Integrated Measures of Biological and Habitat Integrity

Final Report

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FOREWORD

This report represents the second year of a program to integrate an ambient toxicity testing approach with fish community population level metrics. It describes the results of tributary specific toxicity testing and the assessment of a toxicity risk ranking model developed specifically for ambient toxicity testing. It also summarizes results from a parallel study on fish community Index of Biotic Integrity (IBI) investigations and the correlations between the two. The project was undertaken to further our understanding of how toxic contaminants are affecting habitat quality and resource populations in Chesapeake Bay.

ABSTRACT

The goal of this study was to evaluate ambient toxicity conditions in tidal tributaries of Chesapeake Bay, whose watersheds are impacted by existing urban areas and urban development, and assess the results in the framework of a toxicological risk ranking model and a fish community health index. A battery of standardized, directly modified or recently developed water column and sediment toxicity tests were employed with fish, grass shrimp, copepods, amphipods, polychaetes and vascular plants. The study was conducted in coordination with a fish community sampling program. Tests were conducted monthly from April through August 1994 in four tidal tributaries: South River, Severn River, Patuxent River and the Wicomico River (tributary of the Potomac River), which was a nominal reference site. Mortality, reproduction and growth rates in the water column assays did not indicate consistent chemical contamination in any river. These results varied from month to month and from species to species. Water column chemical analyses did not indicate elevated levels of contaminants. The sediment bioassays demonstrated greater responses than water column assays. Sediment in the upper reaches of the South River demonstrated significant toxicity. Peaks of toxicity were also observed at the upper-most Severn River station and the middle Patuxent River station. Sediment chemistry indicated elevated metals levels in the South River. Some metals were above threshold values in the Patuxent and Wicomico Rivers also. The AVS/SEM ratios in pore water were below 1 in all cases. Organic analyses on bulk composite samples demonstrated low level PAH contamination in all four systems. Pore water ammonia was relatively high (> 7mg/l) in all samples.

A toxicity risk ranking model, developed previously, was applied to the laboratory data. The model ranked the South River as the most toxicologically impacted site. The Patuxent and South Rivers were ranked far below the South River, however some specific locations in the Severn and Patuxent Rivers showed indications of sediment contamination. The Wicomico River had the lowest overall risk score. The Patuxent River may require more intense sampling due to it's relatively larger size. The model is tolerant of variable amounts of data between stations. A factor for consistency of results dampens the effect of individual spikes in the data base without masking them altogether. The model can identify trends within and between sampling stations. It can reliably reduce an array of ambient toxicity data into a site-specific metric which is appropriate for comparisons with other metrics, such as IBI or community diversity indices. It does not generate probability limits. The model can document where chemical contamination is contributing to community impacts and also where toxicological impacts are not likely to be contributing to observed population level impairment. The Margalef species diversity index for fish communities sampled by bottom trawl was significantly correlated with toxicological risk scores for sediment.

INTRODUCTION

It is unknown if toxic contaminants directly or indirectly affect fish population levels in Chesapeake Bay. What is known is that many species which depend on the Chesapeake Bay habitat for reproduction are in a more advanced state of decline than those that spawn outside the Bay (Richkus et al. 1991). Clearly, this is due to over-harvest and/or loss of habitat or access to spawning grounds in some cases. It is also clear that some areas (e.g. Baltimore Harbor, Back River) are severely contaminated and others demonstrate localized ambient toxicity (Hall et al. 1994). It is unknown if localized toxic contamination effects influence populations in the Bay as a whole, or if low level, but widespread contamination is a greater problem, or if a combination of the two affect living resource populations. Wise management policy for resource use, including aquatic habitats, wetlands and the watershed requires a simple, yet meaningful method of habitat assessment to gauge the impact of resource utilization in coastal areas. Utilization results in by-products which include not only direct discharges, but also non-point runoff and changes in the hydrologic cycle. Resource use also requires a strategy that applies to management decisions concerning the location of preserves and buffers, development, agricultural use and provisions for public access.

An expanding development in the effort to quantify environmental impacts of toxic contaminants on specific sites and

regions is to employ biological indicators of toxic stress from the ambient environment. An ambient toxicity approach was developed in pilot programs sponsored by the Maryland Department of Natural Resources (DNR) and the EPA Chesapeake Bay Program (Wright et al. 1989; Hall et al. 1991). The objective was to provide a picture of *biologically* significant environmental contamination. The ambient toxicity pilot program field-validated a suite of sensitive lethal and sublethal bioassays for resident aquatic organisms. It has been demonstrated that the bioassays have the ability to detect the presence of toxic effects in contaminated areas, in areas of unknown quality and in areas previously thought to be pristine (Hartwell et al. 1991; Hartwell et al. 1993; Hall et al. 1994).

Measurements of changes in the biodiversity of communities at specific locations are also useful in appraising the ecological effects of toxic contaminants and other habitat alterations. A pilot project to assess the effects of urban development on fish assemblages and water quality in tidal tributaries of Chesapeake Bay was initiated in 1988 by Maryland DNR (Carmichael *et al.* 1992a; 1992b).

It is possible to combine the two approaches to relate quantifiable changes in fish communities to quantifiable toxicological impacts of contamination and begin to address the question of the impact of toxic contamination on populations and communities in specific regions (Jordan et al. 1994; May *et al.* 1992). This information may aid natural resource habitat

protection or restoration efforts by providing additional focus for prioritization of areas for implementation of regulatory programs and growth management plans.

In addition to the regulatory need for site specific estuarine biological measurements, it is useful to be able to represent the condition of complex ecosystems concisely by means of composite indices or simple graphics, so that managers and non-specialists can readily evaluate and compare information, establish goals, and set priorities for remediation. This requires the use of concise, understandable statistics that also are meaningful, representative, reproducible, and can be generated routinely without massive investments in data collection. Indicators are essential for (1) determining priority areas for management, (2) measuring the effectiveness of management actions and progress towards restoration goals, and (3) developing the capability to predict the ecological consequences of management scenarios.

This project is also a component of a larger effort underway in coordination with the Chesapeake Bay Program. The Ambient Toxicity Assessment Pilot Project was initiated in 1990 to address specific commitments in the Chesapeake Bay Toxics Reduction Strategy. Specifically, the commitment states that the signatories will:

> ***Develop and begin to implement a plan for Baywide assessment and monitoring of the effects of toxic substances, within natural habitats, on selected commercially, recreationally and ecologically important species of living resources.***

In addition, the program addresses specific recommendations of the Living Resources Monitoring Plan which calls for;

> *Identification of indicator species, biomonitoring techniques and specific assays suitable for long-term monitoring of ambient habitat toxicity to Chesapeake Bay living resources; recommend specific geographic areas, media and monitoring frequency.*
>
> *Implement ambient habitat biomonitoring based on pilot program recommendations.*

The objectives of this project were to assess ambient toxicity in water and sediment from Chesapeake Bay tributaries whose watersheds are undergoing urbanization and to compare the results with fish community health indices from the same areas.

METHODS

Site Selection

To relate changes in fish assemblages to land use, eight tidal tributaries were categorized according to the dominant land use within each watershed, with special attention paid to areas immediately adjacent to the tributary itself. Evenly-spaced stations were established along the axis of each tributary from its mouth to near the head of tide. These stations were sampled three times each summer in 1989-1992 (July, August, September) with beach seines and bottom trawls deployed near mid-channel. All fish captured were identified and counted. Data from each station were summed over the summer sampling period and an IBI metric was calculated. Salinity, temperature, dissolved oxygen, pH, Secchi depth, and physical habitat characteristics were recorded. Detailed habitat assessments were also conducted at each site. The metric was primarily designed to assess non-point pollution and nutrient enrichment impacts on a system-wide basis.

An association was found between dissolved oxygen and the number of species captured by the bottom trawls (Fig. 1) (Carmichael, et al., 1992a). However, some stations with acceptable dissolved oxygen demonstrated a completely depauperate bottom fauna. Also, fish assemblages in tributaries whose watersheds were dominated by urban development were less diverse than tributaries whose watersheds were dominated by forest and wetlands. The sample stations were located in critical habitats

for living resources, beyond the direct influence of point sources. Information on ambient toxicity and fish community health in these areas provides quantification of the impacts of nonpoint source pollution and sediment contamination on resident populations. Specifically, where low dissolved oxygen or other habitat measures do not predict the observed impaired fish communities, ambient toxicity may provide an explanation for depressed fish populations.

A preliminary study was conducted in four tributaries in 1993. The goal of this study was to test the suite of sediment and water column bioassays and assess the results in the framework of the toxicological risk ranking model. The study was conducted in coordination with the fish community sampling program. Tests were conducted in four tidal tributaries: Curtis Creek, Rock Creek (tributaries of the Patapsco River), Fishing Bay (north of Tangier Sound), and the Wicomico River (tributary of the Potomac River). Mortality, reproduction and growth rates of test organisms in the water column bioassays indicated chemical contamination in Curtis Creek and Rock Creek. These results varied from month to month and from species to species. Survival, reproduction and growth of test organisms in the Wicomico River and Fishing Bay was generally good, but some borderline effects were seen. The sediment bioassays demonstrated greater toxicological impacts than water column assays. The results demonstrated toxic impact in Curtis Creek and to a lesser extent in Rock Creek. Consistent with the biological data,

sediment chemistry clearly showed the that the Curtis Creek and Rock Creek sites were contaminated with heavy metals and PAHs. All four systems had detectable petroleum hydrocarbons present in the sediment.

The toxicity risk ranking model was validated with the laboratory data. The model correctly ranked Curtis Creek as the most chemically impacted site, followed by Rock Creek, and Fishing Bay and Wicomico River, which were essentially equal. The model also identified spatial trends between sampling stations in Curtis Creek. The risk ranking scores were significantly correlated with the diversity index of fish captured in bottom trawls. In addition, the toxicological risk scores correlated with bottom fish community metrics derived from a five year fish sampling data base. The model not only documented where chemical contamination was contributing to community impacts, it also indicates that observed population level impairment in Fishing Bay was not likely to be due to chemical contamination. Based on these studies, four tidal tributaries were selected for paired ambient toxicity/fish IBI sampling to assess the impact of urbanizing watersheds on receiving stream habitat quality (Fig. 2-6).

Severn River

The Severn River watershed is located in Anne Arundel County and covers approximately 51,688 acres. With 36% of this area

developed, it was the most urbanized watershed studied. Residential and forested areas dominate the shoreline, and major highway crossings include Routes 50 and 450 near Annapolis, and Interstate 97 near its headwaters. The river is subject to intense boating pressure, as both the United States Naval Academy and several marina facilities are located at or near its mouth.

South River

The South River watershed is located in Anne Arundel County and covers approximately 43,452 acres. Roughly 25% of the watershed is developed, and about 17% is agricultural. Residential and forested areas compose much of the shoreline, with many public and private marinas and their resultant boating traffic. Major highway crossings include Routes 2, 301, and 50. Land use ratios are nearly identical to those of the Severn River watershed.

Patuxent River

The Patuxent River is bordered on the west by St. Mary's, Charles, Prince George's, and Montgomery Counties, and on the east by Charles, Anne Arundel, and Howard Counties. The watershed is covers approximately 480,660 acres, of which about 11% is developed. The dominant land types are agricultural and forested. Two large reservoirs have been constructed on the

mainstem near Laurel, and United States Naval Testing Centers are located on Solomon's Island and Cedar Point at the river's mouth. The Patuxent River was chosen for this study in part because of its great potential for further urbanization. Fast-growing areas within the watershed include Bowie, Columbia, Crofton, Laurel, and Upper Marlboro among others.

Wicomico River

The Wicomico River is located between Charles and St. Mary's Counties, and drains approximately 61,062 acres. Agriculture and forest make up the dominant land uses at about 30% and 40% respectively. Less than 6% of this watershed is developed. The Wicomico River served as the saltwater reference tributary in Carmichael, et al. (1992b), and consistently demonstrated healthy fish community metrics. It was selected to represent a relatively clean field reference tributary with little direct point source pollution. The Wicomico River was retained from last year's study to serve as an established field reference site and to lend continuity to the Ambient Toxicity project from one year to the next.

Water Column Bioassays

Fish community sampling methods were designed to assess the fish community at its' peak diversity in summer. Ambient toxicity

testing was initiated before the fish community IBI sampling was begun to assess the potential impact of toxic contamination as the fish communities matured and to assess any short term spikes in toxic effects which may be detectable during the late spring and summer.

The following water column tests were conducted on a monthly basis from April through August 1994: 7-d sheepshead minnow (*Cyprinodon variegatus*) survival and growth test; 7-day grass shrimp (*Palaemonetes pugio*) survival and growth test; a copepod *(Eurytemora affinis*) life-cycle survival and reproduction test; and a bacterial luminosity bioassay (Microtox^{R}). Water column tests were also conducted in July and August with the submerged aquatic plant species, sago pondweed (*Potamogeton pectinatus*) which measured growth and reproduction. These bioassays go far beyond the original scope of the proposed project, but the data are included here since they represent a more thorough data base. Fish and grass shrimp bioassays were conducted at DNR's Aquatic Toxicology Laboratory (Glen Burnie, MD). The copepod bioassays were conducted at the University of Maryland, Chesapeake Biological Laboratory (CBL). The vascular plant bioassays were conducted at the Anne Arundel Community College, Environmental Center.

Depth integrated water samples were collected by boat from the four rivers. Standard water quality parameters were measured at the time of collection (Table 1). Samples were taken twice from each site during the course of each 7-day test to provide

fresh renewal water for the bioassays. The sampling interval was four days. Water samples were filtered through 37μm mesh and adjusted to a salinity of 15 ppt. All water was stored in amber bottles at 4°C until use. When the sample salinity exceeded 15 ppt, no adjustment was made. Water for the copepod assays was not salinity adjusted until delivery to CBL for testing.

Heavy metals, acid and base/neutral extractable semi-volatile organic compounds and chlorinated pesticides were analyzed on August samples only. The semi-volatile organic compounds were analyzed by GC/MS, EPA method 625. Chlorinated pesticides and PCBs were analyzed by GC/MS EPA method 608. Arsenic, selenium, silver and thallium were analyzed by furnace or flame AA (EPA method 206.2, 270.2, 272.1 and 279.2 respectively. Mercury was analyzed by cold vapor AA (EPA method 245.1). The other metals (Sb, Be, Cd, Cr, Cu, Pb, Ni, Zn) were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP) (EPA method 200.7).

Fish and Grass Shrimp

Culture and maintenance procedures for grass shrimp and sheepshead minnow used methods contained in APHA 8720 and EPA-600/4-90-027 respectively. Adult sheepshead minnows were fed with Tetramin^{R} and *Artemia* nauplii. Spawning was induced by temperature shift and was timed so the larvae were between 24-48 hrs old when bioassay testing started. The eggs were deposited on spawning mats which were then incubated in high salinity water

(30-40 ppt) to minimize fungal growth and aerated vigorously. Salinity was adjusted to 15 ppt 24-48 hours before hatching.

Grass shrimp larvae were either purchased from commercial suppliers or brooding grass shrimp were collected from a clean site at Ship Point near Calvert Cliffs State Park. They were fed ad libitum with *Artemia* nauplii prior to spawning. They were then transferred to Carolina bowls to spawn. Larvae were separated from adults and incubated between 4-7 days prior to testing.

For both fish and grass shrimp, ten larvae were placed in a 600 ml beaker with 400 ml of ambient water. Four replicates were conducted for all tests. Temperature and photoperiod were maintained constant within an incubator at 20°C and 16:8 L:D. Eighty percent of the test water in each beaker was renewed daily with water from storage. Survival was monitored daily. Dissolved oxygen, temperature, salinity and pH were measured daily. The tests organisms were fed *Artemia* nauplii in the morning before water change and after the water change. The water was aerated if DO levels fell below 60% saturation. At the completion of the 7-day experiments, all larvae were preserved in 8% formalin and stored for no more than four weeks. Following a deionized water rinse, they were dried for up to 24 hr at 100°C and weighed.

Percent survival of the larvae was compared to controls using the t-test following arc sine transformation, or a Wilcoxon rank sum test if the data were not normal. Growth parameters were compared using ANOVA and the Wilcoxon rank sum test. Differences

between means were considered significant at the α=0.05 level.

A 48-h static potassium chloride reference toxicant test was conducted for each species to establish the relative health and sensitivity of test organisms.

Eurytemora affinis

Copepod bioassays were conducted during April through August. Ambient water from each site was filtered through 5um mesh and its salinity adjusted to 10ppt. Reference water was taken from Wachepreague Bay on the Atlantic coast of Virginia each month. It was autoclaved, filtered to 1μm and diluted from its original salinity (33‰) to match the salinity of the test water. Dilutions were made with CBL well water. All media were adjusted to laboratory temperature (24°C) prior to the test.

Animals were obtained from a culture of *E. affinis* which has been maintained at Chesapeake Biological Laboratory for >10 years. The culture is normally maintained at 21°C and 10‰ and is fed every second day on a 1:1 mixture of *Thallasiosira pseudonana* and *Isochrysis galbana*. To segregate young nauplii from the culture, adults were separated using a 200μm Nitex filter and placed in a tank with a 64μm Nitex screen at its base. This tank was placed inside a second container such that eggs passed through the screen into the second tank. Twenty four hour old nauplii from the second tank were concentrated using a 64μm screen and pipetted into plastic weighing dishes. Four replicates with fifteen nauplii were tested in water from each river.

Individual vessels were 400ml beakers with rectangular 63μm Nitex windows 1cm from the bottom. The beakers were immersed in test water in polycarbonate containers. Each beaker contained 200ml of test water. Close scrutiny of the containers was maintained throughout the assay and periodic gentle brushing of algae from the outside of the Nitex window ensured good water exchange.

Water was renewed every second day, at which time the algal feeding mixture (1:1 *T. pseudonana/I. galbana)* was added to achieve a cell density of 4 x 10^5 cells/ml. Algal cell densities were monitored at each feeding and adjustments made such that each system had the same cell density.

Copepod development was followed through one life cycle, e.g. 10-14 days. Numbers of surviving adults were counted and eggs and nauplii and subadults from the F1 generation were also counted at the end of the test.

Percent survival of the larvae was compared to controls using the t-test following arc sine transformation, or a Wilcoxon rank sum test if the data were not normal. Reproduction was compared using ANOVA and the Wilcoxon rank sum test. Differences between means were considered significant at the α=0.05 level.

Potamogeton pectinatus

The techniques used in this study were developed by the U.S Fish and Wildlife Service, Patuxent Wildlife Research Center and the Environmental Center of Anne Arundel Community College (Fleming et al. 1988; Ailstock et al. 1991). Laboratory

propagated stocks of sago pondweed, originally collected from Chesapeake Bay, were weighed and rooted in a nutrient agar medium. Eight replicates were submersed in 750ml of ambient water from each river. Light levels were maintained at 70mol/m^2/s PAR on a 12L:12D cycle. Temperature was held constant at 22 °C. Filtered air supplemented with CO_2 to approximately 3% was continuously pumped into each test chamber. After four weeks, the plants were removed and weighed. The number of rhizome tips on each plant were counted as a measure of reproduction. The plants were then dried and weighed again.

Microtox^R

The Microtox^R assay was performed on water samples from April through August. Each of the two water samples taken each month was tested (except April). The bioassay method exposes a luminescent bacteria (*Photobacterium phosphoreum*) to ambient water samples and measures changes in light output following incubation. Changes in light output are proportional to toxicity (Microbics, 1993). Tests were run as dilution series bioassays for each sample. Samples were filtered through 37μm mesh, adjusted to 15 ppt salinity, and tested at concentrations of 0 (control), 60, 70, 80, 90, and 100 %. Four replicates were conducted at each concentration. Reference water supplied by Microbics Corporation was used as the diluent for the samples and as the control. Incubation time was 15 minutes at 15°C. Light output was then measured with a photometer. Response was measured

as inhibition (or stimulation) of luminosity over time as compared to the control. Luminosity of the bacteria in ambient water was compared to controls using the t-test or a Wilcoxon rank sum test if the data were not normal. Differences between means were considered significant at the α=0.05 level. Lowest Observed Effect Concentrations (LOEC) were determined by Dunnetts' procedure (Microbics, 1993) on inhibitory samples.

Sediment Bioassays

Sediment toxicity tests were conducted using the following tests: 10-d sheepshead minnow (*C. variegatus*) embryo-larval survival and teratogenicity test; 10-d amphipod (*Lepidactylus dytiscus*) survival and growth test; 10-d amphipod (*Leptocheirus plumulosus*) survival and growth test; 10-d polychaete worm, (*Streblospio benedicti*) survival and growth test; and a lettuce and *Spartina alternaflora* seed germination test. The *Leptocheirus plumulosus* bioassays were conducted at the DNR Toxic Aquatic Contaminants Laboratory. The seed tests were conducted at the Anne Arundel Community College. All the other sediment bioassays were conducted by Old Dominion University, Applied Marine Research Laboratory (AMRL).

Sediment samples were collected with a petit ponar grab sampler in April, 1994 from each station for initial grain size analysis. The top two centimeters were retained for testing. In August, 1994, five discreet field samples were collected from each river system (Figs 3-6). The sampling stations were selected

to coincide with fish community assessment trawl sampling stations. The sampling plan does not provide for true field replication for statistical purposes, but does allow a contrast of upstream vs downstream locations. The decision to sample discrete locations throughout each river was based on the intent to assess the condition of the entire system, relative to the fish community indices. This was unnecessary in the water samples since all the systems were tidal. Samples were segregated throughout the collection and toxicological tests. Samples were held out of direct sunlight at 4°C and used within two weeks.

Control sediments for each animal species consisted of native sediments from the area in which the test organisms were collected or naturally occur. Control and/or reference sediments (see below) were tested with each set of test samples. The fine grained reference sediment was obtained from a small tidal creek within the Poropotank River, Virginia. The sand reference sediment was collected from Lynnhaven Inlet, Virginia Beach, Virginia. The control sediment for the *L. plumulosus* bioassays was their laboratory culture sediment.

Particle size analysis of test sites ranged from less than 1 to 99% sand (Table 2). Because of the large range in particle size between test sites, two reference sediments were used with the *L. dytiscus*, *C. variegatus* and *S. benedicti* bioassays. The purpose of these reference sediments was to assess what effect "normal" physicochemical parameters (primarily particle size) would have on the survival of the organism being exposed in the

absence of toxicants. The reference sediments which were used bracketed the sediment particle sizes found at the selected test sites. Reference and control sediments were from the designated sites and are indicated throughout the text as follows:

1) Lynnhaven Sand
2) Lynnhaven Mud
3) Poropotank Mud

Lynnhaven mud was used as the control sediment for *S. benedicti* and *C. variegatus* eggs. Lynnhaven sand was used as the control for *L. dytiscus*. *L. plumulosus* survives well in a wide range of sediment grain sizes. Mud from Fishing Bay on the eastern shore of Maryland was used as the control for this species.

Inorganic contaminants were evaluated concurrently with toxicity tests on a composite sample from each river system. Sediments were analyzed for acid volatile sulfides (AVS) and total organic carbon (TOC). Samples were frozen until analysis, at which time they were thawed, and then homogenized by gently stirring. Samples were analyzed for AVS using the method of DiToro *et al.* (1990). Simultaneously extractable metals (SEM) analysis was conducted on all samples to use with the AVS data in order to determine the potential toxicity of the sediment due to metals. The sample for the SEM analysis was obtained from a step in the AVS procedure. The concentrations of the SEM were

determined by EPA-600/4-79-020 (1979). Cadmium, lead, copper, nickel, and zinc were determined by ICP following USEPA method number 200.7. Mercury was determined by cold vapor generation following USEPA method number 245.1. The concentrations were then converted to micromoles per gram dry sediment and were added together to provide the total SEM value. Pore water samples were extracted by squeezing with a nitrogen press. All pore water samples were filtered then frozen until analyses of ammonia, nitrite and sulfides were conducted. Bulk metals were analyzed by ICP using EPA method 200.1, 200.2 or 200.7. Acid and base/neutral extractable semi-volatile compounds were analyzed on samples composited by river using GC-MS by US EPA method 8270 (SW846).

C. variegatus

Sheepshead adults were maintained in accordance with standard methods and guidance from general literature and U.S. EPA (1991). Animals were cultured at 20ppt salinity at ambient laboratory light and approximately 20^{0}C. Adult breeders were maintained in an 800 liter tank in an elevated "breeder" basket at 20ppt salinity, 25^{0}C, and a 16L:8D photoperiod. Breeders were fed a commercial marine blend flake food 10 times per day and supplemented with newly hatched *Artemia* nauplii twice daily. Eggs were collected daily below the baskets and transferred to clean 4 liter aquaria. These aquaria were then placed into 25^{0}C incubators and aerated. Daily water changes of approximately 90% were performed until the eggs were 48 hours old, when they were

ready for placement into test chambers.

A series of test containers was set up according to ASTM methods (1990). Two centimeters of sediment were placed into each of five replicate, 2-liter test containers with 750 ml of 15 ppt overlying water. Ten embryos were placed into a cylindrical mesh egg chamber. The chamber was then gently placed into the sediment such that the sediment passed through the bottom mesh and was allowed to contact the eggs. Control sediment consisted of Lynnhaven mud. Test containers were monitored daily for oxygen, temperature, and pH. The number of animals, i. e., live/dead eggs, live/dead larvae, and the number hatched was also recorded. Deformed larvae were treated as dead. The larvae were not fed. The test was performed a total of ten days from test initiation or two days post-hatch for all controls, whichever occurred first.

L. plumulosus

Amphipods were maintained in accordance with laboratory methods and guidance from DeWitt et al (1992). Animals were cultured at 15ppt salinity in 10 liter tanks maintained at ambient laboratory light and approximately 20^{0}C. One to two centimeters of native sediment was placed on the bottom of the culture tanks and enriched with a food supplement weekly. The food supplement consisted of approximately 50:50 mixture of ground commercial marine flake food and powdered alfalfa. Fifty percent water changes were performed weekly. Animals were

harvested on a monthly basis and were either used for testing, culture expansion, or simply culled. Culture tanks were aerated gently. Test animals were collected for testing by siphoning the culture sediment from the tanks and passing it through a series of stacked sieves. Those animals which passed through a 1000 μm sieve, but were retained on a 500 μm sieve, were used for the tests. A subset of the test animal population was selected for initial weight measurements.

L. dytiscus

These amphipods were collected from an estuarine site in Virginia Beach and transported to the AMRL for acclimation to laboratory conditions. Salinity was slowly adjusted to 15ppt. Animals were held in their native sandy sediment at least one week prior to initiation of the bioassays.

S. benedicti

Worms were collected in the field, brought to the laboratory and held for at least one week prior to testing. Animals were sieved out of the holding tank and placed into culture dishes 24-hr prior to addition to the test containers.

Procedure

For the polychaete and both amphipod species, a series of test containers was set up according to the methods outlined in ASTM (1990). Two centimeters of sediment were placed into each of

five replicate 1 liter test containers with 700 ml of overlying water. Twenty animals were added to each test vessel and monitored for 10 days at 25°C. Test containers were monitored daily for dissolved oxygen, temperature, and Ph. In the *L. dytiscus* bioassays, the animals were fed 25 mg of ground alfalfa/Tetramin™ cichlid flake food in a 1:1 ratio per test container every three days throughout the duration of the test. At the end of 10 days, animals were sieved from test containers and mortality was recorded. Animals were then preserved for weight measurements. Static, acute, non-renewal, water-only reference toxicant tests were performed for *C. variegatus*, *L. dytiscus* and *S. benedicti* using cadmium chloride. Seasonal changes in sensitivity have been observed previously in *L. dytiscus* Deaver and Adolphson, 1990). Static, acute, non-renewal, water-only reference toxicant tests were performed for *L. plumulosus* using potassium chloride.

Lettuce seed

One hundred seeds were placed in porous bags which were then buried in sediment samples from each sample site. Ten replicates per test were employed. Control tests were done in a sand sediment adjusted to salinities of 0 to 9 ppt. Parallel tests with *Spartina alternaflora* seeds were conducted to assess potential effects of salinity on the lettuce seed bioassay. Seeds were incubated in the sediment for 3 days at 2°C. Percent

germination was recorded at intervals of 2, 7 and 14 days.

Statistical Analysis

Statistical evaluations relative to particle size effects were made based on the response to the reference sediments. Sheepshead egg data were evaluated using analysis of variance ANOVA) contrasts and compared to the controls. Evaluation of total mortality was assessed by combining egg mortality, larval mortality, and unhatched eggs remaining at the termination of the test. Unhatched eggs were included as mortality based upon previous observations and the assumption that probability of hatching and thus survival decreases essentially to zero by test termination i.e. eggs are ecologically dead).

For all other tests, the analyses consisted of ANOVA models with *a. priori* tests of each treatment contrasted to the controls. Arcsine transformations were used for the percent mortality data. Mortality was corrected for particle size effects using the regression equation previously established for *L. dytiscus* % survival = 98.41 - 0.35066 X % Silt/Clay) Hall et. al. 1991). Weight was expressed as percentage of change from the initial weight measurements.

Ranking Model

At the inception of the ambient toxicity program, a ranking scheme was proposed to evaluate the toxicological results on a site by site basis (Hartwell 1989). This scheme has five

components: 1) severity of effect; 2) degree of response; 3) test variability 4) site consistency; and 5) number of measured endpoints. Consistency and the number of endpoints measured are site specific attributes, while severity, response and variability are characteristics of the individual bioassays conducted at all sites. The rational of the ranking system is to quantify environmental risk, not merely to rank presence or absence of toxic effects. Thus, high uncertainty or variability will result in increased risk scores to a similar extent as positive toxic responses.

Severity refers to the degree of effect which the bioassay endpoints measure. Mortality is considered the most severe response followed by impaired reproduction and impaired growth. Other endpoints could be included in the list. The severity factors were arbitrarily set at mortality = 3, reduced fecundity = 2 and reduced growth = 1.

Degree of response is the measure of the proportion of organisms responding in each bioassay regardless of statistical significance e.g. 10% mortality, 15% growth inhibition, etc.). In this regard, it is as important to know what percentage of the organisms responded as it is to know whether it was 'statistically significant'. In the statistical contrasts, mortality was not corrected for control mortality e.g. Abbott's formula) because of inherent uncertainties of the effects of laboratory manipulations on 'non-standard' species in some cases, and no such corrections exist for other bioassay endpoints e.g.

growth). The response values are adjusted for control values in their calculation formulas. Negative values were assigned a value of zero in the model data base. The following equations were used to calculate degree of response values:

Ambient Toxicity Scoring Calculations

% growth response=

{control wt - test wt)/control wt} X 100

% reproduction response=

{control reprod - test reprod)/control reprod} X 100

% luminosity response=

{control lumin.- test lumin.)/control lumin.} X 100

% mortality response=

{test # dead - control # dead)/initial total #} X 100

Variability was expressed as the coefficient of variation of response for each set of laboratory replicates. This parameter reflects the internal variability for each endpoint and sample period. Data were pooled by river or sample date for this purpose.

Consistency refers to the agreement between the various

bioassay endpoints measured at a site. If the results from all tests and/or species agree, consistency is high, and confidence in predicting toxic impacts is high. If half of the results are positive and half are negative, consistency and certainty of toxic impacts is lower. Consistency was calculated as the cube of the difference between 1/2 the number of endpoints and the number of statistically non-significant responses at each site. Statistical significance in this instance refers to typical 'sample site vs control' comparison tests, not a statistical test of the calculated response values.

Consistency = N/2)-X)^3, where N= total number of endpoints and X= number of statistically non-significant endpoints.

When bioassay endpoint values tend to be non-significant N/2 $\leq$ X), the function is negative. When half of the endpoints are significant and half are non-significant N/2 = X) the function is zero. When endpoint values are statistically different than control values the function is positive (Fig. 7). The absolute value is dependent on the amount of data available. Large data sets high N) will have higher extremes. This polynomial function was devised as an additive factor in the equation. It reduces the risk score of a station when most of the test results were not significantly different than controls but increases the risk score when more than half the tests are significant.

The number of endpoints measured at each site refers to the

number of bioassays species) and measured parameters survival, growth, etc.) which are monitored. For statistical and experimental reasons, the number of tests run at each site should ideally be the same. However, given the uncertainties of experimental work, this is not always possible. For example, if mortality is very high, it may not be possible to measure growth.

Each site was ranked by the following scheme; endpoint severity was multiplied by the percent response of the test organisms for each bioassay endpoint, and the coefficient of variation for that test endpoint. The products from all tests were summed for each test site. The sum was adjusted by the site consistency factor and divided by the square root of the number of test endpoints for each site to equalize scores from different sites where different amounts of data may be present.

$$\text{Site Score} = \frac{[\{\Sigma \text{ Severity}) \% \text{ Response}) \text{ Coeff.Var.}\} + \{\text{Consistency}\}]}{\sqrt{N}}$$

There are three possible risk ranking scores which may be calculated; water only, sediment only or water and sediment combined. Since water column bioassays are replicated in the laboratory, a risk score can be calculated for each sampling month or the response scores can be averaged by river over months. This approach allows for an assessment of water column contamination effects on pelagic communities or possibly specific

species. Sediment samples were collected and tested as discrete samples without laboratory replication. Therefore, calculation of a risk score can only be done by pooling the data together by river to calculate the C.V. and consistency factors. The rationale for sampling sediment in this way was the assumption of low temporal variation in sediment relative to the water column and for the purpose of examining sediment contamination effects on a system-wide basis, which is consistent with the IBI community approach. This approach allows for an assessment of sediment contamination impacts on bottom communities and could be contrasted with benthic community metrics as well as bottom trawl survey data.

Sediment and water data may be pooled together by river system to calculate a toxicity risk factor for the whole system. This calculation allows an assessment of toxic contamination on the entire river system with equal weight given to sediment and water column assuming equal data availability). It also has the advantage of combining the data into larger subsets which tends to dampen out individual spikes in the data set. To pool the data, the calculated response results are averaged over months for water and over locations for sediment. The C.V. of the mean responses is used in the risk calculation, rather than the mean C.V. value. Consistency is calculated as before.

A simple toxicity score can also be calculated for each sample. This is the sum of the products of endpoint severity and percent response divided by $\sqrt{N}$. This score is a useful technique

for comparing individual sites and for examining spatial or temporal trends in sediment and water samples. These calculations are also instructive in examining the response of the risk ranking model and its' response to inclusion of the interrelated factors of consistency, coefficient of variability and the number of data points.

$$\text{Toxicity Score} = \{\Sigma \text{ Severity}) \ \% \ \text{Response})\} / \sqrt{N}$$

The risk scores were contrasted to diversity indices (Margalef 1968) and the IBI data. Pearson correlation coefficients were calculated for every combination of toxicological risk score (water, sediment and combined) and fish community index for the 1994 sampling year. Three categories of community index were used, including bottom trawl species diversity, resident (estuarine spawners) species diversity and the overall IBI (Table 3). The resident species data included both bottom trawl and beach seine data. The IBI score effectively incorporates all resident and migratory species in both the trawl and beach seine data. Calculation of an IBI score with only the trawl data would not be effective because it would incorporate an incomplete set of species, relative to the number of metrics in the IBI derivation. The IBI is designed to reflect the diversity and trophic structure of the entire fish community. This also means the IBI score should respond to a variety of factors in the

habitat, including but by no means limited to, toxic impacts.

Briefly, the IBI derivation method includes measures of species richness, dominance, abundance, trophic structure and life history traits. All metrics have been evaluated for correlation with salinity, sampling frequency, consistency and effectiveness of sampling. Nine metrics are used to calculate the IBI (Jordan et al. 1991; Vaas and Jordan 1991) including number of species in trawls, total species, number of species comprising 90% of individuals, total individuals (excluding menhaden), number of anadromous species, number of resident species, % carnivores, % benthivores and % planktivores. Following transformations and salinity calibrations, the individual metric values are ranked between stations and divided into three groups; low, medium and high. A ranking value of 1, 3 or 5 is then assigned to each metric at each station. The ranking values of all nine metrics at each station are then summed to compute the IBI score, which can range from 9 (lowest integrity) to 45 (highest integrity). The Margalef diversity index is calculated as ;

$$I = (S - 1)/ \log N$$

where S = number of species

N = number of individuals

RESULTS

Statistically significant mortality did not occur in water column bioassays with fish or grass shrimp (Table 4). Grass shrimp survival values were lower than normal in the August tests in the South and Severn River bioassays, but were not statistically significant. Growth rates in fish were slightly, but significantly reduced in April in the Severn River water sample (Table 5). The difference was small, but variability was very low. All of the August fish bioassays resulted in poor growth. It is unclear if this is a real effect or a sample handling artifact. Control growth was very good, and all tissue samples were handled and weighed with exactly the same methods and all at the same time. Significant inhibition of grass shrimp growth was not observed (Table 5). The reference toxicant data for sheepshead minnows and grass shrimp are shown in Table 6. The 1994 experiments used Kcl as a reference toxicant for the first time so no historical data base exists for comparison. Results were reasonably consistent from month to month, where comparisons are possible. In the copepod assays, survival to adult stage was significantly reduced in the July tests in the Patuxent River (Table 7). The South River had the lowest overall average survival, but no river was consistently lower than others in all months. Survival was significantly lower than controls in the May Wicomico River test. However, survival and reproduction in May

was reduced in all tests, including the controls. Reproduction, as measured by the number of eggs, nauplii and subadults present, was highly variable between stations and months. Significantly reduced reproduction was observed in June and July samples from the South and Patuxent Rivers, and the Severn River in June. Reproduction in the Wicomico River in May was completely absent (Table 7). Again, the significance of results from May are questionable for all stations. No mortality, growth or reproductive effects were seen in the plant bioassays in July or August (Table 8). The Microtox^{R} assays demonstrated statistically significant inhibition in April, May and August (Table 9a). None of the samples were inhibitory in June or July. The bioassays often showed significant stimulatory results (Table 9a). This may be due to eutrophic conditions in the tributaries. The LOECs for the inhibitory tests are shown in Table 9b. Only one instance of effects below 70 % river water was observed. Due to the experimental design, the true LOEC for the Severn River #2 run is unknown. The water chemistry analyses from the August samples are shown in Table 10. Some metals were above detection limits, but all values were relatively low, and all were below marine ambient water quality criteria.

Survival results from the sediment bioassays with amphipods, worms and sheepshead minnow eggs are included in Tables 11 and 12. High levels of mortality were observed in the amphipod tests. After adjustment for grain size however, only the South River demonstrated elevated mortality levels. Results were not

statistically significant due to high variability. Mortality in the polychaete tests was also highly variable, and statistically significant differences were not observed. Higher levels of polychaete mortality were observed in the Patuxent River relative to controls. Mortality in the South and Wicomico Rivers was marginally elevated. Again, results were not statistically significant. Mortality in the fish bioassays was also highly variable (Table 12). The highest mortality level was observed in the South River. Fish mortality was also elevated above the controls in the Wicomico and Patuxent Rivers. Mortality in the South and Patuxent Rivers was primarily due to hatching failure as opposed to larval mortality. Very distinct patterns of site specific mortality in the amphipod, worm and fish tests are seen when the data are viewed on a site by site basis Figs 8-14. Mortality in the South River is primarily at the upstream sites as opposed to the Patuxent River where peak mortality occurred at the middle site. The most substantial mortality in the Wicomico River was at the downstream station. In this case, mortality was observed in the larval (post-hatch) stage as opposed to the embryos (egg stage). No significant mortality was observed in the *L. plumulosus* bioassays (Table 13). Mortality levels were lower than in the other benthic species, with the exception of the South River. Growth was poor in the Patuxent and South River bioassays (Table 14) however, control growth was also poor. Growth of controls in the *L. dytiscus* and *S. benedicti* tests was also lower than ambient results (Table 15). The results

of reference toxicant tests conducted with *L. dytiscus*, polychaetes and sheepshead minnows are shown in Table 16. Values were below historical levels. No significant effects on germination were observed in sediment bioassays with either the lettuce or *S. alternaflora* seeds (Table 17).

Bulk sediment chemistry results for sediments are shown in Tables 18 and 19. Lead and zinc were above NOAA ER-L levels in the Patuxent, South and Wicomico Rivers long and Morgan 1990). The South River also had chromium levels near the ER-L value. Contamination with routine organic contaminants was relatively low in all systems (Table 19). Data for SEM and SEM/AVS ratios for metals in sediment pore water are shown in Tables 20 and 21. The Patuxent River sediments had the highest SEM levels, primarily due to zinc values, but no SEM/AVS ratios were above 1.0. The South River had the highest ratio of 0.384. Ammonia levels in pore water were relatively high in all samples, including the reference sediments (Table 22). Total organic carbon values are shown in Table 23. The Severn River sample had less than 1% TOC.

The water column risk scores for each sampling period are shown in Figure 15. Results were highly variable between months and stations, with no apparent pattern. Mean water toxicological risk values are shown in Figure 16. Toxicity risk scores in the four rivers are similar, and relatively low level. The toxicity scores for discrete sediment samples are shown in Figure 17. There appears to be a strong upstream to downstream gradient in

the South River. Data from the Severn River demonstrates a localized spike at the upstream station. The Patuxent River has a spike in the middle reach of the river. The Wicomico River scores are relatively uniform, with no extreme peaks. The pooled sediment risk scores are shown in Figure 18. The pooled scores integrate variability and consistency into the scores. These values indicate that the South River has a high risk for sediment toxicity impacts. The risk scores for combined water and sediment data are shown in Figure 19. The South River clearly has the risk highest value. The Patuxent River has the next lowest risk score, which is three times below the South River value. The toxicological risk values for the Severn and Wicomico Rivers are low or negative.

The correlation coefficients for the risk scores and the fish community metrics from 1994 are shown in Table 24. The bottom trawl diversity index was strongly correlated with the sediment toxicological risk score. The combined score, which was dominated by the sediment score also tracks the bottom diversity score, but was not statistically correlated due to increased variability. The resident species diversity index and the overall IBI did not have strong correlations with the toxicological risk scores. The relationships between the toxicological risk scores and diversity indices can be seen in Figures 20-22.

DISCUSSION

The South River bioassays displayed greater toxicological responses than any other tributary. Statistically significant levels of mortality were not observed in the sediment bioassays, primarily due to high variability. However, the South River bioassays generally exhibited the lowest survival rates in both water and sediment bioassays. Significant survival, growth and reproductive effects were seen in water column tests. Low levels of total organic carbon in the sediments, which can indicate potential food shortage stress on test organisms Adolphson and Alden, 1994) did not appear to be a contributing factor. Pore water ammonia did not appear to be a contributing factor. There was a marked difference between the upstream and downstream portions of the river. Most of the observed sediment mortality effects were concentrated in the upper three stations (Figs 8-11). Sediment chemistry did not reveal any specific chemical or suite of chemicals which may be responsible for the observed effects. Metals levels in bulk sediment analyses were marginally higher than other stations, but this result was element specific. Levels of chromium, lead and zinc exceeded NOAA ER-L values. Pore water metals levels were not observed to be the highest of the four rivers. The AVS/SEM ratio was the highest, but was well below 1.0.

The Severn river sediment bioassays demonstrated toxic

effects only at the upper-most station. As in the case of the South River, sediment results were not statistically significant in the pooled data due to high variability. Significant survival, growth and reproduction effects were observed in the water column bioassays. Chemical analyses of composite sediment and water samples were unremarkable.

The Patuxent River results demonstrated both water column and sediment toxicity. Mortality of polychaetes and fish embryos was observed primarily at one station in the middle reach of the river. Significant survival and reproduction effects were observed in the water column bioassays. The Patuxent River had the highest SEM metals levels. It also had the highest AVS level, resulting in a low SEM/AVS ratio. Patuxent River sediments were the only samples in which Cd was present at detectable levels. The levels of lead and zinc exceeded NOAA ER-L levels.

The Wicomico River bioassays resulted in very few toxic responses. Survival, growth and reproduction in the water column bioassays did not show any significant effects except in the May copepod bioassays. All the copepod bioassays, including the controls, had poor survival and reproduction in that particular month. One sediment sample yielded high mortality for fish larvae. Bulk sediment analyses demonstrated that lead and zinc concentrations were above NOAA ER-L levels.

No specific chemical or suite of chemicals has been identified in these samples, which can explain the observed results. The standard suite of priority pollutant chemicals was

analyzed. However, a thousand chemical contaminants have been identified in the Bay USEPA CBLO 1992) and most of these are not analyzed for in standard surveys. In addition, many more organic chemicals are simply regarded as unknowns in GC/MS analyses. Many chemicals can not be analyzed by GC at all. Finally, the chemical analyses are performed on composites of all stations within each river due to cost constraints. Thus, high level concentrations of chemicals from a specific location or locations could essentially be diluted by sediment from cleaner portions of the estuary.

None of the sediment bioassays resulted in statistically significant results. Data from the polychaete, fish and *L. dytiscus* bioassays, which showed site specific responses, had high variability. This is due in part to the great differences in response from different individual stations e.g. upstream vs downstream). Since data must be pooled by river to make statistical tests of river vs control, the mean level of response from a given station may be masked by lack of response at other stations. This is analogous to the analytical chemistry results, where concentration levels from specific stations are diluted by compositing with cleaner sediment from other stations. High variability was not observed in the *L. plumulosus* or plant assays. These bioassays yielded few, if any positive responses in the first place.

No mortality, growth or reproduction effects were observed in any of the vascular plant bioassays. Except for pesticides, the relative sensitivity of plants and animals to environmental

contaminants is largely an unknown factor. However, the sago pondweed has been tested with several individual chemicals and has been shown to be a reasonably sensitive species, especially to herbicides Flemming et al. 1988, 1991, 1993). The potential for seasonal sensitivity of plants to low level contamination is also unknown. The impacts of herbicide runoff would be expected to be lower in late summer than in spring. This aspect of sampling design needs further investigation.

The *E. affinis* data for the month of May are questionable. Survival to the adult stage was lower in May than in other months, including the controls. Reproductive values were drastically lower in May than in any other month for all stations and the controls. The May copepod bioassay results are in large part responsible for the high water risk score for the Wicomico River for that month.

Interesting spatial patterns were observed in the sediment bioassays. Toxicological data from the upper half of the South River demonstrate severe impacts in three out of four of the animal bioassays. The downstream stations do not show above average toxicity values. A more intense evaluation of the South River west of Route 2 is indicated. Only the upper-most station of the Severn River demonstrated high toxicological responses. This is reflected in the fish and *L. dytiscus* survival and growth bioassays. In the Patuxent River, the middle sampling station demonstrated elevated toxicological effects. This was due to the fish embryo and polychaete survival bioassay results. It should

be noted that in spite of the high mortality rate for worms at this site, the highest rate of growth by the worms was achieved at this site also. The only area in the Wicomico River which demonstrated elevated response was at the mouth of the river. Only the fish bioassays demonstrated elevated responses in this region. In the South, Severn and Patuxent Rivers, the impact on fish was primarily on the embryonic (egg) stage. The eggs did not die, but up to 100% failed to hatch. In the downstream Wicomico River station the effect was on larval (post-hatch) survival. The eggs hatched, but the larvae did not survive.

Overall, results indicate that the ambient toxicity bioassay approach is sensitive enough to identify biologically significant contamination. The bioassays demonstrate that water column toxicity is not as severe as localized sediment toxicity but that water column effects are more wide spread and variable over time.

The risk ranking procedure results in comparable risk scores between sites. The use of multiple species, multiple sampling times/locations and a correction for the number of significant data points on a tributary-by-tributary basis results in a robust scoring procedure. The combined toxicological risk scores do not respond strongly to small variations in data availability or isolated spikes. The consistency factor can drive the score negative if there are a large number of nonsignificant endpoints, if laboratory variability is high (as discussed above) or the response values are very low. Inclusion of factors for

variability and response consistency provide additional information on the risk of toxic impact. The consistency factor was designed to act as a counterweight to unusual response values for the purpose of damping out rare spikes while not influencing scores from evenly divided results. It will tend to increase the score of highly polluted sites. Inclusion of the assay specific coefficients of variation in the calculation scheme tends to increase the cumulative score even in the absence of 'statistically' significant results. The simple toxicity scoring method can demonstrate where the specific spatial and/or temporal differences between samples exist.

The combined scores are not merely the sum of the sediment and water scores, due to the interplay between number of data points and the consistency factor. The combined score for the Wicomico River was strongly negative, while the sediment score was just below zero and the water score was over 40. The water and sediment scores for the Severn River were greater than 30 and 10 respectively, but the combined score was less than 10. The South River had multiple high scores in the sediment and statistically significant water column results. The combined score is dramatically different than scores for the other rivers.

Some information is lost in the process of pooling the data to calculate a risk score on a river-by-river basis. This has the advantage of smoothing out individual spikes in the whole data set, but it is instructive to look at the spikes on a sample by sample basis. This approach displays the existence of a

downstream gradient in the Severn River and site specific spikes in the Patuxent and Severn Rivers. Transient spikes, if present from spills or heavy runoff, may also be detectable in the water column bioassays. The ability of the data set to identify isolated hot spots is dependent on the size of the area relative to sampling intensity Gilbert 1987). The Patuxent River sampling stations were distributed over two to three times the distance of stations in the other rivers. Thus the sensitivity of a 'river specific' combined score requires careful application of the data. Large areas of degraded habitat could be missed if the sampling stations are too far apart. The sheer size of the river will affect its' assimilative capacity for environmental degradation. Also, the relative size and complexity of the Patuxent River watershed is greater than that of the other rivers. A more intense examination of the upper South and Severn and the middle Patuxent Rivers may yield clearer pictures of the nature and extent of degraded habitats

The correlation between the toxicological scores and the fish community metrics indicate that the sediment toxicological risk score is strongly correlated with the bottom community diversity index, as opposed to the resident diversity index and the IBI (Table 24). The resident species metrics and the IBI were not well correlated with the risk scores. Similar results were found in the 1993 sampling year (Hartwell et al. 1995). The definition of resident species as estuarine spawners is important in this regard. This metric is dominated by species taken in the

beach seines in terms of number of species and individuals. The 'resident species' are thus not living in close contact with the sediment at the bottom of the channels where the sediment samples were taken. The toxicological data clearly demonstrates that sediment toxicity is a dominant problem in the South River, but that the water column scores were marginal there, as well as the other systems. Consistent with results from 1993, results from the Wicomico River displayed good fish community indices and low toxicological risk scores.

The relatively high fish community scores and low toxicological risk score in the Patuxent River are consistent. However, as indicated above, this system is much larger than any other tributary tested to date, and localized problems may exist, as indicated by the data from the middle station. This station was approximately 1.5 km downstream of the Chalk Point power plant.

The Severn River had a relatively high bottom diversity index but a low resident diversity index and IBI. The low IBI and resident indices in the Severn River system may be due to reasons other than ambient toxicity. This may not be the case in the upper reaches however. Inclusion of this type of system in the correlation calculations affects the results. As illustrated in Figure 21, the low IBI and resident diversity index values from the Severn River (Table 3) introduce scatter in the relationship between those parameters and the sediment risk score. In contrast, the bottom diversity index for the Severn River is

relatively high, consistent with a low sediment risk score. A similar situation in Fishing Bay was observed in the 1993 sampling year (Hartwell et al. 1995). Low IBI scores and relatively low numbers of resident individuals were taken in the Fishing Bay system, but the bottom diversity index was high. None of the toxicological indices in Fishing Bay indicated habitat degradation due to toxic contamination. Low IBI scores in this area may be due to habitat deficiencies, such as the absence of SAV in shallow areas (Carmichael et al. 1992b). A similar scenario appears to be the case for the lower Severn River. The value of the toxicological risk ranking approach is that it was equally able to indicate where toxic contamination is and is not a likely impact, in the face of indications of impaired community health. If this is true, three predictions can be made;

1. Areas with high IBI scores or diversity indices will always have low toxicological risk scores, unless populations have adapted to contaminated conditions.

2. Areas with high toxicological risk scores will always have low IBI scores or diversity indices, unless populations have adapted to contaminated conditions.

3. Areas with low IBI scores or diversity indices may or may not have high toxicological risk scores, depending on the nature of the reason for poor fish communities.

These hypotheses can be tested with a larger data base. As studies progress, more sites will be included in the analyses. As the toxicological data base expands, correlations with a variety of community data bases will be possible i.e. the juvenile seine survey). Additional work needs to be done to examine how well the toxicological risk ranking results from different years can be integrated. In addition, an assessment is needed on the importance of sampling intensity, relative to the size of the river system, on risk score sensitivity.

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Table 1. Physicochemical parameters measured during water collections for ambient toxicity testing from four test stations, from April through September, 1993. Means (SD) for each parameter are given. These values were derived by averaging over the depth of the water column (i.e., bottom, middle, surface), and from two dates within each month on which the samples were collected.

	CURTIS CREEK						ROCK CREEK					
Month	Depth (m)	Temp. (°C)	Sal. (ppt)	DO (ppm)	pH	Tide	Depth (m)	Temp. (°C)	Sal. (ppt)	DO (ppm)	pH	Tide
May	6.0 (0.9)	17.9 (3.4)	3.8 (2.2)	4.84 (3.47)	6.95 (0.61)	Flood/Flood	2.7 (0)	20.1 (1.0)	2.2 (0.4)	5.86 (3.24)	7.10 (0.51)	Flood
June	6.6 (0.2)	20.3 (2.5)	6.5 (1.0)	4.53 (2.94)	6.82 (0.32)	Ebb/Ebb	3.3 (0)	22.1 (1.2)	5.6 (0.2)	6.51 (1.9)	7.10 (0.35)	Flood/Ebb
July	6.2 (1.3)	27.6 (2.4)	6.9 (0.7)	4.42 (4.08)	7.52 (0.61)	Ebb/Ebb	3.5 (0.3)	28.8 (1.3)	6.2 (0.1)	6.19 (3.09)	7.81 (0.59)	Flood/Ebb
August	8.1 (0)	27.5 (0.7)	10.1 (0.5)	ND	7.99 (0.52)	Flood/Flood	3.6 (0.7)	28.5 (1.4)	9.7 (1.5)	ND	7.96 (0.67)	Flood/Ebb
Sept	7.2 (0.5)	25.0 (1.4)	10.2 (1.4)	ND	7.73 (0.26)	Ebb/Flood	4.2 (0.4)	24.2 (1.1)	9.5 (0.7)	ND	8.25 (0.19)	Flood/Flood

ND=no data

Table 1(cont.) Physicochemical parameters measured during water collections for ambient toxicity testing from four test stations, from April through September, 1993. Means (SD) for each parameter are given. These values were derived by averaging over the depth of the water column (i.e., bottom, middle, surface), and from two dates within each month on which the samples were collected.

	FISHING BAY						WICOMICO RIVER					
Month	Depth (m)	Temp. (°C)	Sal. (ppt)	DO (ppm)	pH	Tide	Depth (m)	Temp. (°C)	Sal. (ppt)	DO (ppm)	pH	Tide
April	3.6 (0)	15.8 (0)	11.6 (0.1)	10.25 (0.05)	8.01 (0.12)	Ebb	ND	ND	ND	ND	ND	ND
May	4.0 (0)	23.1 (0.4)	8.7 (0.1)	7.63 (0.31)	7.35 (0.23)	Flood	2.4 (0.3)	20.2 (0.6)	3.2 (0.3)	6.76 (1.06)	7.58 (0.12)	Flood
June	3.0 (0)	24.2 (1.9)	9.3 (0.1)	8.60 (1.16)	7.74 (0.13)	Flood/Low	3.3 (0)	24.2 (0.9)	5.4 (0.7)	7.00 (1.42)	7.71 (0.33)	High/High
July	4.2 (0)	30.8 (0.5)	10.9 (0)	7.05 (0.81)	7.08 (0.10)	Flood	2.6 (0)	30.8 (0.7)	6.9 (0.2)	7.65 (1.30)	8.09 (0.14)	Flood
August	5.1 (0)	26.6 (0.1)	12.5 (0.1)	7.58 (0.08)	8.08 (0.02)	Flood	3.3 (0)	27.2 (1.0)	9.9 (0.1)	5.67 (0.29)	7.78 (0.04)	High/Flood
Sept	3.4 (0.2)	24.8 (1.6)	14.0 (0.2)	8.08 (0.17)	8.11 (0.13)	Flood/Ebb	3.2 (0.2)	25.3 (1.8)	10.3 (0.7)	7.29 (0.44)	7.80 (0.23)	Ebb/Flood

ND=no data

Table 2. Particle size analysis of sediments from test, reference, and control sites used in toxicity tests. Sediments collected 8/8/94 - 8/15/94. ("R" indicates replicates.)

Station	% Sand	% Silt	% Clay
Set #1:			
Patuxent River R1	1.68	85.58	12.74
Patuxent River R2	1.57	63.94	34.49
Patuxent River R3	1.34	55.94	42.71
Patuxent River R4	34.61	39.32	26.07
Patuxent River R5	19.03	51.59	29.17
Severn River R1	6.02	57.50	36.48
Severn River R2	99.19	0.44	0.37
Severn River R3	94.40	3.89	1.71
Severn River R4	84.36	8.89	6.75
Severn River R5	3.50	59.01	37.49
South River R1	3.96	57.70	38.34
South River R2	16.11	52.39	31.50
South River R3	12.46	50.94	36.60
South River R4	3.10	60.28	36.62
South River R5	70.60	18.79	10.61
Wicomico River R1	1.83	57.68	40.49
Wicomico River R2	1.55	57.79	40.66
Wicomico River R3	3.38	56.24	40.38
Wicomico River R4	0.68	59.32	40.00
Wicomico River R5	0.37	58.49	41.14
Poropotank Mud	0.63	60.96	38.41
Lynnhaven Mud	37.23	50.03	12.74
Lynnhaven Sand	97.63	1.25	1.12

Table 3. Summary data of bottom fish diversity index, resident fish diversity index, and river IBI scores vs toxicological risk scores for water, sediment, and water and sediment combined for four stations in the Chesapeake Bay in 1994.

Station	Bottom Diversity Index	Resident Diversity Index	IBI	Water Risk	Sediment Risk	Combined Risk
Patuxent R.	1.991	1.478	34.6	25.15	20.84	42.33
Severn R.	1.836	1.180	26.3	36.33	13.11	4.83
South R.	1.400	1.402	28.0	37.34	149.90	119.08
Wicomico R.	2.037	1.409	31.4	42.85	-2.59	-20.91

Table 4. Summary of survival of fish, *Cyprinodon variegatus*, and shrimp, *Palaemonetes pugio*, after 7-day test, with water samples from four stations in the Chesapeake Bay versus the control in 1994.

Month/ Station	April	May	June	July	August
FISH					
Control	98	100	95	93.3	90
Patuxent	100	100	95	100	90
Severn	100	100	95	96.7	97
South	100	100	97.5	100	90
Wicomico	100	98	97.5	90	100
SHRIMP					
Control	100	/	/	100	95
Patuxent	95	/	/	100	97.5
Severn	95	/	/	100	87.5
South	100	/	/	100	82.5
Wicomico	92.5	/	/	97.5	90

/ Organisms not available.

Table 5. Summary of growth of fish, *Cyprinodon variegatus*, and shrimp, *Palaemonetes pugio*, during 7-day ambient toxicity bioassays with water from four stations in the Chesapeake Bay in 1994. Values are calculated as mean terminal wt. - mean initial wt. (mg).

Month/ Station	April	May	June	July	August
FISH					
Initial Weight	0.139	0.108	0.087	0.029	0.049
Control	0.399	0.271	0.024	0.377	0.434
Patuxent River	0.419	0.324	0.039	0.220	-0.011**
Severn River	0.390*	0.321*	0.066	0.197	-0.023**
South River	0.431	0.308	-0.018	0.236	-0.019*
Wicomico River	0.444	0.309	0.037	0.208	-0.026**
SHRIMP					
Initial Weight	+	/	/	0.183	0.070
Control	0.110	/	/	0.176	0.064
Patuxent River	0.110	/	/	0.173	0.114*
Severn River	0.102	/	/	0.218	0.052
South River	0.090	/	/	0.183	0.040
Wicomico River	0.106	/	/	0.151	0.077

/ = organisms not available
\+ = sample lost, reported vales are terminal weights
* = significant at p = 0.05
** = significant at p = 0.01

Table 6. Reference toxicant data results from 48-hr, water reference toxicant tests with potassium chloride (KCl). All values are nominal.

Date	Species	LC50	CIs (mg/L KCl)
Apr 94	*C. variegatus*	179.24	171.97-186.82
		125.00	107.05-145.96
	P. pugio	52.52	45.92-60.08
May 94	*C. variegatus*	124.97	101.79-153.43
		176.78	nr
Jun 94	*C. variegatus*	50.73	43.65-58.97
Jul 94	*C. variegatus*	71.78	61.73-83.46
	P. pugio	85.18	72.07-100.67
Aug 94	*C. variegatus*	176.78	nr
	P. pugio	67.68	58.20-78.71
	L. plumulosus	> 125.00	

nr = 95% confidence limits are not reliable by Trimmed Spearman-Karber Method.

Table 7. Summary of mortality and reproduction of copepod, *Eurytemora affinis*, from ambient toxicity bioassays using water samples collected from four stations of the Chesapeake Bay in 1994. Values are the mean of four replicates. Values in parenthesis are standard deviations.

Month/ Station	April	May	June	July	August
			SURVIVAL		
Control	2.25 / 5.25[a] (3.34)/(2.95)	5.75 (2.86)	3.25 (1.48)	1.50 (0.87)	3.00 (2.12)
Patuxent	6.00 (4.74)	5.33 (2.49)	3.5 (2.69)	6.50* (1.5)	1.50 (1.5)
Severn	3.75 (1.30)	8.0 (3.08)	6.75 (2.77)	6.00 (3.16)	1.75 (2.05)
South	6.00 (2.12)	7.5 (3.91)	5.25 (2.17)	7.50 (4.09)	2.00 (1.22)
Wicomico	4.5 (3.04)	10.5* (1.12)	4.00 (1.87)	3.75 (1.48)	2.55 (2.60)
			REPRODUCTION		
Control	577.5/350.0[a] (277.48)/(51.02)	162.5 (65.53)	1188.75 (131.29)	1234.75 (230.20)	875.5 (315.99)
Patuxent	445.25 (179.04)	210.67 (80.02)	747.25* (223.57)	738.5* (217.90)	791.75 (381.02)
Severn	665.25 (113.23)	76.5* (81.56)	239.25* 165.76)	1293.0 (563.95)	778.0 (324.02)
South	547.0 (157.74)	310.75 (179.60)	320.0* (207.91)	588.25* (293.27)	1161.0 (145.02)
Wicomico	406.0 (211.93)	0.0** (0.0)	804.75 (274.32)	855.5 (170.37)	982.0 (212.67)

[a] = two controls were used during April due to wide differences in salinities of ambient test water. Salinity of all test water was adjusted to 10 ppt in other months.

* = significant at p = 0.05
** = significant at p = 0.01

Table 8. Growth and rhizome production of *Potamogeton pectinatus* during 4-week ambient toxicity bioassays with water from four stations in the Chesapeake Bay in 1994. Weight values are the mean (SD) of 10 replicates in units of gm/plant.

Station	Initial wt.	Terminal wt.	Dry wt.	#Rhizome tips
		July		
Control	1.28 (0.154)	6.03 (1.113)	0.63 (0.103)	20.7
Patuxent	1.32 (0.149)	6.72 (1.508)	0.69 (0.125)	20.6
Severn	1.33 (0.124)	6.32 (6.317)	0.70 (0.154)	23.0
South	1.24 (0.099)	6.53 (1.272)	0.71 (0.176)	19.2
Wicomico	1.21 (0.165)	6.18 (0.706)	0.66 (0.117)	20.3
		August		
Control	1.33 (0.135)	4.95 (1.428)	0.61 (0.149)	24.7
Patuxent	1.25 (0.149)	4.41 (1.508)	0.51 (0.125)	26.2
Severn	1.32 (0.116)	5.26 (5.262)	0.59 (0.123)	24.3
South	1.30 (0.099)	4.54 (1.272)	0.50 (0.176)	25.0
Wicomico	1.23 (0.136)	4.40 (1.039)	0.50 (0.116)	23.4

Table 9a. Summary of Microtox[R] assay results, expressed as luminosity, for water samples collected from four stations in the Chesapeake Bay in 1994. Results are the mean of four replicates, with standard deviation in ( ). Runs 1 and 2 refer to discrete water collections. Shading indicates inhibitory results.

Month/		April		May		June		July		August	
Station	Run	Ctrl	Amb	Ctrl	Amb	Ctrl	Amb	Ctrl	Amb	Ctrl	Amb
Patuxent	1	92.50 (2.50)	115.25** (6.94)	50.50 (4.15)	39.50* (0.87)	102.75 (9.12)	120.25* (4.15)	93.50 (2.06)	116.75** (4.60)	62.25 (4.44)	12.00** (0.71)
Patuxent	2	#	#	91.00 (6.82)	97.75 (2.95)	86.25 (7.50)	126.75* (11.84)	90.00 (1.22)	99.75 (6.02)	73.75 (1.48)	88.00* (4.74)
Severn	1	73.75 (2.05)	28.25* (3.56)	89.25 (5.31)	65.25** (7.98)	108.25 (14.53)	152.00** (6.28)	95.50 (2.96)	106.50** (2.87)	106.25 (2.28)	66.50** (5.72)
Severn	2	#	#	97.50 (6.73)	104.75 (3.03)	71.75 (6.83)	135.75** (7.46)	84.75 (1.79)	94.75** (1.92)	62.75 (4.15)	34.50** (0.87)
South	1	87.00 (2.55)	70.50** (5.89)	73.75 (3.63)	90.75** (4.92)	109.00 (11.77)	123.75 (19.25)	95.00 (5.39)	105.50 (6.02)	64.50 (5.22)	34.75** (2.86)
South	2	#	#	90.50 (2.69)	106.25** (5.17)	89.50 (13.86)	119.00* (9.14)	79.50 (9.10)	98.25* (3.27)	64.75 (0.43)	87.75* (7.08)
Wicomico	1	98.75 (10.06)	55.25** (5.89)	74.75 (5.40)	42.00** (4.85)	79.75 (15.75)	103.00 (20.94)	80.25 (1.79)	91.00** (3.24)	78.00 (2.74)	86.00* (2.92)
Wicomico	2	#	#	91.75 (3.77)	101.50* (2.96)	75.75 (11.41)	113.75** (2.59)	81.25 (4.32)	94.75** (3.19)	76.25 (1.79)	82.25 (5.76)

Runs 1 and 2 combined for April assays.

* = significant at p = 0.05
** = significant at p = 0.01

Table 9b. Lowest Observed Effect Concentration (LOEC) for dilution series Microtox[R] bioassays which were inhibitory to bacterial luminosity, for water samples collected from four stations in the Chesapeake Bay in 1994. Values are expressed as % river water, diluted with control water.

Month/ Station	April	May	August
Patuxent	/	80	70
Severn Run 1	70	70	70
Severn Run 2	/	/	$\leq$60
South	70	/	90
Wicomico	90	99	/

Table 10. Results of chemical analyses of water samples from four stations in the Chesapeake Bay in 1994 for metals contamination. Units are ug/l.

CHEMICAL	Patuxent	South	Severn	Wicomico	EPA Marine Water Quality Criteria	
					Acute	Chronic
Antimony	2	3	2	4	1500*	500*
Arsenic V	BDL	2	2	3	2319**	---
Chromium VI	6	6	9	4	1100	50
Lead	4	BDL	2	BDL	220	8.5
Selenium	1	2	3	8	300	71

BDL = below detection limit
* = proposed criteria
** = based on LOEL

Table 11. Mortality data for *Lepidactylus dytiscus* and *Streblospio benedicti* at the four stations. Tests were conducted from 08/30/94 to 09/9/94. "(R)" = Reference, "(C)" = Control. "SE" = Standard Error.

Species	Station	% Mortality Unadjusted	SE	Adjusted	SE
L. dytiscus					
	Patuxent River	34.00*	5.10	5.39	4.14
	Severn River	17.00	12.30	9.10	9.10
	South River	44.00*	11.79	23.78	13.28
	Wicomico River	28.00*	2.55	0.00	0.00
	Lynnhaven Sand (C)	3.00	2.00	2.08	1.51
	Poropotank Mud (R)	33.00*	5.83	5.38	4.13
S. benedicti					
	Patuxent River	23.00	10.56		
	Severn River	10.00	2.74		
	South River	17.00	7.18		
	Wicomico River	14.00	3.32		
	Poropotank Mud (R)	10.00	3.87		
	Lynnhaven Mud (C)	5.00	3.16		
	Lynnhaven Sand (R)	14.00	1.87		

* Significantly less than controls ($p < 0.05$).

NOTE: Adjusted *L. dytiscus* and *S. benedicti* survival is percent survival adjusted for predicted particle size effects.

Table 12. Mortality from *Cyprinodon variegatus* at the four stations. Tests were conducted from 08/30/94 to 09/9/94 "(R)" = Reference, "(C)" = Control.

Species	Station	% Mortality	SE	% Hatched	SE	%Dead eggs	SE	%Dead fish	SE
C. variegatus									
	Patuxent River	58.00	13.19	46.00	15.68	32.00	11.58	5.56	5.56
	Severn River	40.00	16.43	62.00	16.55	20.00	3.16	3.57	3.57
	South River	72.00	15.30	30.00	16.43	10.00	8.37	4.76	4.76
	Wicomico River	66.00	15.03	52.00	10.68	20.00	10.20	42.50	17.50
	Poropotank Mud (R)	46.00	14.00	62.00	16.85	22.00	10.68	11.07	7.15
	Lynnhaven Sand (R)	2.00	2.00	100.00	0.00	0.00	0.00	2.00	2.00
	Lynnhaven Mud (C)	42.00	12.81	66.00	13.64	28.00	9.70	10.67	6.86

Note:
% Mortality = (Dead fish + dead eggs at test termination)/(# eggs exposed)*100.
% Dead fish = (Dead fish)/(# hatched)*100
% Dead eggs = (Dead eggs)/(# exposed)*100
% Hatched = (# hatched)/(# eggs exposed)*100

Table 13. Summary of mortality of amphipod, *Leptocheirus plumulosus*, after 10-day test, with sediment samples from four tributaries of the Chesapeake Bay versus the control in August 1994. Units are percent (standard deviation).

Station	% Mortality
Control	12.0 (10.30)
Patuxent	6.0 (7.35)
Severn	8.0 (5.10)
South	22.0 (20.15)
Wicomico	10.0 (7.07)

Table 14. Summary of growth of amphipods *Leptocheirus plumulosus* during 10-day ambient toxicity bioassays with sediment from four tributaries of the Chesapeake Bay in 1994. Values are calculated as mean terminal wt. - mean initial wt. (mg).

	Weight (mg)
Initial Weight	0.029
Control	-0.015
Patuxent River	0.004*
Severn River	0.034**
South River	0.004
Wicomico River	0.011**

* = significant at p = 0.05.
** = significant at p = 0.01.

Table 15. Summary of growth data for *Lepidactylus dytiscus* and *Streblospio benedicti* after 10-day ambient toxicity bioassays with sediment from four tributaries of the Chesapeake Bay in 1994. Values are mean terminal dry weight. "(R)" = Reference, "(C)" = Control.

Site	Number of True Replicates	Weight(mg)	S.E.
L. dytiscus			
Initial	5	0.518	0.016
Patuxent River	5	0.806	0.150
Severn River	5	0.735	0.419
South River	5	1.201	0.808
Wicomico River	5	0.724	0.055
Poropotank Mud (R)	5	0.799	0.161
Lynnhaven Sand (C)	5	0.531	0.031
S. benedicti			
Initial	5	0.103	0.007
Patuxent River	5	0.771	0.394
Severn River	5	0.578	0.037
South River	5	0.650	0.165
Wicomico River	5	0.607	0.054
Poropotank Mud (R)	5	0.581	0.063
Lynnhaven Mud (C)	5	0.529	0.060

* Significantly less than controls ($p < 0.05$).

Table 16. Reference toxicant data results from 96-hr, water only, reference toxicant tests. Cadmium chloride ($CdCl_2$) was used for all organisms.

Organism	LC50 & CIs (mg/L Cd)	Historical Mean	SE
L. dytiscus	1.75 1.47-2.08	4.18	0.510
S. benedicti	2.91 2.30-3.70	4.80	0.703
C. variegatus	0.64 0.53-0.71	0.58	0.056

Table 17. Summary of percent germination (SD) of lettuce and *Spartina alternaflora* seeds following 3-days exposure to ambient sediments from four stations in the Chesapeake Bay, versus the control, in 1994.

Station	Lettuce	*S. alternaflora*
Control	94.52 (5.12)	11.29 (3.34)
Patuxent	94.31 (2.01)	6.75 (3.69)
Severn	93.87 (3.39)	7.34 (2.09)
South	96.68 (1.34)	10.27 (2.92)
Wicomico	92.20 (8.04)	8.55 (4.37)

Table 18. Results of chemical analyses for metals in composite sediment samples from four tributaries of the Chesapeake Bay in 1994. Units are mg/kg.

CHEMICAL	Patuxent	South	Severn	Wicomico	NOAA ER-L	NOAA ER-M
Antimony	ND	ND	ND	1.08	2	25
Arsenic	7.96	11.9	5.91	7.65	33	85
Beryllium	1.31	1.45	0.66	1.69	---	---
Cadmium	1.69	ND	ND	ND	5	9
Chromium	40.0	78.7	33.6	46.2	80	145
Copper	17.2	30.8	12.8	19.7	70	390
Lead	50.8	60.6	23.4	72.3	35	110
Nickel	17.7	15.3	6.69	20.4	30	50
Selenium	2.54	2.19	0.52	4.31	---	---
Zinc	155	219	77.8	141	120	270

ND = not detected

Table 19. Results of chemical analyses for semi-volatile acid/base neutral compounds in composite sediment samples from four tributaries of the Chesapeake Bay in 1994. Units are ug/kg.

CHEMICAL	Patuxent	South	Severn	Wicomico	NOAA ER-L	NOAA ER-M
Fluoranthene	ND	30	70	ND	600	3600
Pyrene	ND	ND	60	ND	350	2200
Butylbenzylphthalene	ND	110	230	560	---	---
Chrysene	ND	ND	60	ND	400	2800
Benzo(a)anthracene	ND	ND	30	ND	230	1600

ND = not detected.

Table 20. Mean SEM metals values for Sediments collected 8/8/94 - 8/15/94.

Site	Cadmium umol/g	Lead umol/g	Copper umol/g	Nickel umol/g	Zinc umol/g	Sum umol/g
Patuxent River	0.036	0.202	0.298	0.155	2.176	2.868
Severn River	0.036	0.069	0.149	0.160	0.912	1.326
South River	0.012	0.064	0.167	0.078	0.814	1.140
Wicomico River	0.172	0.169	0.432	0.445	1.155	1.301
Lynnhaven Sand	0.000	0.000	0.000	0.000	0.013	0.013
Lynnhaven Mud	0.000	0.021	0.036	0.029	0.441	0.527
Poropotank Mud	0.000	0.021	0.000	0.054	0.539	0.613
Detection Limits	0.001	0.004	0.003	0.007	0.005	

* NOTE: Mercury values for all site were < 0.0001 umol/g w/DL of 0.0001 umol/g.

Table 21. Average SEM and AVS values and the SEM:AVS ratio for sediment samples tested in 1994.

	Mean AVS	Mean SEM	Ratio
Patuxent River	31.09	2.868	0.092
Severn River	9.96	1.326	0.133
South River	2.98	1.140	0.384
Wicomico River	11.54	1.301	0.113
Lynnhaven Sand	0.63	0.013	0.021
Lynnhaven Mud	3.17	0.527	0.166
Poropotank Mud	5.46	0.613	0.112

Table 22. Chemical data for pore water samples from the six stations and the references and controls.

Site	Ammonia (mg/L)	Nitrite (mg/L)	Sulfide (mg/L)	Unionized Ammonia (mg/L)
Patuxent River	15.621	0.0165	0.009	0.2493
Severen River	13.486	0.0064	<0.006	0.2152
South River	10.294	0.0058	0.011	0.2580
Wicomico River	25.499	0.0078	0.007	0.4069
Lynnhaven Sand	18.214	2.7851	0.007	0.3644
Lynnhaven Mud	44.009	0.0075	0.495	0.5597
Poropotank Mud	2.545	0.0090	0.006	0.0509

Table 23. Chemical data (TOC) for sediment samples from the four stations and the controls. All data is on a dry weight basis. 8/8/94 - 8/15/94.

Station	Total Organic Carbon (%)
Patuxent River	3.04
Severn River	0.99
South River	2.17
Wicomico River	2.58
Lynnhaven Sand	<0.37
Lynnhaven Mud	1.07
Poropotank Mud	4.26

Table 24. Pearson correlation coefficients and p values ( ) for toxicological risk scores and fish community metrics for four stations in the Chesapeake Bay in 1994.

Risks	IBI score	Bottom Diversity Index	Resident Diversity Index
Water Risk	-0.5366 (0.4634)	-0.1444 (0.8556)	-0.3245 (0.6755)
Sediment Risk	-0.3463 (0.6537)	-0.9626 (0.0374)	0.1816 (0.8184)
Combined Risk	-0.1505 (0.8495)	-0.8787 (0.1213)	0.2985 (0.7015)

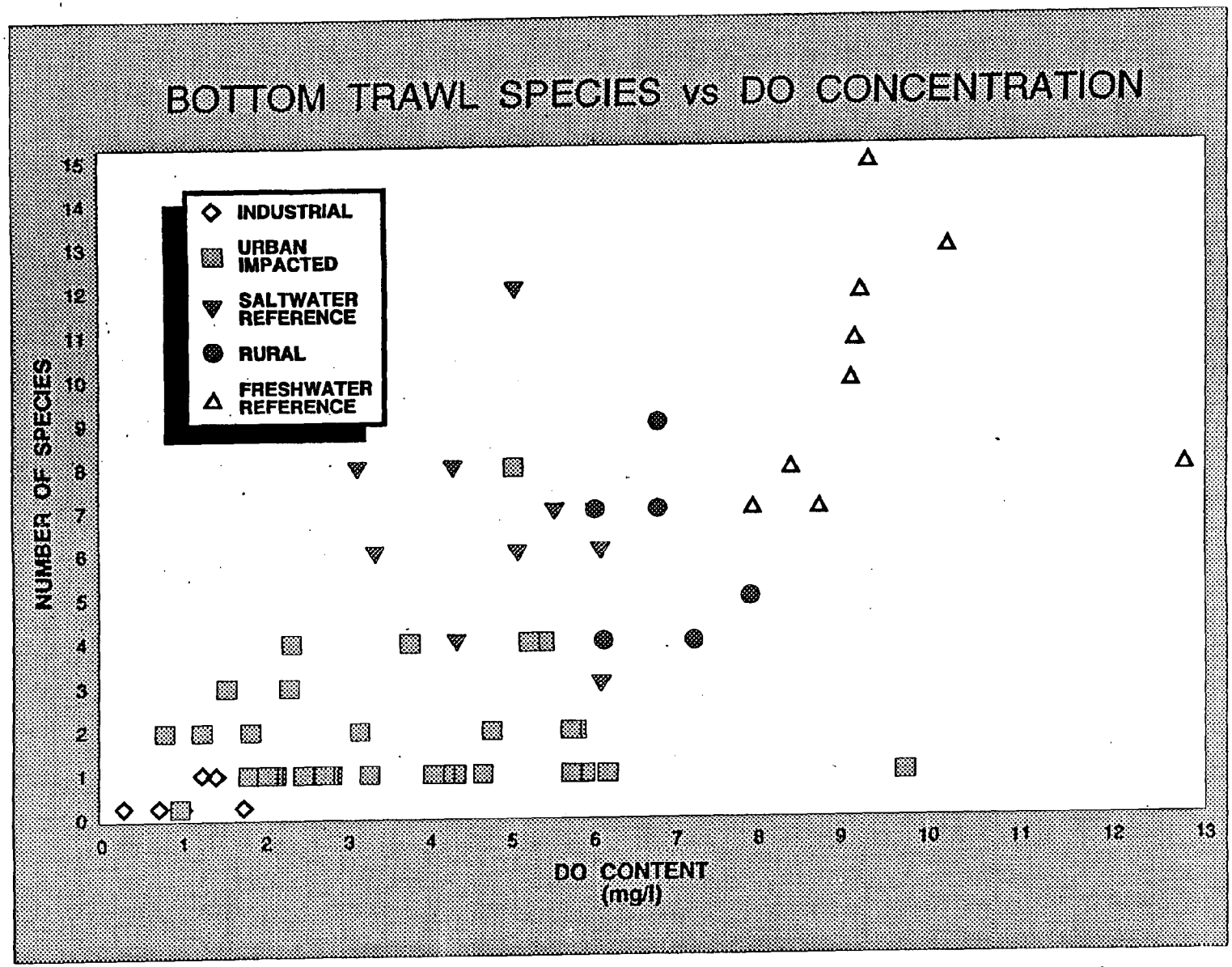

Figure 1. Bottom water dissolved oxygen concentration vs number of species in bottom trawls from Chesapeake Bay tributaries (from Carmichael et al. 1992).

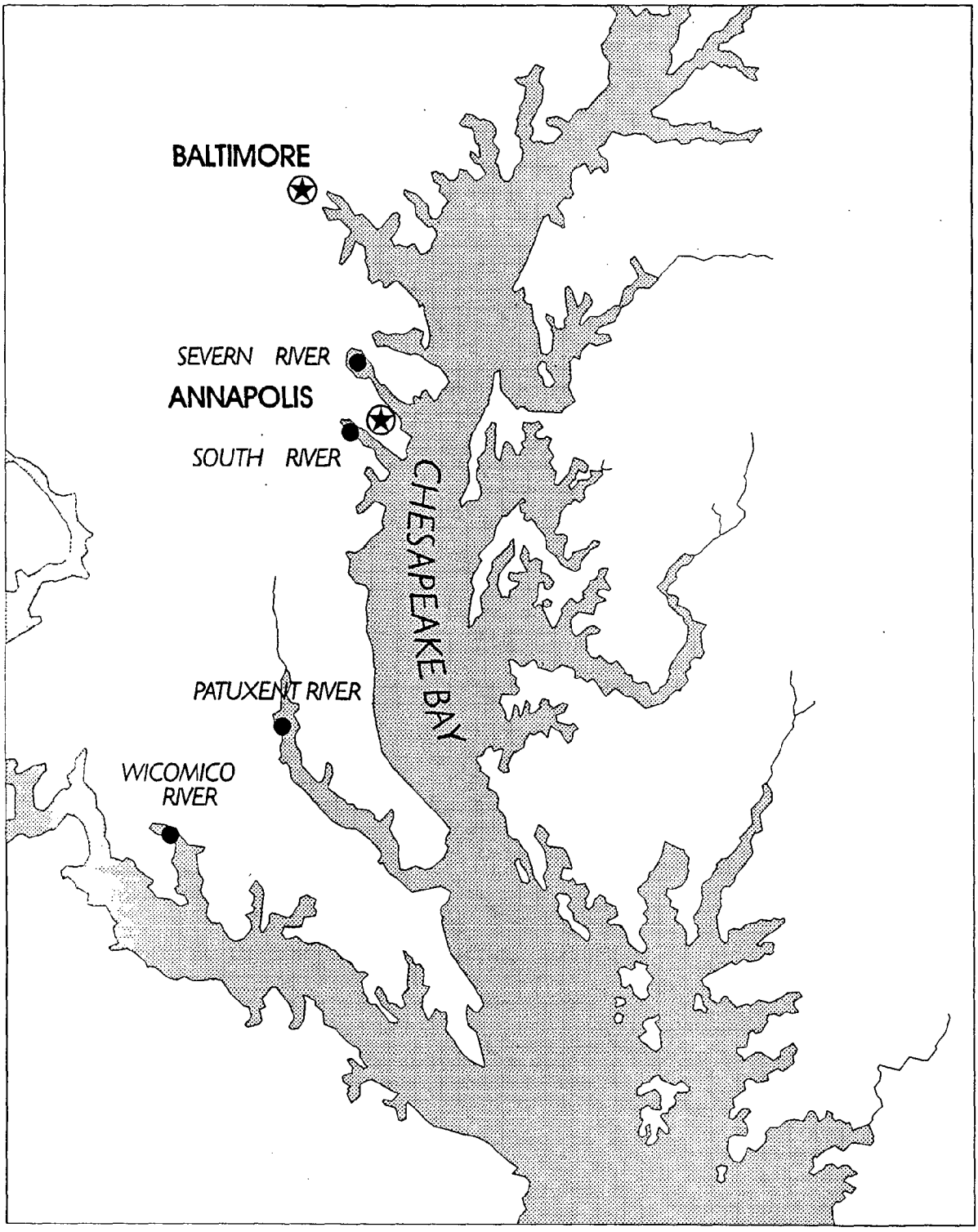

Figure 2. Map of Chesapeake Bay showing the location of four tributaries sampled for ambient toxicity in 1994.

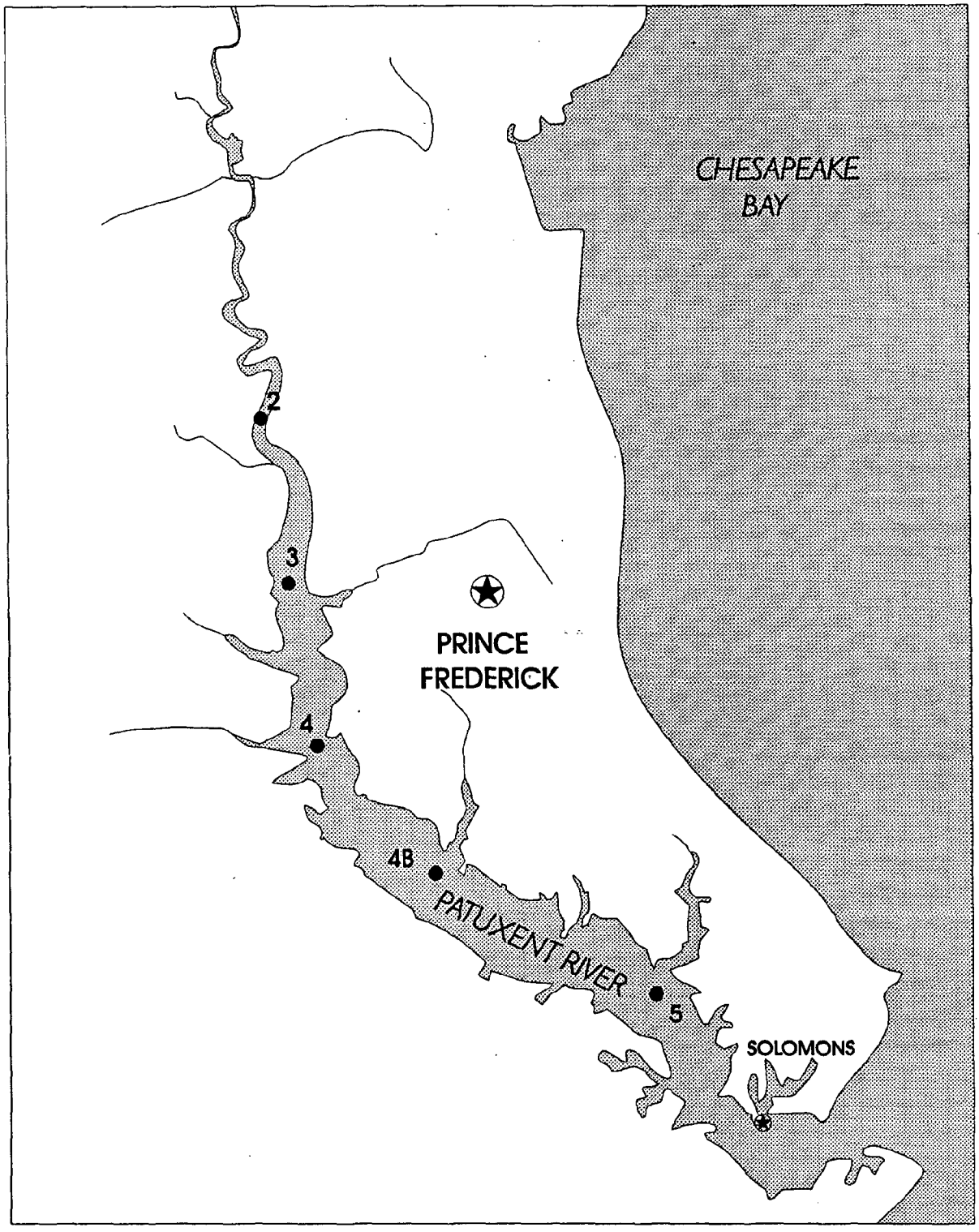

Figure 3. Map of the Patuxent River showing the locations of the 1994 ambient toxicity.

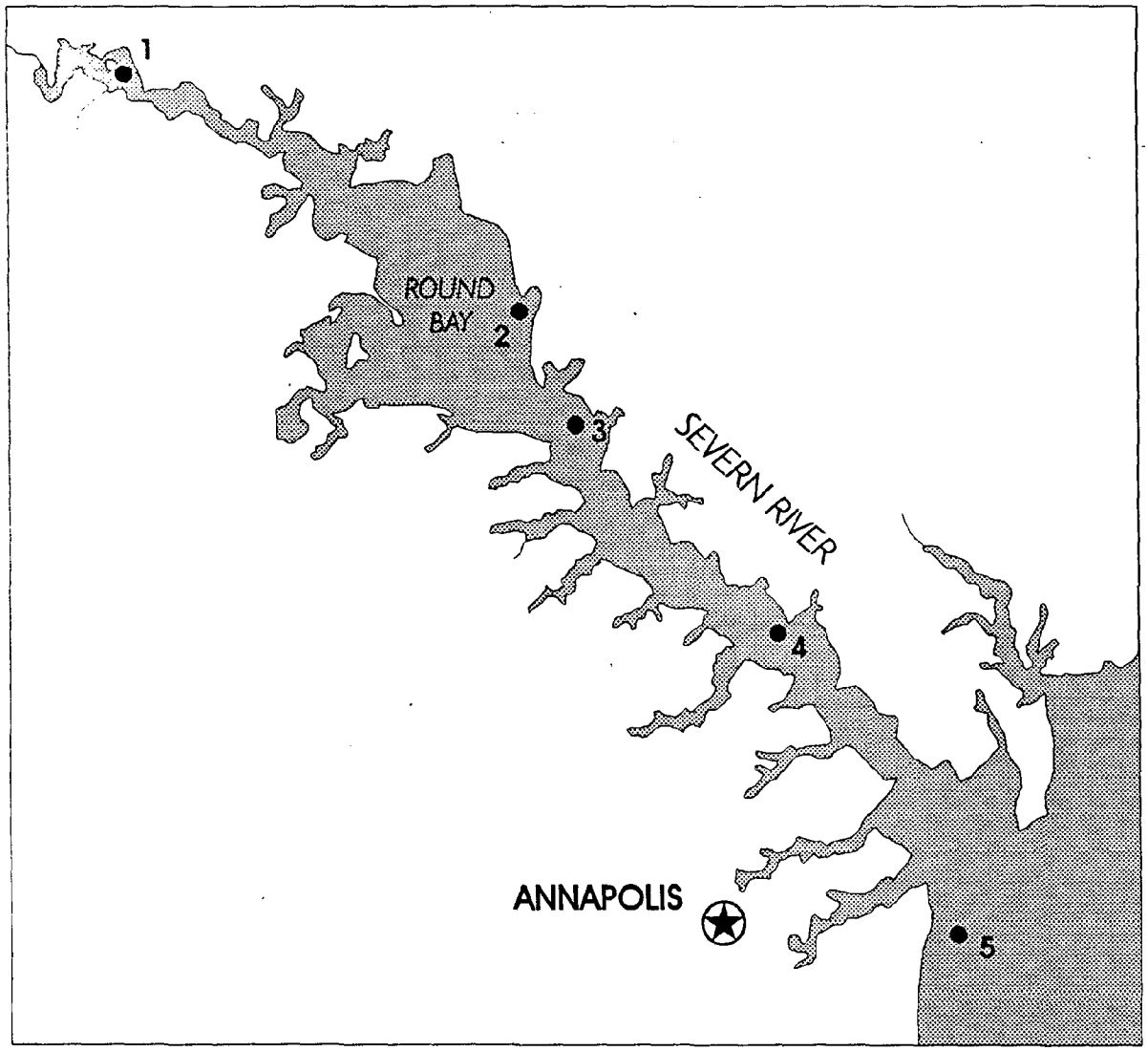

Figure 4. Map of the Severn River showing the locations of the 1994 ambient toxicity sampling stations.

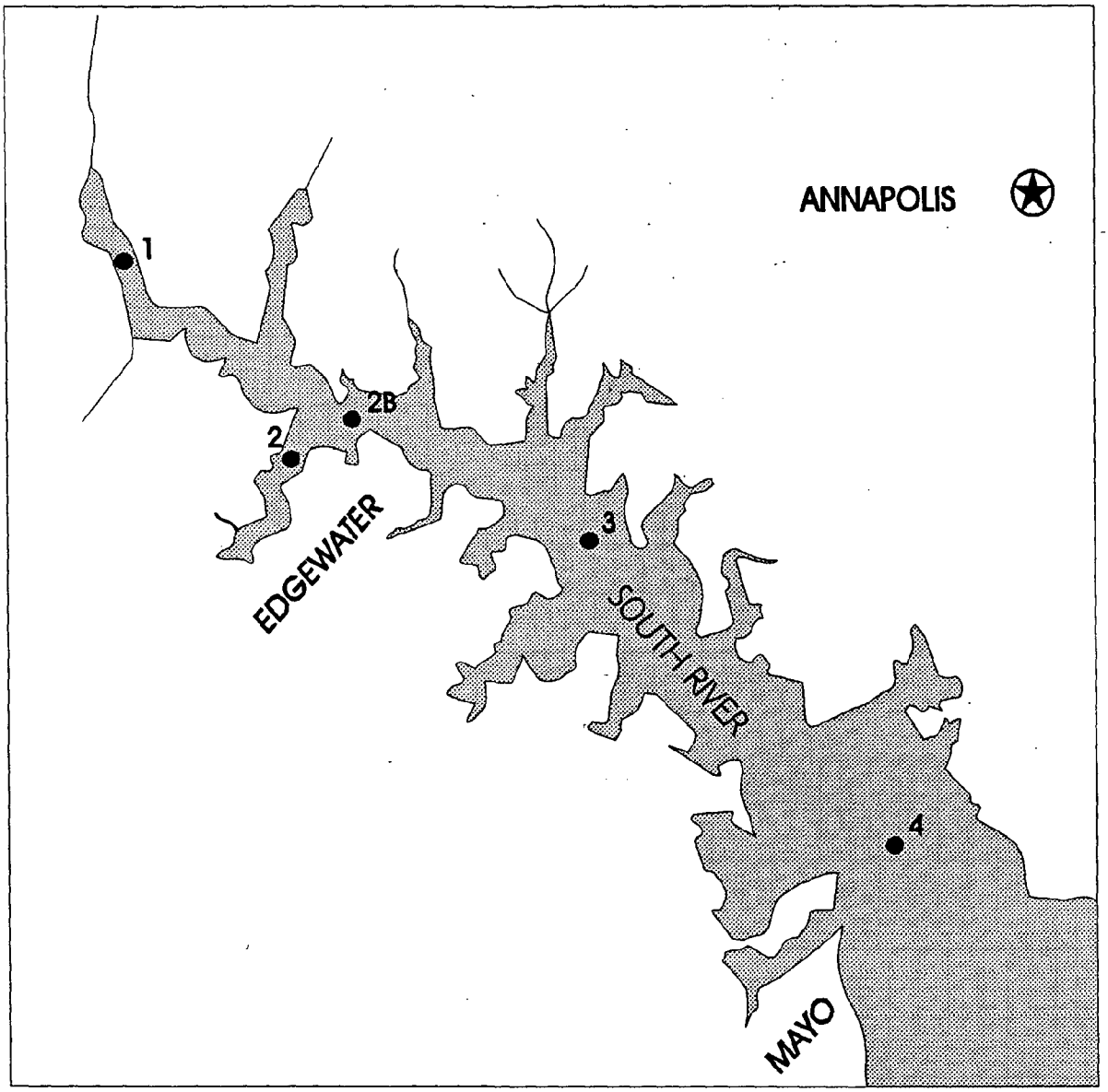

Figure 5. Map of the South River showing the locations of the 1994 ambient toxicity sampling stations .

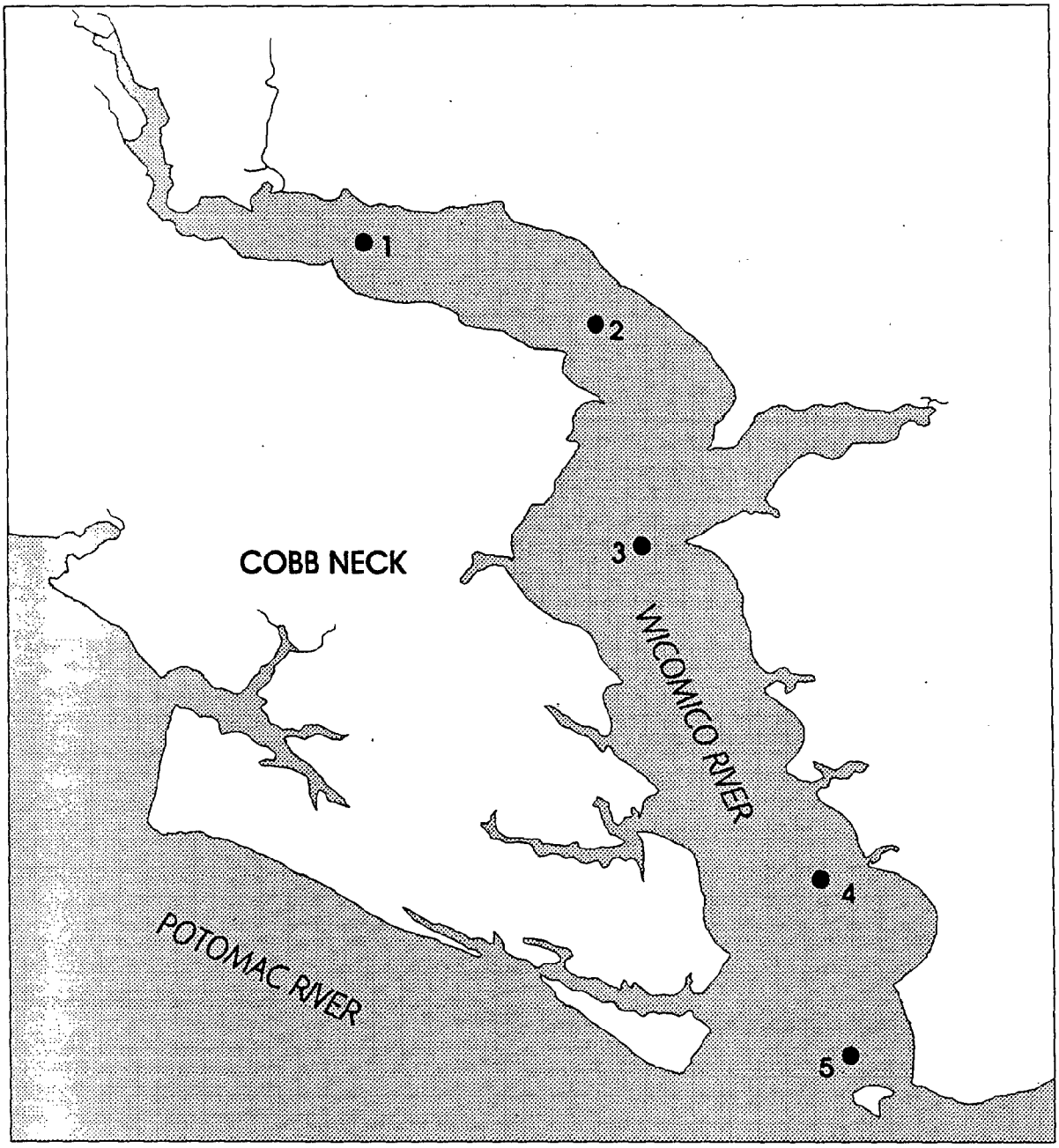

Figure 6. Map of the Wicomico River showing the locations of the 1994 ambient toxicity sampling stations.

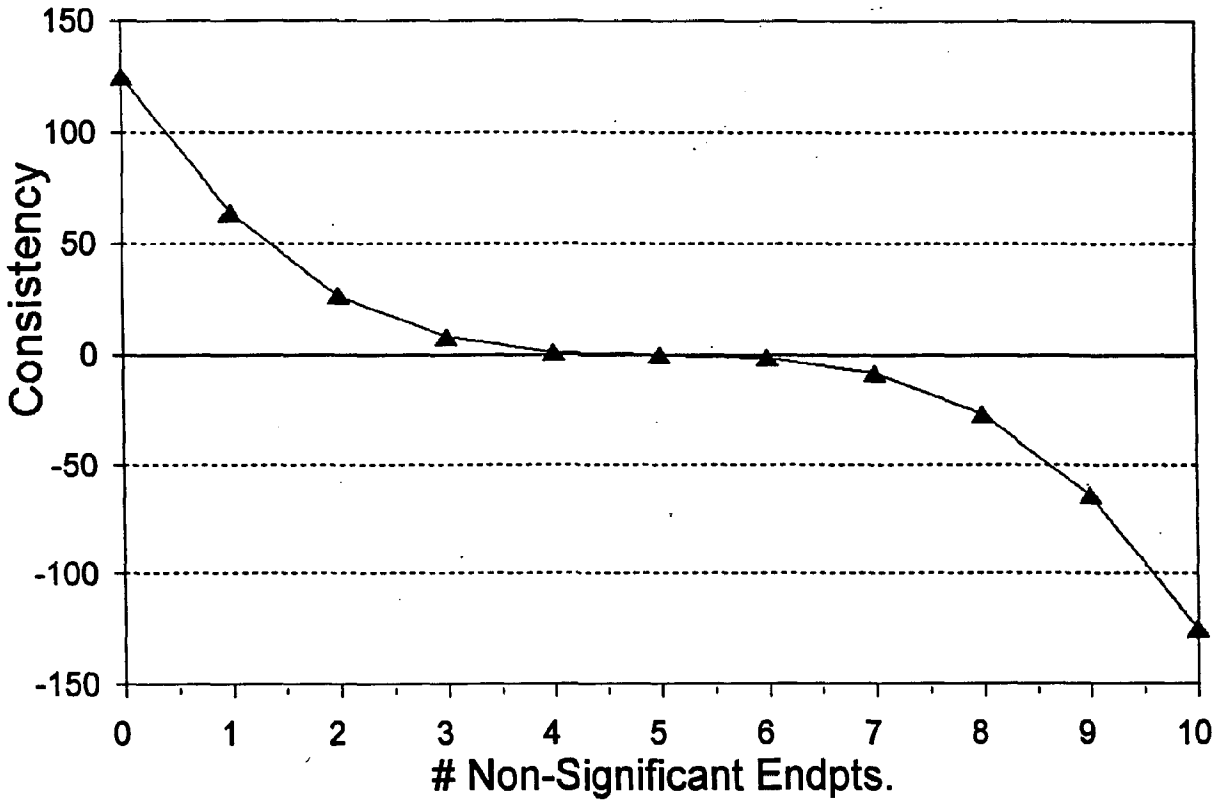

Figure 7. Example consistency values for N = 10 endpoints.

Lepidactylus dystiscus (Mortality)

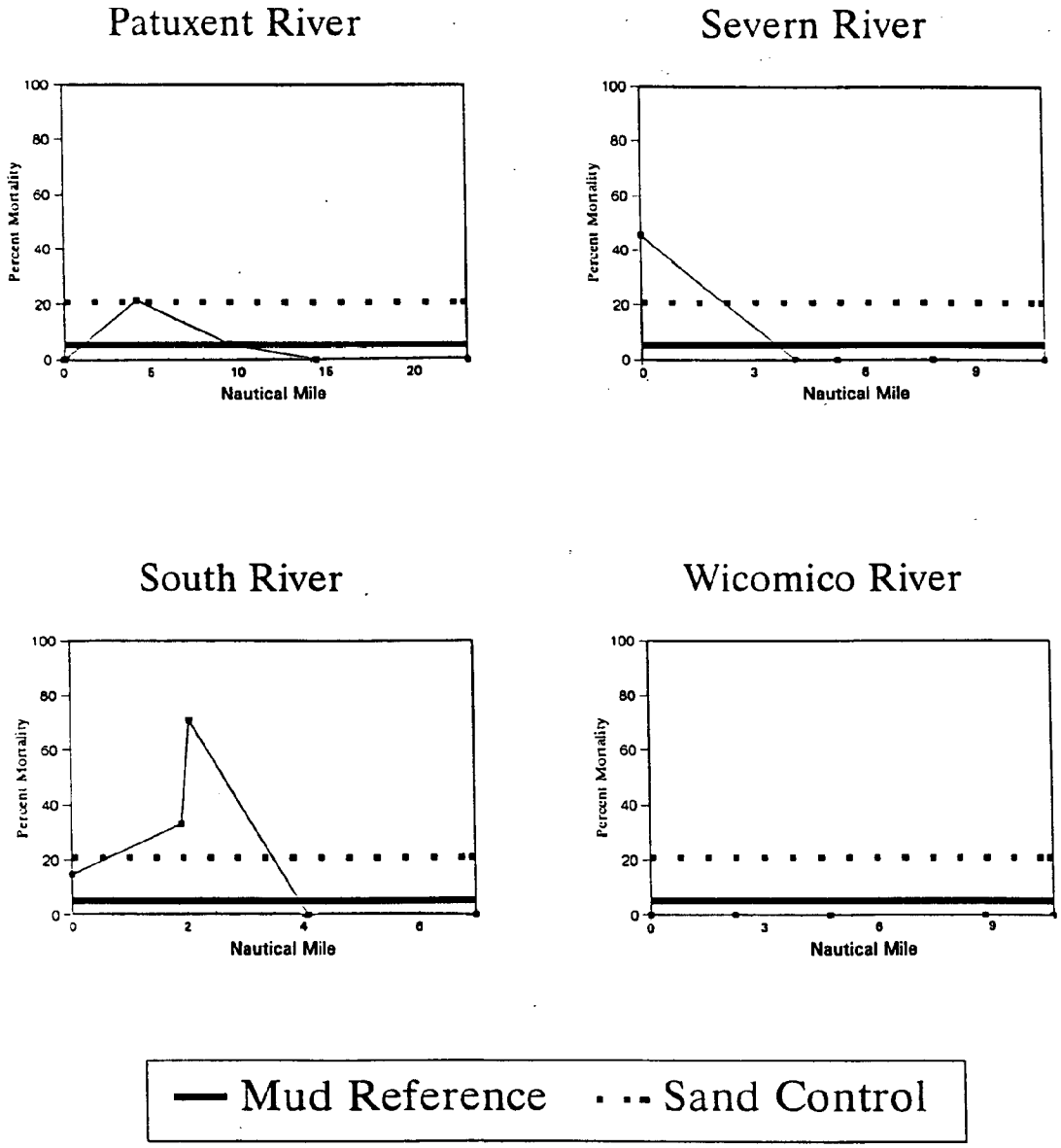

Fig. 8. *Lepidactylus dystiscus* percent mortality. Mortality is plotted against distance between site replicates from up-river to mouth.

Streblospio benedicti (Mortality)

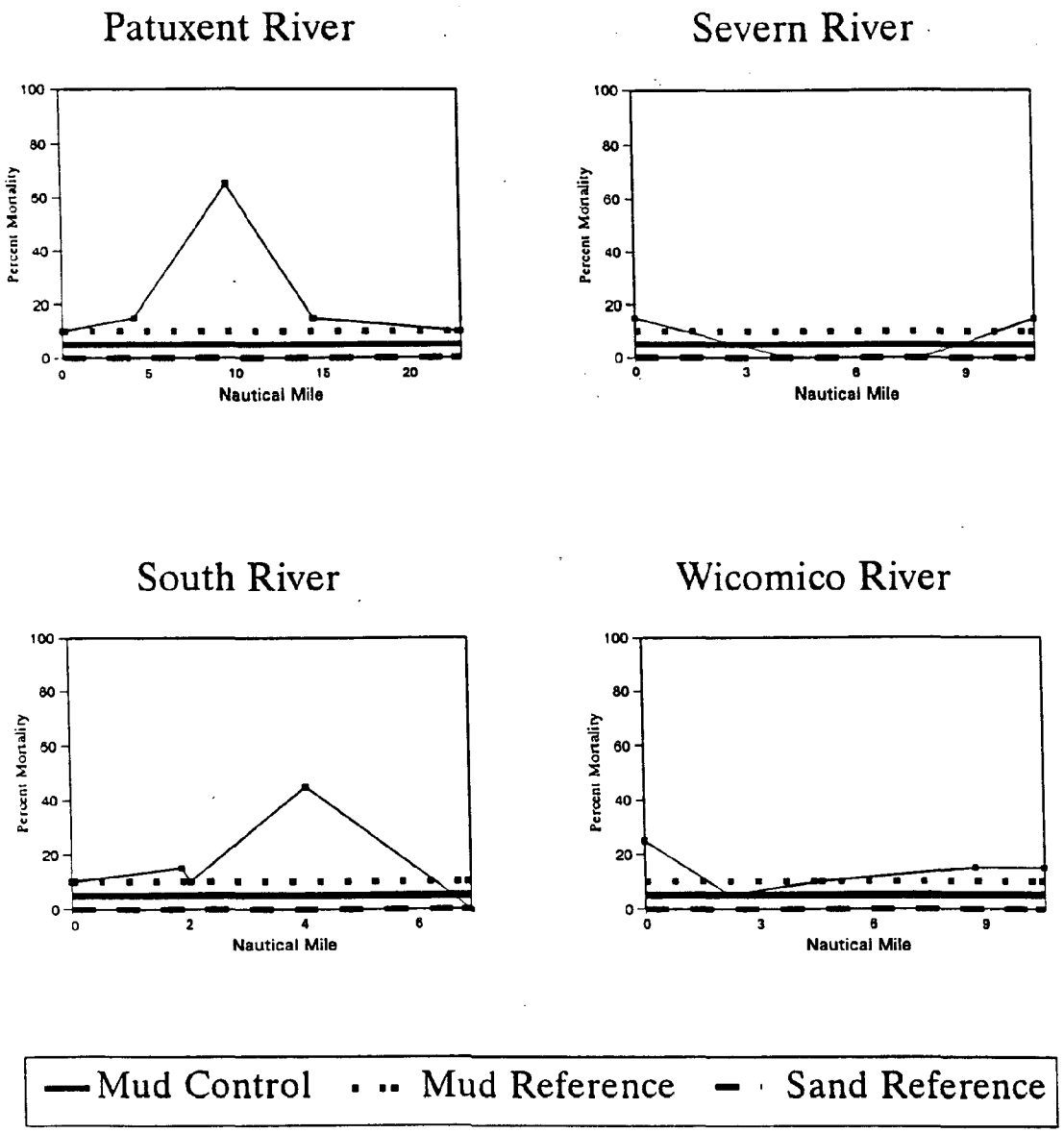

Fig. 9. *Streblospio benedicti* percent mortality. Mortality is plotted against distance between site replicates from up-river to mouth.

Leptocheirus plumulosus (Mortality)

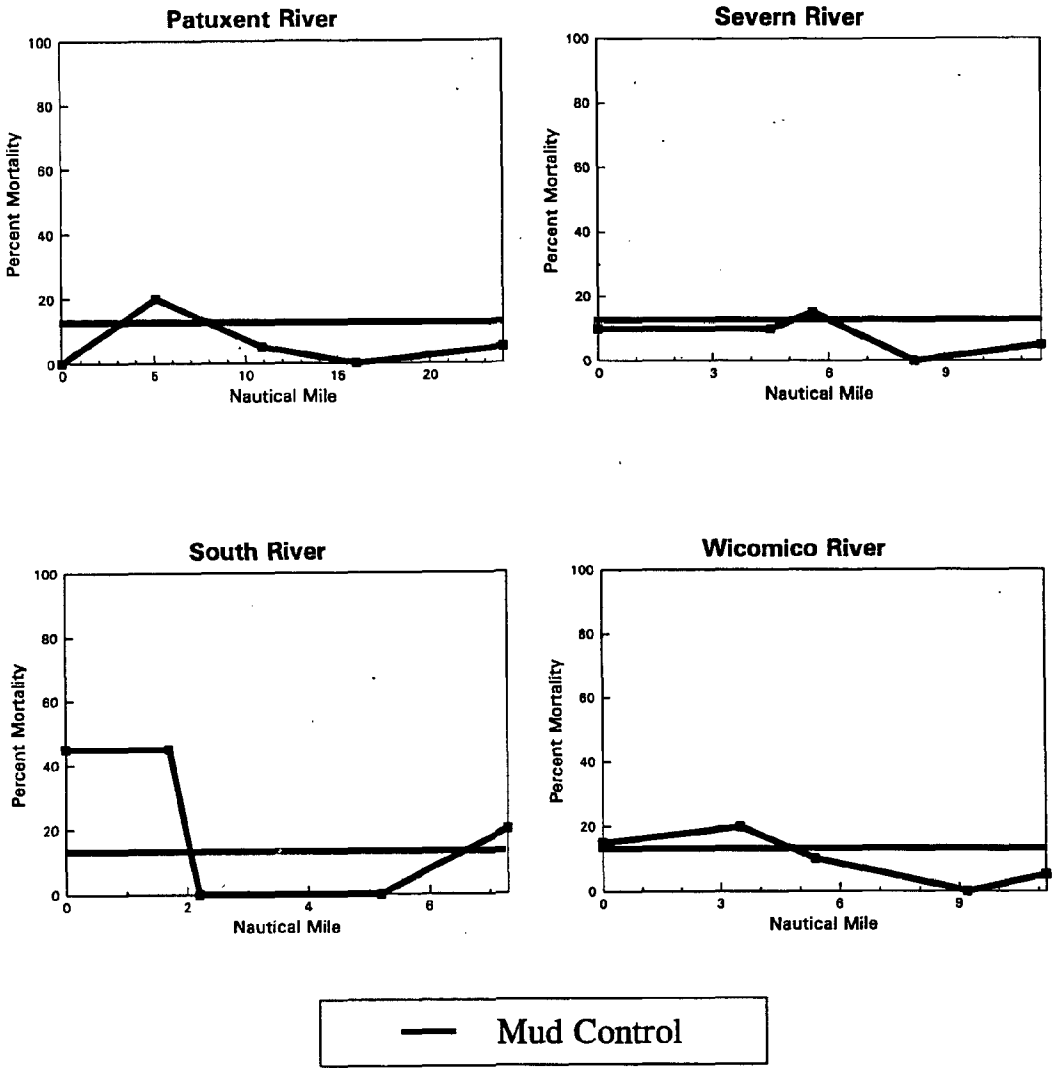

Figure 10. *Leptocheirus plumulosus* percent mortality. Mortality is plotted against distance between site replicates from up-river to mouth. Horizontal baseline is mean control value. Reference sediment is not necessary with *L. plumulosus*.

Cyprinodon variegatus (Mortality)

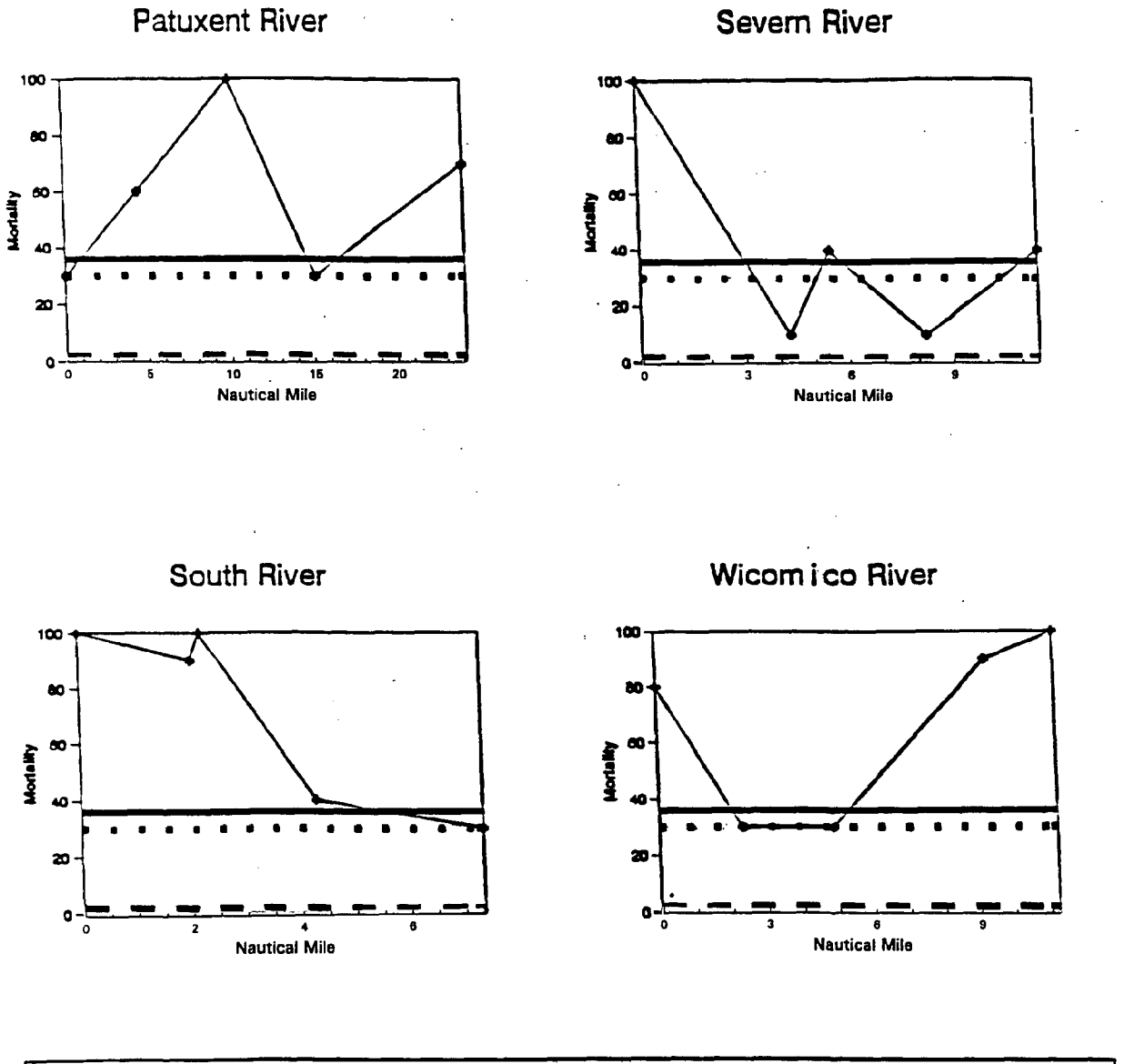

Fig. 11. *Cyprinodon variegatus* percent total mortality. Mortality is plotted against distance between site replicates from up-river to mouth.

Cyprinodon variegatus (Hatching Failure)

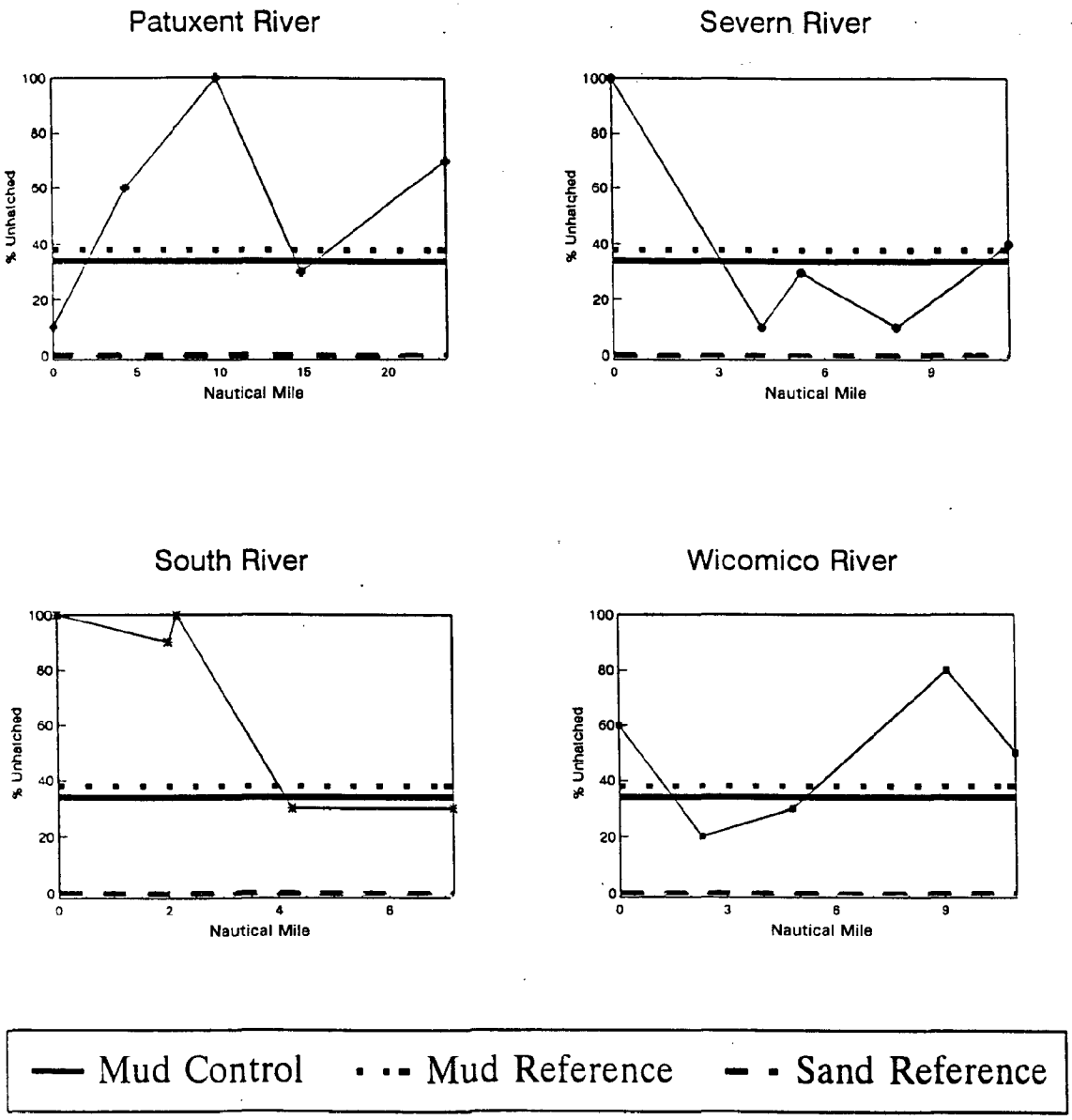

Fig. 12. *Cyprinodon variegatus* percent hatched. Mortality is plotted against distance between site replicates from up-river to mouth.

Cyprinodon variegatus (Percent Dead Eggs)

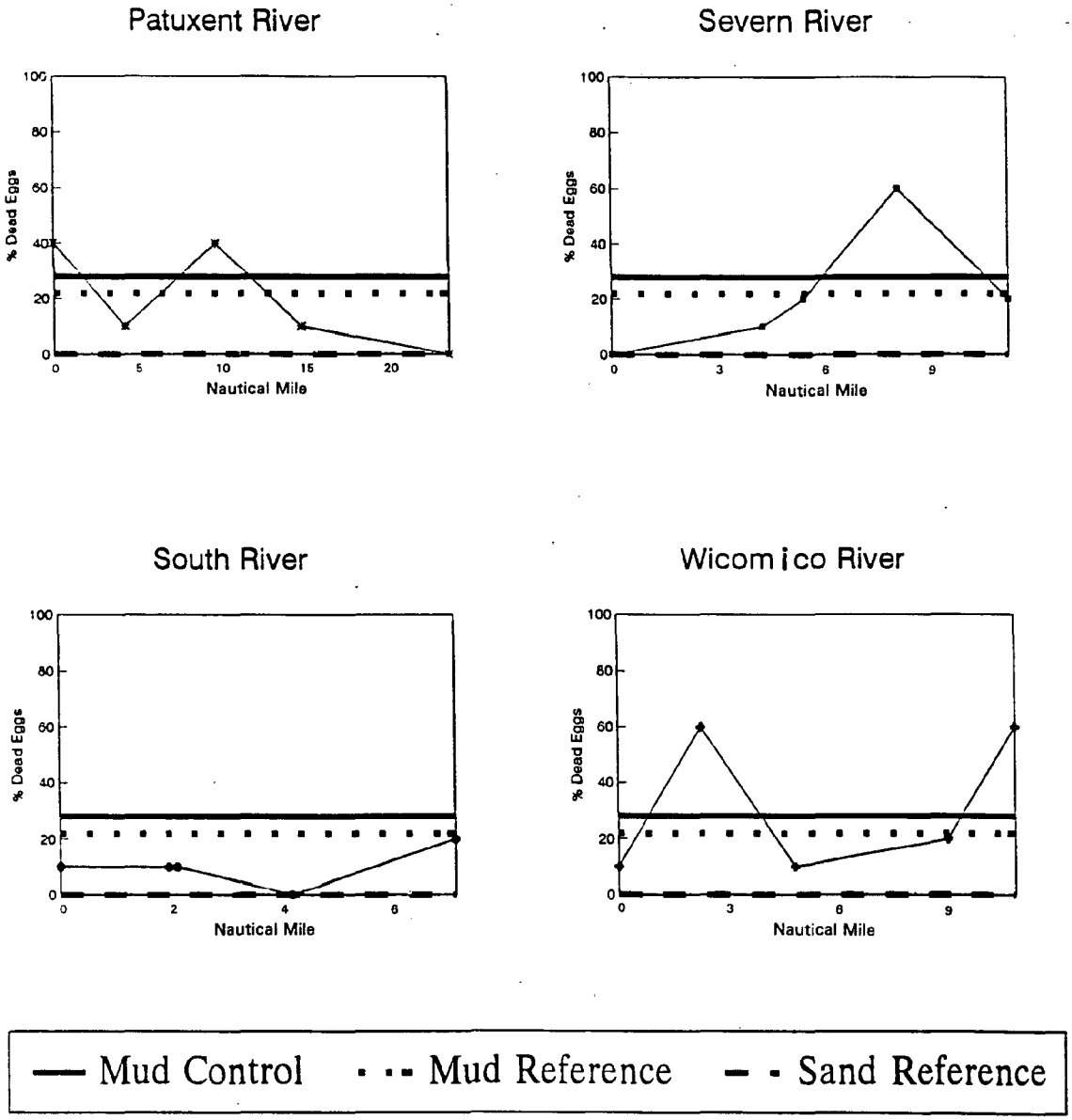

Fig. 13. *Cyprinodon variegatus* percent egg mortality. Mortality is plotted against distance between site replicates from up-river to mouth.

Cyprinodon variegatus (Percent Dead Fish)

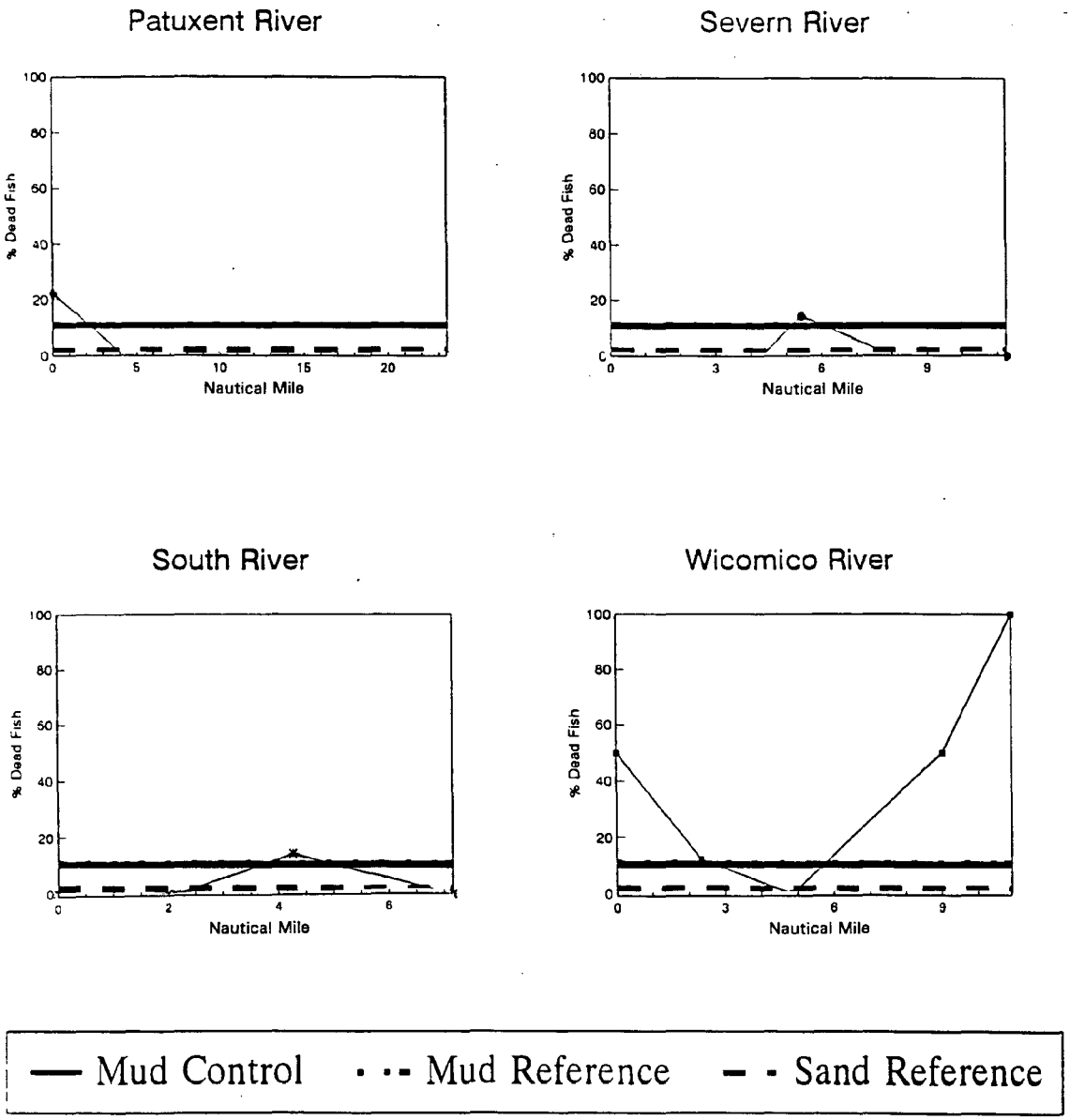

Fig.14. *Cyprinodon variegatus* percent fish mortality. Mortality is plotted against distance between site replicates from up-river to mouth.

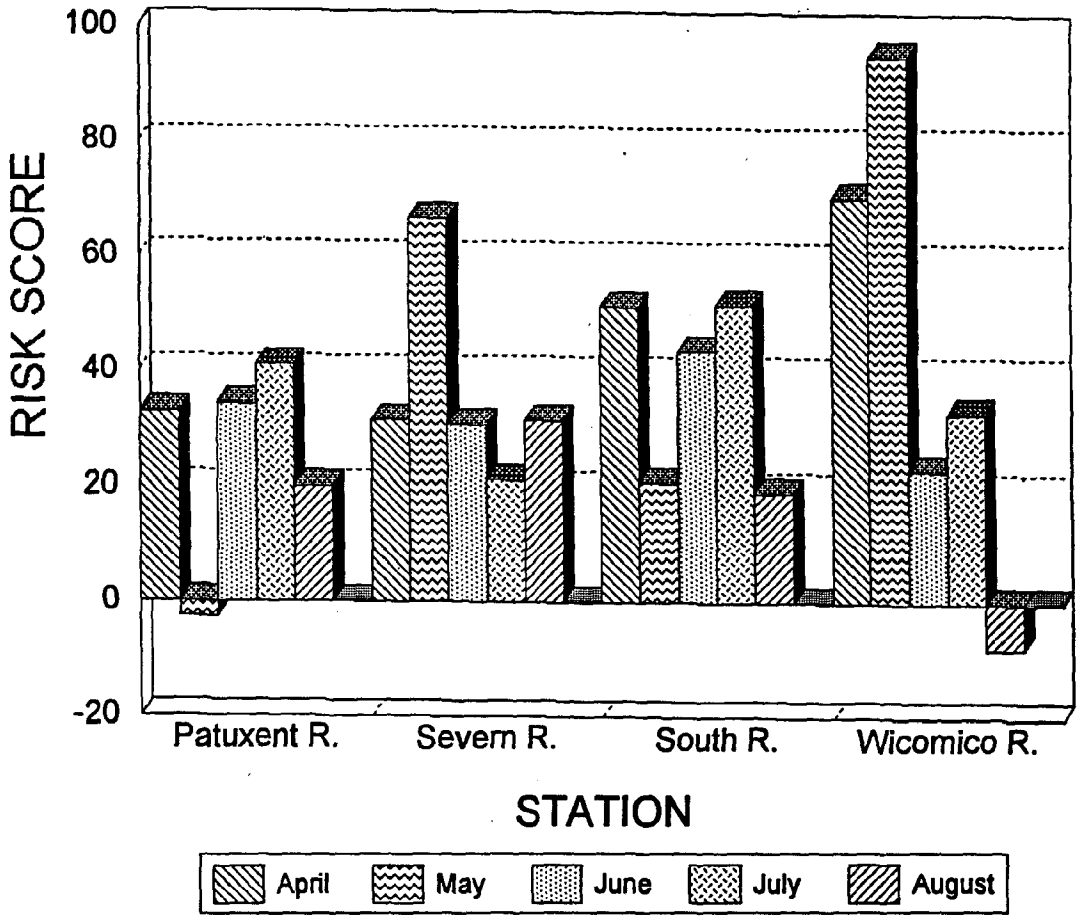

Figure 15. Risk scores for water from four tributaries of Chesapeake Bay sampled in 1994.

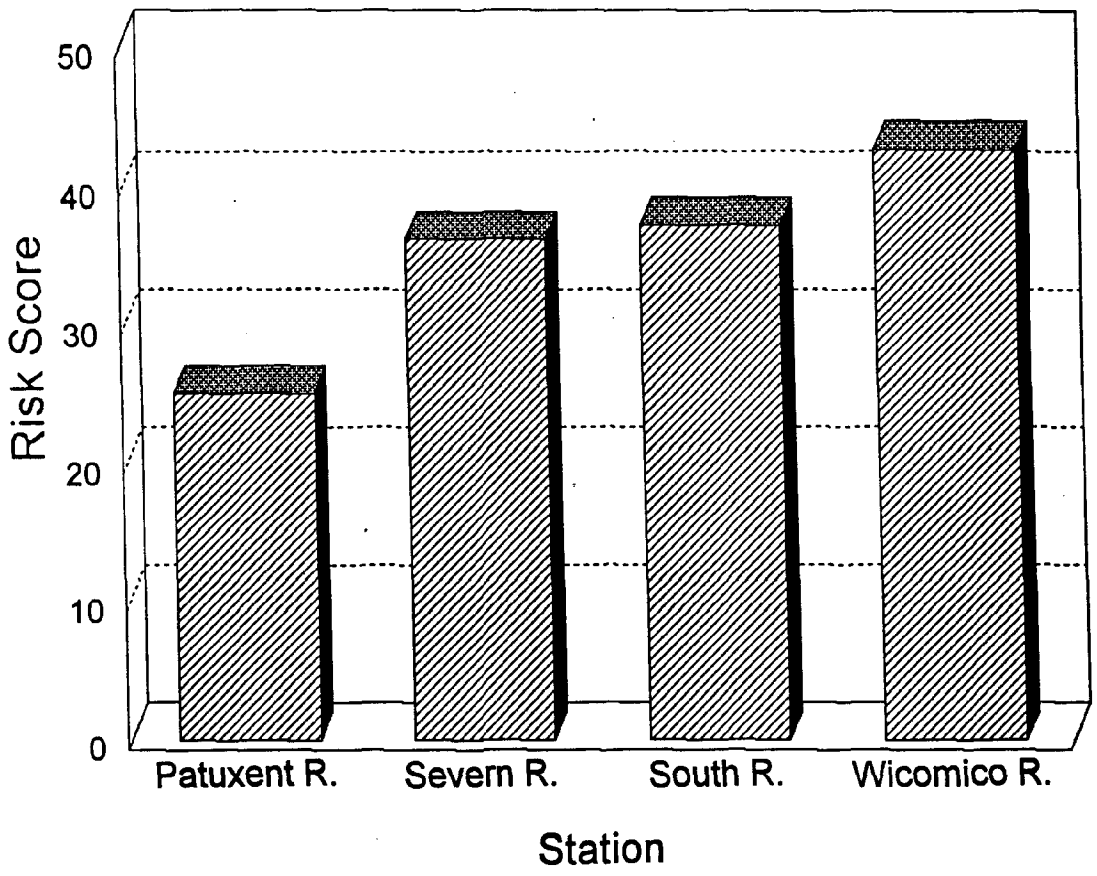

Figure 16. Average risk scores for water from four tributaries of the Chesapeake Bay sampled in 1994.

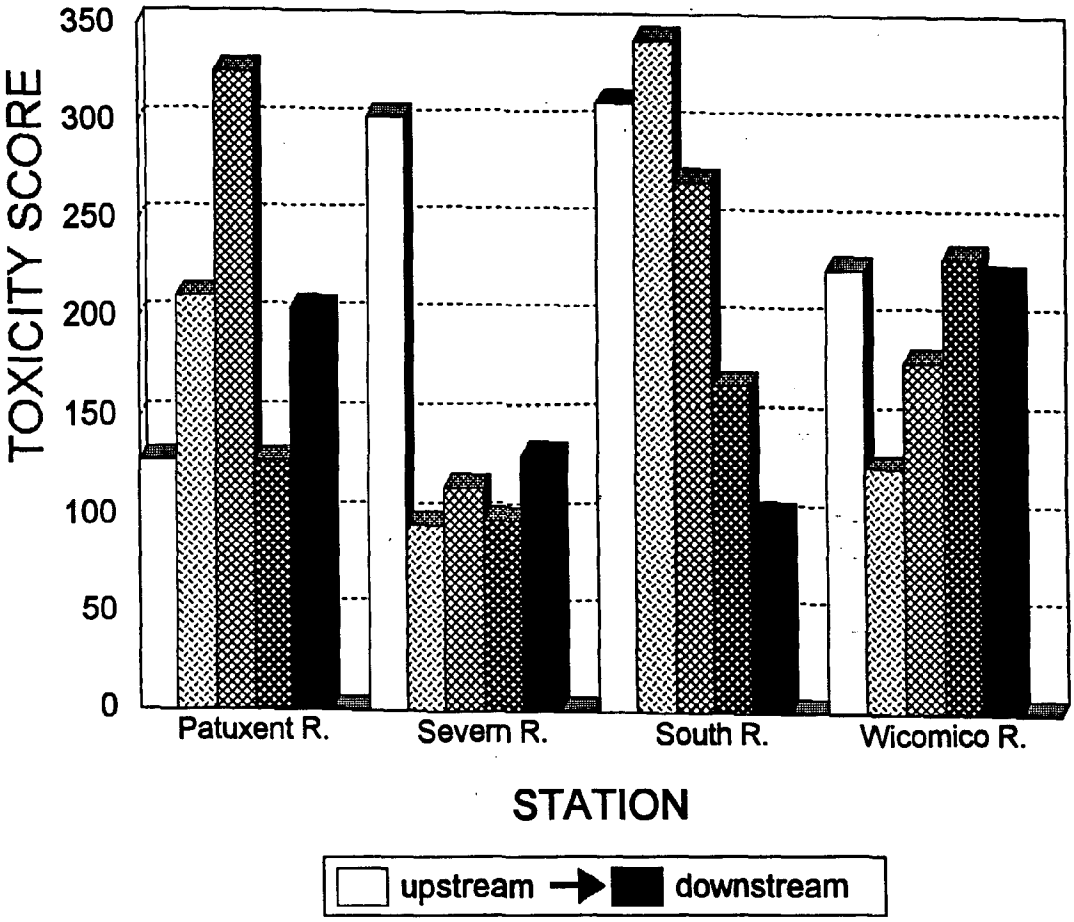

Figure 17. Toxicity scores for individual sediment samples from four tributaries of Chesapeake Bay sampled in 1994. Station scores from upstream to downstream are plotted from left to right.

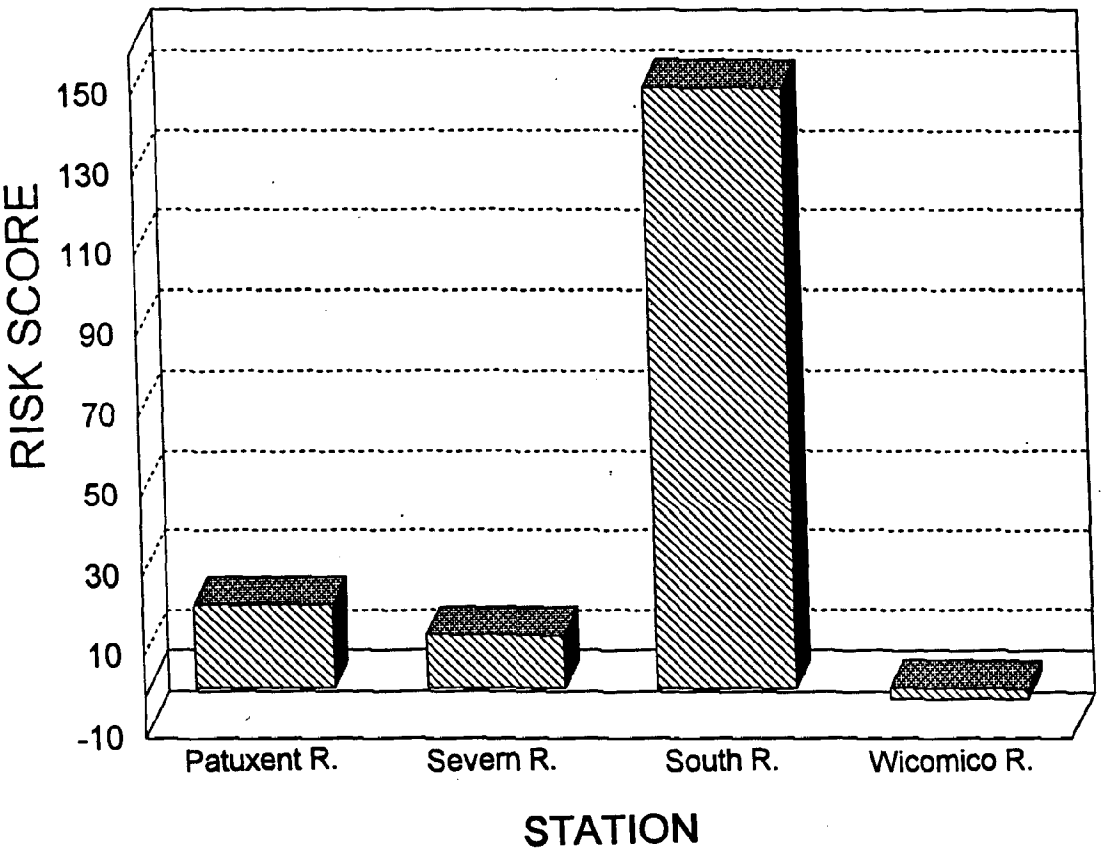

Figure 18. Pooled risk scores for sediments from four tributaries of Chesapeake Bay sampled in 1994.

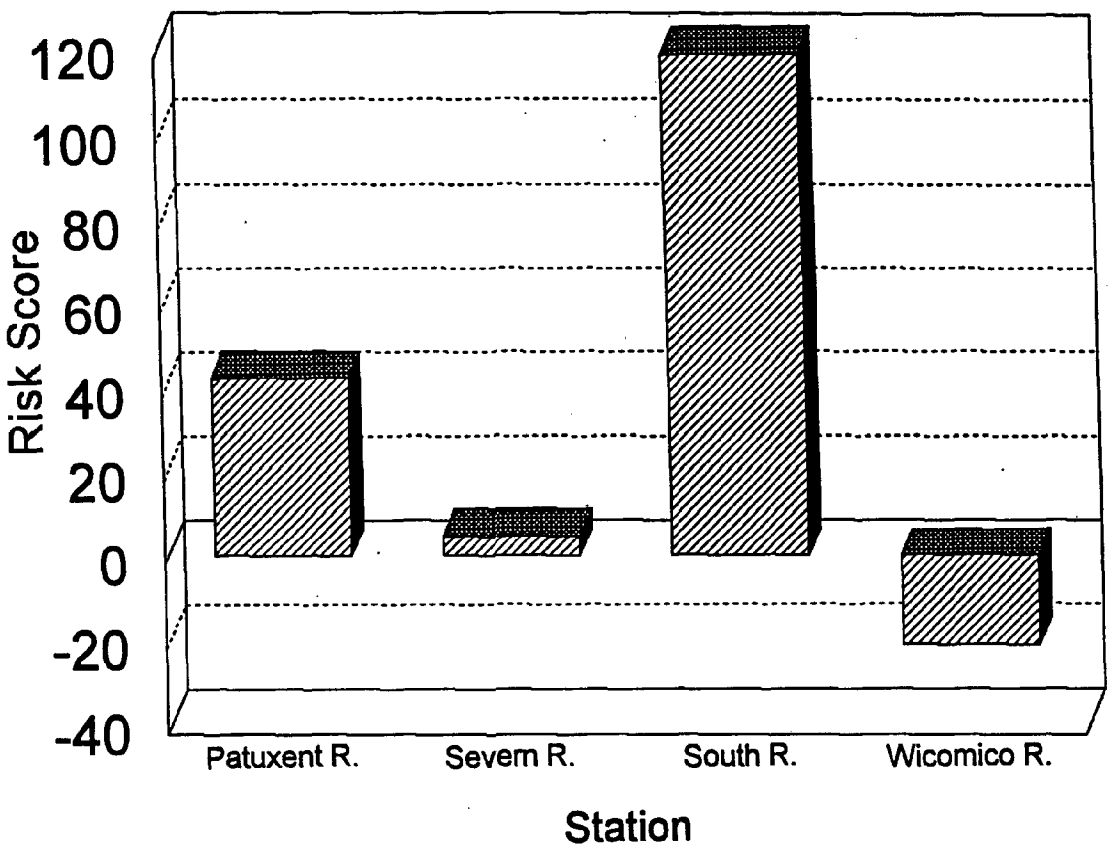

Figure 19. Risk scores for combined sediment and water samples from four tributaries of Chesapeake Bay sampled in 1994.

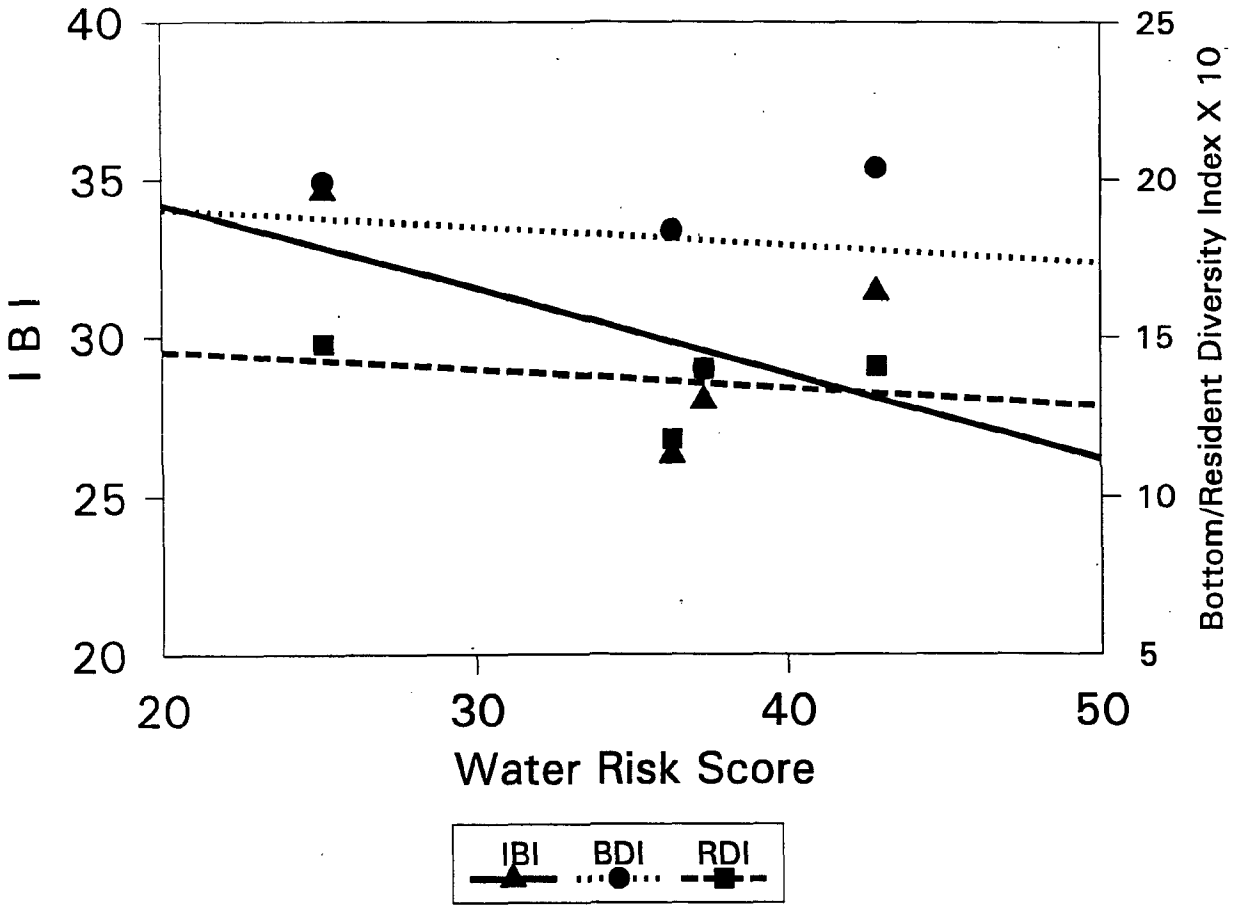

Figure 20. Mean risk scores for water samples from four tributaries of Chesapeake Bay sampled in 1994 vs fish community metrics. (Bottom diversity index - BDI; Resident diversity index - RDI; Index of Biotic Integrity - IBI)

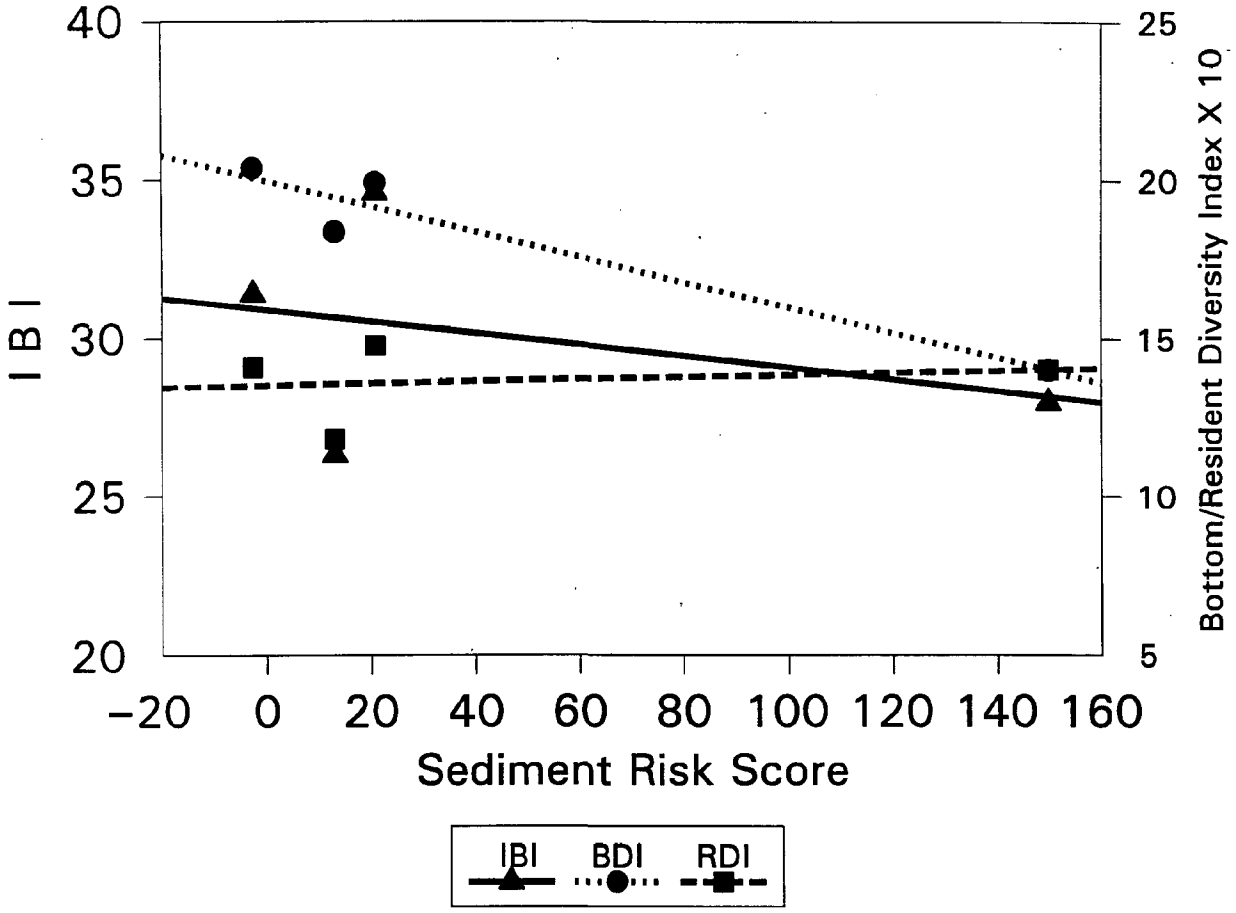

Figure 21. Pooled risk scores for sediment samples from four tributaries of Chesapeake Bay sampled in 1994 vs fish community metrics. (Bottom diversity index - BDI; Resident diversity index - RDI; Index of Biotic Integrity - IBI)

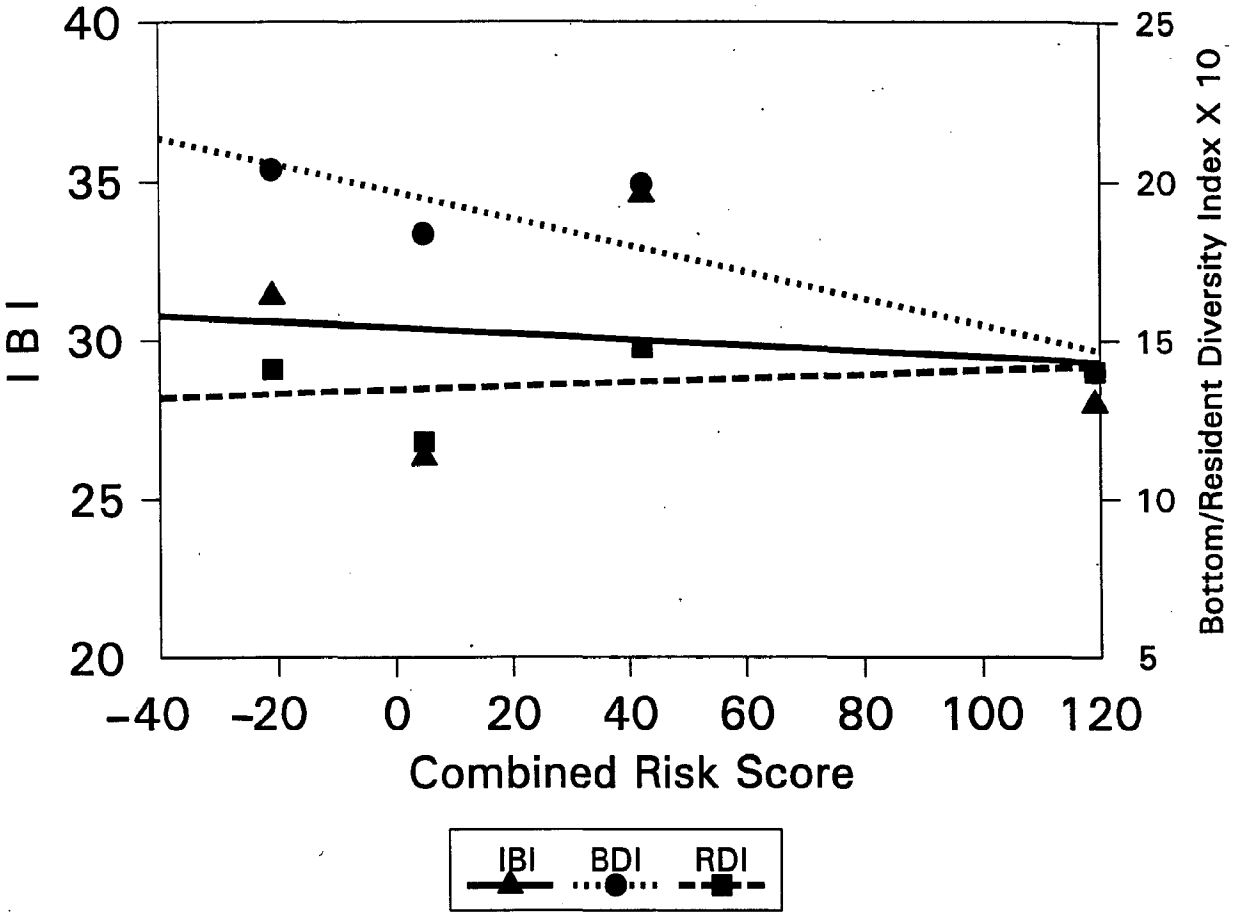

Figure 22. Combined risk scores for water and sediment samples from four tributaries of Chesapeake Bay sampled in 1994 vs fish community metrics. (Bottom diversity index - BDI; Resident diversity index - RDI; Index of Biotic Integrity - IBI)

County ~~City~~ of Ashtabula

LAKE SHORE PARK BOAT LAUNCH RAMP

Ashtabula, Ohio

Final Report

Grant No. NA-7[illegible]-AA-D-CZ085