collected at Stations N and S in April, 1993. Water samples were collected, processed, and analyzed using trace metal clean methods. Samples were collected and filtered in-line (0.45 μm) using a peristaltic pumping system (Flegal et al., 1991; Donat et al., 1994). Samples for total dissolved Cu and Cd were acidified to pH 2 with ultraclean HCl. Water samples for speciation analyses were contained in Teflon bottles and kept cold and dark.

Total dissolved Cu and Cd were determined by differential pulse anodic stripping voltammetry (DPASV) using a thin mercury film, rotating glassy carbon disk electrode

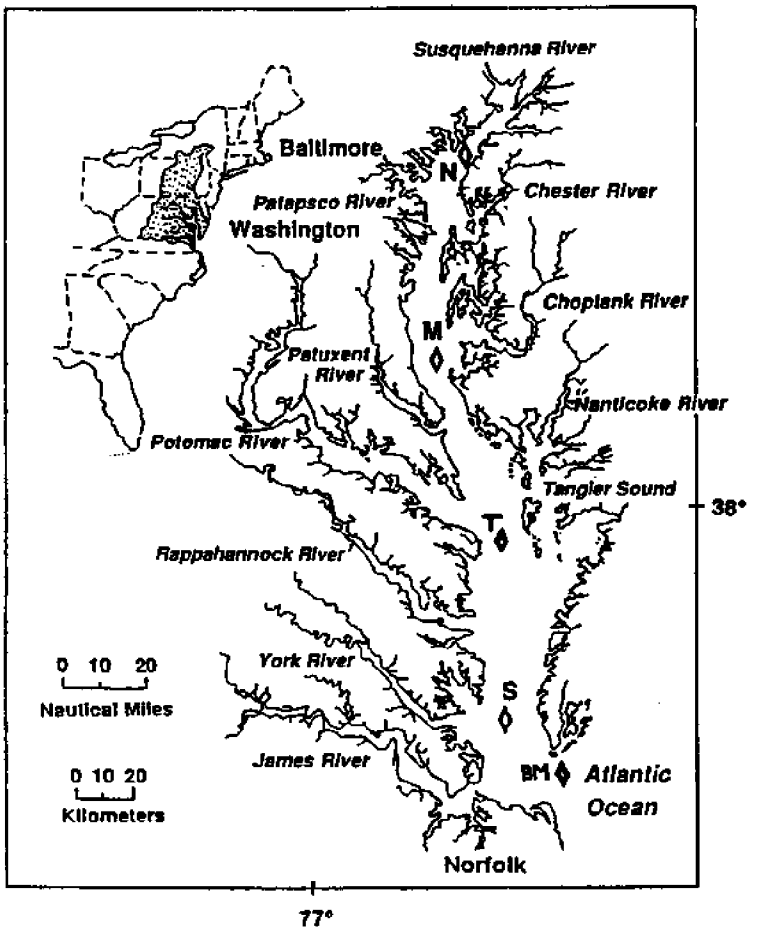

FIGURE 1. Chesapeake Bay Station Locations.

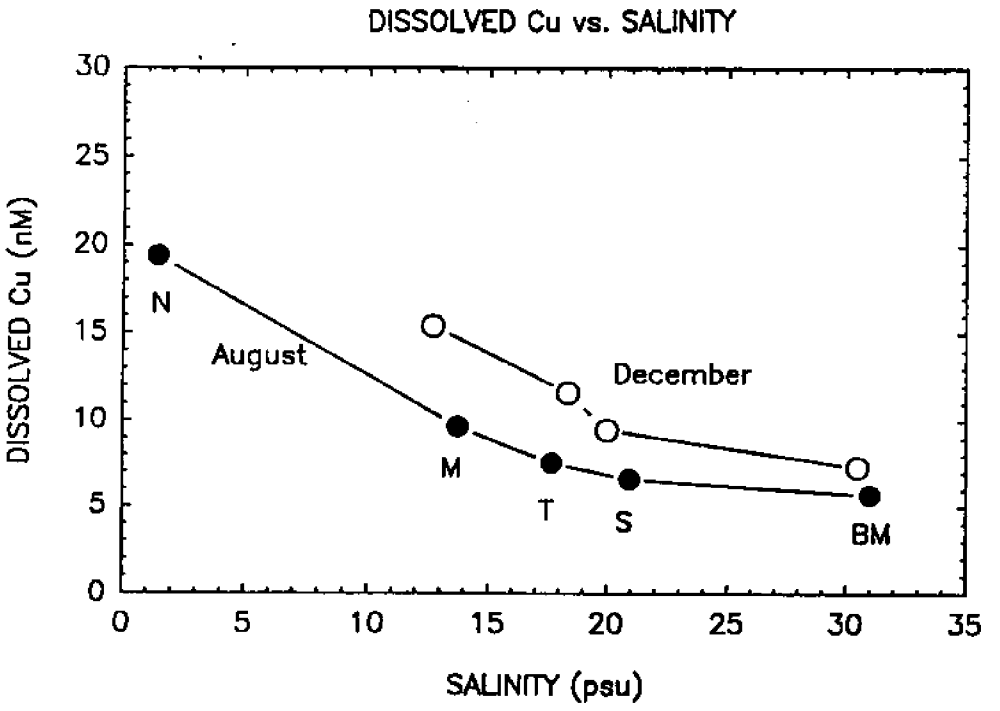

FIGURE 2. Dissolved Copper Concentrations vs. Salinity in August and December 1992.

(TMF,RGCDE), using standard additions, after UV photo-oxidation (Bruland et al., 1985). Total dissolved Cu was also determined on some samples using chemiluminescence detection (Sunda and Huntsman, 1991).

Cu complexation and speciation were determined by DPASV using a TMF,RGCDE (Coale and Bruland, 1988; Donat et al., 1994), and by competitive ligand equilibration/differential pulse cathodic stripping voltammetry (CLE/DPCSV) at a hanging mercury drop electrode, using 8-hydroxyquinoline as the competing ligand (van den Berg et al., 1990; Donat and van den Berg, 1992; Donat et al., 1994).

Cd complexation and speciation were determined by DPASV using a TMF,RGCDE (Bruland, 1992).

RESULTS AND DISCUSSION

Copper - Horizontal and Temporal Trends

Dissolved copper concentrations ranged from 6 to 20 nM and decreased with increasing salinity (down the Bay). Dissolved copper appears to be removed at intermediate salinities. Dissolved Cu concentrations were - 50% higher in December than in August (Figure 2).

Copper occurred predominantly (>98.5%) as organic complexes with three classes of organic ligands, referred to here as L_1, L_2 and L_3 (Figure 3). L_1 is the strongest of these ligands and L_3 is the weakest.

The conditional stability constants of the copper complexes with these ligands are:

Complex	$\log K'_{cond(Cu2+)}$
CuL_1	14.64 ± 0.64
CuL_2	11.10 ± 0.51
CuL_3	8.95 ± 0.73

In August, CuL_1 was the predominant copper complex at Stns. N, T, and BM, with CuL_2 complexes comprising the remainder. CuL_2 complexes were the dominant form at Stn. S, and the speciation was 50%/50% CuL_1 and CuL_2 at Stn. M (Figure 3, top).

In December, CuL_2 was predominant at all stations, with CuL_1 complexes comprising the remainder at these stations (Figure 3, bottom).

While the combined concentrations of the three copper ligand classes were high in the mid Bay (Figure 4), concentrations of L_1, the strongest ligand, decreased with increasing salinity in both August and December, showing evidence of removal in the Bay in August (Figure 5).

L_1 concentrations were generally 2 to 4 times higher than L, concentrations at Stns. M, T, and S, but only slightly higher or equal to the L_1 concentrations at Stns. N and BM (Figure 6).

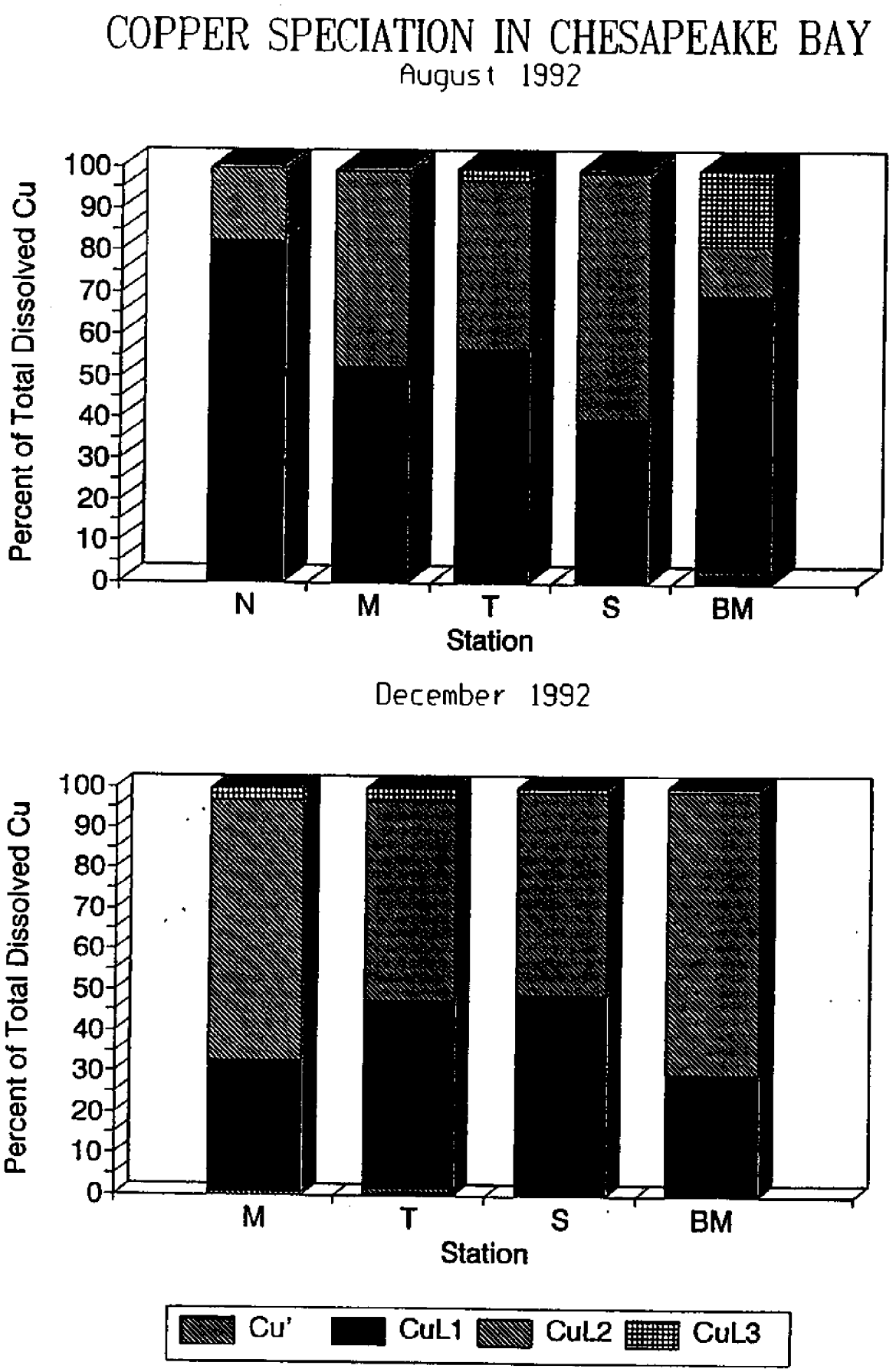

FIGURE 3. Copper Speciation in the Chesapeake Bay:
Top: August 1992
Bottom: December 1992

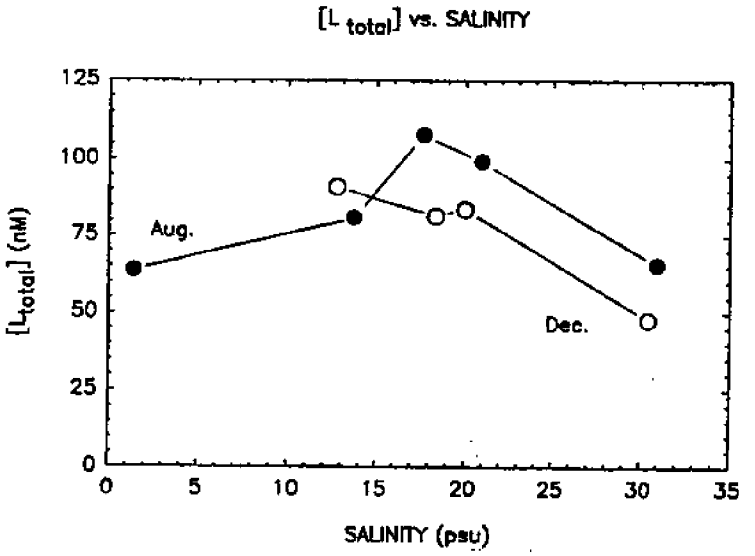

FIGURE 4. Total Copper-Complexing Ligand Concentrations vs. Salinity in August and December 1992.

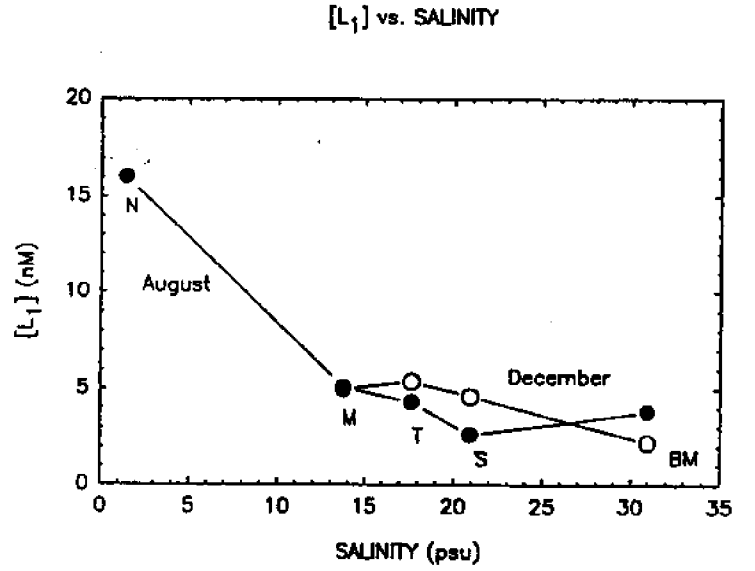

FIGURE 5. L_1 Concentrations vs. Salinity in August and December 1992.

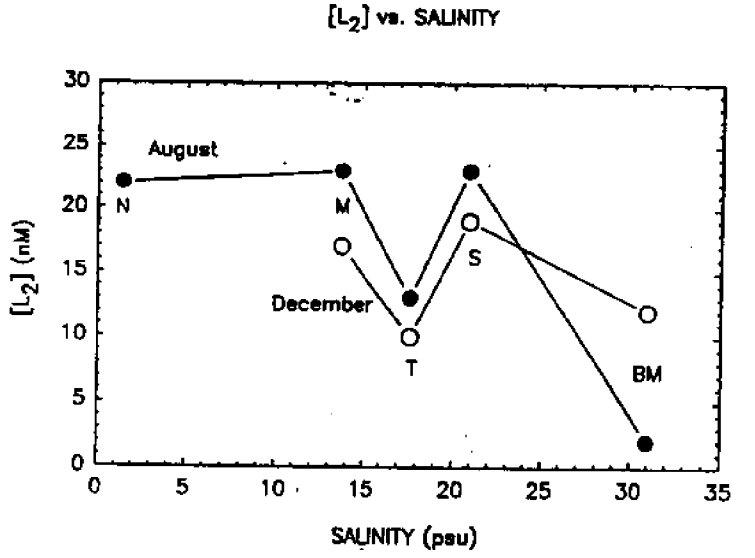

FIGURE 6. L_2Concentrations vs. Salinity in August and December 1992.

L_1 concentrations generally increased with the dissolved Cu concentration (Figure 7). L_1 appears to keep the concentration of free Cu^{2+} buffered at 1-2 pM at most stations However, dissolved Cu concentrations exceeded L_1 concentrations at Stns. M and T in December, causing the free Cu^{2+}, concentration to increase by 15-20 fold (Figure 8).

Copper - Vertical Trends

While dissolved Cu concentrations were relatively constant or only slightly decreasing with depth at Stns. N and S in April, L_1 concentrations increased 3-fold, and L_2 concentrations increased 2-fold (Figures 9 and 10).

Dissolved Cu concentrations exceeded L_1 at the surface at both stations, but L_1, exceeded dissolved Cu near the bottom (Figures 9 and 10). The relative concentrations of L_1 and dissolved Cu appear to cause relatively higher Cu^{2+} concentrations (2.5-5 pM) at the surface, and dramatically decreased concentrations near the bottom (Figures 11 and 12).

Cadmium - Horizontal and Temporal Trends

Dissolved Cd concentrations were generally lower in the mid Bay than in the north and south Bay, and the concentrations were 2 to 5 times higher at Stns. N, M, T, and S in December than in August. Dissolved Cd concentrations at Stn. BM were the same in both August and December (Figure 13).

Dissolved Cd was predominantly organically-complexed (60-70%) by one ligand class ($\log K'_{cond(Cd2+)} = 10.93 \pm 0.60$) only at Stns. N, M, and S in August, and at Stns. T and BM in December. Inorganic Cd (Cd') was significant at all stations in both months (Figure 14).

The Cd-complexing ligand concentration generally decreased with increasing salinity (down the Bay), but showed local increases at Stns. T or S in August, and at Stn. T in December (Figure 15).

The concentration of free Cd" also decreased with increasing salinity, and was slightly lower in August than in December
(Figure 16).

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Sunda, W.G. and S.A. Huntsman, 1991. Mar. Chem., 36: 137-163.
van den Berg, C.M.G., M. Nimmo, P.Daly, and D.R. Turner, 1990. Anal. Chim. Acta, 232: 149.

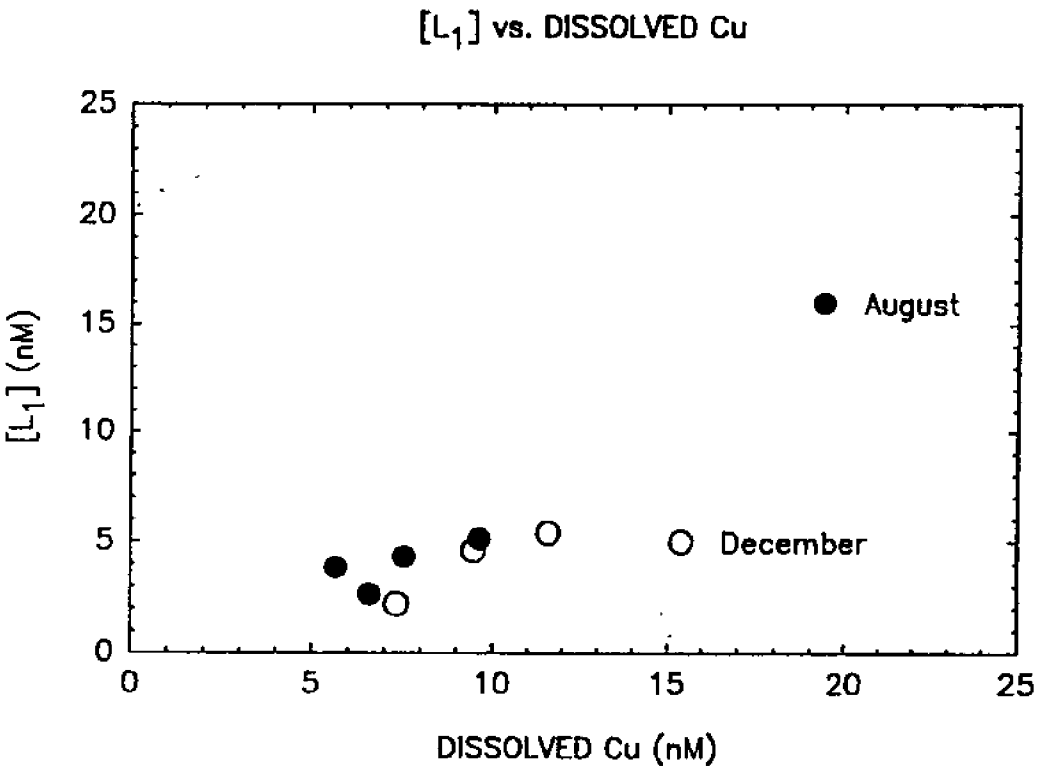

FIGURE 7. L_1 Concentrations vs. Dissolved Cu in August and December 1992.

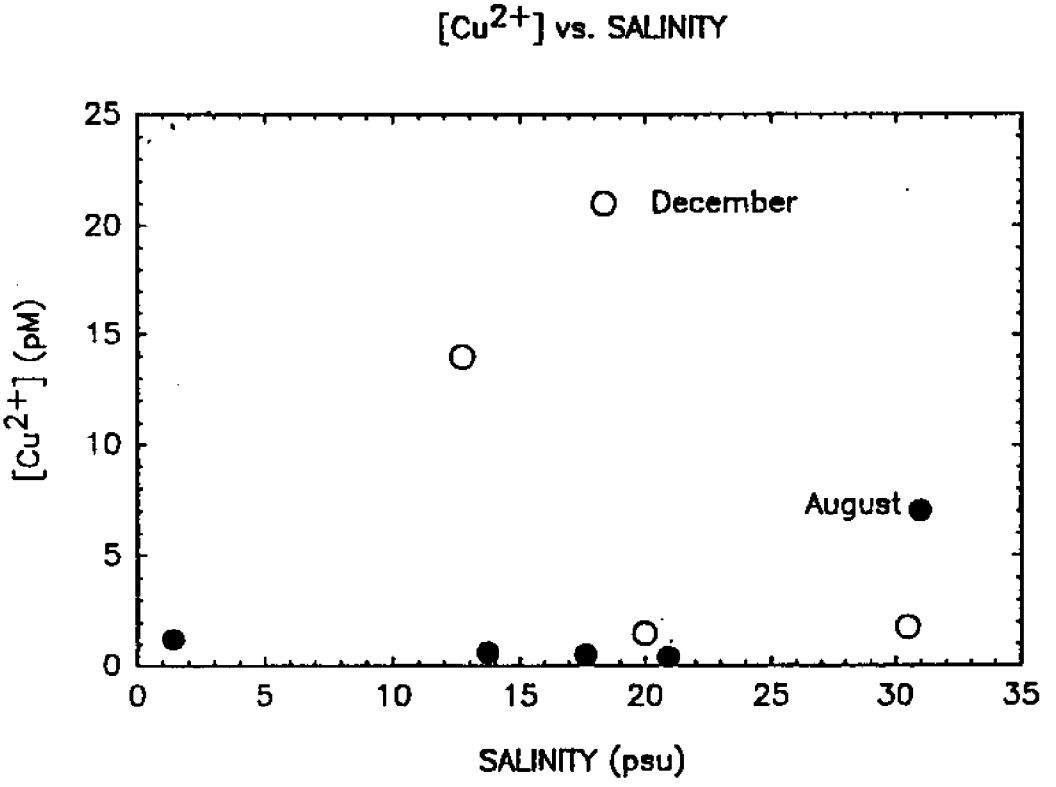

FIGURE 8. Cu^{2+} Concentrations vs. Salinity in August and December 1992.

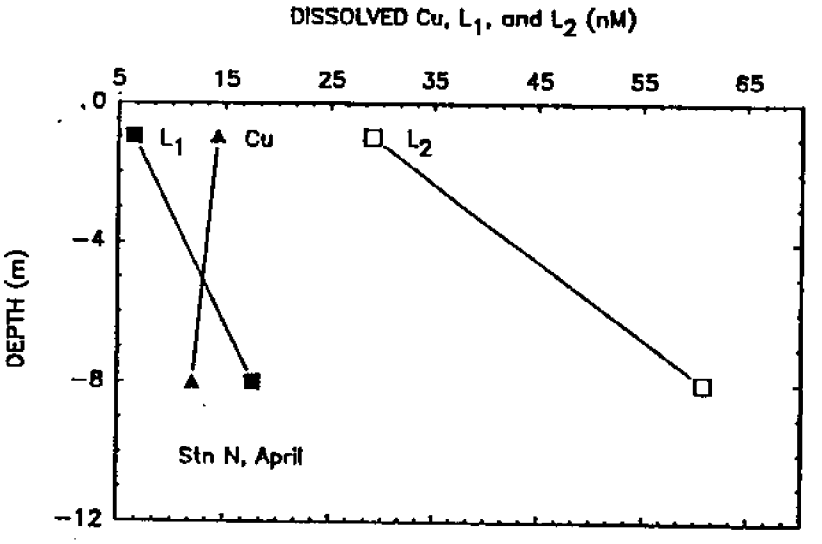

FIGURE 9. Concentrations of Dissolved Cu, L_1 and L_2 in Surface and Bottom Waters at Station N in April 1993.

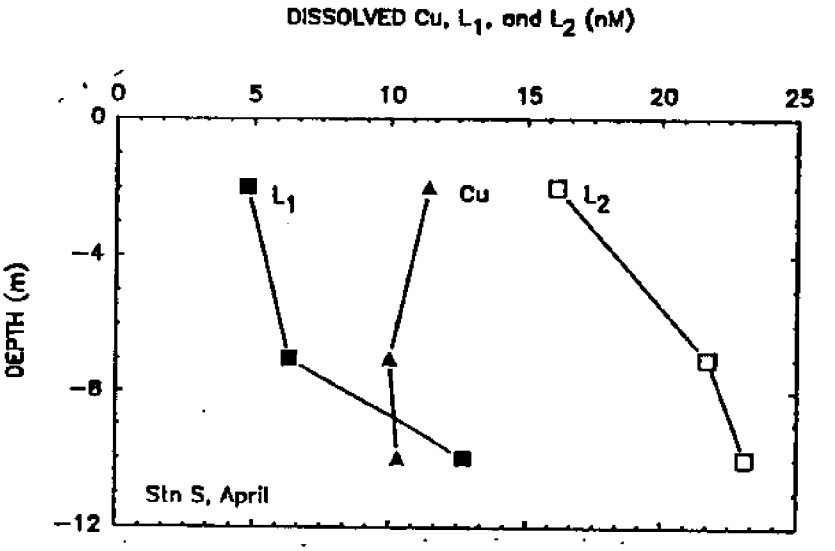

FIGURE 10. Concentrations of Dissolved Cu, LI, and L2 in Surface, Mid-depth, and Bottom Waters at Station S in April 1993.

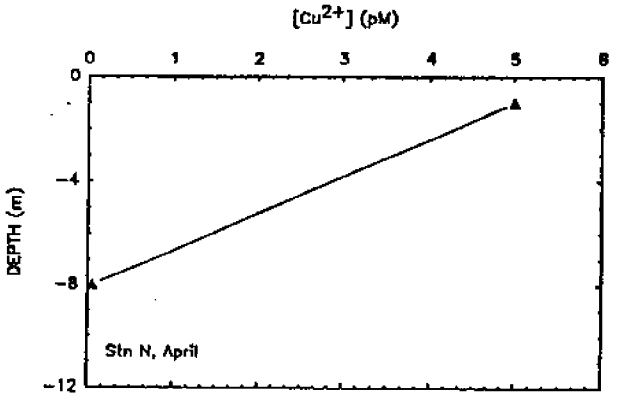

FIGURE 11. Concentrations Of CU2, in Surface and Bottom Waters at Station N in April 1993.

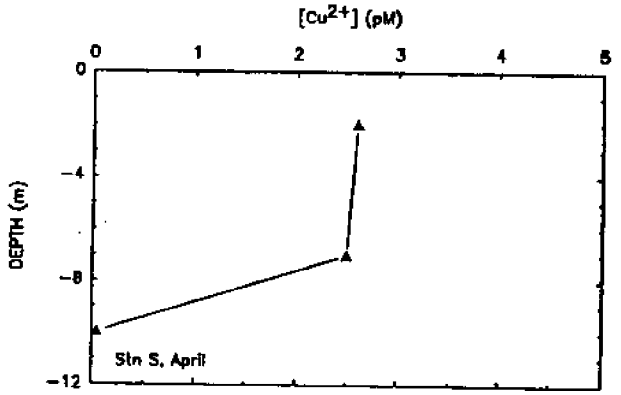

FIGURE 12. Concentrations of Cu^{2+}, in Surface, Mid-depth, and Bottom Waters at Station S in April 1993.

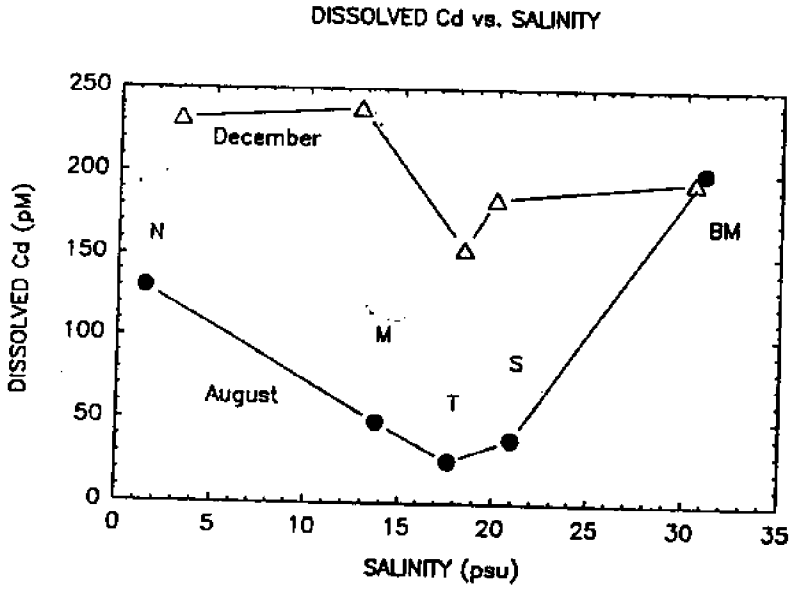

FIGURE 13. Dissolved Cadmium Concentrations vs. Salinity in August and December 1992.

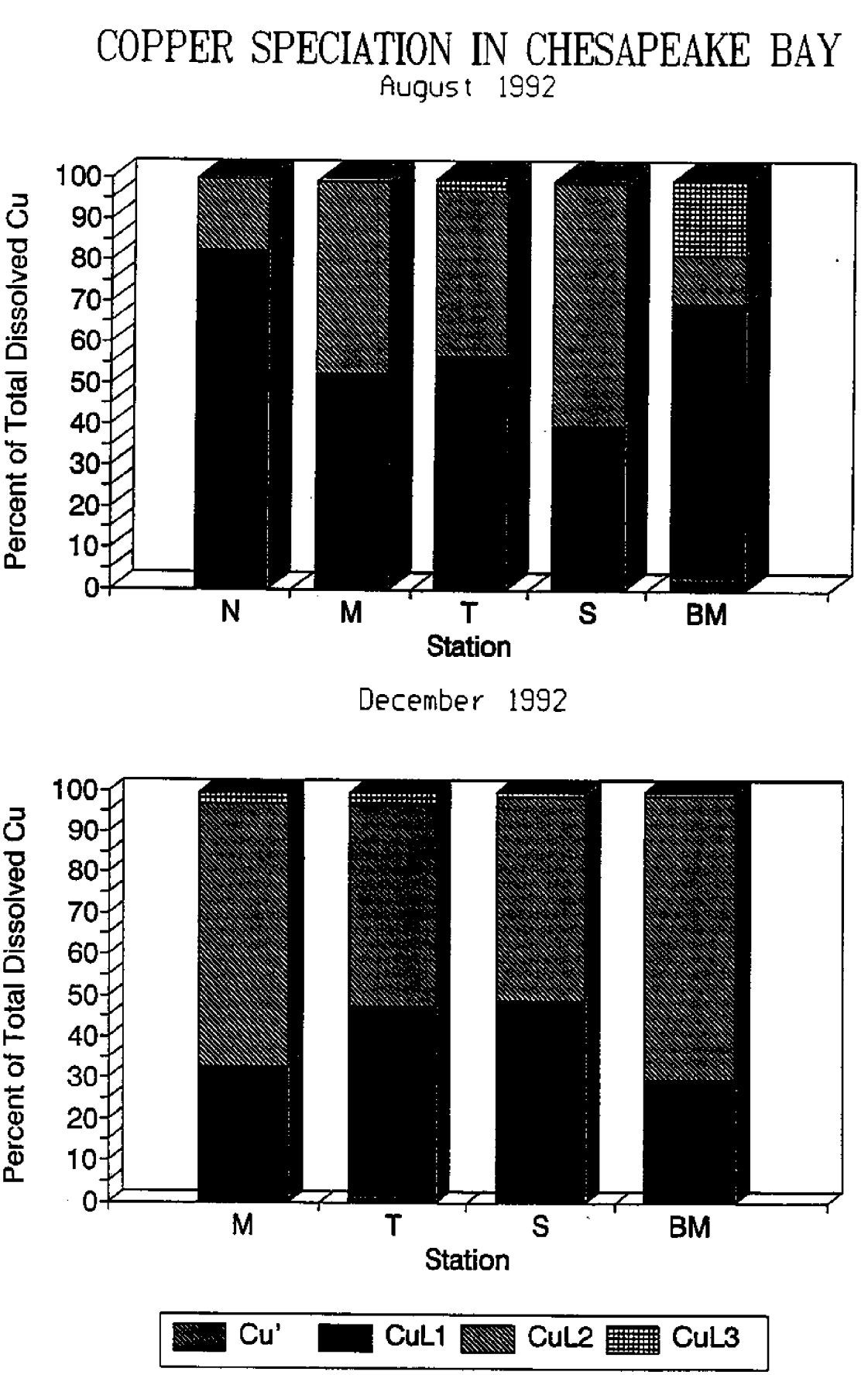

FIGURE 14. Cadmium Speciation in the Chesapeake Bay:
TOP: August 1992
BOTTOM: December 1992

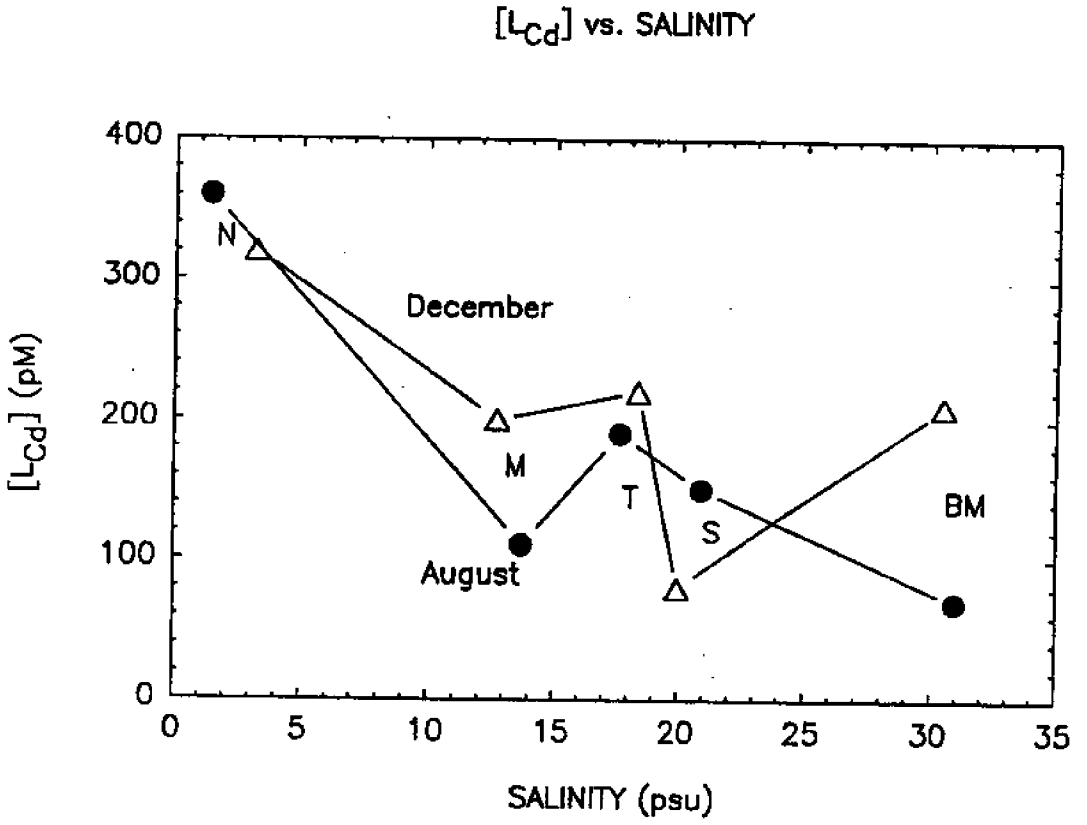

FIGURE 15. Cadmium-Complexing Ligand Concentrations vs. Salinity in August and December 1992.

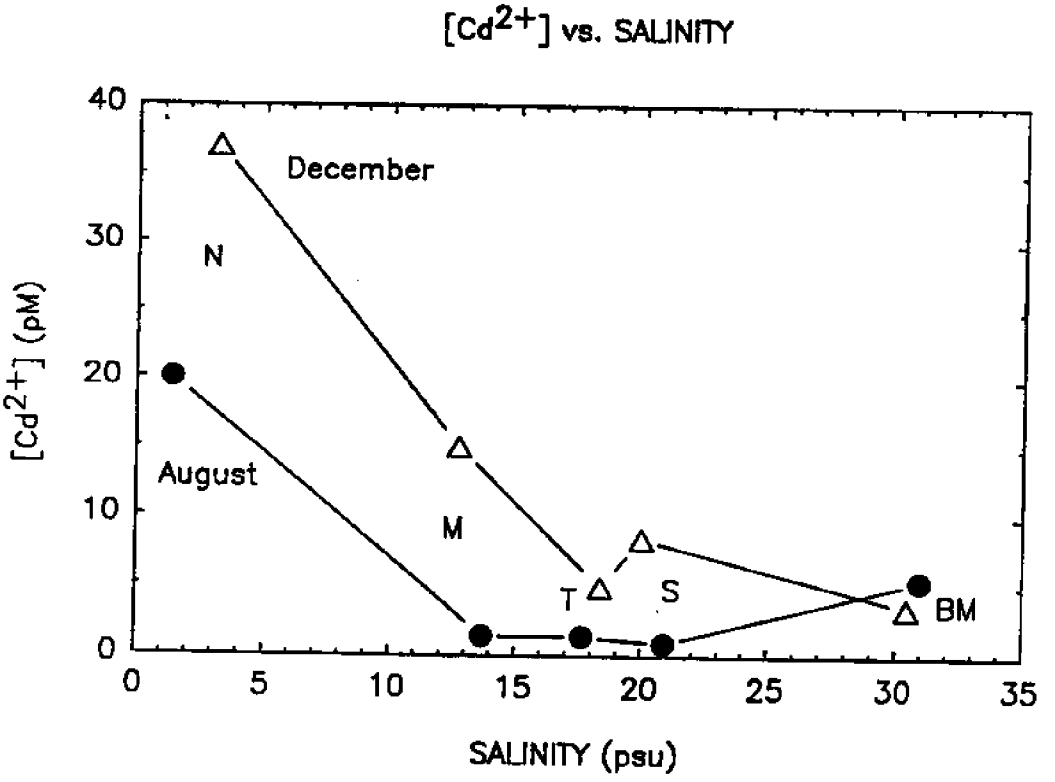

FIGURE 16. Cd^{2+} Concentrations vs. Salinity in August and December 1992.

A Risk Assessment for Diflubenzuron in the Chesapeake Bay [R/CBT-24]

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R. Dawson
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Objectives and Rationale

The CBEEC Toxics Research Program has identified the need for information on the fate and effects of toxic chemical runoff in the Chesapeake Bay catchment area *at environmentally realistic levels*, and the effect which trophic transfer is likely to have on bioavailability (and toxicity). The problem essentially has the elements of a risk assessment in that chemical application is related to exposure concentration which is, in turn, compared with toxicity data. Pesticides are excellent candidates for risk assessment particularly where exposure and toxicity data overlap.

In order to construct a risk assessment, two components are necessary: exposure assessment and hazard assessment. In constructing a model for a non-point-source risk assessment it is important to choose a compound and test organism(s) for which there is a high probability of achieving good quantitative estimates of both of these parameters. There are several reasons why Dimilin and the test species proposed here form an excellent basis for such a model.

Exposure Assessment. Relies for success on accurate data for both environmental chemical loading and distribution of test organisms. In the case of Dimilin, usage in Maryland is carefully monitored and controlled by the Agency responsible for its application (Md. Department of Agriculture). Detailed spatial and temporal records are kept and recorded centrally.

With respect to species distribution, unusually detailed records are available from several sources for both *E. affinis* and *L. plumulosus*.

Hazard Assessment. Data which have been gathered in this laboratory are virtually unique in the Chesapeake system in demonstrating toxicity of Dimilin to a widespread indigenous species (*E. affinis*) at very low levels which have been documented in the ambient aquatic environment. The characteristic cuticular abnormality seen in Dimilin-exposed *E. affinis* (Savitz et al 1994) is likely to prove particularly useful in identifying specific Dimilin toxicity and provide a means of differentiating it from other potentially toxic agents in the water. Spatial and temporal considerations of test-site selection should also minimize complicating

effects of other toxic agents. Information from laboratory experiments will further refine the hazard assessment.

We therefore anticipate a high probability of obtaining precise data for both components of the Risk Assessment. The high, and characteristic, toxicity of Dimilin in this regard, gives the study a good probability of achieving characteristics of a "worst case" model and one which may aid in similarly modelling other non-point source toxics.

The principal components of the model are shown in figure 1. The following considerations apply:

- Pesticide application data for DFB are known with a large degree of accuracy. Such information may be scaled-up to cover the whole state or scaled-down to part of the catchment area.

- Wash-off (from leaves) is climate related and information on this can be obtained from meteorological data, controlled wash-off experiments in the laboratory and field verification.

- Run-off is a function of rainfall and distance from the spray site to the nearest body of water. Part of this may be determined from topographical information although "ground-truthing" is required.

- Chemical half-life is important as particulate partitioning may affect both degradation rate and bioavailability.

- Partitioning between soil particles and interstitial soil water and between sediment particles and river/estuarine water will determine degree of impact on non-target organisms. Both sediment and aquatic tests are being performed to quantify toxicity.

- Degree of impact must also take into account the normal spatial distribution of non-target populations. These may be mapped from known populations, habitat and water quality data.

RESULTS

12.06g DFB/acre were sprayed on the two tests sites totalling 1.88 kg for site #57 and 1.375 kg for site #77. Water and sediment samples collected from test and control sites contained DFB below limits of detection. The limit of detection of DFB analysis was 0.5ng/10μl of extract from solid phase extraction. Field leaf samples contained 7mg DFB/kg dry weight. We found no difference in survival or reproduction between control and test sites.

In lab experiments, the calculated 96hr and 10d LC50 values for aqueous DFB were 2.1 and 1.6 μg DFB/L for and *H. azteca* and 2.0 and 1.0 μg DFB/L for *L. plumulosus*. The 10d LC50 values for sediment bound DFB were 583 and 501 μg DFB/kg dry sediment for

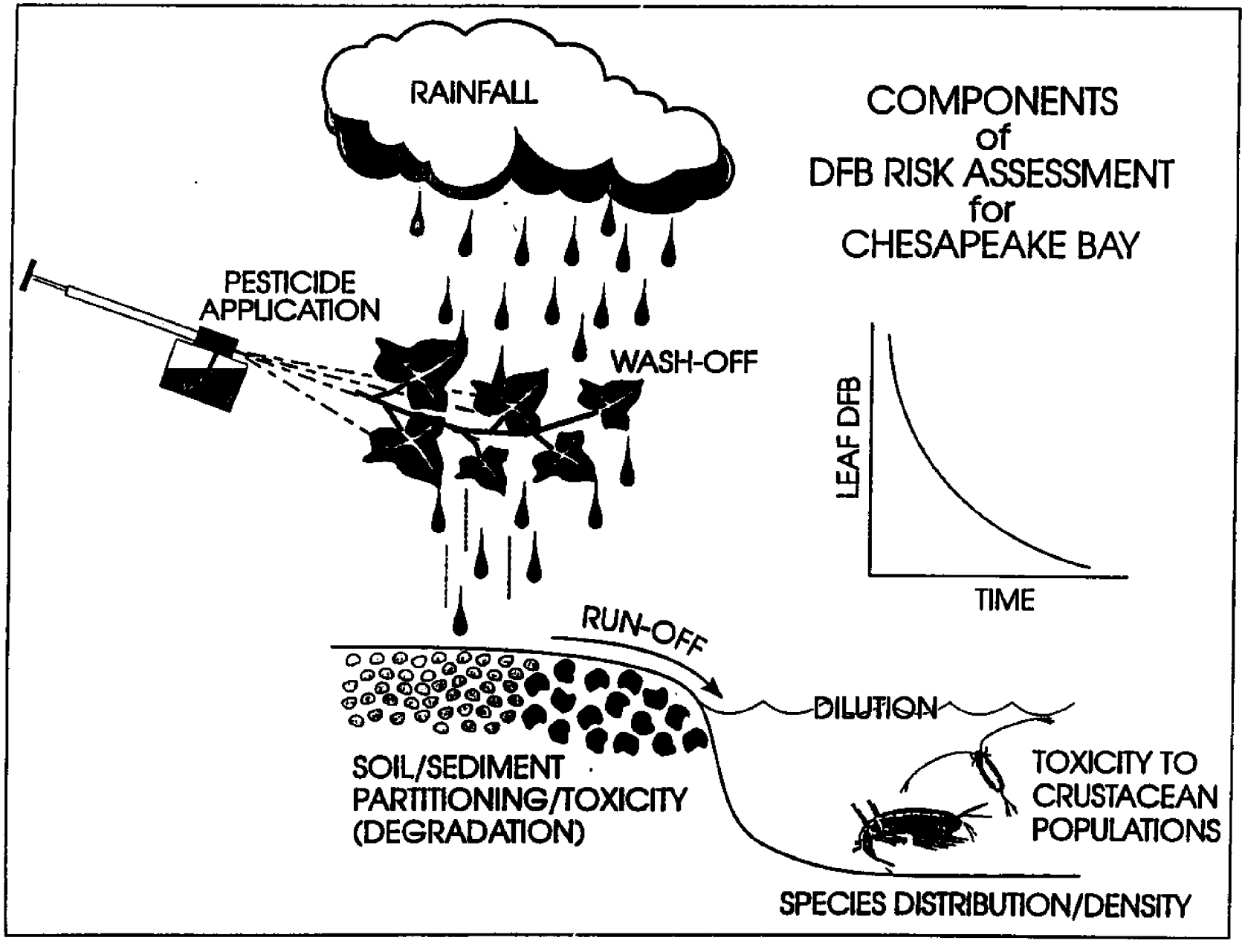

Figure 1 Components of DFB Risk Assessment for Chesapeake Bay.

H. azteca and *L. plumulosus* respectively. Significant reduction in both survival and reproduction in *E. affinis* was found at 0.84 μg/L in salinities ranging from 2ppt to 15 ppt (Figures 2 & 3).

Although the leaves in the wash off experiment had lower levels of DFB than found in the field (0.664 mg/kg dry weight), only 41%, 25%, and 16% of the DFB was left on the leaves after the first, second, and third simulated rainfalls.

The lower limit of detection for Dimilin is 0.5ng, which translates to an environmental concentration of 5ppb for a 100μl injection. This technique easily allows direct analysis at concentrations of 0.5ppb.

DISCUSSION

Aqueous DFB elicits significant effects on survival and reproduction of *E. affinis* at only 0.84 μg DFB/L but we found no effect of salinity on DFB toxicity to *E. affinis*. Survival of *L. plumulosus* and *H. azteca* was reduced between 0.6 and 1.2 μg DFB/L. After a 96 hr exposure and subsequent transfer to DFB-free water for 6 days, survival of *L. plumulosus* and *H. azteca* was reduced at 1.2 μg DFB/L. Sediment bound DFB appears to be less important to toxicity than aqueous DFB to these two amphipods based on 10 day survival rates.

Although 59% of the DFB initially on the leaves was washed off after the first rain event, the leaves were allowed to dry for only 45 minutes after application with DFB. According the MDA guidelines, DFB must be allowed to dry on a spray site for a minimum of three hours or the site must be resprayed for insufficient DFB will be retained on the leaves to be toxic to Gypsy moth larvae. Because the leaves in our study were dried for only 45 minutes, these data cannot represent field conditions. However, the fact that only 16% of the initial DFB was present after three rain events is significant. This represents a major pathway of introduction of DFB into surrounding water bodies.

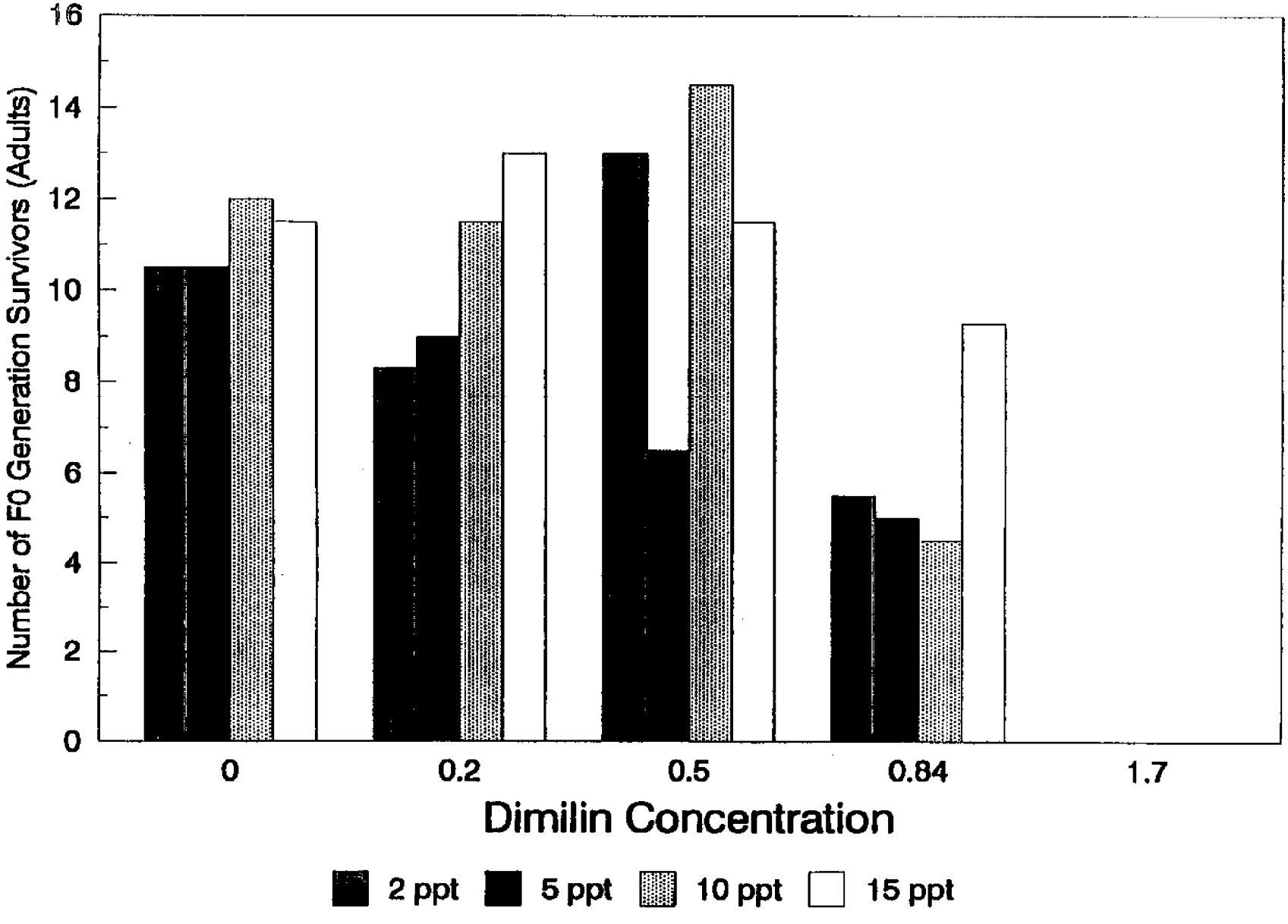
Number of F0 Generation Survivors (Adults)
16
14
12
10
8
6
4
2
0
0
0.2
0.5
0.84
1.7
Dimilin Concentration
2 ppt
5 ppt
10 ppt
15 ppt

Figure 2.

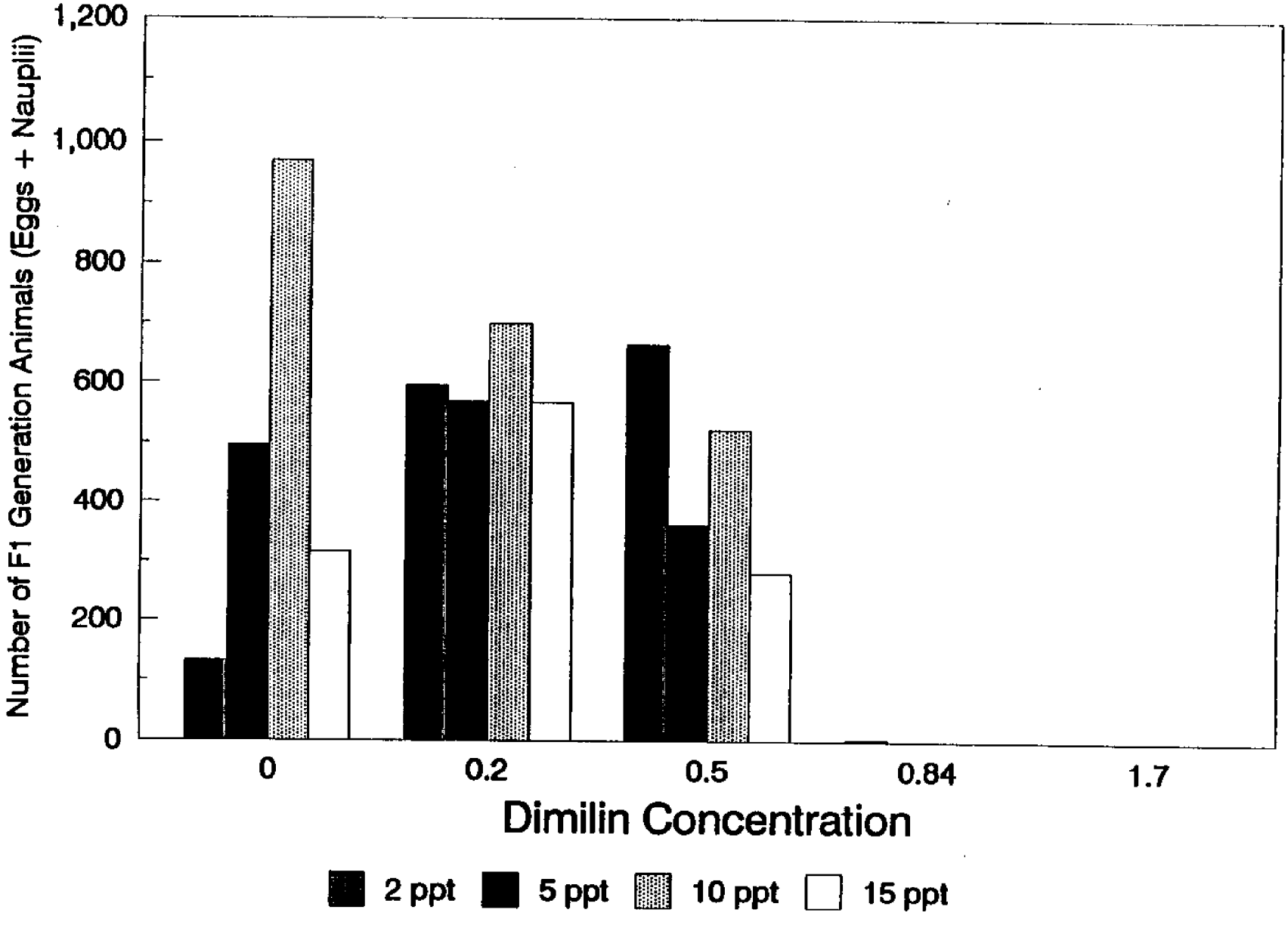
Number of F1 Generation Animals (Eggs + Naupli)
1,200
1,000
800
600
400
200
0
0
0.2
0.5
0.84
1.7
Dimilin Concentration
2 ppt
5 ppt
10 ppt
15 ppt

Figure 3.

ROLE OF PLANKTON IN CONTROLLING THE PARTITIONING AND TRANSPORT OF HYDROPHOBIC ORGANIC CONTAMINANTS IN CHESAPEAKE BAY [R/CBT-8]

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Rodger Dawson
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OBJECTIVES

In field collections of surficial sediments, sediment trap material, and four size fractions of suspended particles, the concentrations and distributions of hydrophobic organic contaminants (HOCs) have been quantified. Specifically, the objectives of the field component of this study were to document the spatial and temporal variability in HOC partition coefficients to suspended detritus, phytoplankton, and zooplankton, and to relate this variability to physical (i.e., resuspension, density stratification), chemical (i.e., organic geochemical composition of particles, hydrophobicity of HOCS), and biological (i.e., bloom versus non-bloom plankton, zooplankton *versus* algae) factors. Parallel laboratory studies have examined the speciation and binding of contaminants to phytoplankton and the role of specific organic pools (e.g., lipids) in the speciation of HOCs in estuarine waters.

RATIONALE

Field Study.

Anthropogenic chemicals enter estuaries from a variety of point and diffuse sources. In order to predict the impact of these chemicals and to design remediation strategies, the fundamental ecosystem processes which determine contaminant reactivities, bioavailabilities, and ecosystem residence times must be understood. The estuarine geochemistry of many chemical contaminants, including persistent, bioaccumulative organics such as polychlorinated biphenyls (PCBs), chlordanes, and polychlorinated dibenzodioxins and furans, are largely determined by their high affinities for natural particles. To a first approximation, these hydrophobic organic contaminants (HOCS) are transported along with estuarine solids, and their inventories and residence times are controlled by particle transport processes. The extent of HOC-particle associations is dictated by basic properties of the chemical (i.e., aqueous solubility) and of the natural solid (i.e., organic content, size, lability). In addition to influencing their geochemical behaviors, uptake of HOCs by phytoplankton introduces these chemicals into the estuarine food web. Until recently, HOC uptake by phytoplankton was thought to be a rapid, reversible partitioning process. Recent

data, however, indicate that HOC-algal associations are very complex, depend upon the chemical composition of the aquatic solids, and can only likely be accurately predicted using kinetic models.

Existing predictive models of HOC associations with natural solids are mainly derived from laboratory studies of contaminant sorption to soils and sediments (e.g., Karickhoff et al. 1979; Schwarzenbach and Westall, 1981). In those studies, highly concentrated suspensions of soils or sediments (e.g., g/L) were mixed with relatively elevated concentrations of HOCs until an apparent equilibrium was achieved. These studies determined that sorption equilibrium was apparently achieved within hours to days, and that the extent of HOC association depended inversely upon HOC solubility and directly upon the solid's bulk organic carbon content. Based upon those studies, HOC-solids associations have been described as a reversible equilibrium between HOCs dissolved in solution and partitioned into the solid organic matrix. At equilibrium, the sorbed HOC concentration (C_s, μg/Kg) is proportional to the dissolved HOC concentration (C_d, μg/L), with the ratio of these concentrations defined as a partition coefficient ($K_d = C_s/C_d$). Because the extent of partitioning depends upon the solid's organic matter content, Karickhoff *et al.* (1981) introduced an organic carbon-normalized distribution coefficient ($K_{oc} = K_d/fOC$; foc = solid fraction organic carbon). Numerous semiempirical relationships between K_{oc} and HOC properties have been published (Elzerman and Coates, 1987 and references within).

Although these HOC partitioning models are widely used to estimate HOC associations with suspended solids, including plankton, recent studies demonstrate that these semiempirical equations fail to predict HOC distributions measured under realistic field conditions. Baker *et al.* (1991) compiled distribution coefficients of polychlorinated biphenyl (PCB) congeners measured under a variety of field conditions, and concluded that existing equilibrium partitioning models are unsuitable for estimating the extent of HOC association with aquatic solids. Regression analyses showed that the PCB congener partition coefficients were poorly correlated with the octanol-water partition coefficient (K_{ow}), with r^2 ranging from 0.0 to 0.6 and slopes from 0.0 to 0.36. Two possible reasons why equilibrium partitioning models cannot explain observed HOC distributions are: the presence of HOC-binding colloidal material results in an overestimation of dissolved HOC concentrations; and slow HOC uptake and release rates relative to changing particle composition results in continual disequilibrium. Several investigators have argued that field measurements include colloidally-bound HOC in the dissolved fraction, thereby overestimating C_d and underestimating, K_s (Gschwend and Wu; 1984; Baker *et al.* 1985). Because the magnitude of this artifact depends upon the contaminant hydrophobicity, the "three-phase" argument explains the observed poor relationship between HOC K_d and K_{ow} values. Several investigators have attempted to eliminate the colloidal artifact by measuring dissolved HOC via gas purging (Yin and Hassett, 1985; Servos and Muir, 1989). We are currently employing a gas equilibration system to control the dissolved HOC concentrations in our laboratory plankton exposure experiments.

Karickhoff (1980) noted that although the sorption of HOCs to sediments appears rapid, desorption rates are much slower, and argued that HOC uptake occurs via a two step

process, with rapid adsorption to the surface followed by slower transport into the particle. Despite Karickhoff's kinetic description, the uptake of HOCs by plankton continues to be modeled as an equilibrium partitioning process. Recent studies of Skoglund and Swackhamer (1991) suggest that HOC partitioning rates into algae are slow relative to the rates at which the algal carbon pool changes in magnitude and chemical composition. Under slow growth conditions, PCB congener partition coefficients eventually reach those predicted by equilibrium partitioning models, with the more hydrophobic congeners requiring longer to reach equilibrium. Skoglund and Swackhamer interpret these data as evidence that algae accumulate HOCs via a two step process similar to that proposed by Karickhoff (1980).

Finally, much of the variability in field-measured HOC distributions may result from uptake by a complex mixture of particles suspended in the water column. The seasonal production of plankton carbon and episodic resuspension and erosional events drive large spatial and temporal fluctuations in both the concentration and chemical composition of estuarine particles. Given the apparently slow uptake and release rates of HOCs from aquatic solids, it seems likely that particle transport dynamics continually disrupt the partitioning equilibrium, and that a kinetic description of HOC uptake and release is required.

Laboratory Study

Adequately tracking hydrophobic organic contaminants through aquatic food webs requires a thorough description of the contribution of contaminants from lower (phytoplankton and zooplankton) to higher trophic levels by feeding and through dissolved phase exposure. Consequently, several investigators have proposed trophic transfer models that attempt to predict what the levels of contaminants will be at each level of a given food web (e.g., Thomann 1981, 1989; Connolly and Tonelli, 1985; Connolly and Pendersen, 1988). Quantitative descriptions of contaminant accumulation in estuarine food webs include those of polychlorinated biphenyls in the Hudson River (Thomann *et al.* 1991) and kepone in the James River of the lower Chesapeake Bay (Connolly and Tonelli 1985). These models have successfully described the annual averages and long-term changes in contaminant inventories in the upper trophic levels. However, these models were essentially developed using a "top down" approach, beginning with measured amounts of contaminants in upper trophic levels and then estimating the transfers through phytoplankton and zooplankton needed to support those levels. Therefore, organic contaminant levels in phytoplankton and zooplankton are basically used as fitting parameters in order to provide an observed fish concentration.

In general, trophic transfer models take the form (Thomann, 1989; Thomann *et al.*, 1992; Connolly and Tonelli, 1985):

$$dB_i/dt = k' W_i C_w + A_{i-1} W_i (B_{i-1}/W_{i-1}) - E_i B_i$$

where dB_i/dt (mg /organism-time) is the time dependent change in contaminant body burden, k' is the lumped water to organism mass transfer coefficient (L/day-kg wet weight), W_i is the wet weight of an organism (kg) at the trophic level, C_w is the water concentration (mg/L), A is the assimilation efficiency of the i-1th tropic level (mg contaminant adsorbed/ mg ingested), B_{i-1} is the body burden of the i-1th trophic level, W_{i-1} is the wet weight of the i-1th

trophic level and E_i is the contaminant excretion rate (day^{-1}). This equation states that there are two routes of exposure for organisms, one from water and one from food. Further modifications of this model also include the effects of growth, which is a dilution term. Organisms lower down the food chain have a greater proportion of contaminant entering via the dissolved phase because of larger surface to volume ratios and a lesser concentration of contaminant in the next lower trophic level. The contribution reaches a maximum with phytoplankton, which have no contribution from food. Therefore, it is important to understand the transport and or modification of pollutants at their primary entry point in the food chain, phytoplankton and zooplankton.

The model results of Thomann (1989) and field results of Oliver and Niimi (1988) specifically implicate lower trophic levels, especially phytoplankton, as crucial mediators of the eventual fate of hydrophobic organic contaminants (HOC) in fish. For instance Thomann (1989) states; "...the model results are particularly sensitive to assumptions made in chemical efficiency, phytoplankton BCF (bioconcentration factor), and top predator growth rates..." Therefore, there exists a clear need for further information regarding the disposition of HOCs in plankton, especially in terms of amounts and rates of transfer.

Unfortunately, field phytoplankton and zooplankton HOC data have not been forthcoming in the literature and those that are available (e.g., Oliver and Niimi, 1988; Hargrave *et al.*, 1992; Knickmeyer and Steinhart, 1989; Swackhamer and Skoglund, 1993) show levels to be extremely variable both temporally and spatially. In addition, our own data from Chesapeake Bay (Ko *et al.*, 1993), Lake Baikal (Kucklick *et al.*, 1993) and the Great Lakes (Baker *et al.*, 1991) also demonstrate this variability, strongly suggesting that contaminant levels in zooplankton and especially phytoplankton are influenced by growth rates and lipid stores and therefore may be kinetically limited. The results of two cruises in the mesohaline Chesapeake Bay where PCBs were measured in the 10 to 64 μm and >202 μm size classes (representing phytoplankton and zooplankton, respectively) demonstrate the high variability between surface and bottom zooplankton and especially phytoplankton PCB concentrations during the fall of 1991. We suspect that these differences are partially caused by nonequilibrium with dissolved phase PCBs because of differences in growth rates. Indeed, Swackhamer and Skoglund (1993) point out the importance of phytoplankton growth in their work as the major factor inhibiting thermodynamic equilibrium between dissolved PCBs and algal cells. Therefore, any attempt at measuring uptake of HOCs in these organisms must take a kinetic (time dependent) approach.

Several other investigators have examined partitioning of HOCs to plankton in a laboratory setting (Geyer *et al.*, 1981; Wang *et al.*, 1982; Lederman and Rhee, 1982; Malhot, 1987; Autenreith and DePinto, 1991; Swackhamer and Skoglund, 1993). In general these studies attempted to measure the algal BCF by adding a suite of compounds and monitoring, usually as a function of time, the resulting algal concentration. While these studies have provided meaningful data on the magnitude of algal uptake and BCFS, they do not provide the type of area-based mass transfer coefficient needed for a synoptic food chain accumulation model. The reason for this is that the dissolved-phase concentration is not known during the course of the experiment either because of immediate uptake by the algae or association with colloids (Baker *et al.*, 1986; Chiou *et al.*, 1987; Landrum *et al.*, 1987; Pankow and

McKenzie, 1991). In addition, few if any studies have included an estimation of cell surface areas, which is intrinsic to a flux calculation, i.e.,

$$dC/dt = D\ A\ DC$$

where dC/dt is the flux of contaminant into the algal cell from water, D is the mass transfer coefficient, A is the cell surface area and DC is the concentration gradient from water to algae. Therefore adequate description of the flux from water to plankton requires a knowledge of both the truly dissolved (unassociated) contaminant concentration and the organism's surface area.

RESULTS

Field Study

Detailed discussion of the results of our field study are described in Ko and Baker (1993), Johnston (1993), and Johnston and Harvey (1993).

Characterization of particles. In the mesohaline Chesapeake Bay, the concentrations of total suspended particles (TSP) in the surface water during our 5 cruises were 4.1$\pm$1.6 mg/L, which are typical of the eutrophic estuary reflecting the high organic production and resuspension in the shallow water. By comparison, TSP concentrations are 0.5-2.0 mg/L in Lake Michigan (Eadie & Robbins 1987) and <0.5 mg/L in open ocean (Bishop *et al.* 1978). The seasonal variation of TSP concentrations in the surface water are inversely proportional to those in bottom water. In the surface water, TSP concentrations in summer (6.6 $\pm$ 0.4 mg/L; N=4) were higher than those in fall (2.68$\pm$ 0.76 mg/L; N=6). In contrast, bottom water concentrations of TSP in summer (3.0$\pm$ 1.5 mg/L; N=4) were lower than that in fall (17.7$\pm$ 5.6 mg/L; N=8). The increased surface suspended particle concentration in summer is coincident with the peak of maximum primary production. The high amount of solids near the bottom in fall was due to sediment resuspension driven by intensive storms. High variation of the bottom TSP may be caused by tidal current (Sanford 1992).

The source and distribution of particles in the surface and bottom water column were estimated by the fraction of organic carbon (foc) in the particles. The foc of the bottom particles (6.2-28.2 %) were significantly lower ($p < 0.0\ 1$, N = 10) than that of the surface suspended particles (30.8-32.8 %), except in spring. While the suspended particles settle to the bottom water, the fraction of organic carbon (foc) in the particles may be decreased by degradation and diluted by resuspended sediments that have lower carbon content. The temporal and vertical distribution of particle and organic carbon concentrations indicate that the major components of suspended particles in the surface water result from surface organic matter production, while the suspended solids in the bottom water are the mixture of sediment resuspension and the settling particles from the surface.

Size distribution of particles. The method used to separate particles by size in this study may not be fully efficient. Some particles smaller than the filter mesh may be caught in the filter as the filter pores became clogged after pumping. Also, the nonuniform shape of the filtered particles may decrease the size fraction efficiency, depending on their random direction when

impacting the filter pore. However, the main components of filtered samples in each size have been identified by microscopy. In the mesohaline waters of the Chesapeake Bay, the dominant particles were < 10 μm, including nanophytoplankton, detritus, bacteria, and resuspended silt. Between 78 and 85 % of total suspended particles in the surface water of the mid-bay were 10μm in summer and fall. During the spring bloom, dominated by the large diatoms *Cyclotella* sp. and *Thalassiosira* sp. (Sellner & Brownlee 1986), the < 10 μm particles still comprised 50% of TSP. The bottom particles were also predominantly < 10 μm. In spring and summer, the particle size distribution in the bottom water was similar to that in the surface water, reflecting particle settling. In the fall, intensive storms may drive coarser sediment upward and decrease the percentage of the fine particles in the bottom water. However, the 10μm particles still comprised 50% of TSP. Small particles sinking slowly can remain in suspension for long periods of time.

It has been reported that nanoplankton (< 10 μm) are responsible for most of the primary production and TSP concentration in mesohaline waters of the bay (McCarthy *et al.* 1974, Van Valkenburg *et al.* 1978, Malone *et al.* 1992). Transferring to a higher trophic level (e.g., zooplankton grazing) may pack these fine particles and raise their settling velocities, however Brownlee & Jacobs (1987) found that zooplankton graze and remove a very small fraction of the available phytoplankton production in the mesohaline Chesapeake Bay. We believe that phytoplankton remain largely ungrazed and stay suspended in the water column. Assuming a particle density (s) and the kinematic viscosity (v) of 2.6 mg/cm^3 (silts) and 0.01 cm^2/sec, respectively, the settling velocity of 10 a μm spherical particle is 0.3 m/hr estimated by Stoke's settling law. Since the density and shape factor of nanoplankton are smaller than we assumed, their actual settling velocity should be much slower. The particle components of 10-64 μm and 64-202 μm size fractions overlapped and were mainly composed of phytoplankton and detritus. Large particles (>202 /μm) representing zooplankton, colonial phytoplankton, fecal pellets and large detrital aggregates are relatively rare (0.5%-2.8%) among the total suspended particles, but they sink more rapidly and hence are presumed responsible for the transport of surface materials through the water column to the bay sediment.

Speciation and concentration of HOCs in water column. The 70+ PCB congeners measured in this study are the main components of the PCB mixtures Aroclor 1242, 1254, and 1260. The concentrations of total PCB (t-PCB) including particulate and dissolved phases collected from the mid-bay in the surface water (0.74 to 0.95 ng/L) were lower than that in the bottom water (1.23 to 2.85 ng/L). Overall, this contaminant level was lower than the water samples collected in the other areas. The t-PCB concentrations in the dissolved phase were relatively constant temporally and vertically, ranging from 0.54 to 0.67 ng/L (average $=0.60\pm0.05$ ng/L). Even though the dissolved t-PCB concentrations were similar in surface and bottom waters, the congener pattern was different. In the bottom water the lower chlorinated PCBs (higher solubility) have higher concentration in the dissolved phase; however, in the surface water PCB concentrations were dominated by the tetra- and pentachlorobiphenyl congeners. The different patterns between surface and bottom waters implied that the dissolved PCBs were not simply transported by diffusion or advection of the water mass, but were determined by contaminant-particle association as well as particle transport.

The t-PCB concentrations associated with suspended particles varied from 48 to 87 ng/g (average = 65±20 ng/g) in the surface water and from 43 to 146 ng/g (average = 95+52 ng/g) in the bottom water. The high variation of particulate PCB concentration in the water column was irregular temporally and spatially. This variation may be caused by rapid particle exchange processes in the shallow bay water, including high production, rapid degradation, and significant resuspension. Particulate t-PCB concentrations in the surface and bottom waters were higher than those in the boundary layer water (32.2 ng/g), in the sediment floc (16.3 ng/g) or in surface sediment (10 ng/g), implying either that PCBs are released from particles during sinking or that relatively uncontaminated sediments dilute the concentration in bottom waters. The carbon content of particles also decreases with depth in the water column.

The distribution of PCB congeners in the particulate phase was similar to that in the dissolved phase, particularly in the bottom water. Through the water column, the low concentrations of less chlorinated PCB congeners both in particulate and dissolved phases increased in the bottom water. The particulate t-PCB concentrations were elevated in the > 202 μm particles, which were dominated by zooplankton except one bottom sample collected during a storm (Oct. 1990). The high concentration of t-PCB in the largest particles may be due to plankton grazing. During the storm, > 202 μm particles in the bottom water were mostly resuspended coarser sediment particles. Thus, the relatively low surface areas of these large particles may cause the low concentrations of t-PCB. The concentrations of t-PCB in the larger particles (> 10lμm) in the surface water were higher ($p<0.01$, $N=16$) than those in the bottom water except the 10-64 μm size fraction samples collected in spring. Alternately, t-PCB concentrations in < 10 μm particles of the bottom water (97±47 ng/g) were significantly higher ($p<0.01$; $N=6$) than those in the surface water (51±22 ng/g), implying that t-PCB concentrations in large suspended particles (> 10μm) settling from surface water were diluted by resuspended sediment but that the < 10 μm sediment particles were enriched with PCBS. High concentrations of PCBs in the < 10 μm particles in the bottom water may be caused by greater surface areas and carbon contents in the fine particles.

The total PCB concentrations (ng/L) in the bottom waters were higher than those in the surface water due to much larger suspended solids concentrations in the bottom waters. Also, the total particulate PCB concentrations in bottom waters were higher than in the surface water since PCBs were enriched in < 10 μm particles in the bottom water and < 10 μm particles were dominant in the water column. The particulate t-PCB concentrations are only slightly related to the organic carbon fraction in the suspended particles, with a correlation coefficient (r^2) of 0.35. However, higher correlations were found ($r^2= 0.49$) if two extremely high t-PCB concentrations in surface >202 μm particles were excluded. The > 202 μm particles dominated by zooplankton may accumulate PCBs primarily by grazing rather than by physical sorption. The correlation between the particulate t-PCB concentrations and the organic carbon fraction in particles of the bottom water ($r^2= 0.77$) was markedly higher than that in the surface water ($r^2= 0.39$). This improved correlation may be explained by slow sorption kinetics. The major component of the suspended particles in the surface water is "fresh" organic matter which provides ample opportunity for HOC sorption (due to their higher lipid concentrations) but the organic production may be too fast to let the contaminant

reach complete sorption. Therefore, their organic carbon content does not correlate to the t-PCB concentration well. In contrast, the solids near the bottom are "older" particles settling from the surface water or biogenetic particles resuspending from the surface sediment. These particles have been in contact with PCBs longer, resulting in PCB sorption which is closer to equilibrium.

PAHs. The concentrations of individual PAH (in dissolved and particulate phases) among 13 PAHs ranged from 0.10 to 2.87 ng/L and from 0.41 to 4.76 ng/L in surface and bottom respectively. The surface water PAH concentrations were enriched in spring (phytoplankton bloom) but in the bottom water their concentrations were elevated in fall 1990 (storm resuspension). Most of the PAH concentrations in the bottom water declined between Oct. 1990 to Oct. 1991. The concentrations of 13 individual PAH in dissolved phase ranged from 0.02 ng/L to 2.08 ng/L, which were not significantly different ($p<0.05$, $N=39$) between the surface and bottom waters. Higher concentrations of dissolved PAHs were found for some low molecular weight PAHs (e.g., fluorene and phenanthrene) while the dissolved concentrations of the rest of the PAHs in our study were below 0.5 ng/L.

The concentrations of PAHs associated with the suspended particles ranged from less than ten to several hundred ng/g dry weight. Higher particulate PAH concentrations were found during the spring bloom, indicating that the high production of organic matter may contribute to the PAH distribution and transport in the Chesapeake Bay. The particulate PAH patterns in the surface water, bottom water, boundary layer waters, sediment floc, and surface sediment were similar, indicating that the PAHs may have fast chemical sorption process and be transported in the water without significant alteration. By quantitative comparison, particulate PAH concentrations decreased in the water column, boundary layer water, sediment floc, and surface sediment, perhaps due to desorption of PAHs through the decreasing organic carbon content in the sediment particles.

The PAH concentrations in each size fraction of particles were highly variable. However, the expected results of plankton grazing for contaminants in $> 202\mu m$ particles of surface water were not found for PAHs even in the spring with high production. In contrast, in bottom water every PAH elevated in $>202\ \mu m$ particles all of the time, suggesting that the fast chemical sorption of PAH to the particle's surface may overcome the biological accumulation in the surface water. The mechanism of PAHs enriched in $>202\ \mu m$ particles of the bottom water is still unknown, but may relate to the two-step sorption of contaminant/particle matrix association. These results suggest that the PAHs absorbed to the particle surface faster than PCBS, but the second-step sorption may be restricted tightly.

Overall, the total concentrations of 13 PAHs in each sized suspended particles poorly related to their organic carbon content ($r^2=0.005$) in the surface water. As discussed in the previous results of the PCB section, the correlation between the concentrations of PAHs and the organic carbon content in bottom particles ($r^2=O.41$) was higher than in the surface particles.

Laboratory Study.

To initially characterize the exposure reactor, an experiment was conducted to determine the time required for the system to reach equilibrium with respect to air-water exchange.

Concentrations of all the congeners in the water and air were constant after 7 days, indicating that PCB transfer from air to the dissolved phase reached equilibrium in 7 days. PCB transport from the air to the dissolved phase can be calculated using the following equation:

$$F = k_{ol} \cdot [(C_a/H') - C_d]$$

where the flux (F) equals to the concentration gradient at the air-water interface multiplied by the mass transfer coefficient (k_{ol}). The concentration gradient is the difference between the instantaneous air concentration (C_a)and the equilibrium concentration (C_a/H') where H' equals the Henry's Law constant ($C_{a,eq}/C_{d,eq}$). At equilibrium, the concentration of congener 118 in the dissolved phase (C_d) was 164±11 ng/L (N=5) and its concentration in the air phase (C_a) was 0.36±0.04 ng/L (N=5). Using the time-variable concentration of congener 118 in the dissolved phase, we estimated the mass transfer coefficient of PCB 188 to be 0.1 to 0.3 m/day, in close agreement of published values of 0.03 to 0.3 m/day (Mackay and Yuen, 1983).

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THE ROLE OF PLANKTON IN CONTROLLING THE PARTITIONING AND TRANSPORT OF HYDROPHOBIC ORGANIC CONTAMINANTS: ZOOPLANKTON FEEDING AND EXCRETION [R/CBT-10]

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OBJECTIVES

To determine the role of zooplankton in the processing of hydrophobic organic contaminants (HOCS) in Chesapeake Bay, we conducted experiments on feeding and excretion, and measured HOC content of the Bay's dominant zooplankton. These were performed in contrasting seasons to evaluate temporal variation in biological control of HOC transport.

RATIONALE

Zooplankton, through their feeding activities, can often determine particle concentrations, fluxes and size spectra in estuarine waters. These particle parameters, in turn, determine the fate of HOCs, which are mostly associated with particulates. The production of rapidly sinking zooplankton fecal pellets is a primary means by which HOCs can be transported to the sediment. Thus, biological factors are probably as important as physical and chemical means affecting the transport and fate of toxics in aquatic ecosystems.

PROJECT RESULTS

We began research (September 1990 - December 1992) to elucidate the role of zooplankton in the transport of HOCs in Chesapeake Bay. Our research objectives were to measure zooplankton abundance and fecal pellet production. Using this data in collaboration with Baker *et al.* we proposed to measure the amount of lipids and HOCs in zooplankton (>200 μm) and zooplankton fecal pellets in order to assess the inventory of HOCs in the water column and to estimate the flux of HOCs to the bottom via zooplankton fecal pellets.

We had proposed three cruises in 1990-1991. Because the project was scaled back due to budget reductions, we only participated in cruises in October 1990. We have just begun our second year of funding (1 January 1992). We will participate in cruises this spring, summer and fall.

Our cruise to the Hooper Island Station, in the mesohaline portion of Chesapeake Bay on 18 October 1990 was abbreviated because of high winds. A second cruise on 30 October 1990 was calmer, allowing us time to conduct day/night sampling, fecal pellet production experiments and sample from three depth strata. Zooplankton were collected with a pump sampler. Zooplankton biomass was determined from direct measurements with a C-H-N analyzer.

Table 1. Zooplankton Biomass at Hooper Island Station

Date	Time	Depth ($mg\ C\ m^{-3}$)			Integrated ($mg\ C\ m^{-2}$)
18 October 1990		0 - 7m	8 - 16m		0 - 16m
	1300	10.8	18.8		244.6
30 October 1990		0 - 7m	8 - 13m	14 - 16m	0 - 16m
	1240	0.5	0.9	2.9	17.5
	1850	1.5	3.6	7.8	55.6

The average weight of the dominant copepod, *Acartia tonsa* was 5 μg C. Thus the estimated abundance of zooplankton was 49,000, 3,600 and 11,200 animals per m^2 on 10/18 (1300), 10/30 (1240) and 10/30 (1850). The higher zooplankton abundance on 10/30 may be due to the different tidal stages sampled (nearly slack high at 1240 and slack low at 1850), the water possessing more "upstream" zooplankton at 1850 and being more "diluted" with lower Bay waters at 1240.

Analysis of copepod fecal pellets during October were as follows: 0.16 μg dry weight/pellet, 0.11 μg C/pellet, 69% C/dry weight, C/N of pellets averaged 7.7. We conducted fecal pellet production experiments on 30 October by incubating 10 *Acartia* in 1 liter jars containing surface Bay water for various time periods (Figure 1). We found that approximately 1 pellet/copepod/hour are produced under these conditions. Using the average pellet C biomass and our estimates of zooplankton abundance, we estimate that the production of fecal pellets was 130 and 20 mg C/m^2 /day on 10/18 and 10/30. These potential fecal pellet production rates are roughly 10% of the carbon flux estimated at the station with sediment traps by Baker and Harvey during our cruise. Comparing the ratio of C/dry weight of the ambient seston to that found in the traps, they found the trap ratios to be more similar to sediments rather than suspended particulate matter. Thus resuspension during our cruises in October likely dominated the catch in the sediment traps resulting in high apparent flux rates and low contributions from fecal pellets.

As part of the monitoring program conducted by the Maryland Department of the Environment, zooplankton are measured each month at a number of stations in Chesapeake Bay. We have graphed their abundance estimates of *Acartia* at the mid-Bay station

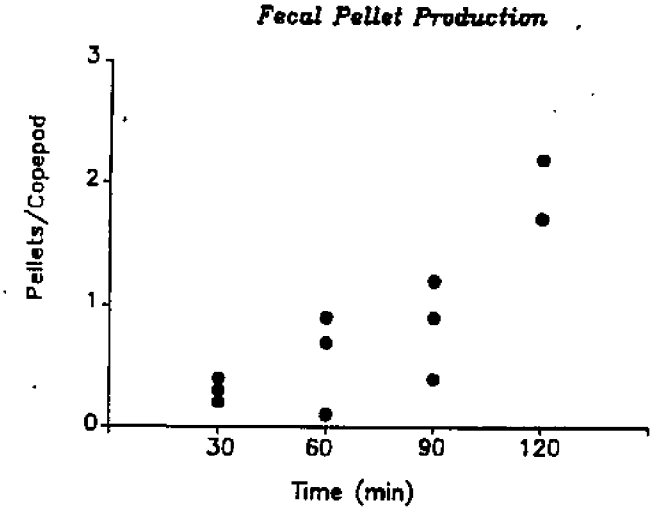

Figure 1. Fecal pellet production over time of *Acartia tonsa* incubated in surface seawater from Chesapeake Bay on 10/30/90.

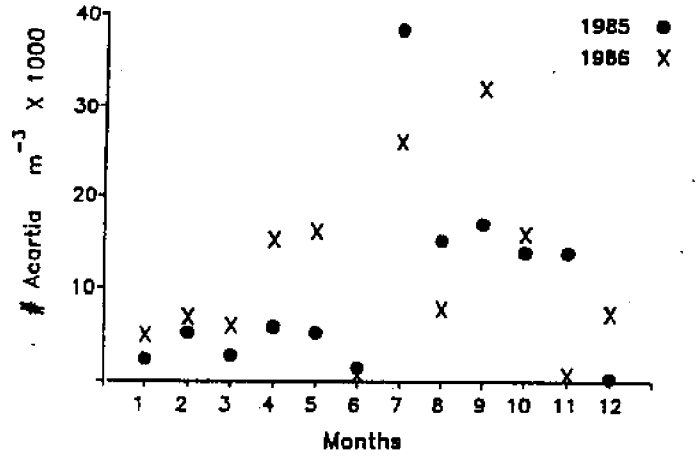

Figure 2. Abundance of *Acartia* from the mesohaline portion of Chesapeake Bay in 1985 and 1986. Data from Maryland Department of Environment monitoring program, prepared by Versar, Inc.

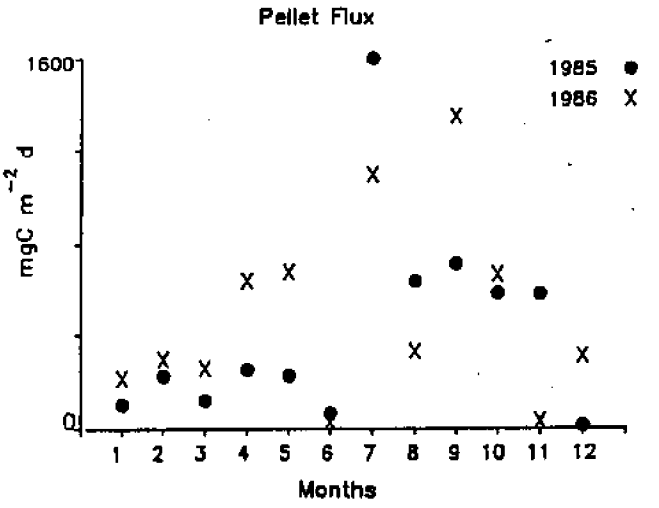

Figure 3. Potential fecal pellet production rates in mesohaline portion of Chesapeake Bay, assuming a 16m water column, a production rate of 1 fecal pellet/hour and an average fecal pellet biomas of 0.11 μg C/pellet.

(MCB4.3C) near our Hoopers Island station for 1985 and 1986 (Figure 2). If we assume a 16m water column, an average fecal pellet production rate of 1 pellet/copepod/h and an average fecal pellet C of 0.11 μg C/pellet, we can estimate the potential flux of carbon via fecal pellets (Figure 3). Potential flux rates over the two years range from 11 to 1616 mg C/m^2/day. Maxima in potential pellet production occur in July, September (Figure 3). These rates can be the major source of carbon flux measured in sediment traps (Boynton et al. 1988) over the same period.

Clearly these approximations are over simplifications of fecal pellet production rates. Other copepod species (e.g., *Eurytemora*, *Centrovages*) can also be abundant and contribute to fecal pellet flux. The production rate of fecal pellets is influenced by temperature, food quality and food quantity. At the higher concentrations of phytoplankton that occur in Chesapeake Bay in spring and summer, fecal pellet production rates would likely be greater than we found in October (Figure 4). In the remaining year of the grant we will measure fecal pellet production rates under a variety of conditions so that we can better understand the factors which influence fecal pellet production rates. We hope to produce algorithms which predict fecal pellet production rates from data on particle concentration and temperature. These predictive equations could then be used with data on zooplankton abundance such as that from the zooplankton monitoring program of the Maryland Department of the Environment, to estimate the potential flux of fecal pellets in different areas of Chesapeake Bay over the year.

Preliminary data on HOC distributions in plankton size fractions indicate increasing concentrations with larger particles. Thus the zooplankton are bioaccumulating HOCs from smaller sized particles (Table 2). Increased amounts of neutral lipids, principally as triacylglycerals are found in larger particles, particularly the $>$ 200 μm zooplankton. HOC associations will be most closely related to these neutral lipid concentrations rather than the polar lipids which predominate in the small size particles ($<$ 10 μm). Harvey found an average of 7.3 ng lipid/fecal pellet in our October study. Our fecal pellet flux rates during the 2 cruises could thus be extrapolated to estimate lipid fluxes via fecal pellets of from 0.6 to 8.6 mg lipid/m^2 /day.

Table 2. HOC concentrations in plankton size fractions in October, 1990 (data from Baker).

HOC Component	64 - 200 μm	$>$ 200 μm
	(ng/g dry wt)	
Phenanthrene	74	236
Anthracene	48	200
Fluoranthene	284	581
Pyrene	325	509
Benzo-a-Anthracene	336	588
Benzo-e-Pyrene	607	994

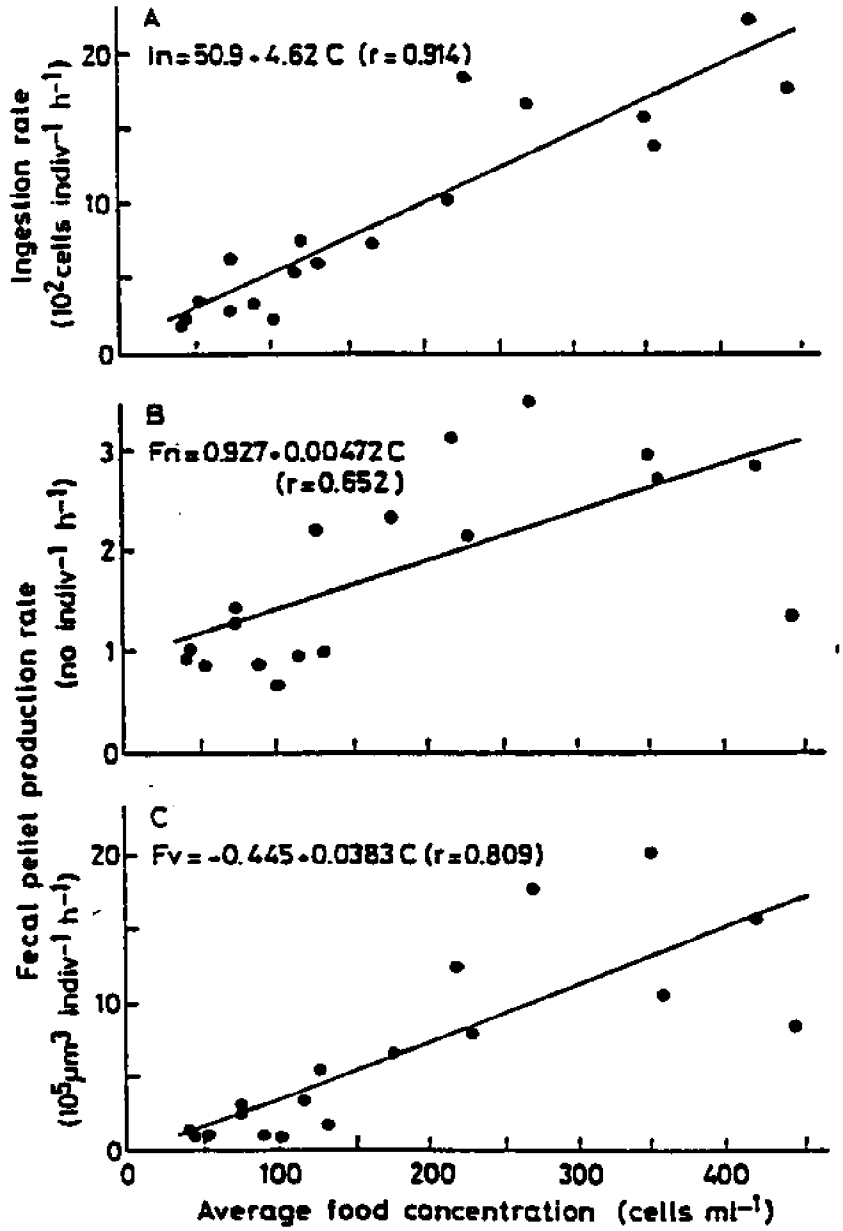

Fig. 4. Relationship between the concentration of *Thalassiosira decipiens* and (A) the number of cells ingested, (B) the number and (C) total volume of fecal pellets voided by adult female *Calanus pacificus pacificus*. From Ayukai and Nishizawa 1986.

Our estimates of phytoplankton growth and mortality are summarized in Table 3. In all three experiments, microzooplankton appeared to be the main grazers on phytoplankton, since their grazing rates equalled or exceeded growth rate estimates. This suggests that the main fate of phytoplankton in mesohaline Chesapeake Bay in both late summer and fall is to be eaten by microzooplankton (20-200 μm size range). Although there are some differences among pigments (fucoxanthin growth sometimes exceeds grazing by microzooplankton; see also McManus and Ederington-Cantrell, 1992), this pattern seems to hold generally across most of the phytoplankton taxa seen in the bay.

The importance of microzooplankton as significant grazers of phytoplankton in the bay suggests the possibility of greater water column cycling of particle-bound contaminants. Microzooplankton, as particle feeders, process both living and detrital material suspended in the bay. The zooplankton community as a whole (including copepods and other larger zooplankton) is capable of filtering the entire bay clear of particles many times over in the time it takes the bay to flush itself physically. Only the continuous production of particles by biological and inorganic processes keeps the bay's particle load high. Unlike the larger zooplankton, whose feeding results in the production of large, rapidly-sinking fecal pellets, which have the potential to transport contaminants to the sediments, the microzooplankton produce only dissolved excreta or very fine egested particles, particles that are likely to stay in suspension for longer periods. Thus in a system like Chesapeake Bay, where the predominant grazers appear to be very small zooplankton, particle-bound contaminants may remain in the plankton, cycling through the food web, for a longer time than they would in a system dominated by larger, fecal pellet-producing zooplankton or large benthic filter feeders.

Table 3. Dilution experiment results: phytoplankton growth rates and microzooplankton grazing rates in the mesohaline portion of Chesapeake Bay during the October 1990 and August 1992 cruises of the Toxic Trophodynamics Program. Total growth and grazing is represented by values for chlorophyll-a (in boldface). Rates for other pigments represent estimates of differential growth and grazing for different phytoplankton taxa. Asterisk (*) is for separate incubations enriched with nutrients (5 micromolar ammonium + 1 micromolar phosphate). Specificity of pigments as markers for different phytoplankton groups is as follows: chlorophyll-b (chlorophytes); chlorophyll-c (chrysophytes, diatoms, dinoflagellates, and cryptophytes); fucoxanthin (diatoms and chysophytes); alloxanthin (cryptophytes); zeaxanthin (cyanobacteria); peridinin (dinoflagellates).

Date	Pigment	Growth (d^{-1})	(s.e.)	Microzooplankton grazing (d^{-1})	(s.e.)
30 Oct 1990	**chl-a**	**0.07**	**(0.08)**	**0.17**	**(0.04)**
	chl-b	0.12	(0.22)	0.05	(0.08)
	chl-c	-0.12	(0.03)	-0.11	(0.05)
	fucoxanthin	0.39	(0.05)	0.22	(0.03)
	diadinoxanthin	0.18	(0.11)	-0.16	(0.03)
	alloxanthin	0.01	(0.06)	0.04	(0.04)
	zeaxanthin	0.64	(0.25)	0.75	(0.02)
	peridinin	-0.56	(0.15)	-0.49	(0.03)
30 Oct 1990*	**chl-a**	**0.18**	**(0.34)**	**0.18**	**(0.02)**
	chl-b	0.19	(0.28)	0.12	(0.04)
	chl-c	0.06	(0.28)	0.01	(0.03)
	fucoxanthin	0.33	(0.27)	-0.02	(0.01)
	diadinoxanthin	-0.12	(0.33)	-0.28	(0.00)
	alloxanthin	0.11	(0.47)	0.15	(0.01)
	zeaxanthin	0.25	(0.17)	0.45	(0.00)
	peridinin	-0.57	(0.35)	-0.37	(0.03)
12 Aug 1992	**chl-a**	**2.10**	**(0.04)**	**2.35**	**(0.07)**
	chl-c	1.96	(0.31)	2.19	(0.09)
	fucoxanthin	2.08	(0.04)	2.22	(0.05)
	zeaxanthin	0.92	(0.05)	1.25	(0.10)
	peridinin	-0.75	(0.07)	0.56	(0.30)

IMPORTANCE OF DINOFLAGELLATE BLOOMS IN THE TRANSPORT OF CARBON AND TOXIC TRACE ELEMENTS IN CHESAPEAKE BAY [R/CBT-13]

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INTRODUCTION

To date, we have sampled a number of bloom events, including the spring diatom blooms in the Patuxent River and in Chesapeake Bay, the spring *Prorocentrum* bloom in the Patapsco River, the summer dinoflagellate (*Gymnodinium/Gyrodinium*) bloom in the Patuxent, and the winter *Katodinium rotundatum* bloom in the Patuxent.

Samples from these blooms have been analyzed for trace element concentrations, phytoplankton and microzooplankton species composition and cell density, nutrient concentrations, chlorophyll-a, and particulate carbon and nitrogen concentrations.

Experiments to examine feeding rates and trace element uptake have been performed in the microcosms, in conjunction with the winter *Katodinium* bloom.

Plankton biomass and trace element content

In each of the blooms examined, biomass of phytoplankton was greater in the blooms than in surrounding non-bloom regions; however, the degree of difference varied considerably. For example, cell densities during the *Prorocentrum* bloom in the Patapsco River were approximately twice that in surrounding regions. In non-bloom regions, phytoplankton were dominated by the diatom, *Thalassiosira* sp., and had lesser densities of *Prorocentrum* sp. During the *Gymnodinium/Gyrodinium* bloom in the Patuxent River, densities within the bloom were 3 to 5 times those in non-bloom areas. Differences between densities in the winter *Katodinium* blooms in the Patuxent River were even larger--on the order of 10 times higher. Particulate carbon and chlorophyll-a concentrations were also elevated in bloom regions, with the degree of enrichment similar to that seen for cell density.

Zooplankton distributions from bloom to non-bloom regions were also examined for the three dinoflagellate blooms. During the *Prorocentrum* bloom in the lower Patapsco River, highest net microzooplankton densities were observed during the deployment of the sediment trap in the area of highest dinoflagellate densities. Whole-water microzooplankton densities were also high during the deployment (1.7-2.5 10^4 L^{-1}) and continued to increase throughout the bloom, even at the mouth of the estuary where diluted portions of the bloom were observed. The increase might reflect a response of the smallest grazers to production in the

dinoflagellate, or the transfer of bloom production to bacteria. Another possibility that must be considered is that the increase simply reflects increasing densities accompanying increasing water temperatures and intrusions from the Bay. Mesozooplankton densities also increased through time (0.7-1.6 10^3 m^{-3}), likely the normal seasonal increase in copepods observed at this time of year.

Zooplankton responses to the August *Gymnodinium/Gyrodinium* bloom were even less apparent. Whole water and net microzooplankton were slightly elevated in the bloom region relative to the non-bloom area, but the differences were likely within counting error for both size fractions. Mesozooplankton densities did increase in the bloom region, reaching 1.6 10^4 m^{-3} versus only 4 10^3 m^{-3} outside the bloom.

Largest differences in zooplankton abundances between bloom and non-bloom regions were noted during the 1994 *Katodinium* bloom. In February, net microzooplankton densities in the bloom surface waters averaged 1580 L^{-1} (primarily the rotifer *Synchaeta baltica*) during 3 sampling periods; densities in non-bloom areas averaged 294 L^{-1}. In addition, microzooplankton densities in whole water samples were also higher in the blooms, averaging 1880 L^{-1} versus 1060 L^{-1} at the non-bloom site. These data suggest bloom production was supporting a large (for winter) microzooplankton community. Mesozooplankton were also higher in bloom regions than areas of the estuary with lower dinoflagellate densities and copepod grazing was high on the dinoflagellate. These results support previous observations by Sellner and colleagues that the dominant copepod at this time, *Eurytemora affinis*, readily ingests the bloom-former, perhaps providing the crustacean with an abundant resource in late winter-early spring.

Trace element content of the seston (suspended material) and sediment trap contents varied with bloom location, and to some extent with bloom type and season. Copper and cadmium concentrations were greatly elevated in the Patapsco River samples; however, arsenic contents were similar in both the Patapsco River and the Patuxent River. Within the Patuxent River, arsenic contents displayed some seasonal differences, being lower in the winter.

Carbon and trace element flux

Particle formation rates and settling were considerably higher, ranging from 1.5 to 5 times higher in bloom areas relative to non-bloom areas (20-30 times higher during the winter *Katodinium* bloom). Flux of carbon and associated trace elements, As, Cd, and Cu from the surface was similarly elevated. Fluxes were higher in the Patapsco River, presumably because of higher trace element concentrations, but the difference between bloom and non-bloom regions was greater in the Patuxent.

Much of this material apparently settled to the sediments. An examination of surficial sediment underlying the two stations in the Patuxent River during the summer *Gymnodinium/Gyrodinium* bloom resulted in higher trace element concentrations in sediments in the bloom region than those in the non-bloom region. Because of the large size of this bloom, however, the two stations were considerably separated in space, and differing physical characteristics were responsible for some portion of the difference. In fact, when

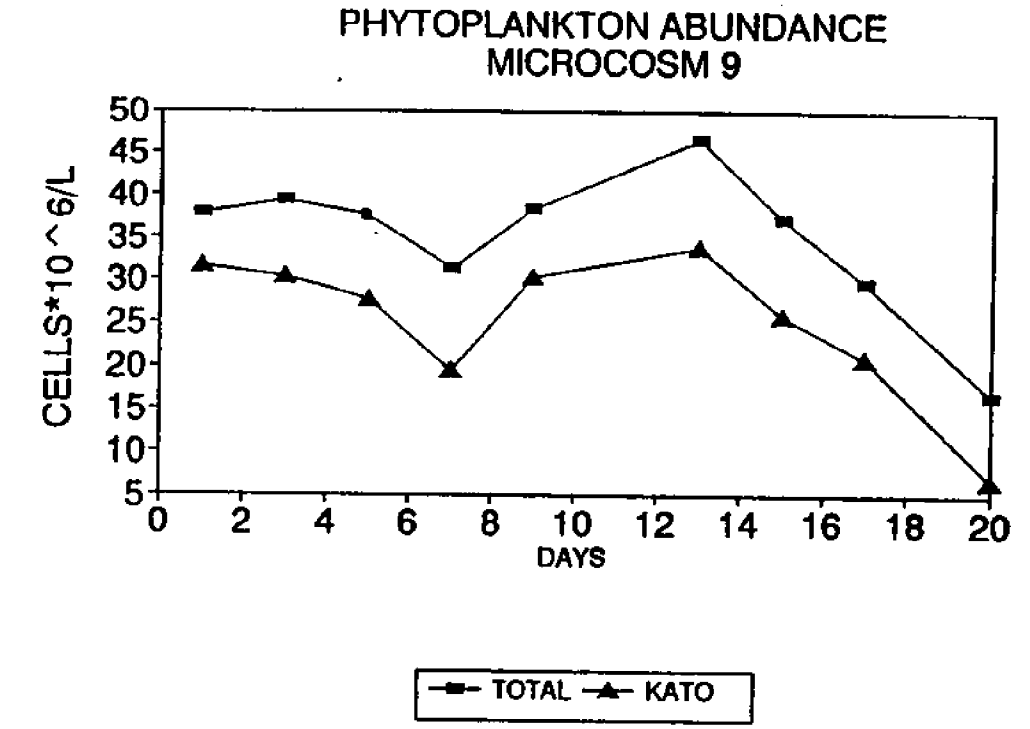
data are normalized to iron content, only Cu was significantly elevated in the bloom site. During the winter *Katodinium* bloom, As and Cd contents of sediments were higher under the bloom region; Cu contents were similar in the bloom and non-bloom regions. Normalization to iron content essentially removed differences between As and Cd in bloom and non-bloom regions.

Feeding experiments

Bloom assemblages (predominately [65-70%] *Katodinium*, but also containing a variety of chrysophytes and small flagellates) were placed into microcosms and exposed to both ambient and elevated (2-3X ambient) trace element concentrations. Phytoplankton exposed to elevated conditions had significantly higher contents of As, Cd, and Cu--2-3 times contents seen in blooms maintained under ambient conditions.

Microcosm experiments with bloom and non-bloom assemblages pre-screened through 53 μm mesh indicated a strong linkage between bloom levels of *Katodinium* and two small oligotrich ciliates (Figure 1). Densities of the ciliate increased (k=0.3) through the course of the enclosure studies, likely in response to an abundant food supply (the dinoflagellate or the bacteria and flagellates accompanying the bloom) as well as elimination of the larger copepod and rotifer grazers that would limit densities of the ciliates *in situ*. Over a two week period, ciliate numbers reached densities approximately 2 orders of magnitude higher than those noted in the field during the experiment. The tremendous accumulation of the ciliates in the absence of larger zooplankton further supports the importance of top-down zooplankton grazing in processing *Katodinium* bloom production and the accompanying small heterotrophs.

Eurytemora affinis collected from the bloom region of the Patuxent River were fed from microcosms containing the two different bloom assemblages for 4 days. Copepods exposed to elevated trace element concentrations exhibited higher trace element content relative to copepods fed the ambient assemblage at the end of the experiment. Copepods exposed to elevated concentrations in the water only (not allowed to feed on the bloom assemblage but maintained in water with the elevated trace element concentrations) showed elevated Cd content, similar to fed animals, and a less elevated Cu content. Thus, trace element uptake of Cu was from the bloom assemblage, suggesting that trophic transfer of Cu is occurring.



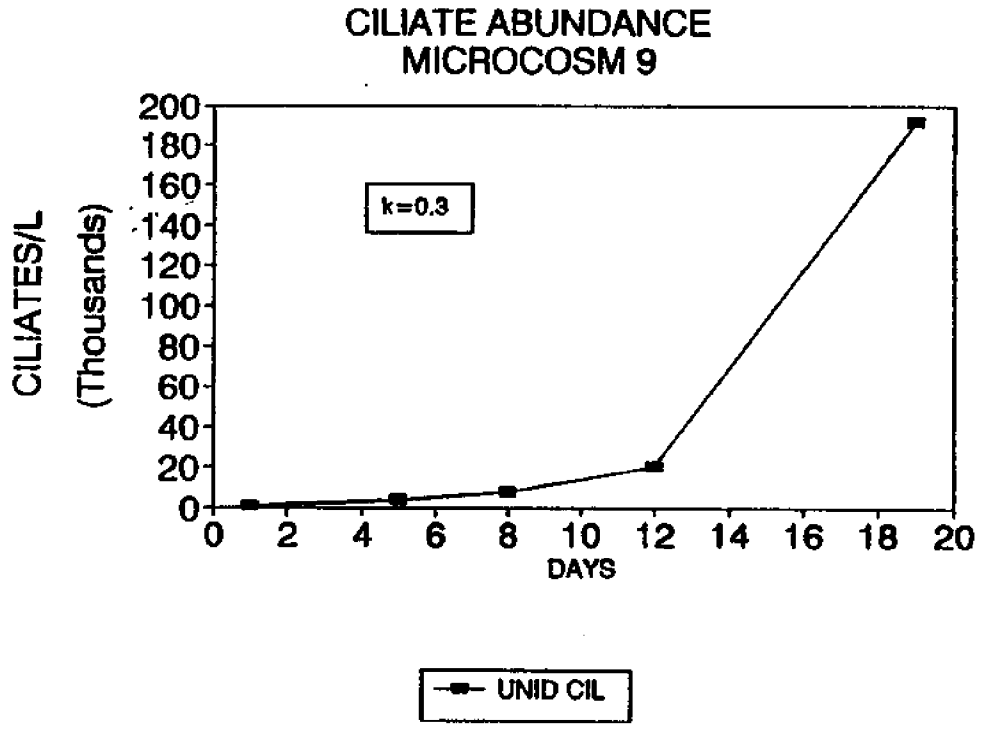


Figure 1. Abundance of *Katodinium rotundatum* (KATO) and total phytoplankton density (TOTAL) and abundance of an unidentified ciliate (bottom figure) in microcosm experiments.

UPTAKE OF DISSOLVED AND PARTICULATE-ASSOCIATED TOXICANTS BY THE EASTERN OYSTER [R/CBT-16]

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PROJECT OBJECTIVES

The objective of the proposed research is to determine how the partitioning of toxic substances among dissolved and particulate phases affects bioaccumulation by a benthic suspension feeder, and how quantification of the phase-dependent bioavailability could be applied in environmental risk assessment. We will establish the role that ingestion of organic and inorganic particulate matter plays in toxicant uptake by oysters. Our study design involves three laboratory experiments to investigate how changes in the concentrations of dissolved organic matter, particulate organic matter (i.e., food particles), and particulate inorganic matter (i.e., ingestible but non-nutritive particles) affect bioaccumulation of PCB by individual oyster spat. We will determine the relative bioavailability of PCB associated with each of these fractions, and if chemical quantification of PCB concentrations within these fractions is an accurate predictor of bioaccumulation potential by the oyster.

PROGRESS

The first of the three research components, DOM effects on PCB bioavailability, has been completed in its entirety. This component consisted of two experiments measuring uptake of PCBs and a third experiment measuring the rate of depuration from contaminated tissues. Tissue lipid data, which are critical to interpretation of results, were somewhat delayed but are now available. Later experiments build upon the results of this first component, and we are now integrating the results into our current work. We are now pursuing the second and third research components involving the bioavailability of particle-bound PCBS. Progress was slowed somewhat during the first half of 1994 because of an intense teaching load, but we are now able to devote full-time to this research project, and anticipate no difficulty in finishing the work within the current project time period (through December 1994).

ACCOMPLISHMENTS

The DOM research component has indicated that DOM does not reduce PCB bioavailability to the extent anticipated. Most literature data shows that the bioavailability of organic pollutants is reduced by DOM, although this is not true for all compounds. The fact that our results for the bioavailability of a tetrachlorobiphenyl are atypical may be due to the species under investigation. Oysters are known to utilize DOM to partially meet their nutritional needs, and it may be that in doing so they accumulate the associated PCBs. A graduate student who has worked extensively on the effects of DOM on PAH bioavailability is now pursuing this aspect of the study.

We have had very good success in maintaining Eastern oysters under laboratory conditions at UC-Berkeley. The mortality rate has been essentially zero. We also have been successful in culturing the algal food (*Isochrysis galbana*) and can easily meet the oysters nutritional needs with the laboratory culture alone. This capability is critical to our current efforts requiring an adequate supply of ^{14}C-PCB-labelled algae.

CONTAMINANT FLUX FROM SEDIMENTS: IMPACT ON CHESAPEAKE BAY FOOD WEBS [R/CBT-20]

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INTRODUCTION

There has long been a presumption by both the public and scientific community that chronic releases of contaminants are responsible for a perceived degradation of the environment. However, there is very little scientific evidence for such a conclusion, since conclusively assigning blame for any trend in living resources is extremely difficult. Given the enormous variability of such resources due to climate, weather, fishing pressure, and other unknowns, along with the lack of suitable control systems for comparison, such perceptions must be treated with at least a little skepticism. Moreover, single species acute and chronic toxicity studies ordinarily suggest that orders of magnitude higher concentrations of contaminants than are found in the environment are necessary to cause significant deleterious effects.

With the implementation of regulations and programs controlling the release of pollutants into surface waters, we have seen and can expect a gradual and continued decrease of many contaminants in our rivers, lakes, bays, and estuaries. However, as pollutant loading of the water column decreases, the major sink for pollutants--the sediments--may exert an important influence on overlying water quality. The sediments in the Chesapeake Bay, as in any other coastal systems, contain elevated amounts of toxic substances and are an enormous potential repository for continuing inputs of toxic substances. Toxic substances present in sediment are not completely removed from the environment, as they can be returned to the water column by diffusion or the action of benthic invertebrates. A crucial question is whether or not the sediment bound contaminants represent a significant threat to the ecosystem.

The concept behind the study reported here was to develop an experimental system to examine the sub-acute effects of sediment-bound toxic contaminants on estuarine food webs. The experimental system allows us to determine the rates at which toxic pollutants flux between the sediment and the water column, to expose natural plankton systems to contaminants released from those sediments, and to determine the effects of those contaminants on the plankton. We will also use the resulting planktonic communities to feed populations of zooplankton and other filter feeding animals, and determine how any resulting changes in the phytoplankton community structure and contaminant accumulation affect the growth and reproduction of the herbivores. We believe that this experimental design will be able to detect subtle changes in the function of the estuarine ecosystem caused by the presence of contaminated sediments.

OBJECTIVES

Overall Objectives

The overall objective of the research program proposed here is to test the hypothesis that contaminated sediments, and the effects of anoxia on contaminated sediments, can influence the phytoplankton and herbivore communities in Chesapeake Bay. Our research program is broken down into two subprojects of one year duration which will test supporting links of this hypothesis.

1993 Objectives

In the first year we have tested the hypothesis that contaminated sediments in the absence of anoxia can produce changes in the phytoplankton and zooplankton similar to the changes we have observed with phytoplankton microcosms dosed with low concentrations of arsenic or copper. This is an important link in the chain of causality in the proposed hypothesis. We have whether microcosms receiving water which has interacted with clean sediment develop a phytoplankton community that differs from systems with either no sediment or contaminated sediment.

1994 Objectives

In the second year of the project, we tested the hypothesis that the occurrence of anoxia over contaminated sediments causes greater deleterious effects (or at least different effects) on estuarine food webs than contaminated sediments exposed to normal surface waters. Experiments paralleled the first year's design, with the addition of coupled systems using anoxic sediments. This is particularly significant in that the largest changes in trace element flux have been in response to the presence of anoxic water in contact with the sediment.

RESULTS

Experiments for both the 1993 and 1994 Objectives have been completed. For the 1993 seasons experiments, all the samples have been analyzed and the data tabulated, whereas we are still in the process of analyzing the samples and data from the 1994 experiments. Therefore, the following results will refer to 1993 experiments.

Chemical Effects

Arsenic Concentrations. In the no sediment microcosms, arsenic concentrations remained near their initial concentrations for the first two weeks, then decreased sharply. In the Chesapeake Bay microcosms, there was initially a rise in the total arsenic concentration through the first two weeks, which leveled off and even declined through the remainder of the experiment (Fig. 1). In this experiment virtually all the arsenic was found in the oxidized arsenate form.

Copper Concentrations. The were no significant changes in the copper concentrations of either the control or the Chesapeake Bay sediment treatments. However, in the Baltimore Harbor sediment treatment, copper concentrations increased from approximately 1.5 to nearly 4.0 μg/l during the experiment (Fig. 2).

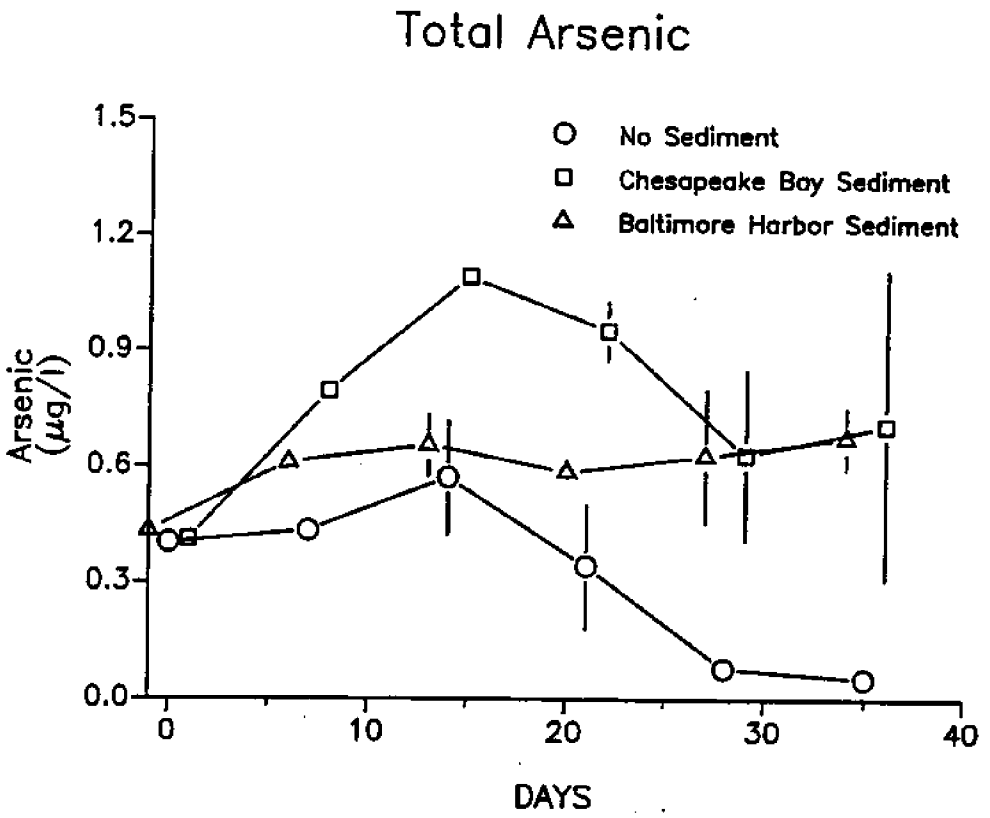

Fig. 1. Changes in total dissolved arsenic concentration in the 1993 experimental microcosms. Mean ± standard deviation of duplicate microcosms.

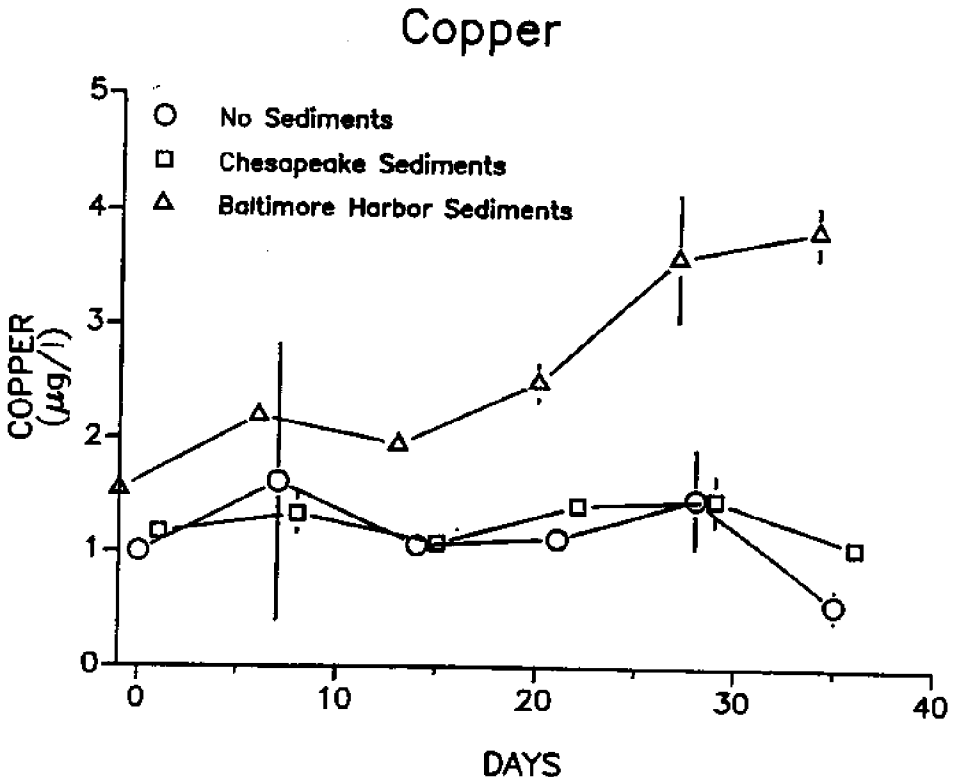

Fig.2. Changes in total dissolved copper concentrations in the 1993 experimental microcosms. Mean ± standard deviation of duplicate microcosms.

Cadmium Concentrations. Cadmium showed a very interesting sequence. In the first week, concentrations rose in all treatments from the levels of about 0.04 μg/l initially present, to approximately 0.1 μg/l in the control and Baltimore Harbor sediment treatments, and greater than 0.2 μg/l in the Chesapeake Bay sediment treatment (Fig. 3). We are not yet sure of the origin of the cadmium causing this rise, but at present, we speculate that it is cadmium remineralized from the suspended matter in the water initially filling the microcosms. Given the weight of suspended matter in the water column (50 mg/1) and the measured concentrations of cadmium in the suspended particles (1-2 μg/g), there is sufficient cadmium in the particles to provide the necessary amounts. Some cadmium may also have come from sediment resuspended in the initial fill of the microcosm. After the first week, cadmium declined to concentrations at or below the initial concentrations, although the no sediment microcosms remained below the other treatments throughout the decline.

Biological Effects

Phytoplankton Abundance and Community Composition. Phytoplankton density as measured by *in vivo* fluorescence (Fig. 4) declined precipitously after the beginning of the experiment. In part, we presume this was due to grazing by the copepods (*Acartia*), which grew up in the microcosms in the course of the experiment (see below). In general, the treatments were in general agreement, however, there was a period from about day 8 through day 15 where the Baltimore Harbor Sediment treatment was consistently below the other two treatments. Near the end of the experiment (day 33) the control treatment showed a large increase in fluorescence due to the growth of a group of haptophytes, however, the haptophytes were also seen in the other treatments, and there is some evidence that they were starting to come up in the other treatments as well. In general, phytoplankton community succession (Fig. 5) followed similar patterns in all three treatments, although there were some differences, such as a greater number of pennate diatoms in both sediment treatments than in the control, and lower numbers of cyanophytes in the Chesapeake Bay treatment.

Copepod Densities. Copepods grew rapidly in all the microcosms, reaching densities of nearly 1000 individuals per liter (summing both adults and copepodites) in two weeks (Fig. 6), and declined thereafter. There are no consistent differences between the treatments, except that the Baltimore Harbor sediment treatment retained somewhat greater numbers during the final three weeks of the experiment.

Microzooplankton Densities. Microzooplankton densities (Fig. 7) started at relatively high values, about 2 X 10^4/L, but declined rapidly. However, they showed a second peak in abundance in the third week reaching nearly 1 X 10^4/L. As with phytoplankton, the type of microzooplankton dominating the assemblage changed through time, but there were no large differences between treatments.

Oyster Growth. Oysters grew slightly, but consistently throughout the experiment (Fig. 8). There were no significant differences in the growth rates of the different treatments, however, both the Chesapeake Bay and Baltimore Harbor treatments were approximately equal, and slightly greater than the control.

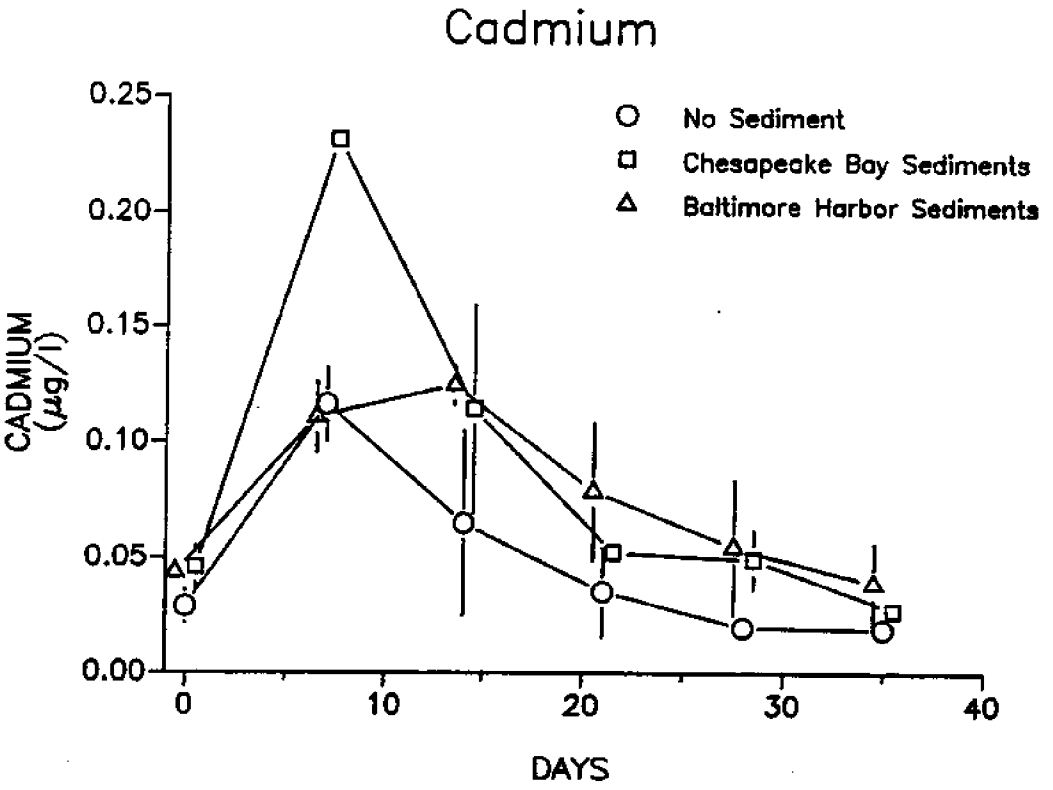

Fig.3. Changes in total dissolved cadmium concentrations in the 1993 experimental microcosms. Mean ± standard deviation of duplicate microcosms.

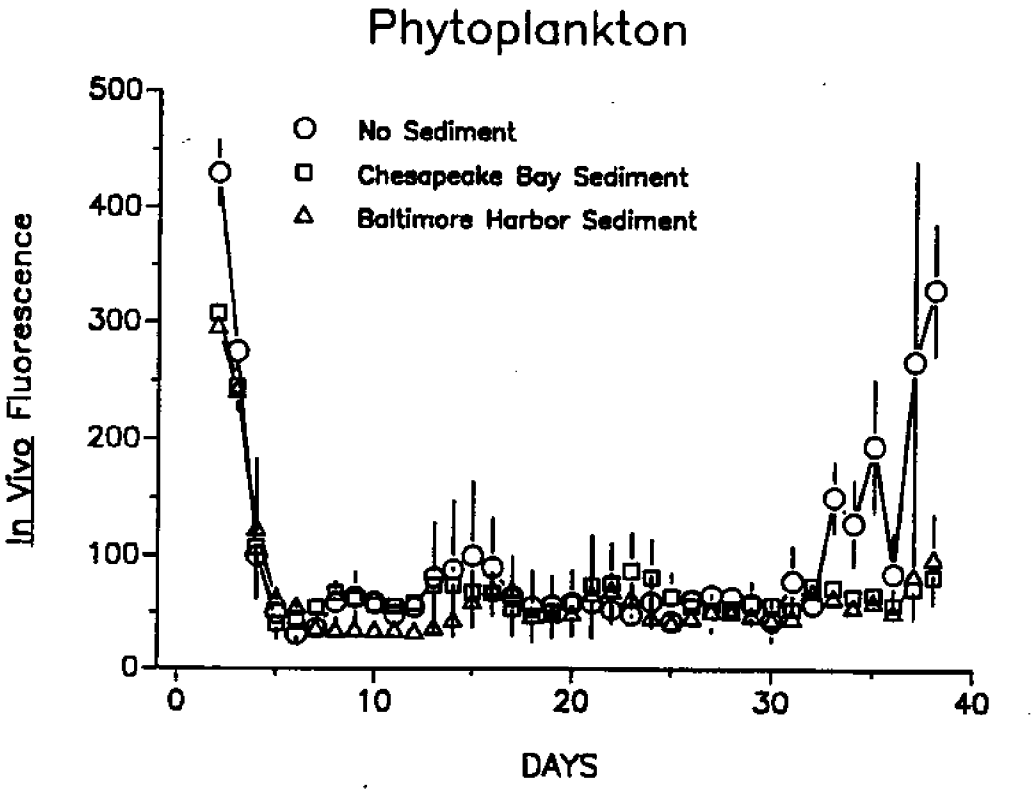

Fig. 4. Phytoplankton density (as measured by in vivo fluorescence) in the 1993 experimental microcosms. Mean ± standard deviation of duplicate microcosms.

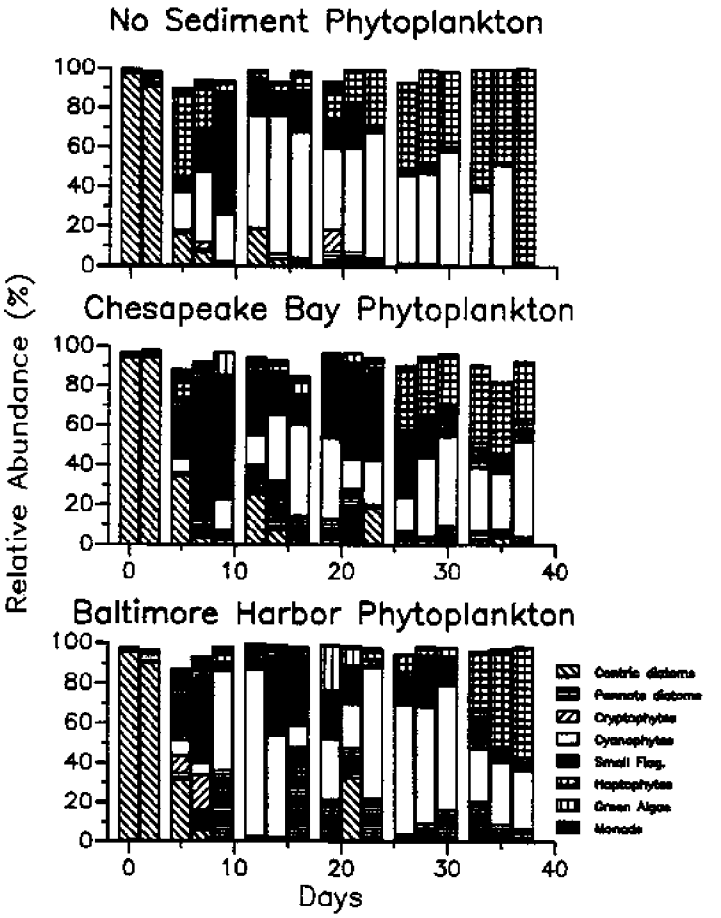

Fig. 5. Phytoplankton community composition in the 1993 experimental microcosms. Mean of duplicate microcosms.

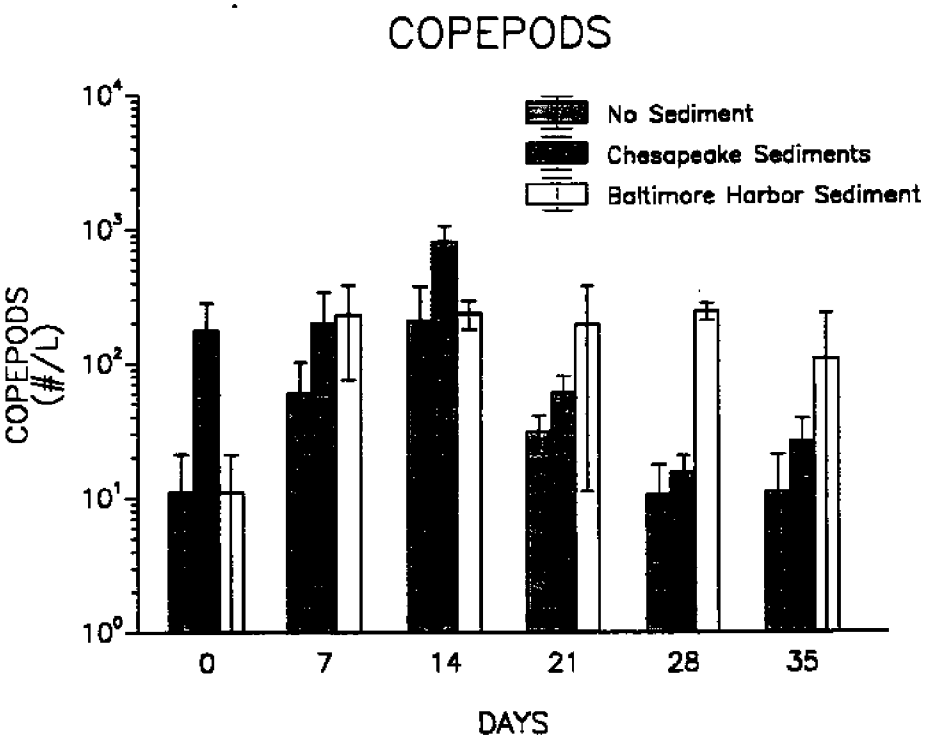

Fig. 6. Copepod densities in the 1993 experimental microcosms. Mean ± standard deviation of duplicate microcosms.

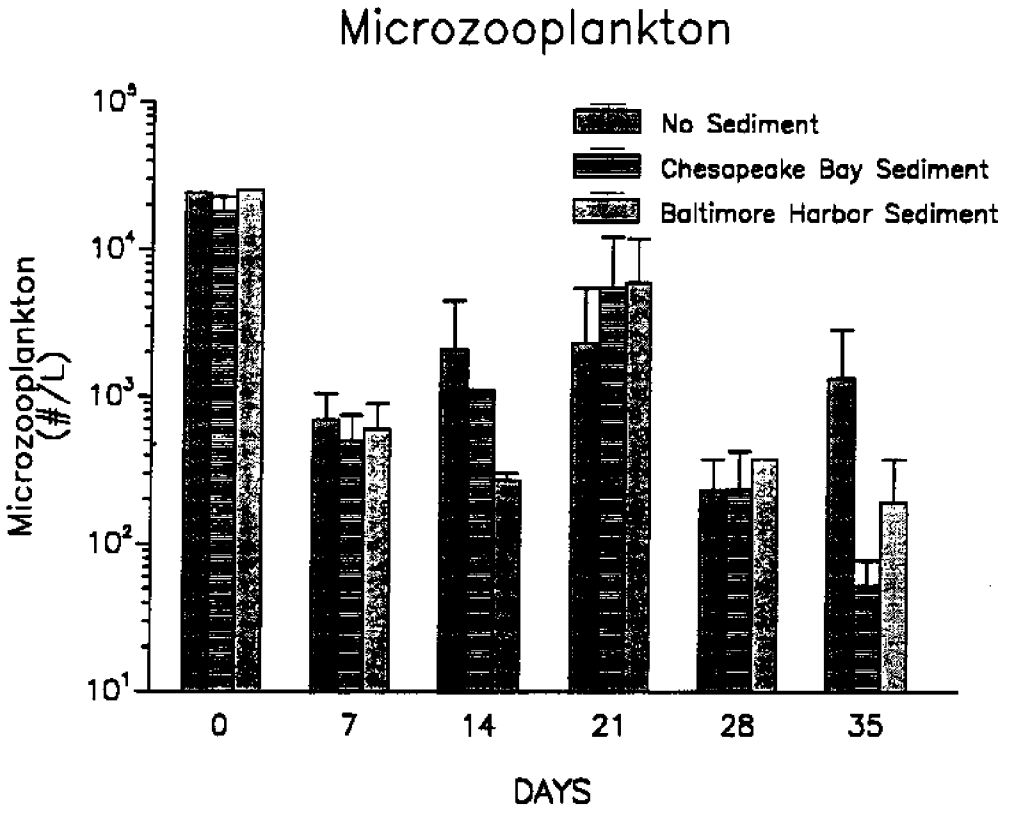

Fig. 7. Microzooplankton densities in the 1993 experimental microcosms. Mean ± standard deviation of duplicate microcosms.

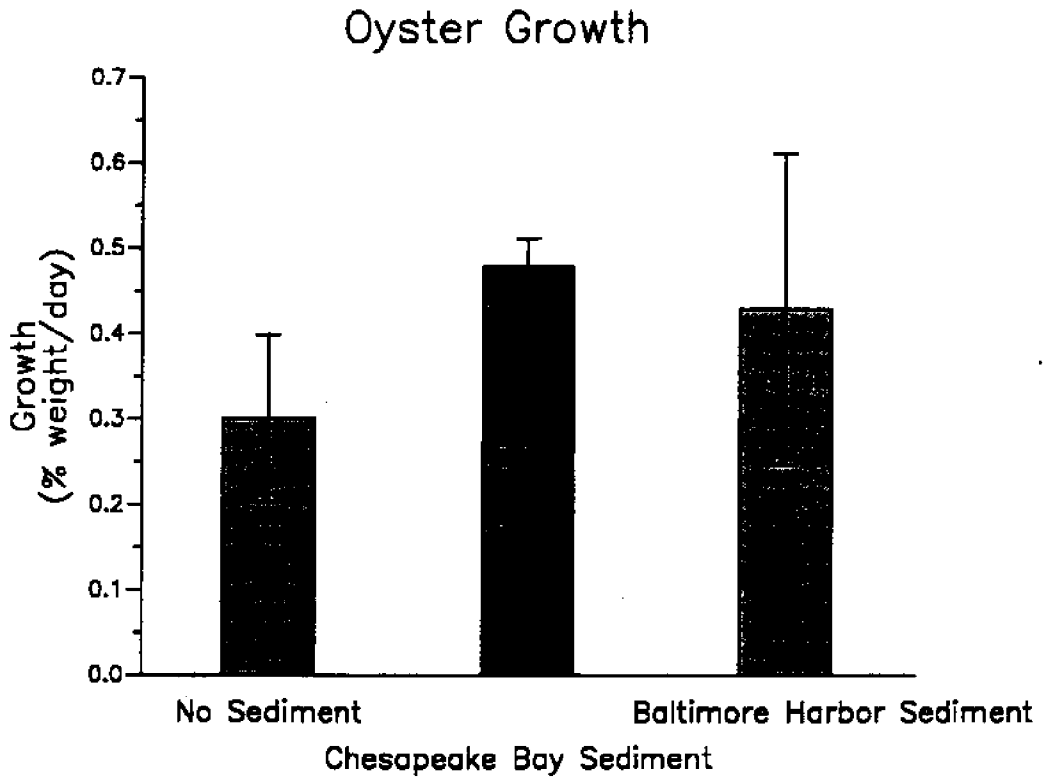

Fig. 8. Oyster growth rates in the 1993 experimental microcosms. Mean ± standard deviation of duplicate microcosms.

SUMMARY

The experimental system showed significant results in terms of the flux of materials from the sediment. These fluxes were small but significant from a geochemical viewpoint, in that they could significantly alter the concentrations of metals in the water column of Chesapeake Bay over the course of a season. The biological communities showed very little or no response to the relatively minor trace element perturbations caused by the sediment.

ORGANIC CONTAMINANT METABOLITE PRODUCTION, ELIMINATION, AND BIOAVAILABILITY IN BENTHIC MACROFAUNA OF LOWER CHESAPEAKE BAY [R/CBT-25]

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RATIONALE

Our work (Schaffner and Dickhut, 1991) and that of others (e.g., McElroy 1985&1990), illustrates the strong capability of a variety of benthic macrofauna to accumulate and transform organic contaminants, in particular, polycyclic aromatic hydrocarbons (PAHS) and low molecular weight polychlorinated biphenyls (PCBs). Bioaccumulation and biotransformation vary both within and among major taxa, and with the organic contaminant (OC) physical-chemical properties (e.g., desorption rate, diffusivity, octanol/water partition coefficient - K_{ow}) (Dickhut *et al.*, 1994). Moreover, large fractions of various organic contaminants in certain benthic macrofauna exist as metabolic products (Dickhut *et al.*, 1994; McElroy 1985&1990) and little is known about the bioavailability and effects of these substances. Metabolites resulting from benthic macrofauna biotransformation of OCs in sediments can potentially adversely affect the organisms directly or be transferred through trophic interactions with subsequent effects on consumers. Thus, to fully understand the effects of organic contaminants on benthic organisms and the influence of benthic biota in the transfer of toxic substances to other aquatic species, it is necessary to quantitatively evaluate the production, binding and elimination of OC metabolites in benthic macrofauna.

OBJECTIVES

This research emphasizes quantification of the rates of OC uptake, and subsequent metabolite production, binding and elimination by selected benthic macrofauna from lower Chesapeake Bay. Our effort will allow determination of the disposition of OC parent compound and metabolites within the benthic region and delineation of associated risks after initial exposure of the benthos to organic pollutants. Using Chesapeake Bay macrofauna as representative estuarine species our objectives are to:

[1] quantitatively evaluate the rates of uptake, transformation, binding and elimination of a series of representative OCs;

[2] examine the rates of uptake and metabolism of representative OCs as a function of food source material;

[3] determine the fate of OC metabolites produced for a series of representative organic contaminant parent compounds.

APPROACH

OC bioaccumulation/transformation experiments are conducted using individual species of benthic macrofauna and defaunated Chesapeake Bay sediment. Sediment augmented with radiolabeled organic contaminants is used during the uptake phase of the experiments, while sediment spiked with nonradiolabeled contaminants is used during the elimination phase. Four to six sampling times are used to evaluate rates of organic contaminant uptake, transformation, and metabolite binding in the selected benthic macrofauna.

At each sampling time, replicate samples are collected to assess sampling and analytical variance. Sediment samples are immediately extracted, while animals are allowed to evacuate their guts for 4 hr prior to sample processing. Samples are then extracted and analyzed with an analytical protocol we have developed to quantify OC parent compounds, polar and conjugated metabolite fractions, and nonextractable, cellular bound OC fractions in aquatic organisms and sediments. Briefly, OCs and degradation products are extracted from the sample using a combination of methanol, dichloromethane, and water. After extracting the sample twice, the sample residue is removed via centrifugation and reextracted with dichloromethane. Subsequently, the residual sample is then dried and combusted at 1000°C with evolution of bound radioactive chemical as $^{14}CO_2$ or tritiated water which is trapped in phenethylamine and quantified using liquid scintillation counting (LSC). The solvent extracts are fractionated into organic and aqueous soluble components and subsequent radioactivities determined via a combination of high performance liquid chromatography (HPLC) and LSC. Parent compounds and polar metabolites associated with the organic fraction are resolved using HPLC; polar metabolites elute prior to parent compounds on a reversed phase column which allows for separation and LSC analysis. Evaluation of radioactivity in the aqueous fraction results in quantification of secondary, conjugated metabolites.

FINDINGS TO DATE

We have performed a set of experiments to evaluate the rates of uptake, transformation, binding and elimination of a model organic contaminant benzo[a]pyrene (BaP) and its metabolites in an estuarine benthic amphipod *Leptocheirus plumulosus*. The results from this experiment indicate that uptake of BaP occurs rapidly with a steady state body burden of pollutant approached within the first few hours of exposure to contaminated sediments (Fig. 1). This rapid accumulation of BaP by *L. plumulosus* is thought to be due to sorption of the contaminant to the surface of the organism. The rates of formation of aqueous soluble metabolites of BaP, and binding of contaminant pools to cellular material, are also rapid in *Leptocheirus* (Fig. 1).

Elimination of BaP and primary metabolites (organic fraction) from *L. plumulosus* occurs rapidly as well (Fig. 1). Fast uptake and concurrent elimination indicates that large amounts of contaminants are processed by the organism when exposed to polluted sediments.

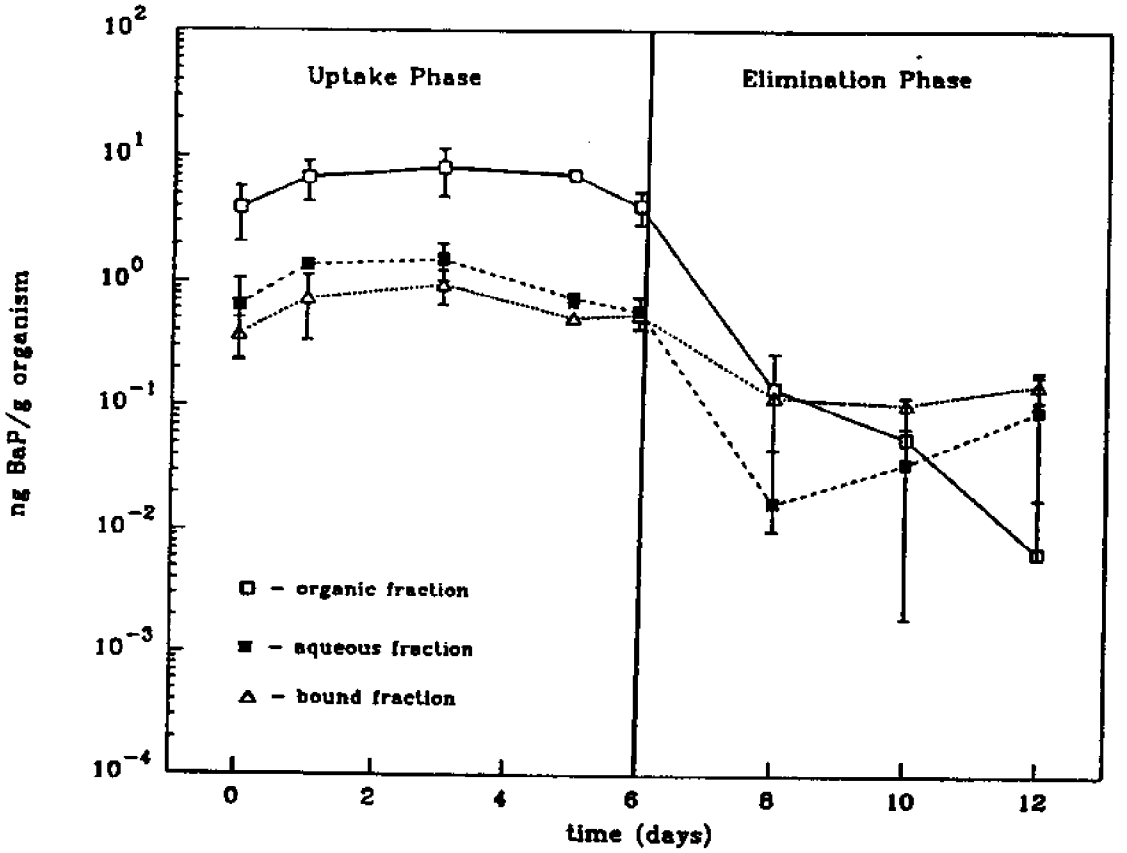

Fig. 1. Uptake, production of metabolites, and elimination of benzo[a]pyrene (BaP) and metabolites from *Leptocheirus plumulosus*. Organic fraction = BaP + primary metabolites, aqueous fraction = conjugated (secondary) metabolites, bound fraction = OC/metabolite associated with cellular material.

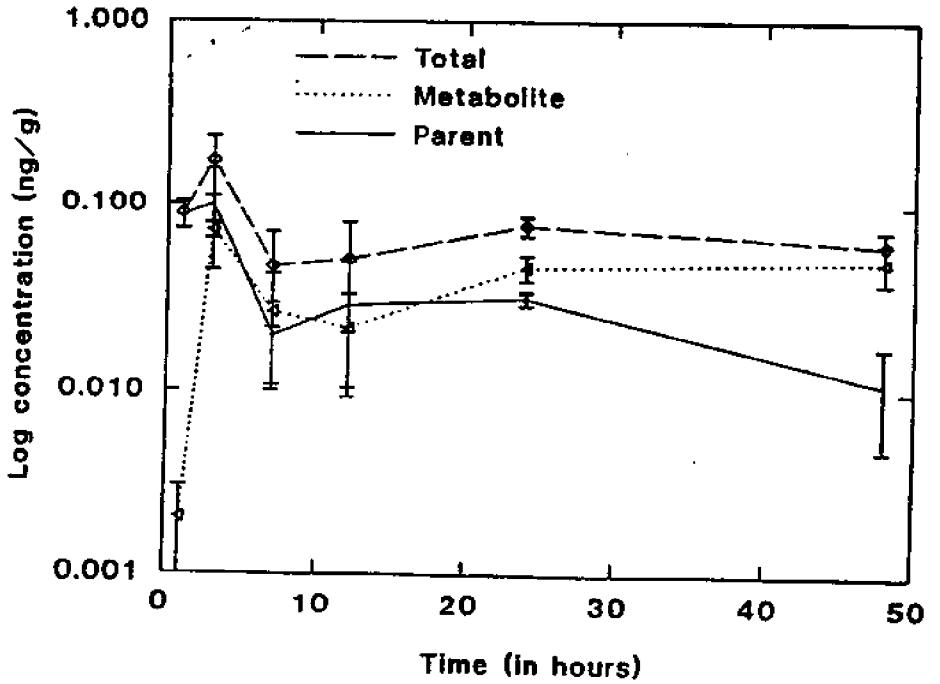

Fig. 2. Production of metabolites and elimination of pyrene and metabolites from spot (*Leiostomus xanthurus*) after ingestion of contaminated food. Parent = pyrene + primary metabolites, metabolite = conjugated (secondary) metabolites, total = parent + metabolite.

Rapid processing rates by organisms of potentially toxic substances increases overall exposure to contaminants within the body, and thus, the possibility of adverse effects.

From the data collected in the experiment described above, and that from our previous experiments (Schaffner and Dickhut, 1991), we are developing a bioaccumulation/ transformation model to predict contaminant and corresponding metabolite body burdens in benthic macrofauna. This kinetic model predicts contaminant and metabolite pools in the macrofauna from a variety of concurrent first order processes occurring within the organisms. Important mechanisms determining contaminant and metabolite levels within the macrofauna include: uptake of parent compound from sediments, elimination of parent compound and metabolites from the organism, formation of metabolites, and loss of contaminants from the sediments.

We have also initiated experiments to evaluate the rates of contaminant elimination and metabolite formation in demersal predators. Pyrene spiked food was fed to spot (*Leiostomus xanthurus*). The rates of formation of metabolites, and elimination of parent compound and metabolites, were determined (Fig. 2). As with *L. plumulosus* metabolites rapidly form, and within 24 hr after ingestion, dominate the organism body burden of contaminant. The organism body burdens of OC and metabolites in the predators can thus be modeled similarly to that of the benthic macrofauna. In subsequent experiments, we will evaluate the transfer of a variety of OCs with varying physical-chemical behavior, and their biotransformation products, from benthic macrofauna to demersal fish. The occurrence of metabolism in both predator and prey organisms adds complexity to evaluating and modeling the food chain transfer of OCs. Nonetheless, through sequential experimentation we will determine the parameters required for modeling organic contaminant transfer through the benthic food web of Chesapeake Bay.

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ROLE OF SEDIMENT ASSOCIATED POLLUTANTS IN INFECTIOUS DISEASE SUSCEPTIBILITY IN THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA* [R/CBT-5]

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OBJECTIVES

The four objectives of this study are to examine:

1) the behavior of selected polycyclic aromatic hydrocarbons (PAHs) in the proposed sediment/water exposure system;
2) the *in vitro* effects of WSFs, derived from creosote-contaminated Elizabeth River sediments and laboratory generated sediment-sorbed PAHs on oyster hemocyte activities;
3) the dosages and duration of exposure to PAH-contaminated sediment required to increase the oysters' susceptibility to *Perkinsis marinus* (Dermo); and
4) the assimilation of selected PAHs from laboratory-contaminated sediments by oysters.

RATIONALE

Pollutants have been observed to affect several immune system components of aquatic organisms in the laboratory. However, reports of their effects on actual susceptibility to infectious disease have generally been anecdotal in nature. Recently, we reported that exposure to pollutants, derived from estuarine sediments heavily contaminated with polycyclic aromatic hydrocarbons (PAHs), affected the susceptibility of oysters to Dermo in a dose-related manner.

Additional data on the mode(s) of action of dissolved compounds derived from contaminated estuarine sediments are needed. Both *in vitro* and *in vivo* approaches will be employed. Information regarding the effects of sediment sorbed contaminants are also required. Therefore, specific PAHs, commonly present in contaminated Chesapeake Bay sediments, will be tested to examine their affect on disease resistance and potential mode of action. Thus, these studies address issues regarding not only toxic effects, but also bioavailability and disposition of toxic compounds of concern in the Chesapeake Bay system.

CHARACTERIZATION OF MATERIALS USED IN EXPOSURES

Oysters - Test oysters used in all but the current ongoing exposure study (see Experiment III below) were obtained from a commercial source in Maine, USA. Oysters for Experiment III were obtained from Deep Water Shoals, VA, an area of low Dermo prevalence.

Elizabeth River Water Soluble Fraction (ER-WSF) - The ER-WSF was obtained by stirring filtered York River water with creosote contaminated Elizabeth River sediments for one hour, allowing particulates to settle overnight and removing the remaining suspended sediment by filtration.

PAH amended sediment - Material for use in PAH-amended sediment studies was collected from a relatively pristine freshwater tributary of the York River. To obtain a more reproducible matrix, sediment was sieved before use (approximately 90% of the particles remaining were less than 50 μm and about 50% less than 10 μm). Oysters rapidly removed these small sediment particles from suspension. PAHs selected for study [fluoranthene, pyrene, chrysene, benzo(a)pyrene, benzo(e)pyrene and benzo(ghi)perylene] have low water solubilities and preferentially bind to sediments. They also are dominant contaminants in creosote contaminated Elizabeth River sediment. As expected, the target PAHs remained associated with the sieved material when amended sediment was added to filtered York River water, under the proposed exposure conditions. The initial sediment PAH load was 20 mg/kg (wet weight basis). Sediment and water were separated by filtration and the PAHs therein determined. Water concentrations ranged from a maximum of 2 μg/l (ppb) for fluoranthene to less than 0.2 μg/l for benzo(ghi)perylene. Details of specific experiments in which oysters are exposed to pollutants are provided below.

APPROACH AND FINDINGS TO DATE

I. In vitro effects of exposure on oyster hemocyte viability and activities.

A. Effect of ER sediment-sorbed pollutants: The utility of chemiluminescence (CL), phagocytosis, and chemotaxis measurements are limited since sediment particles aggregate with hemocytes when added to hemocyte suspensions. Therefore, only viability of hemocytes exposed to ER sediment-associated pollutants was assessed here. Exposure of hemocytes to these pollutants for 4 h resulted in some cell deaths, although the results were not statistically different from controls (Fig. 1A & 1B). Hemocyte viability was assessed by neutral red and trypan blue assays.

B. Effect of ER-WSFs: Previously, we demonstrated a trend toward immunomodulation (CL response, phagocytosis, and chemotaxis) in hemocytes exposed to ER-WSFs, although results were not statistically different from controls. This work was repeated with additional replication. Results agreed with our previous observations (see below).

1. **Cell viability:** Similar to the case in which hemocytes were exposed to contaminated ER sediment, ER-WSFs did not cause significant hemocyte mortality until after 24 hr of incubation with a 50% dilution of the ER-WSFs (Fig. 2).

2. **CL, phagocytosis and chemotaxis responses:** ER-WSFs appeared to affect the CL (Fig. 3), phagocytic (Fig. 4A) and chemotactic (Fig. 4B) responses of the hemocytes when exposed to 30 and 50% dilutions of WSFs. However, the results were not statistically different from the control ($p > 0.05$, One-Way ANOVA). The phagocytic index decreased with incubation time ($p < 0.05$, Two-Way ANOVA).

II. *In vivo* exposure of oysters to sediments amended with selected PAHs in the laboratory to assess immune system effects (no laboratory Dermo challenge).

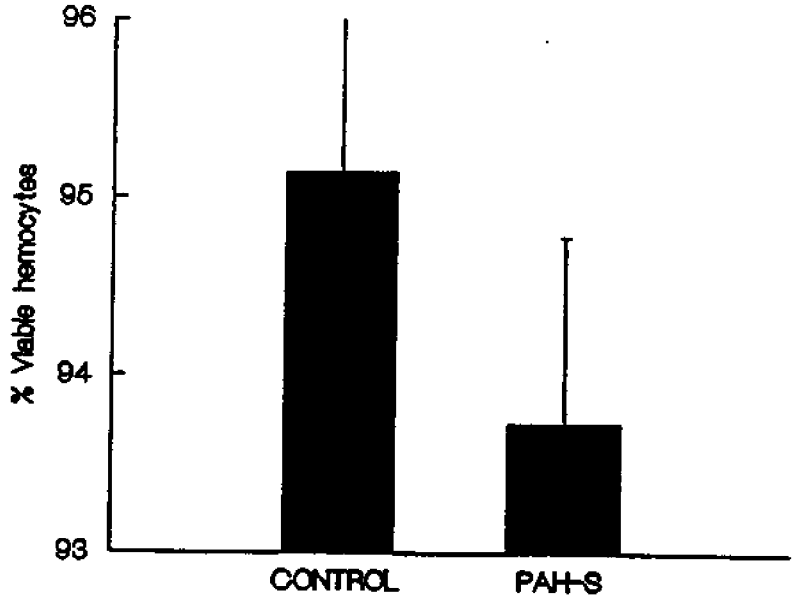

Fig. 1A. viability of *In vitro* PAH-S exposed hemocytes determined by trypan blue assay. PAH-S = PAHs contaminated sediment. N=2

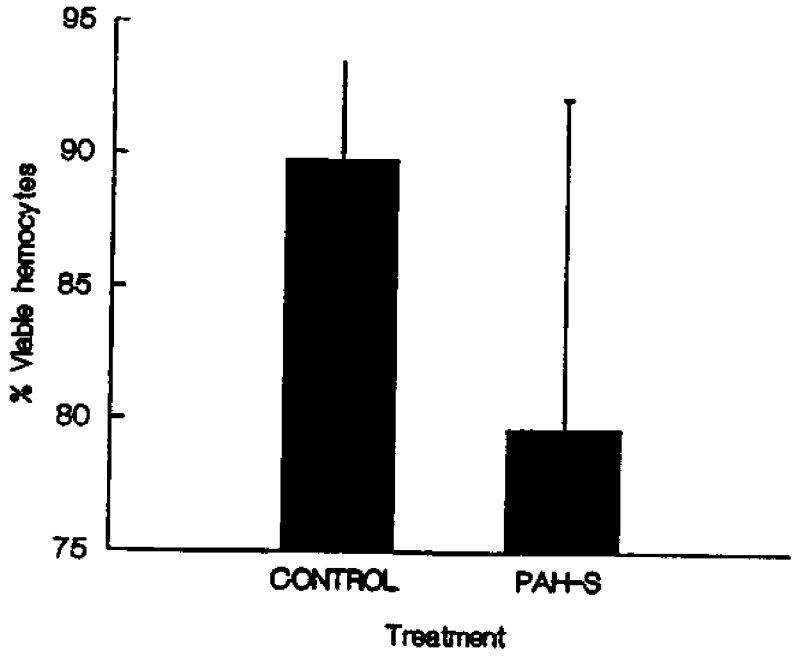

Fig. 1B. viability of *In vitro* PAH-S exposed hemocytes determined by neutral red assay. PAH-S = PAHs contaminated sediment. N=2

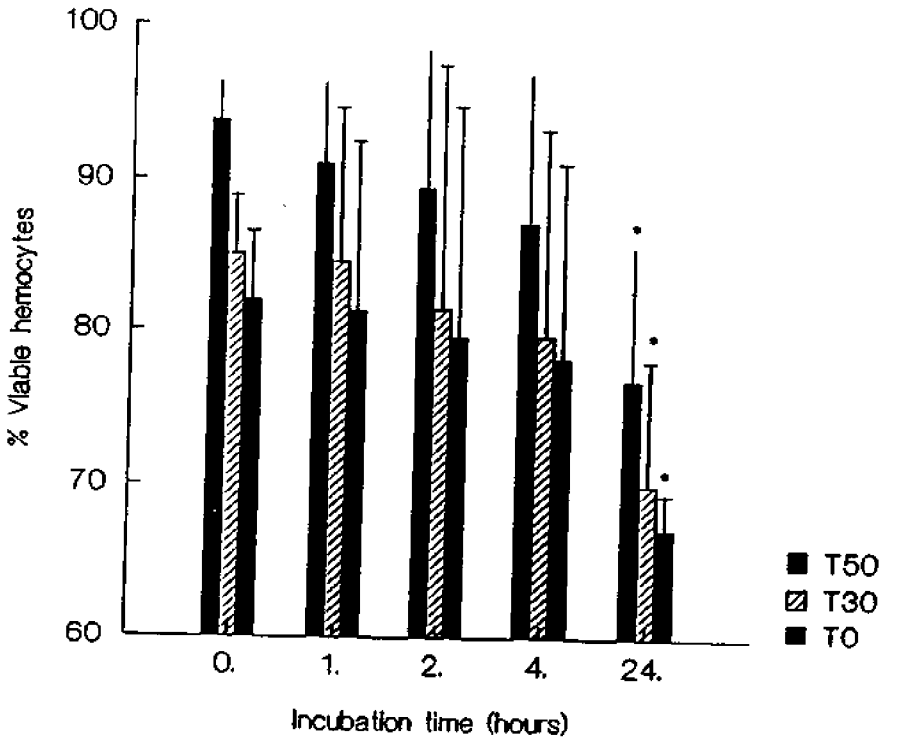

Fig. 2. Viability of *in vitro* WSF-exposed hemocytes determined by trypan blue. T50, T30 and T0 are 50%, 30% and 0% WSF respectively. "*" indicates significance at $p < 0.05$. WSF = Water soluble fraction. N=4-10

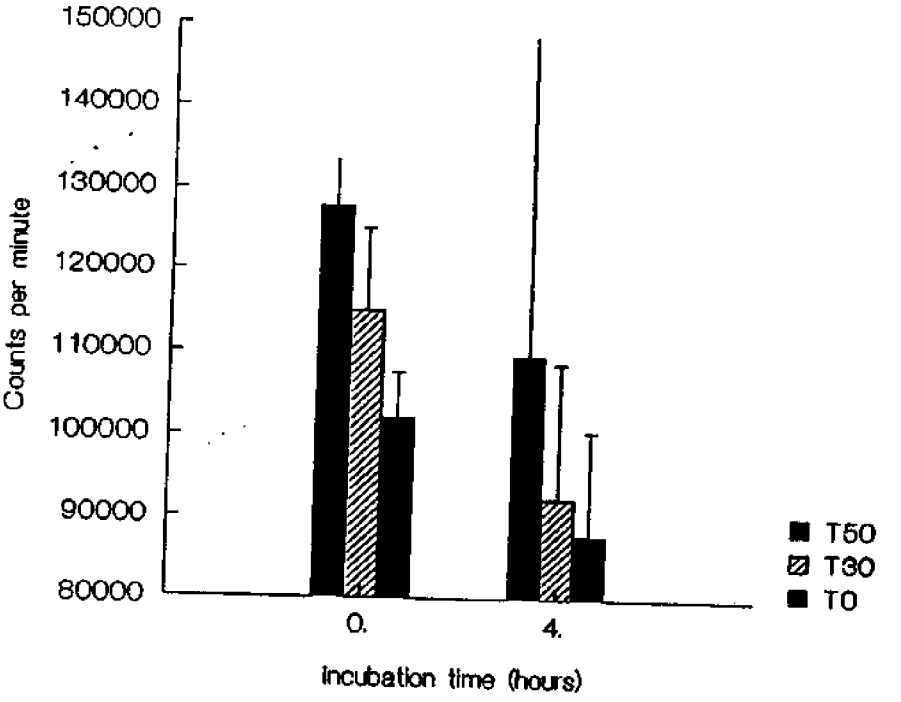

Fig. 3. Chemiluminescence of *in vitro* WSF-exposed hemocytes. T0, T30 and T50 are 0%, 30% and 50% WSF respectively. WSF = Water soluble fraction. N=4.

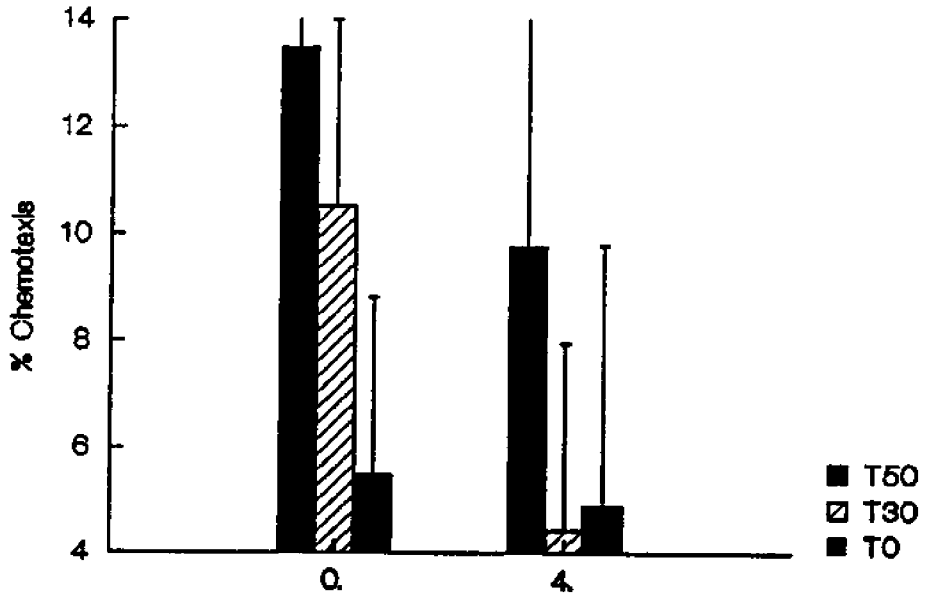

Fig. 4A. % Phagocytosis by *in vitro* WSF-exposed hemocytes. T0, T30 and T50 are 0%, 30% and 50% WSF respectively. WSF = Water soluble fraction. N=3.

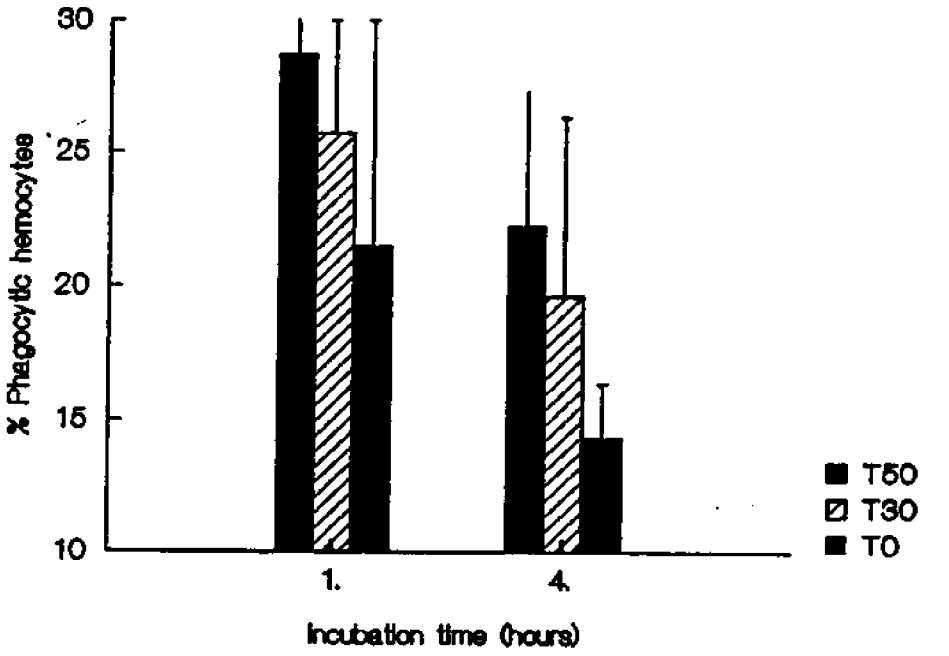

Fig. 4B. Chemotaxis of *in vitro* WSF-exposed hemocytes. T0, T30 and T50 are 0%, 30% and 50% WSF respectively. WSF = Water soluble fraction. N=4.

Oysters were maintained in individual 2 l glass jars with aerated, filtered York River water and were fed algal paste twice daily. Two test groups were used, each contained 20 oysters. Oysters in the PAH-exposed group were provided, on a daily basis, 5.0 ml of a sediment slurry containing 5 μg of each target PAH (PAH-S), while control oysters received an identical aliquot of uncontaminated sediment (C-S). Water was changed daily and dissolved oxygen, salinity and temperature monitored. Thirty days after initiation of sediment exposure, ten oysters from each treatment were sacrificed to examine PAH accumulation. Hemolymph samples were taken from the remaining ten oysters in each treatment. In addition to measuring hemocyte CL, phagocytosis, and chemotaxis, the incorporation rate of ^{3}H-thymidine (DNA precursor), ^{3}H-urine (RNA precursor) and ^{3}H-leucine (protein precursor) in hemocytes were evaluated to determine if syntheses of these important cellular macromolecules were affected by exposure to PAH-amended sediments. Three replicates of 10^6 pooled hemocytes, from PAH exposed or control oysters, were incubated with 1 μCi labeled thymidine, uridine and leucine for three hr. Exposure of oysters to sediments was then continued for an additional 30 days. Subsequently, hemolymph was sampled from the remaining oysters and the above parameters measured.

RESULTS:

A. *Incorporation of ^{3}H-labeled DNA, RNA and protein precursors:* Exposure to sediment associated PAHs for 30 days appeared to significantly impair ($p < 0.05$, Student-T test) the hemocytes' ability to synthesize DNA, RNA and protein (Fig. 5). After 60 days of exposure to sediments, the overall uptake of these three precursors by hemocytes declined in both control and PAH-containing sediments and no significant differences were observed between test groups.

B. *Chemiluminescence:* CL responses in hemocytes from PAH-amended and control sediment exposed oysters were quantified by integrating the area under the CL curve (expressed as counts per minute). Exposure of oysters to sediment associated PAHs for 60 days significantly reduced the CL response in hemocytes ($p < 0.05$, Students T-test, Fig. 6).

C. *Phagocytosis:* Phagocytosis of laboratory cultured Dermo merozoites and zymosan (yeast cell extracts) by hemocytes from PAH-S exposed and control oysters was determined. Differences in phagocytic index (number of hemocytes that phagocytosed and/or associated with at least one merozoite or zymosan particle/total number of hemocytes) was assessed using Two-Way ANOVA. PAH-S exposure significantly decreased phagocytic activities of the hemocytes ($p < 0.05$, Fig 7A).

D. *Chemotaxis:* Both laboratory cultured *P. marinus* merozoites and zymosan were used as stimulants for chemotactic assays. Results indicated that 60 days of PAH-S exposure significantly suppressed the chemotactic response of the hemocytes ($p < 0.05$, two-way ANOVA, Fig. 7B).

E. *Filtration, feeding rate and DO:* Filtration rates of PAH-contaminated particles by the oysters were significantly lower compared to those given uncontaminated control sediment, in oysters sampled 4.5 hrs after addition of sediments (Fig. 8). No differences in dissolved oxygen were noted between the water dosed with PAH-S and control sediments.

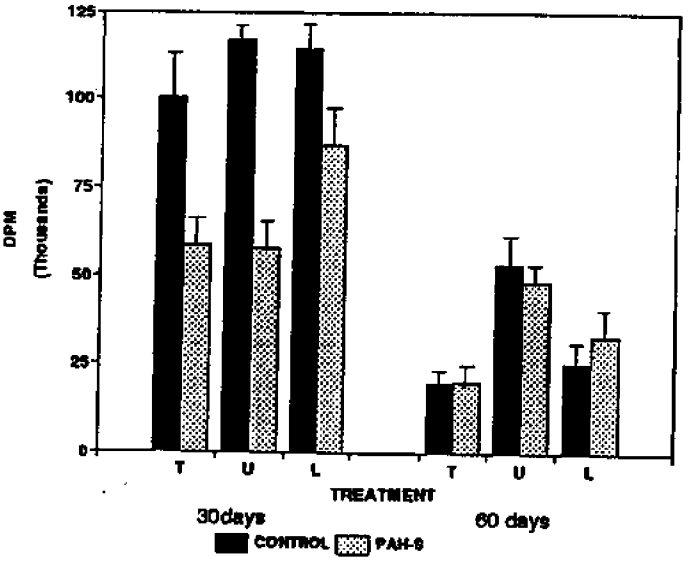

Fig. 5. DNA, RNA and protein synthesis by hemocytes from oysters exposed to PAH-S or control sediment. T=Thymidine, U=Uridine, L=Leucine. PAH-S = PAHs contaminated sediment. N=3.

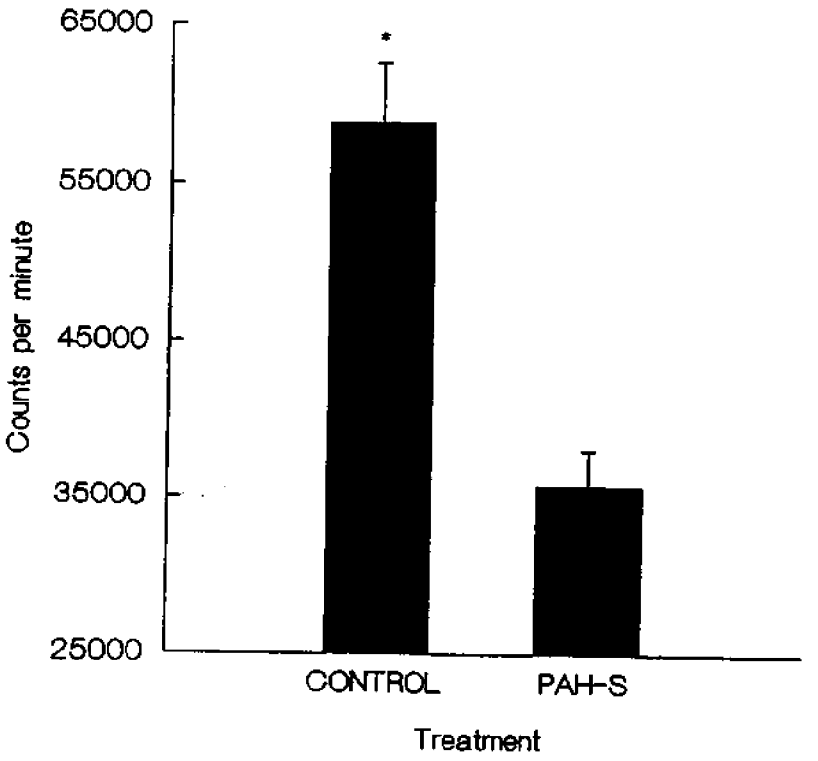

Fig. 6. CL of pooled hemocytes from oysters exposed to PAH-S or control sediment. "*" indicates significant difference ($p < 0.05$) between treatments. PAH-S = PAHs contaminated sediment. N=3.

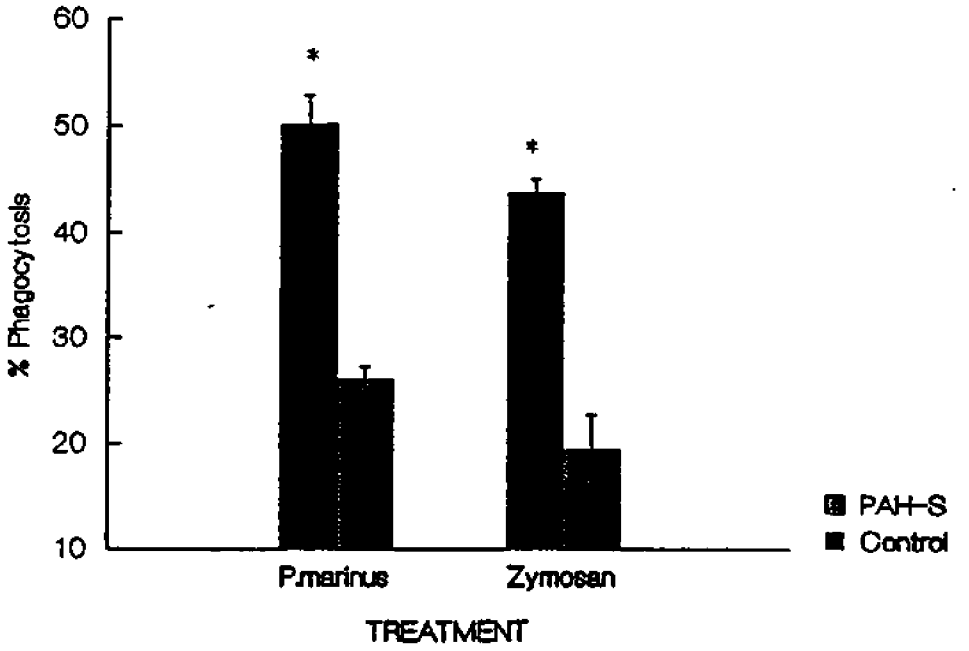

Fig. 7A. % phagocytosis by pooled hemocytes from oysters exposed to PAHs or control sediment. "*" indicates significant difference between treatments PAH-S = PAHs contaminated sediment. N=3.

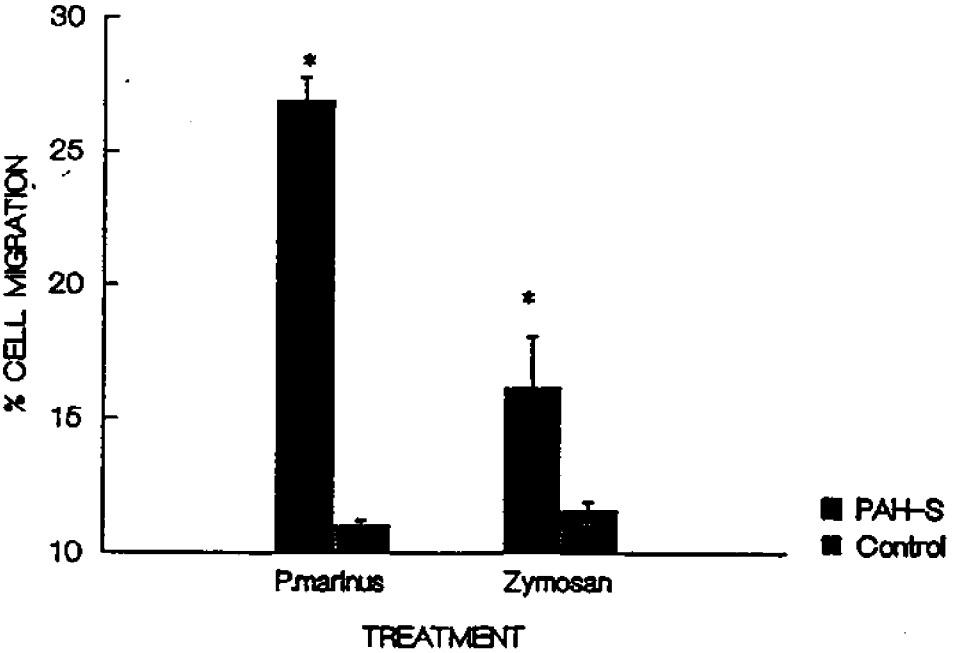

Fig. 7B. Chemotaxis of pooled hemocytes from oysters exposed to PAHs or control sediment. "*" indicates significant difference between treatments PAH-S = PAHs contaminated sediment. N=3.

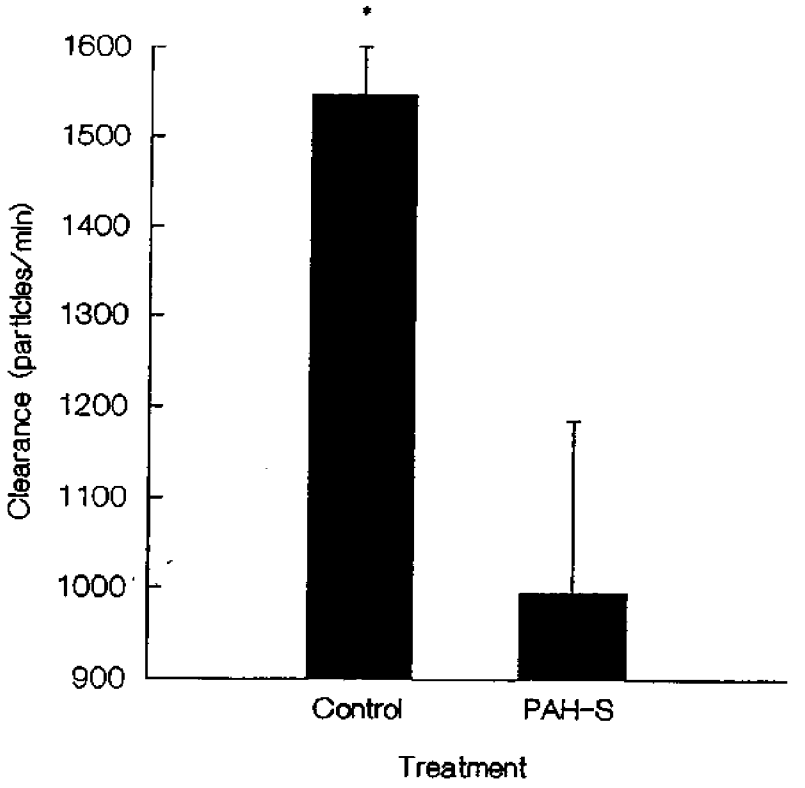

Fig. 8. Clearance of sediments by oysters exposed to PAH-S or control sediment. "*" indicates significant difference ($p < 0.05$) between treatments. PAH-S = PAHs contaminated sediment. N=4 oysters.

F. Bioaccumulation of PAHs from sediments: Improved methods for the analysis of the target PAHs are under evaluation. Supercritical fluid extraction (SFE) is being examined as a means to remove PAHs from sediment and shellfish samples. HPLC on a C_{18} column, with fluorescence detection, is being evaluated as a more sensitive and selective alternative to GC/FID. Analysis of oysters using conventional soxhlet extraction and HPLC/fluorescence show that PAH, in the mg/kg range (wet weight basis), are being accumulated by the oysters (Fig. 9).

III. Dosages of contaminated sediment required to increase susceptibility of oysters to Dermo.

An experiment has been initiated to determine whether specific sediment-borne PAHs increase disease susceptibility. Three test groups (60 oysters per group) are being exposed daily to either 10 ml C-S, 5 ml C-S plus 5 ml PAH-S (5.0 μg of each target PAH) or 10 ml of PAH-S (10.0 μg of each target PAH). Oysters are being maintained in individual 2 liter glass jars. Thirty days after initiation of sediment exposure, 50% of the oysters from each treatment (PAH-exposed and controls) were challenged with freshly isolated *P. marinus* merozoites (10^3 /oyster). This experiment will conclude in December 1994.

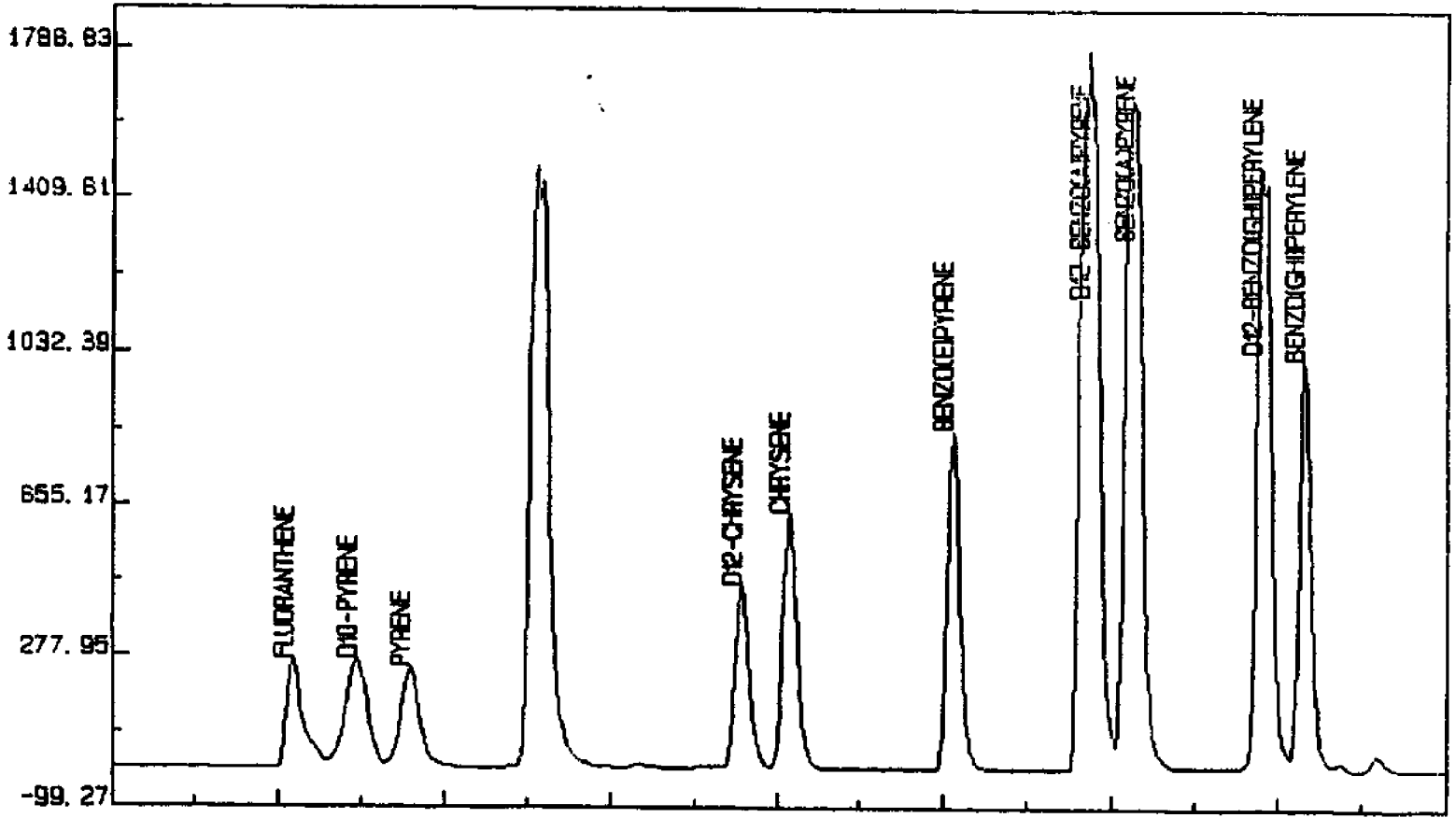

Fig. 9. HPLC/fluorescence chromatogram of an extract of an oyster exposed to sediment spiked with fluoranthene, pyrene, chrysene, benzo(a)pyrene, benzo(e)pyrene and benzo(ghi)perylene. The deuterated internal standards shown were added just prior to soxhlet extraction.

USE OF FISH AND OYSTER CELL CULTURES IN THE STUDY OF TOXIC EFFECTS OF CHEMICAL POLLUTION IN THE CHESAPEAKE BAY [R/CBT-6]

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RATIONALE

The need for assessment of the toxicity of xenobiotics released into the aquatic environment of the Chesapeake Bay has stimulated the search for bioassays from which reliable information can be obtained quickly. In the last decade, there has been an increasing interest in the use of *in vitro* cell assays as alternatives to the classical 96 hr-LC_{50} determination using living fish. A major advantage of using cell assays in assessing the toxicity of a chemical is that it allows the elucidation of the mechanism of action.

Among the Chesapeake Bay contaminants, polynuclear aromatic hydrocarbons (PAH) have received special attention due to their abundance and possible association with liver cancers and immune dysfunction in exposed fish. Some of the high molecular weight PAH can be transformed into electrophilic metabolites that bind to cellular macromolecules, thus inducing a variety of genotoxic effects. On the other hand, low molecular weight PAH compounds target the cellular membrane or some vital organelles causing toxic cell damage.

The aim of this study was to examine the feasibility of using cultured cells of fish and oysters in a short-term assay to assess the cyto- and genotoxic effects of PAH compounds and PAH-polluted sediments.

RESEARCH OBJECTIVES

The overall objective was to standardize cell culture systems from fish and from oysters and use them to assess the biohazardous effects of Elizabeth River sediments in areas dominated by PAH contamination and individual PAH compounds.

APPROACH

As hepatocytes are the primary target of toxic chemical aggression, our laboratory has developed protocols to culture hepatocytes of Chesapeake Bay fishes. Some of these cultures have developed into immortal cell lines. Cells of the digestive diverticulum of the Eastern oyster (*Crassostrea virginica*) were also cultured using a special synthetic medium. Confluent monolayers were exposed to a series of tenfold dilutions of the PAH-compound for 96 hrs. The effects of exposure were calorimetrically measured using four techniques:

1) Crystal violet staining which determines the confluence of the cell sheet; 2) Uptake of neutral red, a supravital stain, and its accumulation in the lysosomes of viable, uninjured cells; 3) The MTT assay that is based on the reduction of the soluble yellow MTT tetrazolium salt to a blue insoluble MTT formazan product by mitochondrial succinic dehydrogenase; and, 4) ^{3}H-thymidine/uridine uptake which measures the rate of nucleic acid synthesis.

Genotoxic effects were evaluated by chromosomal damage, induction of micronuclei, and anaphase aberration.

SUMMARY OF FINDINGS

Comparison between the Endpoints of Toxicity

Our results have clearly shown that cell attachment to the substrate (measured by crystal violet staining and neutral red uptake) is superior to other tested endpoints for cytotoxicity. In the addition, readings of both techniques were automated using a Photometer/ELIZA-plate reader. In the case of oyster cells, the uptake of neutral red and of ^{3}H-uridine were superior to all other cytotoxic endpoints.

The results also indicated that anaphase aberration using fish hepatocytes is the test of choice to assess PAH-induced genotoxic effects.

Assessment of the Toxicity of Elizabeth River Sediments

All Elizabeth River sediments were toxic to cultured fish and oyster cells. The degree of cytotoxicity differed, however, from one sediment to the other. The highest cytotoxicity was obtained with the Station 217-sediment [10^5 Tissue Culture Cytotoxic $Dose_{50}$ ($TCCD_{50}$/ml of the organic sediment extract)]. At high dilutions, two of the sediments (217 and Atlantic Wood) showed significant increases in cell proliferation. One other sediment (Craney Island) inhibited cell division at low concentrations and was cytotoxic at higher concentrations. In general, a sediment extract was considered cytotoxic if it contained more than 10^2 $TCCD_{50}$/ml of the sediment extract. By combining cytotoxic and genotoxic tests, we were able to demonstrate four different responses following exposure to contaminated sediment extracts: a) non-cytotoxic/non-genotoxic (this was only found in the control York River site), b) cytotoxic/genotoxic such as 217 and Atlantic Wood sediments, c) genotoxic/non-cytotoxic, d) cytotoxic/non-genotoxic.

A linear correlation was found between the total PAH and cytotoxicity (r^2=O.87). This correlation was more evident with the concentrations of the low molecular weight PAH (r^2=O.96).

On the other hand, no correlation could be found between genotoxicity and total PAH in the sediment. A barely significant correlation was found between the concentration of benzo(a)pyrene and visible chromosomal macrolesions (r^2= O.7 1).

Assessment of the Toxicity of Individual PAH compounds

Screening 16 PAH compounds using hepatocytes indicated that naphthalene is the most

toxic chemical (9.8 x 10^8 $TCCD_{50}$/ml), followed by phenanthrene (5.5 x 10^5 $TCCD_{50}$/mi). Methylcholanthrene induced the highest genotoxic effects (53 anaphase aberration/2000 cells) and 35 micronuclei/5000 cells.

Interestingly, benzo(a)pyrene at low concentrations (10_{-12}-10_{-4} M) stimulated the cell division, i.e., the number of cells per well increased. Higher concentrations were, however, toxic.

METABOLISM OF PAH IN CULTURED HEPATOCYTES

In order to form confluent monolayers, hepatocytes need 8-10 days. Hence, it was important to determine whether hepatocytes were capable of metabolizing high molecular weight PAH after being in culture for over 10 days.

Tritiated benzo(a)pyrene [B(a)P] was incubated with the five hepatocytes (up to I 1 days in culture) for 24 hrs after which the culture supernatants were analyzed with HPLC. Nine metabolites were identified, the most predominant of them is the B (a)P-9, 10-dihydrodiol.

Experiments were performed to determine the correlation between the median tissue culture cytotoxic dose ($TCCD_{50}$) and median lethal dose (LC_{50}) of Elizabeth River sediment extracts using juvenile spot (*Leiostomus xanthurus*) have shown a positive correlation (r^2=O.93). Moreover, in eight out of 16 sediment extract samples, the $TCCD_{50}$ were at least two logs higher than their LC_{50} indicating a higher sensitivity of the *in vitro* cytotoxicity assays.

RESULTS

The overall conclusion of the results obtained to date suggests that assessing toxic effects of sediments using cell culture techniques is both an accurate and cost effective means to assess the consequences of PAH pollution.

EFFECTS OF TRACE METALS AND ORGANIC POLLUTANTS ON STRESS-INDUCED PROTEINS IN OYSTER LARVAE AND SPAT: A MOLECULAR APPROACH [R/CBT-17]

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RATIONALE

The oyster population in the Chesapeake Bay has decreased dramatically during the last three decades. This decrease coincides with an increased release of municipal and industrial waste into the bay. Toxic chemicals present in this waste appear to be harmful to oysters, and are believed to be partially responsible for the population decline. Two classes of chemical pollutants present in this waste pose the greatest threat to oysters. These are: (i) trace heavy metals such as As, Cd, Cr, Cu, Hg, Sn, and Zn; and(ii) organic compounds such as pesticides, phthalate ester, polynuclear aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). High levels of both types of chemicals have accumulated in the sediment of the bay. Since oysters are sedentary bottom dwelling organisms, they are exposed to extremely high concentrations of these toxic substances. Although there are sensitive methods available for measuring the levels of heavy metals or organic pollutants accumulated in oyster issues, it is still unclear as to how these chemicals affect the developmental processes and the physiology of these organisms. Therefore, in order to restore oyster fisheries in the bay, it is important to: (i) study the interaction of these chemical pollutants with oysters at various developmental stages, and (ii) determine their associated biological effects.

It is well known that exposure of prokaryotic and eukaryotic cells to heat shock results in the synthesis of a set of cellular proteins with apparent molecular weights of 110K, 80-90K, 69-70K and 16K-28K. These proteins have been called heat shock proteins. These heat shock proteins are now referred to as stress-responsive proteins since they are also induced by a variety of stimuli such as heavy metals, pesticides, viral infection, and inflammatory agents. Studies conducted in mammalian cells, fish cells, insect cells, yeast, and *E coli* reveal that some of these stress-responsive proteins are highly conserved. Although the biological roles of these proteins are not fully understood, they have been implicated in several major biological phenomena such as embryogenesis and differentiation, viral infection, growth rate and metabolism, and protection from phenocopy induction and thermotolerance. Therefore,

the stress-responsive genes can not only be used as stress indicators in aquatic organisms but also serve as a model system for studying the biological effects of organic and inorganic chemical pollutants.

RESULTS

This project was funded at a much reduced amount compared to the original request. The agency funded this project at $40,000 for one year to generate results for feasibility studies. For this reason, two objectives were set to be achieved in this year. These objectives were: (i) Are there stress-responsive proteins induced in oyster larvae and spat under heat and other stress? (ii) Can one clone cDNA of the stress-responsive proteins? The results of the project are summarized below.

Several specific proteins were induced by heat treatment in larvae and spat of oysters. The molecular weights of these heat-induced proteins are in the ranges of 70 KDa, 40 KDa and 20 KDa. While most these proteins were induced in both larvae and spat, some of these proteins were either larval-specific or spat specific. Similar patters of protein synthesis were observed when oyster larvae or spat were incubated in sublethal levels of heavy metals or pesticides. However, due to low levels of protein synthesis, the overall protein patters were not very conclusive.

In order to improve the sensitivity, we decide to develop a series of stress-responsive cDNA by molecular cloning. From a cDNA bank prepared from RNA of heat-induced oyster larvae, a heat-induced cDNA clone was isolated. Nucleotide sequence determination revealed that this cDNA encoded a protein homologous to Hsp-70. In addition, we have developed a quantitative hybridization method for determining the levels of hsp7O mRNA in oyster tissues following heat shock treatment or exposure to other stressors.

A step toward identifying more molecular markers that may be responsive to stress as well, we have isolated a portion of cDNA for *c-myc* and insulin-like growth factor (IGF) by PCR amplification. The expression of *c-myc* and IGF genes in oyster larvae and spat under various stress conditions will be investigated.

INTERACTION OF COPPER AND CADMIUM WITH MICROBIAL BENTHOS BIOFILM AND EFFECTS ON OYSTER LARVAL SET [R/CBT-19]

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RATIONALE

We proposed to study heavy metal bioconcentration by microbial biofilms because it has been well documented that many marine microbial biofilms are comprised primarily of anionic exopolysaccharide (EPS), that such films cover nearly all marine surfaces, and are a significant part of neritic sediments. These biofilms have been found to sequester numerous cations including toxic metals. Microbial biofilms are also beneficial to invertebrate set-- i.e., microfouling precedes macrofouling. Thus, metals of concern such as Cd and Cu may be bioconcentrated on surfaces that are also important for oyster larvae to initiate metamorphosis, a process shown by several laboratories to be extremely sensitive to the presence of heavy metals.

OBJECTIVES

a) Increase the sensitivity and resolution of Cd and Cu analysis to study the behavior oftrace levels of these metals in biofilms and their effects on oyster larval metamorphosis.
b) Assess Cd and Cu concentrations in natural waters and biofilms.
c) Determine Bioconcentration Factors (BCF) of autochthonous Chesapeake Bay and laboratory strain biofilms.
d) Determine the effects of dissolved Cd and Cu on oyster larval search and set.
e) Determine the effects of natural and laboratory strains of biofilm on oyster larval set both in the presence and absence of metals.

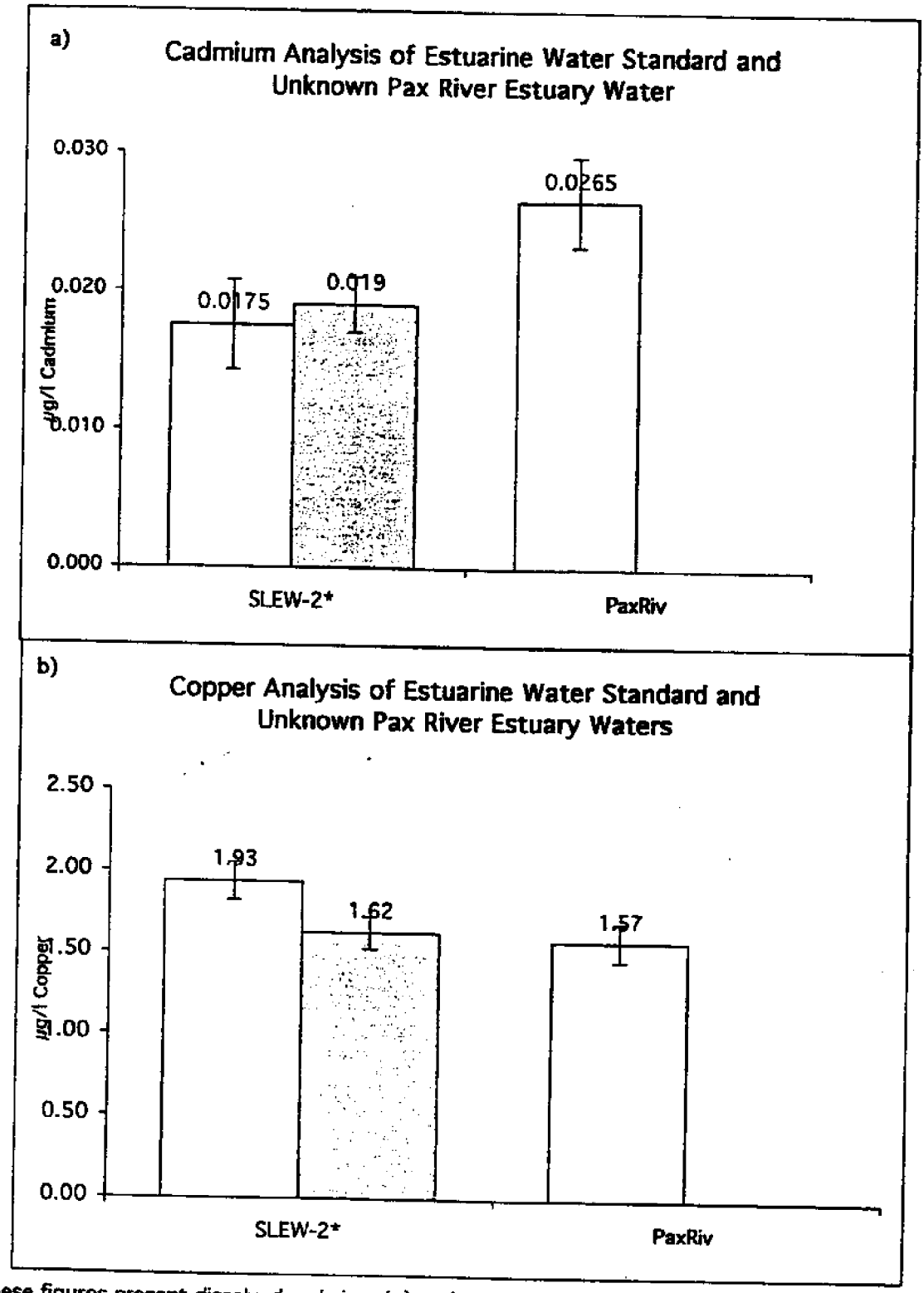
OVERVIEW

We have greatly increased the sensitivity and resolution of our Cd and Cu analysis in Chesapeake Bay waters by adapting an organic extraction technique developed by Brueland et.al. (1979), in collaboration with Dr. Jim Sanders and Dr. Fritz Riedel at the Academy of Natural Sciences Estuarine Research Laboratory (Table 1). Sample waters containing ng/l concentrations of Cd and Cu have been analyzed using this technique in conjunction with graphite furnace atomic absorption spectroscopy (GFAAS) at levels that are relevant in Chesapeake Bay (Table I & Fig. 1).

Tabel 1. Dissolved Cadmium and Copper concentrations in natural waters:

Site	Cd µg/l (ppb)	Cu µg/l (ppb)	Source
Chesapeake Bay, USA	0.007-0.10	0.10-4.70	NBS (1985)
Estuarine Water, England	ND	1.920-5.715	Van den berg et.al. (1986)
Inner Oslofjord, Norway	ND	0.520-1.420	Hassle et.al. (1981)
San Diego Bay, CA	0.060-0.190	0.882-2.772	Flegal et.al. (1993)
Shark Bay, Australia	0.060-0.50	0.330-29.20	McConchie et. al. (1988)
South San Francisco Bay, CA	0.055-0.165	1.386-4.60	Flegal et.al. (1993)
Gulf of St. Lawrence, Canada	0.010-0.031	ND	Cossa (1988)
Atlantic Ocean	0.0003-0.046	0.070-0.108	Danielsson et. al. (1985) & Bruland & Franks (1983)
Pacific Ocean	0.0007-0.054	0.034-0.192	Mart et. al (1982), Nurnberg & Mart (1985), & Bruland (1980)
Atlantic Ocean Interstitial Waters	0.10-0.90	0.600-10.80	Kosov &Demidova (1986)
Pacific Ocean Interstitial Waters	0.10-0.20	0.100-5.60	Kosov & Demidova (1986)
Baltic Sea	0.016-0.058	0.318-5.080	Cossa(1988) & Brugmann (1981)
Irish Sea	0.016-0.033	1.016-2.476	Cossa (1988) & Constant et. al. (1984)
Mediterranean Sea, Israeli Coast	0.0006-0.0029	1.5-3.2	Roth & Hornung (1977)
Tasman Sea	0.0032-0.0062	0.060-0.101	Mart et.al. (1982)

Fig. 1: Cadmium and Copper in Patuxent River Estuary



These figures present dissolved cadmium (a) and copper (b) concentrations in the Patuxent River estuary near Benedict, Maryland. In each assay n=3. Error bars represent ± the Minimum Significant Difference. *SLEW-2 is a standard referrence water

Our findings estimate concentrations of Cd ranging from 0.01-0.04 μg/l and Cu concentrations ranging from 1-3 μg/l in the Patuxent River estuary (Fig. 1). Autochthonous biofilm Bioconcentration Factors (BCF, a vol./vol. ratio) for Cd range from 420-3600 times ambient dissolved metal concentrations (Fig. 2), and BCF for Cu range from 240860 (Fig. 3).

We have developed powerful bioassays to determine the effects of dissolved heavy metals on *Crassostrea* larval search behavior and metamorphosis. Concentrations normally found in Chesapeake Bay of both Cd (0.007-0.1 μg/l) and Cu (0.9-5.714 μ/l) (Table 1) had little effect on oyster larval behavior or metamorphosis (Fig. 4 and Table 2 respectively). However, dissolved Cd concentrations as low as 1 μg/l significantly reduced (to 14% $\pm$ 4% vs. 27%$\pm$ 4% controls; $p \leq 0.05$) oyster larval metamorphosis on biofilms (Fig. 5). This concentration is 10-50 times greater than commonly found dissolved in the Chesapeake Bay water column (Table 1), but equivalent to that found in sediment waters (Table 1).

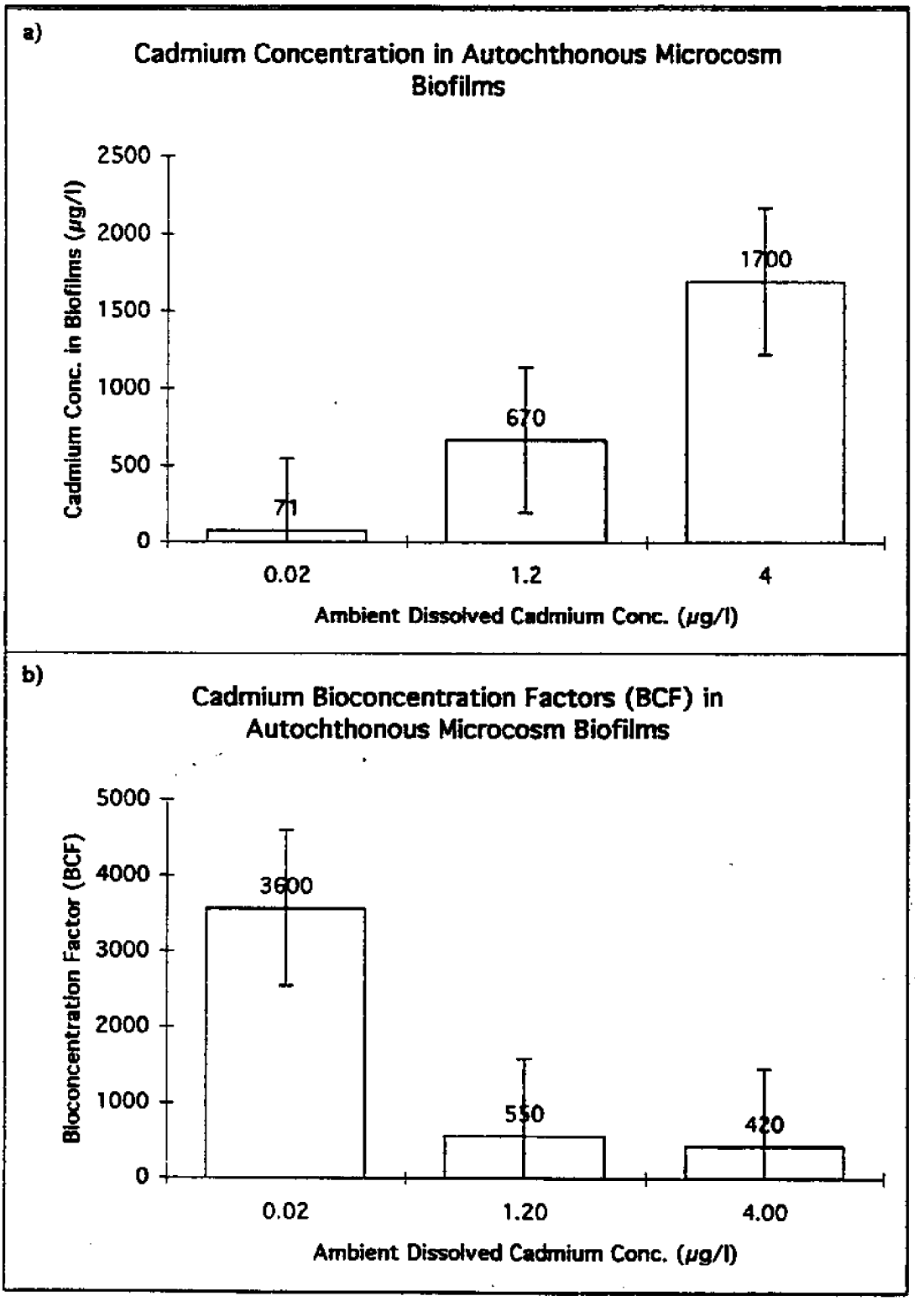
To examine these very low but environmentally relevant concentrations, we have developed technology to assess Cd and Cu at trace levels and synthesize trace-metal clean control waters. This technology uses both organic extraction methods and chelating resins. What we have found is that dissolved Cd and Cu in the Chesapeake Bay water column do not inhibit oyster larvae search or metamorphosis (Figs 1 and 4, and Tables 1 and 2). It is important to note, however, that dissolved metal concentrations in interstitial waters are commonly 10- 100 times greater than water column concentrations (Table I). Dissolved Cd concentrations in interstitial water of as little as 0. 1 μm/l and biomagnified 3600 times (Fig. 2) to 360 μg/l could inhibit oyster development (Figs. 4 and 5 and Table 3) assuming the Cd was made available to the organism. Most importantly, we now have the means to look at this question. We plan to continue these studies using coincident Cd and Cu to look at the effects of combinations of the two metals on oyster larval behavior and metamorphosis, and to pursue studies of dissolved metal concentrations in benthic waters.

METHODOLOGY

Sensitive metal detection procedures were developed for natural and synthetic water analysis of Cd and Cu using the technique developed by Brueland *et.al.* (1979) (Table 4). Biofilms are digested in Optima grade nitric acid and the digest brought up to volume in 1% trace metal grade nitric acid. Both water and biofilm are analyzed using GFAAS in conjunction with The Academy of Natural Sciences Estuarine Research Laboratory in St. Leonard's, Maryland. Biofilm Bioconcentration Factors are calculated by dividing the concentration of metal in biofilm (as a function of film wet volume) by the concentration of ambient dissolved metal. All biofilms are grown on vigorously acid-washed Teflon or LDPE.

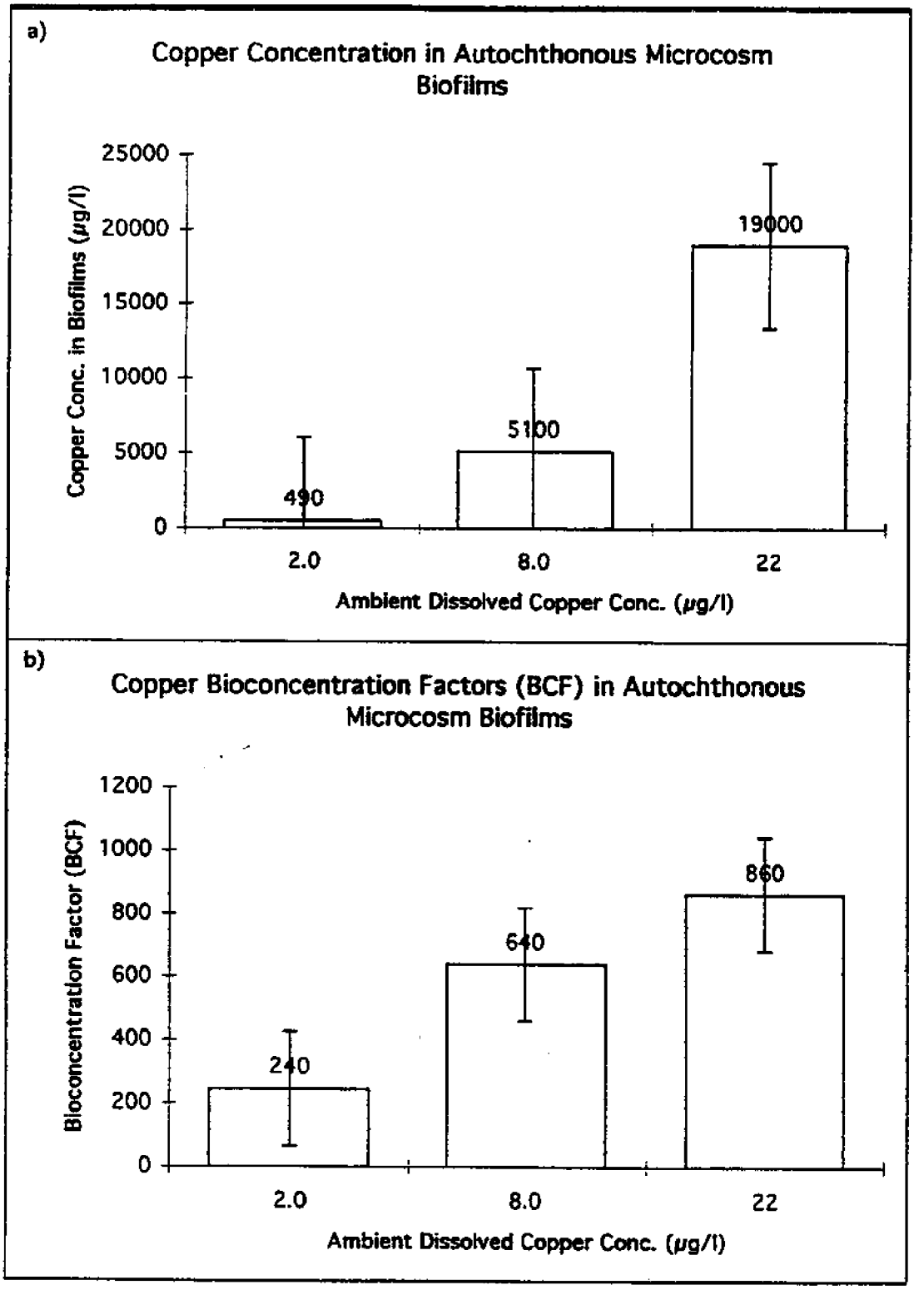
Oyster larvae bioassays are of two types: chemically induced and biofilm induced. In chemical induction, larvae are pre-exposed to the metal for a discrete period of time in inverted Teflon separatory funnels bubbled with air. Larvae are then rinsed in clean water and transferred to 24-well plates where they are treated with either 33μM epinephrine to induce set or 100 μM DOPA to induce search behavior. Wells containing epinephrine are incubated for twelve hours then rinsed and refilled with clean water; metamorphosis rates are

Fig.2: Cadmium concentration (a) and Bioconcentration Factors (b) in autochthonous biofilms from Patuxent Estuary microcosms



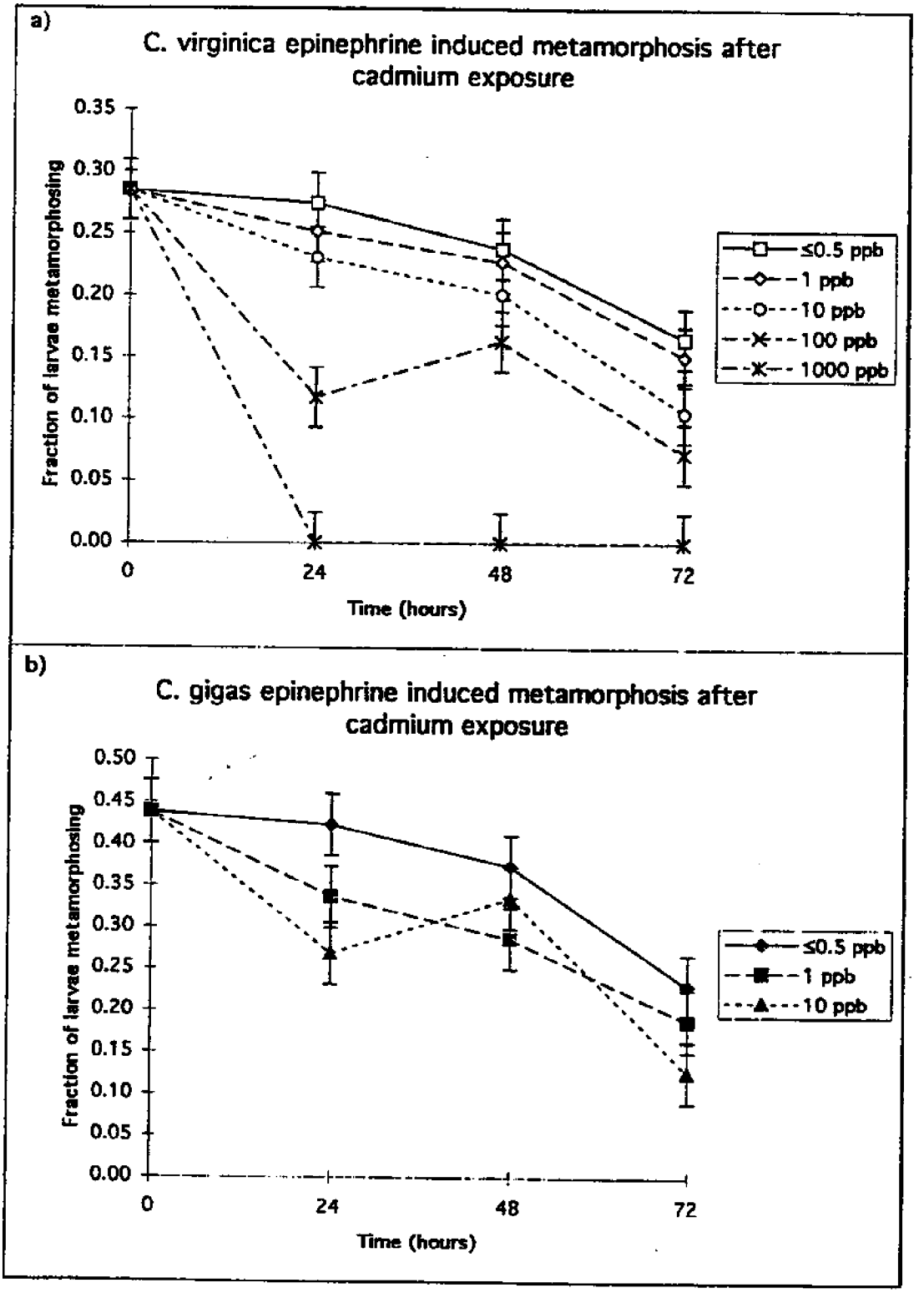
GFAAS analysis of biofilms grown in ANS Laboratory microcosms in various cadmium concentrations. BCF is a vol./vol. comparison. Error bars represent ± Minimum significant Diference, n=4.

Fig. 3: Copper concentration (a) and Bioconcentration Factors (b) in autochthonous biofilms from Patuxent River estuary microcosms



GFAAS analysis of biofilms grown in ANS Laboratory microcosms in various copper concentrations. BCF is a vol./vol. comparison. Error bars represent ± Minimum Significant Difference ($p \leq 0.05$), $n=4$.

Fig. 4: Effects of cadmium on *Crassostrea virginica* and *C. gigas* larval metamorphosis after epinephrine induction



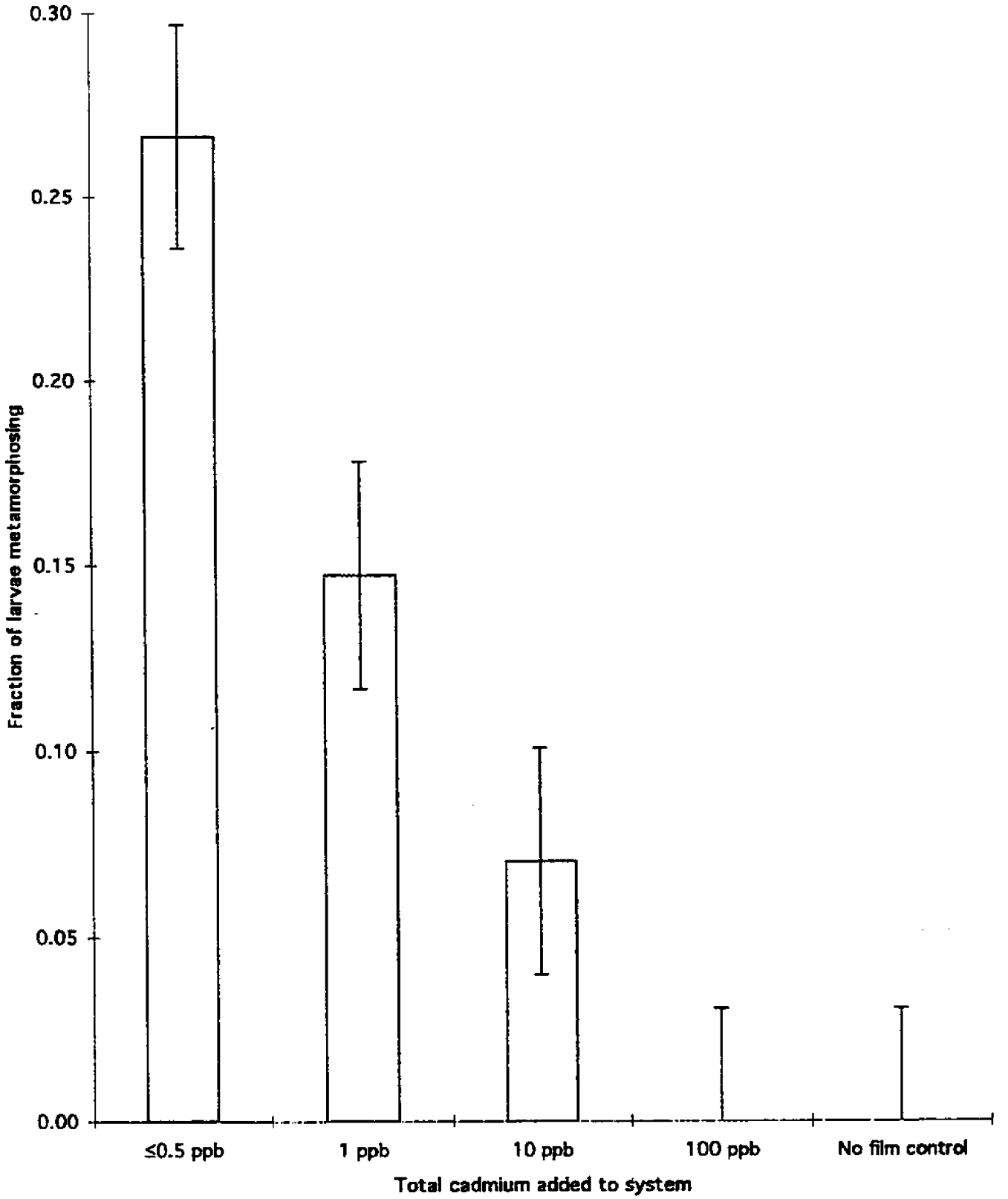
Shown are pooled results of ten replicate experiments. n=4 and error bars represent ± Minimum Significant Difference. Two-Way ANOVA detected significant time and treatment effects.

Table 2. Effects of copper on larval development of oysters, *Crassostrea gigas* and *C. virginica*, including swimming activity, searching behavior, metamorphosis and set on biofilm.

Oyster	Total Cu[a] (ppm)	Cu^{2+} (ppm)[b]	Swim[c]	Search[d]	Meta.[e]	Set on Film[f]
C. gigas (37‰ S)	0	0	++	++	++	++
	0.1	4.4E-4	++	++	++	+
	0.5	2.2E-3	++	++	++	0
	1.5	6.6E-3	++	++	+	0
C. virginica (24.7‰ S)	0	0	++	++	++	++
	0.1	9.7E-4	++	++	++	+
	0.5	4.9E-3	++	+	++	0
	1.5	1.5E-2	+	0	0	0

[a] Absolute metal concentration in seawater.
[b] Estimated concentrations of free ionic species according to metal speciation program (WQ4F) at specific salinity.
[c] Swimming activity in 96 hours. ++, > 80% vs control; +, 35-80% vs Control; 0, < 35% vs control.
[d] L-dihydroxyphenylalanine (L-DOPA) induced searching behavior after 96 hours of metal exposure.
[e] Epinephrine (EPI) induced metamorphosis. Metals were added with EPI for 4 hours exposure, then the water containing EPI was removed and replaced by water containing only appropriate concentration of metals.
[f] Biofilms of 5 day cultures of Hyphomonas MHS-3 and PM-1. Marine broth was replaced with MBL seawater amended with metals prior to addition of competent oyster larvae.

Fig. 5: C. virginica metamorphosis on MHS-3 biofilms exposed to cadmium



Shown are pooled results of five replicate experiments. n=5, error bars represent ± Minimum Significant Difference, ANVA detected significant differences between treatment means ($p \leq 0.5$)

Table 3. Inhibitory Effects of Cadmium on Invertebrates:

Organism	Type of Test	Min. Inhibitory Conc. (µg/l)	Min. Conc. Tested (µg/l)	Source
Crassostrea gigas	96hr LC_{50}	85	10.0	Watling (1982)
C. gigas	48hr EC_{50} Embryo Development	611	1.0	Martin et.al. (1981)
C. gigas	epinephrine induced metamorphosis rate	10	1.0	This study
C. cuculiata	96hr LC_{50}	80	10.0	Watling (1982)
C. margaritacea	96hr LC_{50}	75	10.0	Watling (1982)
C. virginica	48hr LC_{50}	3800	5.0	Calabrese et.al. (1973)
C. virginica	epinephrine induced metamorphosis rate	10	1.0	This study
C. virginica	biofilm induced metamorphosis rate	1	1.0	This study
Callianassa australiensis	96hr LC_{50}	490	1.0	Ahsanullah et.al. (1981)
Campanularia flexuosa	colony growth rate	200	1.0	Stebbing (1976)
Cancer magister	96hr LC_{50}	247	1.0	Martin et.al. (1981)
Echinometra mathaei	fertilization success	20	5.0	Ringwood (1992)
Isognomon californicum	larval growth	1	1.0	Ringwood (1992)
Mytilus edulis	48hr EC_{50}	1200	1.0	Martin et.al. (1981)

Table 4. Organic Extraction Preconcentration of Trace Metals in Seawater

1) ~250 gm acidified seawater added to 250ml Teflon separatory funnel

2) Buffer to pH 4 w/ammonium acetate

3) Sample extracted with Ammmonium pyrolidine dithiocarbamate (APDC) and Diethyldithiodicarbamate (DDDC) into chloroform

4) 7.5M nitric acid added to chloroform phase to initiate dithiocarbamate degradation and back-extract metals from chloroforom to acid phase

5) acid phase derived into 10 ml LDPE collection vial

6) back-extract evaporated to dryness

7) further oxidize with concentrated nitric acid

8) redissolve residue in 2 ml warm 1M nitric acid

Modified from Brueland et. al. (1979).

determined after seven to ten days. Wells containing DOPA are monitored for 30 seconds every five minutes, beginning immediately after DOPA addition. Rates of search behavior are determined by counting individuals crawling on substrate with foot extended. In biofilm induction bioassays, larvae are not pre-exposed to metal, but rather replicate biofilms are grown, then introduced to fresh water spiked with metal for an equilibration period of 24 hours. Then larvae are introduced to the biofilm/water system. Rates of metamorphosis are determined after seven to ten days.

THE EFFECTS OF ENVIRONMENTAL CONTAMINANTS ON THE PROGRESSION OF *PERKINSUS MARINUS* INFECTION IN THE EASTERN OYSTER [R/CBT-22]

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OBJECTIVES

The major objectives are to determine (1) if chronic exposure of *Crassostrea virginica* to aquatic contaminants will alter the progression of *Perkinsus marinus* infection, and (2) if modulation of disease progression can be correlated to changes in oyster immunocompetency.

RATIONALE AND APPROACH

Although there is evidence that exposure of *C. virginica* and other bivalves to sublethal concentrations of xenobiotics causes immunosuppression, documentation of actual alterations in microbial pathogenicity produced by chemical stressors is meager. Initially the effects of the mammalian immunotoxin, and aquatic contaminant, tributyltin (TBT) on the progression of *P. marinus* will be determined. Analysis of oyster cellular immunity as influenced by experimental exposure protocols, will provide complementary information on the basis of chemically altered disease progression. Knowledge gained from this project should find practical application in shellfish management by contributing to understanding the effects of environmental pollutants on the severity of a major disease of oysters.

The basic approach was to compare the progression of *P. marinus* infection in TBT-exposed oysters to that recorded in unexposed oysters. Disease progression was determined by periodic assessment of infection in the hemolymph or via the total body-burden method. Experimental infection was to be via laboratory cultured *P. marinus*, but this method was revised as described below; tissue levels of TBT were followed by gas chromatography. The hemocytic immune parameters measured included the generation of antimicrobial oxyradicals, the ability to phagocytize, and the production of lysozyme. Oysters from four treatment situations were examined: (1) no TBT, no infection, (2) no TBT, *P. marinus* infected, (3) TBT-exposed, uninfected, and (4) TBT-exposed, *P. marinus*-infected.

PROGRESS TO DATE

Establishment of Experimental *P. marinus* Infection.

By the use of cultured cells we hoped that experimental infections could be produced and experiments conducted without regard to seasonal effects on the natural abundance of *P. marinus*. To this end, the efficacy of delivery by feeding and mantle cavity injection of 10^5, 10^6 and 10^7 cultured parasites was studied. This cumulative dose per oyster was reached after ten consecutive exposures to *P. marinus* in aqueous suspension or after a single injection into the mantle cavity. *P. marinus* was not detected in hemolymph five weeks after dosing. Ten weeks post-exposure the oysters were analyzed for the parasites by the total body-burden method; prevalences in the 10^5, 10^6 and 10^7 dose groups were 0, 0, 20 % respectively per os, and 20%, 30%, 30% respectively by injection. The levels of infection were categorized as very light. In light of these results, it was decided to use *P. marinus* cells isolated from heavily infected oysters to infect experimentally the oysters in the study.

TBT Dosing Protocol: Preliminary Study.

As expected, TBT concentrations in our static renewal exposure situations were cyclic; aquaria were spiked every 24h to bring the water concentration to ~100pptr. TBB was also administered in food using algae that had been equilibrated with TBT-spiked water. During the nine week experiment TBT tissue concentrations increased steadily, regardless of route of administration. However, especially during the first 6 wk, oysters receiving TBT by both water and algae routes simultaneously showed the most rapid increase in body burdens (Figure 1). Based on the results of the preliminary study, oysters will be dosed by both water column and algae routes. This should give the most rapid TBT accumulation rate and is representative of the dominant routes of TBT exposure in the environment. The nominal water concentration is representative of exposure levels and cyclic nature of exposure that could be encountered in the Chesapeake Bay.

TBT Effects on Oyster Hemocytes: Immunocompetence.

TBT exposure for 1 hr at a wide range of concentrations (0.5ppb -1ppm, nominal) produced no lethal effects on the hemocytes, on the basis of trypan blue exclusion assays. Analysis of TBT concentrations in the exposure medium indicated: control = ND = <0.003ppb; 0.5ppb = ND; 5.Oppb = 0.5ppb; 50ppb = 10ppb; 500ppb = I 10ppb; and 1 ppm = 240ppb (nominal = analyzed value). The necessity of knowing the actual vs. the nominal TBT exposure concentrations is apparent. Although not cytolethal at these levels after 1 hr exposure, the higher TBT concentrations produced significant reduction of luminol-dependent chemiluminescence (Figure 2). Figure 3 shows that longer exposure (20hr) produced a more marked suppression of chemiluminescence, even though TBT-induced hemocytic lethality was still minimal (<85%). Hemocytic production of chemiluminescence (CL) in response to phagocytic stimulation (by the addition of zymosan) is a quantitative indication of the immune status of the cells. The hemocytes' main means of killing ingested pathogenic microbes is via the actions of certain lysosomal hydrolases and reactive oxygen intermediates (ROIs); CL, as measured with the probe luminol, quantifies activity of a major hemocyte defense mechanism: the myeloperoxidase/hydrogen peroxide pathway. In Figure 2 a dose-dependent CL inhibition is seen for the higher TBT concentrations. This provides a strong rationale for our planned in vivo experiments by directly demonstrating

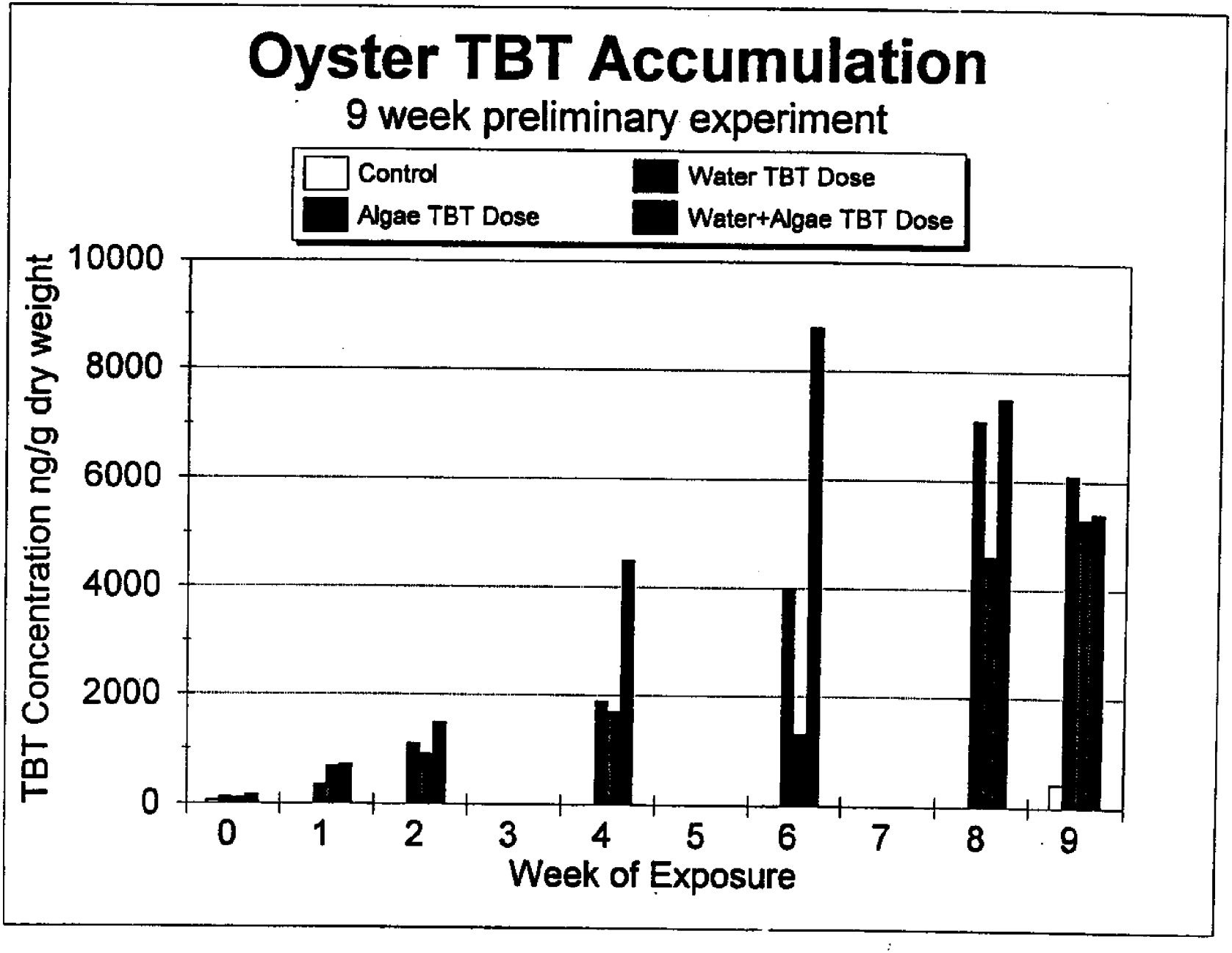
Oyster TBT Accumulation
9 week preliminary experiment
Control
Water TBT Dose
Algae TBT Dose
Water+Algae TBT Dose
TBT Concentration ng/g dry weight
10000
8000
6000
4000
2000
0
0
1
2
3
4
5
6
7
8
9
Week of Exposure

Figure 1

OYSTER CL: EFFECT OF 1 HR TBT EXPOSURE

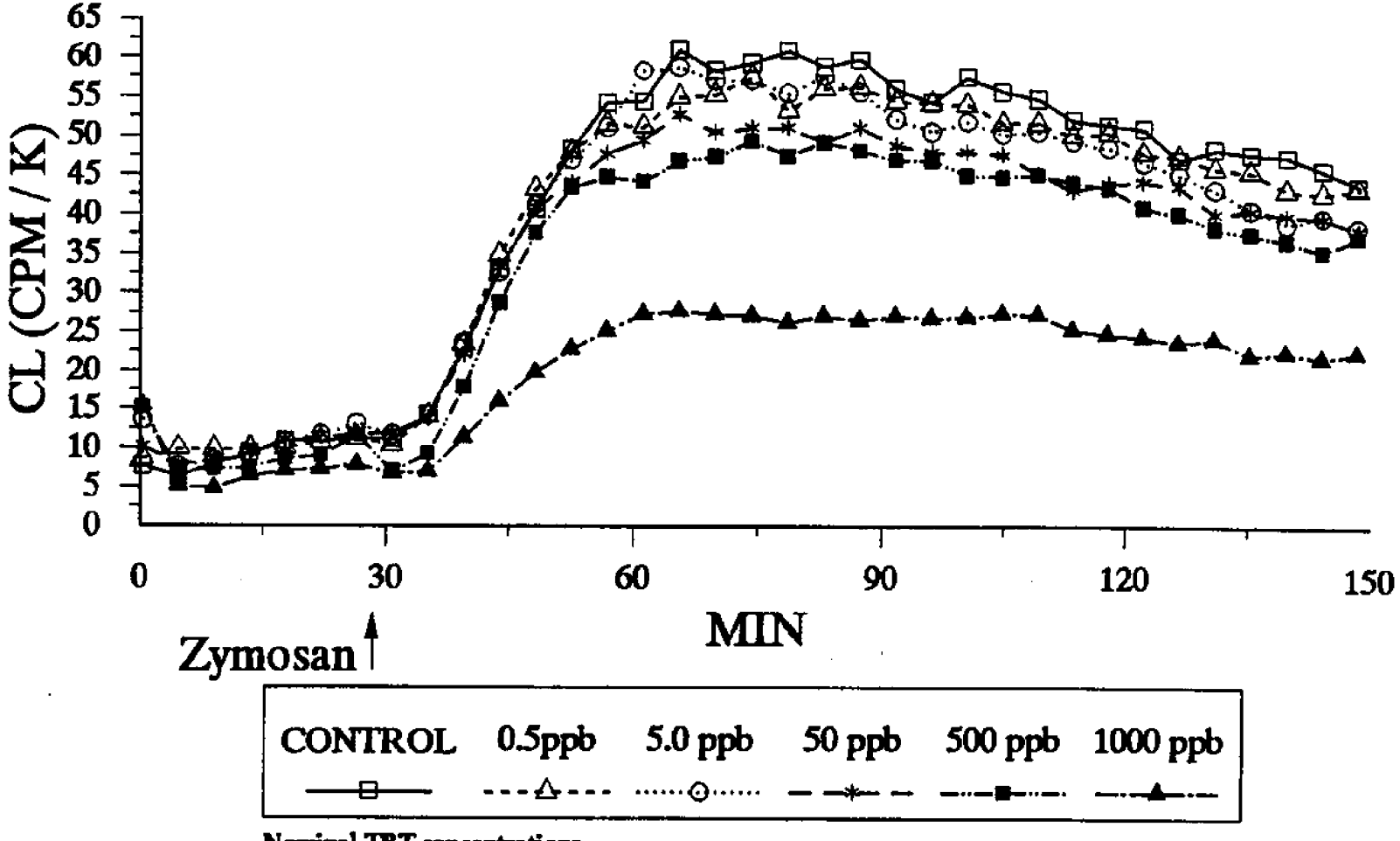

Figure 2

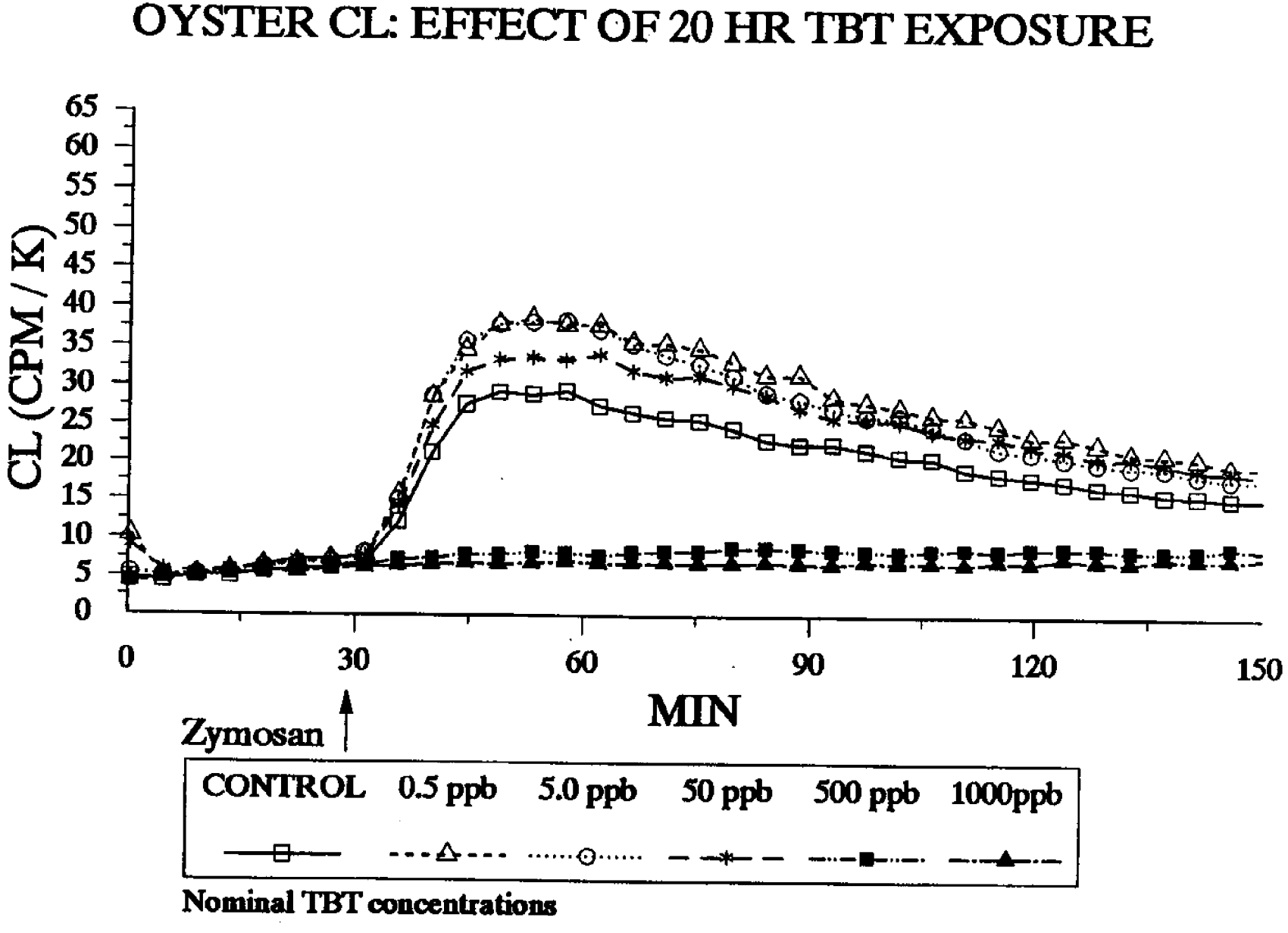
OYSTER CL: EFFECT OF 20 HR TBT EXPOSURE
CL (CPM / K)
65
60
55
50
45
40
35
30
25
20
15
10
5
0
0
30
60
90
120
150
MIN
Zymosan
CONTROL
0.5 ppb
5.0 ppb
50 ppb
500 ppb
1000ppb
Nominal TBT concentrations

Figure 3

immunomodulation by TBT at sublethal concentrations. The implications of immunosuppression regarding altered resistance to parasitic disease will be explored further during the course of this study.

Effects of TBT on Immunological Parameters and Disease Progression: Preliminary Results.

Oysters were divided into four treatment groups as listed above. TBT spiked water was added with each water renewal (3 x per wk), TBT-dosed algae were fed daily to the appropriate exposure groups. Oysters were exposed to infective cells isolated from heavily infected oysters six times in a one week period. TBT exposure was carried out for 4 wk prior to *P. marinus* exposure, and TBT exposure was continuous during the study. The first sample was taken 3 wk post disease challenge, oysters were analyzed for disease prevalence and intensity, TBT content, and immune-parameters. Hemolymph diagnosis showed no infection in uninfected, TBT-exposed (UE) or uninfected, TBT-unexposed (UU) groups; *P. marinus* prevalence was 25 % in infected, TBT-unexposed (IU) and 50% in infected, TBT exposed (IE) oysters, infections were light in these groups. Similar differences in *P. marinus* prevalence among the groups were seen using the Ray tissue culture method, which is slightly more sensitive than the hemolymph method. Circulating hemocyte numbers, phagocytic activity and CL activity showed no statistically significant differences among the control and experimental groups. Serum lysozyme activity has not yet been determined. Clearly, the immune-parameters cannot distinguish such low *P. marinus* infection levels; TBT effects were not seen either, but these data cannot be interpreted because TBT tissue levels have not yet been determined. At this time, the entire study is at a very preliminary stage. The infections will have progressed and TBT tissue levels increased by the time of subsequent samples.

CONCLUSIONS

TBT has been shown to be immunosuppressive at sublethal doses when administered in vitro to *C. virginica* hemocytes. The procedural and design details have been worked out, allowing initiation of the first large-scale in vivo experiment, data from which are currently being collected and analyzed. There are preliminary data indicating in vivo immunotoxicity: the prevalence of *P. marinus* infection is enhanced in TBT-exposed oysters; however, the results of this project are too incomplete to draw meaningful conclusions at this time.

METALLOTHIONEIN IN MARINE COCCOID CYANOBACTERIA: CLONING, TRANSCRIPTIONAL ANALYSIS AND APPLICATION TO THE ASSESSMENT OF METAL STRESS IN NATURAL COMMUNITIES OF PICOPLANKTON IN CHESAPEAKE BAY [R/CBT-23]

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OBJECTIVES AND RATIONALE

The objective of this program is to determine how toxic metals impact at the cellular and molecular biological levels upon selected components of the picoplanktonic community in Chesapeake Bay. Marine coccoid cyanobacteria, *Synechococcus* spp. are prominent members of this community and are appropriate models for the development of molecular biological indices of metal exposure and stress. Recent studies with closely related freshwater cyanobacteria have described the sequence and basic regulation of a putative metallothionein gene (*smt*A) in these organisms (Robinson et al 1990, Gupta et al 1993). The gene is induced by acute metal exposure and undergoes a novel re-arrangement upon long-term chronic exposure. Together, these molecular attributes make this gene a good candidate as a sensitive molecular bio-marker of toxic exposure and stress.

Sensitive indices for toxic stress derived from laboratory studies should lead to an improved capability to examine toxic effects in estuarine picoplankton. This capability will enhance the current understanding of how metal toxicity impacts phototrophs and ultimately is propagated into the community as a whole.

APPROACH

I have employed a two-part approach to develop a bio-indicator of metal stress in the picoplankton. The first portion involves the isolation and cloning of the metal responsive, metallothionein (MT) locus (*smt*AB) from a typical estuarine *Synechococcus* strain. These efforts are conducted in conjunction with studies of the effect of key metals upon physiological and molecular biological attributes of the cells. Basic investigations are essential to understanding the framework within which metals impact these cyanobacteria. The two lines of research will merge in a series of studies examining metal regulation of the *smt*A gene in marine *Synechococcus* spp. These will in turn, serve to orient analyses of field populations of picoplankton from impacted and non-impacted sites in Chesapeake Bay.

PROGRESS TO DATE

Isolation and cloning of smtA.

Early results from our efforts to isolate the MT gene from marine *Synechococcus* sp. WH 5701 have been promising. Initial efforts employed short oligonucleotide probes derived from the published sequence of *smt*A. These fragments complemented sequences flanking a 200 bp

region encoding a portion of the gene. The oligonucleotides were end-labeled using polynucleotide kinase and the resulting radioactive probes hybridized to chromosomal following standard Southern blotting procedures. DNA from two marine strains (WH 5701 and WH 7803) was examined. Portions of the data obtained from the probe to the 5' end of the fragment are summarized in Table 1. Clear bands were noted for both *Synechococcus* spp. examined and even the limited data presented here reveals some level of polymorphism between the strains. Similar results were found for the probe to the 3' end of the fragment. Based upon these results, an effort was made to amplify the *smt*A gene from the marine

Table 1. Hybridization of an oligonucleotide probe complementing a conserved site in the 5' end of the smtA gene to specific fragments in genomic digests from two marine *Synechococcus* species.

Enzyme	*Synechococcus* spp. Fragments (kb)	
	WH 5701	WH 7803
Bam HI	41	—
Eco RI	14.7	8.4
Hind III	4.0	11.5
Xba I	6.0	---

strains. Standard polymerase chain reaction protocols were employed using template DNA from both strains in conjunction with the oligonucleotides used in this case as primers. Preliminary results point to a weak amplification of a fragment in the 250 bp range. Efforts to amplify this fragment are presently continuing. Once amplified and cloned this fragment will be used as a probe to screen a genomic library from *Synechococcus* sp. WH 5701. Because of an unexpected loss of viability in my original WH 5701 library, I have been forced to re-construct it. I am presently in the final stages of library construction using a new high efficiency vector (Lambda Dash 11). Isolation of clones will proceed as soon as the library is completed.

Toxicity studies:

An essential part of the first phase of this program has been to determine the baseline toxicity of a suite of metals to marine *Synechococcus* sp. WH 5701. Very little is known in this regard. I have examined the effects of six metals (Cu, Cr, Cd, Hg, Pb and Zn), on the growth of the organism. Studies were conducted in two fully defined seawater media. It is important to note, that in all experiments metals were added to cultures with an equivalent amount of chelating agent (in this case EDTA) to prevent ancillary changes in the speciation of other trace elements (Sunda and Huntsman ,1992) Initial studies were done in ASW (Wyman, Gregory and Carr, 1985) the standard culture medium used in my laboratory. Both cadmium and zinc inhibited cell growth in concentration of 5 μM and above (Figures 1A and 1B). Mercury on the other hand was extremely toxic. Cell death and lysis was observed with concentrations as low as 10.0 nM (data not shown). Three other metals have had relatively little effect upon cell growth. These include Cu, Pb and Cr. Early studies examining Cu exposure to cells grown in ASW revealed that there was no growth inhibition at

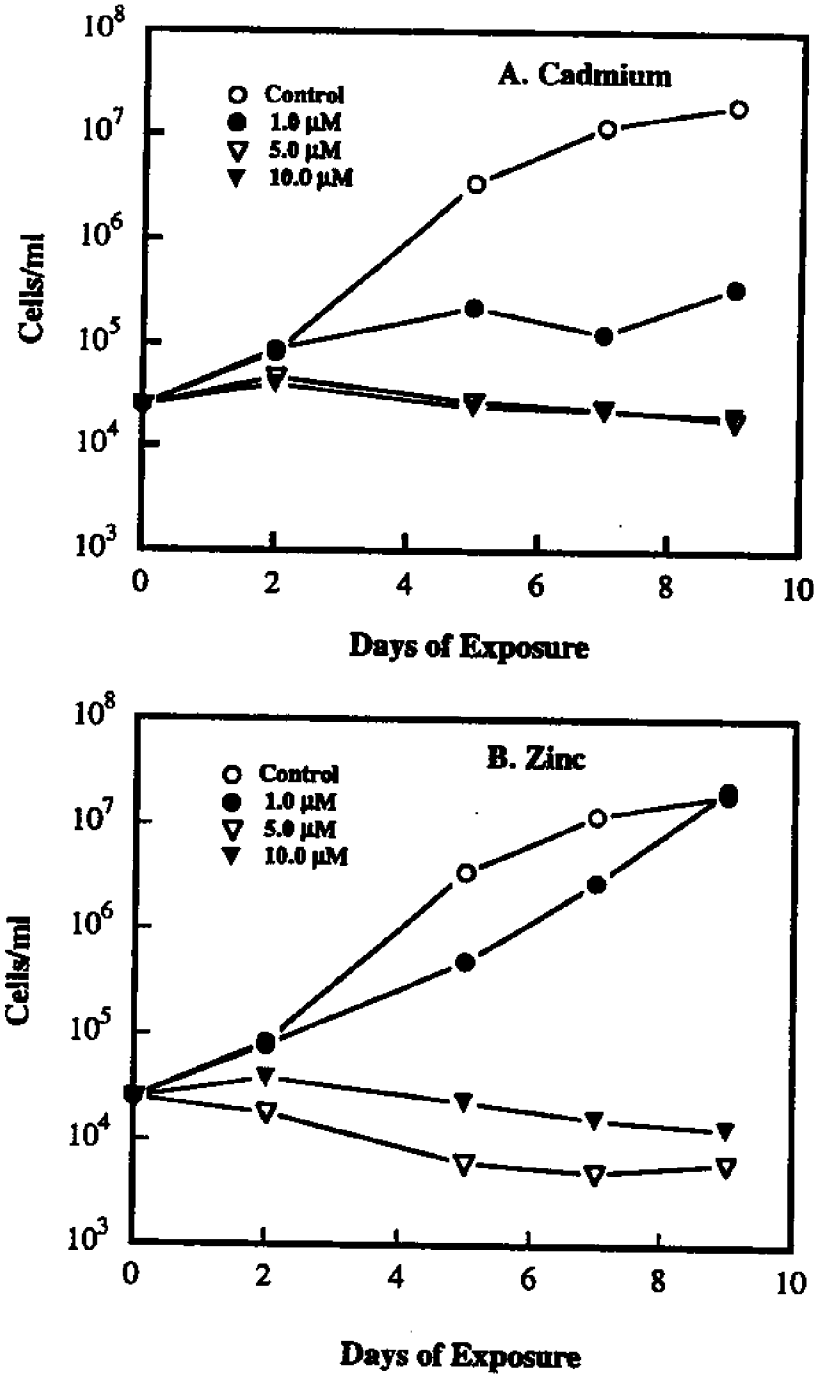

Figure 1. Growth of Marine *Synechococcus* sp. WH 5701 in ASW medium after the addition of cadmium or zinc. Metal additions made with equal concentrations of chelator (EDTA). Cell density assessed by direct counts of autofluorescent cells immobilized on 0.4 µm filters

concentrations of 1.0 and 5.0 μM. (Figure 2A). Growth was inhibited somewhat after 6 days when the cells were exposed to 10 μM Cu. These experiments were repeated in a separate medium, (AQUIL) which is designed specifically for trace metal studies with marine phytoplankton (Morel et al., 1976). In this case toxicity was observed at Cu concentrations of 5.0 μM (Figure 2B). The apparent loss of cells observed in the latter portion of the experiment in the control and 1.0 μM Cu treatments (Figure 2B) is attributable to nitrogen depletion in the medium. AQUIL is significantly less enriched in nitrate than is ASW. Similar experiments conducted with both Pb and Cr revealed that the cells have a very high tolerance for these metals. Neither inhibited growth of *Synechococcus* sp WH 5701 in ASW or AQUIL at the 10.0 μM level (data not shown). Increasing the concentration of Pb or Cr 5-fold failed to invoke a response (Figure 3). Studies with higher concentrations of Cu were also conducted. In these experiments some inhibition of growth was noted at 20 μM with clear decreases and complete inhibition at 30 and 50 μM respectively (Figure 3). The results from the experiments with Cu over this range of concentrations and in different media accentuate the importance of metal speciation and hence metal availability to the cells. (Ref).

CONCLUSIONS

Studies of this type are presently being completed. They provide a baseline understanding of toxic effects that will now be expanded to examinations of variations in physiological and molecular characteristics during exposure to sub-lethal concentrations. I am now conducting the first of these studies targeting variations in cellular pigment, total protein and RNA content in response to additions of Cu, Cd and Zn. Cultures are sampled over short and long time scales after metal exposure. Total RNA purified from these treatments will be probed for rRNA content using a 16S rRNA specific probe. Changes in rRNA content provide a very sensitive and accurate indicator of alterations in physiological state that can be observed well before growth rate changes occur. In addition, as soon as the *smt*A gene probe is available variations in the transcription of this gene will be examined as well. Experiments of this type are essential to establish the basic patterns of cellular response. In particular, the molecular methods will be used to determine what minimum exposure provokes a cellular response. These studies are important and will enable us to "calibrate" how our probes will be used in upcoming studies of field populations later in 1995.

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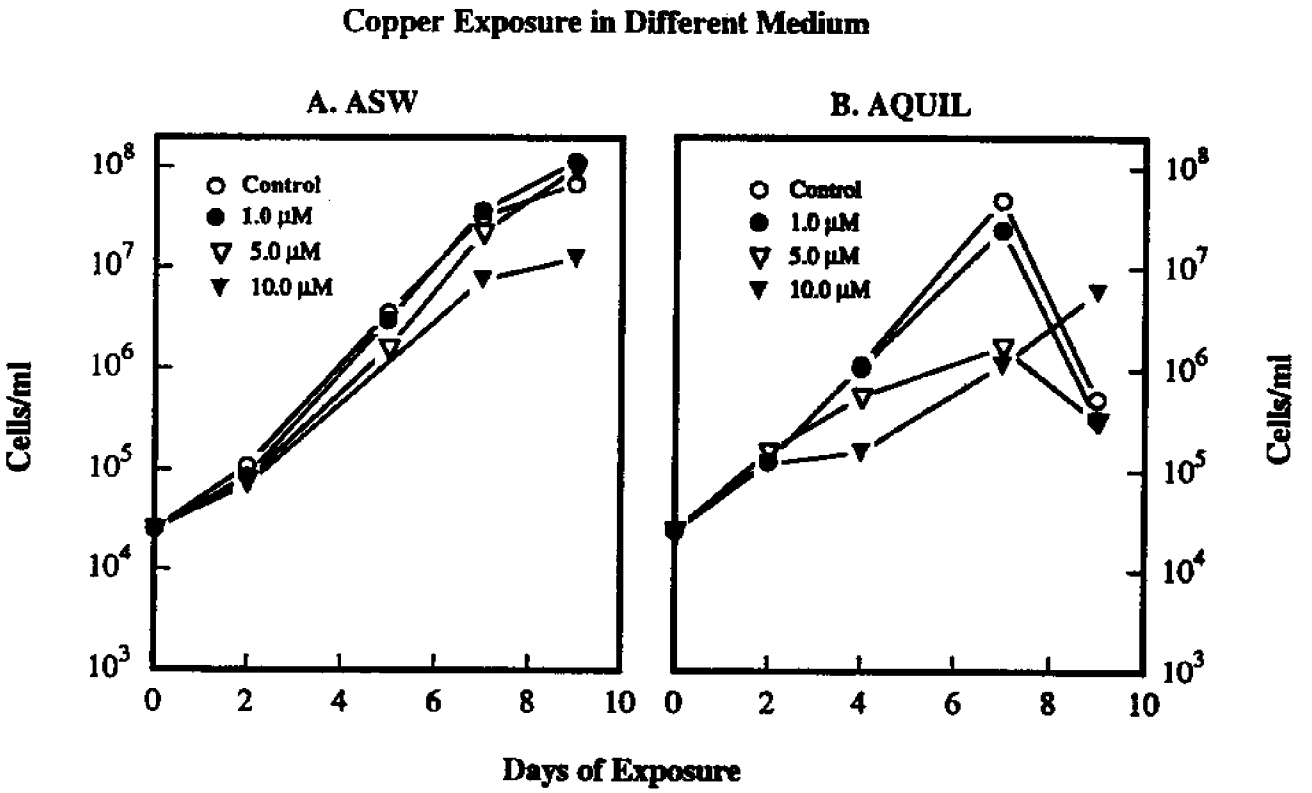

Figure 2. Growth of Marine *Synechococcus* sp. WH 5701 in different media after the addition of copper Metal additions made with equal concentrations of chelator (EDTA). Cell density assessed by direct counts of autofluorescent cells immobilized on 0.4 µm filters

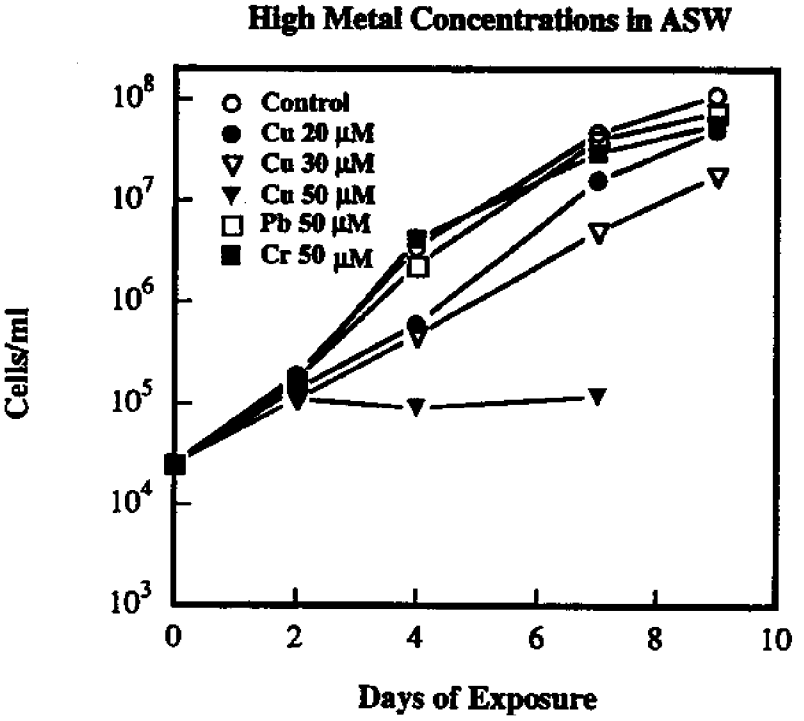

Figure 3. Exposure of marine *Synechococcus* sp. WH 5701 to high concentratrations of metals in medium ASW. Culture density assessed as in Fig. 2.

IMPACT OF SEDIMENT-ASSOCIATED CONTAMINANTS ON BENTHIC SPECIES IN CHESAPEAKE BAY: IMPLICATIONS FOR CARBON AND CONTAMINANT TRANSFER IN FOOD WEBS [R/CBT-31]

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INTRODUCTION

The goal of preliminary experiments conducted during 1994 was to test methodology described in our original CBEEC proposal. Specifically we were most concerned with questions of whether test organisms would survive and grow in our proposed experimental apparatus, and how large an experimental system would be required. Therefore, for the 1994 preliminary experiments, we examined effects of tank size (and therefore test organism density) and age of test organisms (for mummichogs only) on contaminant uptake, survival and growth.

Survival

Survival of both mummichogs and grass shrimp was high in most experimental tanks. Mummichog survival appeared to vary with both tank size and larval age, ranging from 100% in the medium and large tanks stocked with 18 dph larvae to 35% in small tanks stocked with 9 dph larvae. Some of the mortality of small larvae was due to predation by nereid polychaetes foraging in mm-deep pools on the sediment surface at low tide. Grass shrimp survival ranged from 100% in the small and large tanks to 75% in the medium tanks.

Growth

The size of test tanks affected growth of both mummichogs and grass shrimp in preliminary experiments. At the end of the experiment, mummichog larvae tested in large tanks were larger and greater in weight than those tested in smaller tanks (Table 1). One-way analysis of variance indicated significant tank-size effects on fish standard length and wet weight; dry weight differences were not statistically distinguishable although there was a 2-fold difference in mean weights between large and small tanks (Table 1). Dry weights of grass shrimp, but not wet weights or lengths, varied significantly among test tank sizes (Nested ANOVA, Table 1).

Table 1. Growth of mummichogs and grass shrimp in experimental tanks during preliminary experiments. Statistical results are for main effects only. All measurements are (x±SE, [n]). Mummichog experiments were analyzed with one-way ANOVA; grass shrimp experiments were analyzes as a nested design ANOVA. nd = no data available; na = not applicable.

Test organism	Growth measurement	Start size	Small tank(s)	Medium tank(s)	Large tank(s)	F	df	P
mummichog (18 dph)	standard length (mm)	13.1±0.3 [20]	14.7±0.4 [15]	15.4±0.4 [21]	16.5±0.7 [20]	7.63	3	0.0002
	wet weight (mg)	43.0±2.8 [20]	42.7±4.7 [15]	57.6±4.7 [21]	79.7±11.1 [20]	6.50	3	0.0006
	dry weight (mg)	9.3±0.6 [20]	7.4±0.8 [15]	15.9±6.2 [21]	14.3±2.0 [20]	1.21	3	0.313
mummichog (9 dph)	standard length (mm)	nd	12.3±1.8 [6]*	12.8±1.1 [14]	16.1±0.9 [14]	3.51	2	0.042
	wet weight (mg)	nd	44.5±18.9 [6]*	37.6±8.0 [14]	81.9±13.5 [14]	4.18	2	0.025
	dry weight (mg)	nd	8.5±3.8 [6]*	7.1±1.5 [14]	14.2±2.4 [14]	3.23	2	0.053
grass shrimp	body length (mm)	na	25.8±0.8 [12]	27.8±1.3 [9]	28.7±1.3 [12]	0.71	2	0.558
	starting wet weight (mg)	na	134.3±15.6 [12]	133.9±17.2 [12]	136.4±14.9 [12]	0.00	2	0.998
	end wet weight (mg)	na	135.8±14.4 [12]	182.9±30.7 [9]	204.9±27.9 [12]	1.03	2	0.456
	end dry weight (mg)	na	27.4±27.5 [12]	43.4±8.2 [9]	47.5±6.3 [12]	1.52	2	0.349

* One larvae lost during retrieval from test tank.

Uptake of trace elements from sediments

Trace element uptake varied between element and species. Arsenic was not incorporated by mummichogs, however larger fish contained more arsenic than did smaller fish throughout the experiment (Table 2). Mummichogs did accumulate significant quantities of copper, with small fish having higher burdens than large fish. Cadmium was also accumulated, leading to significantly higher burdens than at the start of the experiment (Table 2). Grass shrimp accumulated significant quantities of arsenic during the experiment (Table 2), tripling their body burdens. Grass shrimp also accumulated significant quantities of copper, and exhibited slight, but not significant increases in cadmium content (Table 2).

DISCUSSION

Results of our preliminary experiments clearly demonstrated that our methods are suitable for the growth and survival of test organisms, and indicate the potential for interesting differences among species in their potential for accumulating sediment contaminants that appear to be related to feeding modes.

Observations of mummichog and grass shrimp behavior in experimental tanks indicated the potential for transfer of contaminants through direct contact with sediments as well as through prey and exposure to dissolved contaminants in the water column. Feeding by mummichogs was primarily from the substrate, and involved taking mouthfuls of sediment along with prey and then expelling sediment and water past their gills through their gill rakers. In the process, sediment is heavily disturbed, which may affect release of contaminants to the water column. Grass shrimp also appeared to feed primarily from the substrate and disturbed the sediment surface, especially by swimming activities during low tides. Because *Coullana* was added only weekly to experiments, there was sufficient time for these prey to accumulate contaminants while in test tanks, and then pass these contaminants to their predators.

Trace element uptake was significant in many cases. Arsenic was accumulated only by grass shrimp. This difference in uptake is likely related to the trophic state of the two consumer species. Grass shrimp are omnivores, and will feed on plant detritus and algal material, as well as animal material. Arsenic compounds in plants are generally available for uptake, and can be accumulated through feeding, while arsenic in animal material is not. Mummichogs, feeding primarily on animals, are unlikely to accumulate significant quantities of arsenic. While some differences were noted, both test organisms accumulated both copper and cadmium, and exhibited elevated levels at the end of the experiment. With the small number of samples collected, we could not separate the effect of tank size. However, grass shrimp were analyzed from each tank, and little difference between tanks was noted.

Implications of preliminary tests for experimental design during 1995-1996

Tank size: Because survival and growth of both mummichogs and grass shrimp was greatest in large tanks, we will use these 8-core tanks (4 cores per side) in experiments conducted during 1995-1996. Although prey abundances supplied to mummichogs and shrimp was sufficient for survival and growth, we anticipate using higher densities of the benthic

Table 2. Trace element content before (initial) and after (final) the microcosm feeding experiment.

Test organism	Sample type, size	Arsenic	Cadmium	Copper
Mummichog, small	final n=3	2.92 ± 0.25	0.18 ± 0.05	6.41 ± 1.8
Mummichog, large	initial n=5	4.76 ± 0.70	.12 ± .02	2.93 ± 0.16
	final n=3	5.80 ± 1.06	.19 ± .03	4.69 ± .20
grass shrimp	initial n=5	3.29 ± 0.26	.55 ± .20	102 ± 4.2
	final n=6	10.4 ± 1.19	.63 ± .21	160 ± 6.1

harpacticoid copepod prey in future experiments. Since we now have *Coullana* in culture, scaling up to culture quantities needed for experiments will be a quicker process than we faced during 1994.

Tidal cycle: Survival in "puddles": It is clear from the high survival (up to 100%) that larvae are not killed by the simulated low tides. Thus we are able to restrict fish to small muddy puddles similar to microhabitats they occupy in the field when the tide recedes.

Number of "low tides" in experimental tanks: Depending on geographic location (including outside the Chesapeake Bay region), local topography, and the behavior and depth of an individual, larval and small juvenile mummichogs can be restricted to the marsh surface and mm-deep puddles 0 - to at least 86% of the day. Even in an area with two low tides each day, the total number of hours an individual is exposed will vary; a total 6-hr exposure as in our preliminary experiments is a reasonable choice from a biological perspective. A single, longer duration low tide, rather than 2 briefer low tides also conserves prey that might be lost and greatly reduces the cost of experiments by reducing the quantity of chemicals used to produce contaminant-free salts.

Although we believe our choice of a test "tidal cycle" was reasonable, we recognize that the time organisms are restricted to the marsh surface is a potentially important variable that may affect uptake of contaminants from the sediment. Therefore, during 1995-1996 we will use two tidal treatments resulting in 0 h and 6 h duration sediment exposures in our experiments. All tanks will exchange the same volume of water daily, but a several cm deep layer of water will be retained over mummichog sediment cores in the 0-h exposure treatment. Thus, each year we will test 2 tidal durations X 2 sediment types X 3 replicates per treatment.

Age of grass shrimp: As in these preliminary experiments, we propose to use small nonreproductive adult grass shrimp in 1995-1996 experiments. These animals remain in intimate contact with the sediment during much of the day, and feed mostly from the sediment surface. They are therefore more appropriate for these experiments than planktonic larvae.

Prey: As in these preliminary experiments, we intend to introduce copepods into experimental tanks at least 1 week prior to addition of mummichogs and grass shrimp. *Coullana* has approximately a 2-week generation time.

ECOSYSTEM PROCESSES IN RELATION TO TRANSPORT, PARTITIONING, AND EFFECTS OF ORGANIC CONTAMINANTS IN CHESAPEAKE BAY: A SIMULATION MODELING STUDY [R/CBT-21]

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PROJECT OBJECTIVES

The objective of this study is to test the hypothesis that planktonic and benthic-pelagic coupling control the speciation, transport, bioavailability, bioaccumulation, and toxic effects for synthetic organic contaminants in Chesapeake Bay. The development of numerical simulation models of key ecological processes enables us to: integrate data collected by various investigators in the Bay region; test hypotheses about interactions between trophic dynamics and contaminant fate/effects; provide a basis for further elaboration of the toxics modeling effort; foster interaction and synthesis among investigators working on problems of toxics cycling in the Bay region; and provide information needed for Ecological Risk Assessment.

RATIONALE

It has become clear that to properly assess fate and transport of HOCs in aquatic environments, the physiology, trophic dynamics, and metabolism of plankton and benthic ecosystem components must be considered.We use simulation modeling techniques to determine how HOC cycling and transfer in the environment is influenced by biological processes, sorption, biomagnification, and deposition to sediments. Additionally, fundamental ecological processes may be impacted by toxic stresses associated with contaminants, and we simulate impacts in our models and predict their effects in the natural system. A complex ensemble of biological, chemical and physical processes controls the dynamic behavior of HOCs in estuaries such as Chesapeake Bay. Simulation models which incorporate key ecological interactions and appropriate chemical kinetics, equilibrium reactions and toxicological relations for such contaminants are useful tools for sorting out inherent complexity. Such models provide information needed for effective scientific integration and for risk assessment to establish sound management policies. This research provides a

framework for combining existing, incoming, and future ecosystem process data on planktonic trophic dynamics, sediment geochemistry, and benthic-pelagic coupling with data on HOC dynamics such as chemical kinetics, surface chemistry, equilibrium relations, and toxicological effects generated from CBEEC. Current information will contribute to a risk assessment analysis, and our simulation model will provide a mechanism for integrating current and future scientific studies.

RESULTS

Our model studies revealed that HOC concentrations increase along food chains as expected and that differences among congeners were generally predictable from K_{ow} values. Simulations of HOC behavior showed remarkable consistency with field data based on assumptions about size relations for functional groups. Model experiments also revealed that HOC concentrations associated with planktonic and benthic communities are clearly dynamic properties far from chemical equilibrium. Each congener behaved differently in terms of its bioaccumulation at various levels in the trophic chain, and there was generally good agreement between model results and field data. Under conditions of constant external inputs, organism-bound HOC pools reached pseudo-equilibrium conditions within 6-10 months. When input conditions were varied at seasonal or greater frequencies, however, plankton biomasses varied accordingly and HOC pools never reached equilibrium within one year simulations.

Simulation of acute toxicity effects for general and taxon-specific herbicides and for pesticides specific both for copepods and planktivorous fish showed that effects on phytoplankton production were most pronounced on diatoms and were attenuated along trophic pathways. Seasonally concentrated herbicide additions had greatest impact on the plankton community when applied in the early spring; summer additions had much smaller effect on copepod abundance. Inhibition of copepod ingestion had substantially greater impact on both zooplankton and phytoplankton abundances than did proportionally similar increases in copepod mortality. We also investigated the top-down effects of reductions in fish predation which resulted in substantial increases in copepod abundance, in turn, causing smaller decreases in phytoplankton biomass.

The ecosystem process model has been calibrated using data from the mesohaline region of mainstem Chesapeake Bay. Model behavior faithfully reproduces seasonal dynamics for key variables and processes in the plankton and benthic communities. For example, the spring diatom bloom is clearly differentiated from summer conditions dominated by flagellate growth. The characteristic bimodal pattern of copepod growth is captured with peaks occurring in March and August. Sedimentation of particulate organic matter also exhibits two seasonal peaks--in April and in August. Simulation experiments have illustrated that plankton community trophic interactions control the timing and magnitude of POM deposition.

Extensive review of the general literature and data availability specific for Chesapeake Bay has provided a synthesis of relevant information necessary for our continued development of the models and has assisted us in compiling a list of data requirements which may guide future field investigations. Foremost among these information needs is quantitative

descriptions of kinetic behaviors for uptake and depuration of HOCs and other contaminants. In addition, there is a clear need for data which describes HOC concentrations associated with specific functional groups of organisms (e.g., bacteria, ciliates, flagellated protozoa) in the plankton community as opposed to simple distinction by size-class. In general, we have found that there is also incomplete description of major ecological processes and organism groups for areas of highest concentrations of toxic contaminants. Similarly, descriptions are available for specific key processes or in situ concentrations for specific contaminants (or congeners) for specific areas of the Bay; however, there is no region or contaminant for which a more complete description is available. We strongly recommend future CBEEC research should focus on selected contaminants and specific Bay regions.

Appendix 1.

CHESAPEAKE BAY ENVIRONMENTAL EFFECTS STUDIES
TOXICS RESEARCH PROGRAM
FUNDED PROJECTS

AIR-WATER PARTITIONING AND TRANSFER

R. M. Dickhut
Air/water partitioning and mass transfer properties of toxic organic chemicals.
R/CBT-1 1990-1991

R. M. Dickhut
Determination of the volatile/absorptive exchange of hydrophobic organic contaminants across the air/water interface of lower Chesapeake Bay.
R/CBT-18 1993-1994

SEDIMENT-ASSOCIATED RESUSPENSION AND TRANSPORT

G. T. F. Wong
Particle-reactive radionuclides as analogues of particle-reactive pollutants in the Chesapeake Bay.
R/CBT-2 1990-1991

D. J. P. Swift
Residence time of particle reactive pollutants in the coastal sea bed: control by resuspension and sea bed mixing processes.
R/CBT-3 1990-1991

L. D. Wright, J. D. Boon, J. P. -Y. Maa, and L. C. Schaffner
Dynamics of sediment resuspension: Bay-stem plains of the lower Chesapeake Bay.
R/CBT-4 1990-1991

L. P. Sanford, J. P. Halka and J. M. Hill
Resuspension and transport of sediment associated toxics in the northern Chesapeake Bay.
R/CBT-9 1991-1992

SEDIMENT FLUX PROCESSES

G. F. Riedel, J. G. Sanders, R. W. Osman, and C. C. Gilmour
The role of benthic infauna and fluctuating oxygen concentrations in the flux of toxic trace elements from Chesapeake Bay sediments.
R/CBT-7 1990-1991

D. G. Capone, J. W. Gooch, and J. E. Baker
Microbial degradation of chlorinated hydrocarbons under alternating redox conditions in Chesapeake Bay.
R/CBT-11 1992

J. C. Cornwell, D. J. Burdige, and W. R. Boynton
Direct measurements and biogeochemical controls of sediment-water flux of trace metals from estuarine sediments.
R/CBT-12 1992-1993

L. C. Schaffner and R. M. Dickhut
Role of benthic communities in sediment-associated toxic organic chemical fate and transport in lower Chesapeake Bay.
R/CBT-15 1992-1993

CONTAMINANT SPECIATION AND EXPOSURE

J. R. Donat
The speciation of dissolved copper and cadmium in Chesapeake Bay.
R/CBT-14 1992

D. A. Wright and R. Dawson
A risk assessment for Dimilin use in the northern Chesapeake Bay: a model study for non point-source runoff.
R/CBT-24 1994-1995

R. P. Mason and D. A. Wright
Factors controlling the food chain accumulation and transfer of inorganic and methylmercury in Chesapeake Bay organisms.
R/CBT-27 1995-1996

J. S. Weis and P. Weis
Impacts of CCA pressure-treated wood structures in Chesapeake Bay.
R/CBT-29 1995-1996

UPTAKE AND TROPHIC TRANSFER

J. E. Baker, H. R. Harvey and R. Dawson
Role of plankton in controlling the partitioning and transport of hydrophobic organic contaminants in Chesapeake Bay.
R/CBT-8 1990-1991

G. B. McManus and M. R. Roman
Role of plankton in controlling the partitioning and transport of hydrophobic organic contaminants in Chesapeake Bay: zooplankton feeding and excretion.
R/CBT-10 1990-1991

J. G. Sanders and K. G. Sellner
Importance of dinoflagellate blooms in the transport of carbon and toxic trace elements in Chesapeake Bay.
R/CBT-13 1992-1993

D. P. Weston, D. L. Penry, R. I. E. Newell, and J. E. Baker
Uptake of dissolved and particle-associated toxicants by the eastern oyster.
R/CBT-16 1992-1993

G. F. Riedel, J. G. Sanders, and C. C. Gilmour
Contaminant flux from sediments: impact on Chesapeake Bay food webs.
R/CBT-20 1993-1994

R. M. Dickhut and L. C. Schaffner
Organic contaminant metabolite production, elimination, and bioavailability in benthic macrofauna of lower Chesapeake Bay.
R/CBT-25 1994-1995

L. C. Schaffner and R. M. Dickhut
Role of Benthic macrofauna in trophic transfer of organic contaminants (PAHs, PCBs) to demersal predators.
R/CBT-28 1995-1996

J. G. Sanders and G. F. Riedel
Impact of sediment-associated contaminants on benthic species in Chesapeake Bay: implications for carbon and contaminant transfer in food webs.
R/CBT-30 1995-1996

CONTAMINANT-ORGANISM RESPONSE INTERACTIONS

F.-L. Chu and R. Hale
Role of sediment associated pollutants in infectious disease susceptibility in the Eastern oyster, Crassostrea virginica.
R/CBT-5 1990-1991

M. Faisal
Use of fish and oyster cell cultures to study toxic effects of chemical pollutants of the Chesapeake Bay.
R/CBT-6 1990-1991

T. P. Chen and G. Roesijadi
Effects of trace metals and organic pollutants on stress-induced proteins and metallothionein in oyster larvae and spat: a molecular approach.
R/CBT-17 1992

R. Weiner and M. Walch
Interaction of copper and cadmium with microbial benthos biofilm and effects on oyster larval set.
R/CBT-19 1993-1994

R. S. Anderson and E. M. Burreson
The effects of environmental contaminants on the progression of Perkinsus marinus *infection in the Eastern oyster.*
R/CBT-22 1994-1995

J. G. Kramer
Metallothionein in marine coccoid cyanobacteria: cloning, transcriptional analysis and application to the assessment of metal stress in natural communities of picoplankton in Chesapeake Bay.
R/CBT-23 1994-1995

J. G. Sanders, D. L. Breitburg, G. F. Riedel, and C. C. Gilmour.
Impact of Sediment-Associated Contaminants on Benthic Species in Chesapeake Bay: Implications for Carbon and Contaminant Transfer in Food Webs.
R/CBT-31 1994-1995

F.-L. Chu, R. Hale and W. Vogelbein
Role of sediment associated pollutants in infectious disease susceptibility in the eastern oyster, Crassostrea virginica.
R/CBT-30B 1995-1996

MODELLING FRAMEWORK

W. M. Kemp, J. W. Gooch, and J. E. Baker
Ecosystem processes related to transport, partitioning and effects of organic contaminants in Chesapeake Bay: a simulation modeling study.
R/CBT-21 1992-1993

C. J. Madden, J. R. Kucklich and J. Baker
Quantitative Evaluation of Contaminants in a Chesapeake Bay Region of Concern: A Simulation model of exposure and bioaccumulation in Baltimore Harbor.
R/CBT-26 1995-1996

Appendix 2.

CBEES TOXICS RESEARCH PROGRAM
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Appendix 3.
CHESAPEAKE BAY ENVIRONMENTAL EFFECTS STUDIES
TOXICS RESEARCH PROGRAM

WORKSHOP

6-7 December 1994

Waterman's Hall
Virginia Institute of Marine Science
College of William and Mary
Gloucester Point, Virginia

sponsored by

NOAA CHESAPEAKE BAY ENVIRONMENTAL EFFECTS COMMITTEE
and
CHESAPEAKE BAY PROGRAM
SCIENTIFIC AND TECHNICAL ADVISORY COMMITTEE

AGENDA

Tuesday 6 December

8:00 CONTINENTAL BREAKFAST

8:20 WELCOME . . . . . Taylor
Dr. Dennis Taylor, Dean/Director of the Virginia Institute of Marine Science.

8:30 WORKSHOP OBJECTIVES . . . . . Gillelan
Bess Gillelan (NOAA Chesapeake Bay Office) will present the objectives of the workshop, outlining issues to be addressed.

8:45 TOXICS RESEARCH PROGRAM FINDINGS
Jim Sanders (Academy of Natural Sciences) will moderate this session which focuses on integration of findings of toxics research sponsored by the Chesapeake Bay Environmental Effects Studies.

OVERVIEW . . . . . Baker
Joel Baker (Chesapeake Biological Laboratory) will present an overview of toxic research issues in Chesapeake Bay.

TRANSPORT AND FATE PROCESSES

Air-water Partitioning and Transfers . . . . . Dickhut
Rebecca Dickhut (Virginia Inst. Marine Science) will summarize findings from her projects (R/CBT-1 & R/CBT-18).

Sediment Associated Resuspension and Transport . . . . . Swift
Don Swift (Old Dominion Univ.) will summarize findings from Wong (R/CBT-2), Swift (R/CBT-3), Wright et al. (R/CBT-4), and Sanford et al. (R/CBT-9).

Sediment Flux Processes . . . . . Riedel
Fritz Riedel (Academy of Natural Sciences) will summarize findings to date from Riedel et al. (R/CBT-7), Capone et al. (R/CBT-11), Cornwell et al. (R/CBT-12), and Schaffner & Dickhut (R/CBT-15)

10:00 BREAK

10:15 SPECIATION AND TROPHIC TRANSFER

Speciation and Trophic Transfer of Contaminants . . . . . Sanders
Jim Sanders (Academy of Natural Sciences) will summarize findings to date from Baker et al. (R/CBT-8), McManus & Roman (R/CBT-10), Sanders & Sellner (R/CBT-13), Donat (R/CBT-14), Newell et al. (R/CBT-16), Riedel et al. (R/CBT-20), Sanders et al. (R/CBT-PD-94-2), and Dickhut & Schaffner (R/CBT-25).

EXPOSURE AND EFFECTS

Chemical Contaminant Exposure . . . . . Wright
David Wright (Chesapeake Biological Laboratory) will summarize findings to date from Wright & Dawson (R/CBT-24) and Mason & Wright (R/CBT-29).

Contaminant-Organism Response Interactions . . . . . Anderson
Robert Anderson (Chesapeake Biological Laboratory) will summarize findings to date from Chu & Hale (R/CBT-5), Faisal (R/CBT-6), Chen & Roesijadi (R/CBT-17), Weiner & Walsh (R/CBT-19), Anderson et al. (R/CBT-22), Kramer (R/CBT-23), and Chu et al. (R/CBT-PD-94-1).

Modeling Framework . . . . . Kucklich
John Kucklick (Chesapeake Biological Laboratory) will summarize findings to date from Kemp et al. (R/CBT-21) and discuss the direction of future modeling work.

12:30 LUNCH (at VIMS)

1:30 BASINWIDE TOXICS STRATEGY . . . . . Batiuk
Richard Batiuk will present an overview of the *Chesapeake Bay Basinwide Toxics Reduction and Prevention Strategy* with an emphasis on the Directed Toxics Assessment objectives and commitments.

1:50 MANAGEMENT/SCIENTIFIC FRAMEWORK . . . . . Alden
Ray Alden will introduce the management/scientific framework as called for in the *Chesapeake Bay Basinwide Toxics Reduction and Prevention Strategy* under Directed Toxics Assessments. Dr. Alden will lead discussion of this topic at 7:30 p.m.

2:10 RESEARCH DIRECTIONS IN SUPPORT OF STRATEGY IMPLEMENTATION
Frank Dukes (Institute for Environmental Negotiation, Univ. of Va.) and Richard Batiuk (EPA, Chesapeake Bay Program) will introduce the format and issues for discussion during the remainder of the workshop.

2:30 Breakout Sessions
coffee/drinks available to take to break-out discussions

Jim Sanders (Academy of Natural Sciences) will lead discussion among researchers.

Ron Klauda (MD DNR) will lead discussion among management participants.

5:30-7:30 SOCIAL (at VIMS)

7:30 MANAGEMENT/SCIENTIFIC FRAMEWORK . . . . . Alden
Ray Alden will lead a discussion of the proposed management/scientific framework, as called for in the Directed Toxics Assessments commitment in the draft *Basinwide Toxics Reduction and Prevention Strategy*.

Wednesday 7 December

8:00 CONTINENTAL BREAKFAST (at VIMS)

8:20 OPENING COMMENTS . . . . . Rickards

8:30 TOXICS RESEARCH IN CHESAPEAKE BAY: FINDING COMMON GROUND

Introduction to the session . . . . . Dukes/Batiuk/Gillelan

8:50 Report from the Management Breakout Group . . . . . Klauda

9:10	Report from the Research Breakout Group	Sanders
9:30	Report from the "Framework" discussion	Alden
9:50	Finding Common Ground	Dukes/Batiuk/Gillelan
10:30	BREAK	
10:50	Finding Common Ground (cont)	
12:00	LUNCH (at VIMS)	
1:00	Finding Common Ground (cont)	
3:00	Wrap-up & Adjourn	

Appendix 4.
ATTENDEES - 94 WORKSHOP

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