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Year Two Report

A Pilot Study for Ambient Toxicity Testing in Chesapeake Bay

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Year 2 Report

A Pilot Study for Ambient Toxicity Testing in Chesapeake Bay

Lenwood W. Hall, Jr.
Michael C. Ziegenfuss
Steven A. Fischer
Ronald D. Anderson
William D. Killen

University of Maryland System
Maryland Institute for Agriculture and Natural Resources
Agricultural Experiment Station
Wye Research and Education Center
Box 169
Queenstown, Maryland 21658

Raymond W. Alden, III
Emily Deaver

Old Dominion University
College of Sciences
Applied Marine Research Laboratory
Norfolk, Virginia 23529-0456

Jay W. Gooch
Nikki Shaw

University of Maryland System
Chesapeake Biological Laboratory
Box 38
Solomons, Maryland 20688

FOREWORD

This pilot study was designed to evaluate ambient toxicity in the Chesapeake Bay watershed by using a battery of water column, sediment and suborganismal tests. A team of scientists from three Chesapeake Bay research laboratories worked jointly to complete this goal. Water column toxicity studies were directed by Lenwood W. Hall, Jr. of the University of Maryland System's Agricultural Experiment Station, sediment toxicity tests were managed by Raymond W. Alden, III of Old Dominion University Applied Marine Research Laboratory, and suborganismal tests were directed by Jay Gooch of the University of Maryland System's Chesapeake Biological Laboratory. This report summarizes data from the second year of this two-year study. The following government agencies were responsible for supporting and/or managing this research: Maryland Department of Natural Resources (CB-90-001-005), Maryland Department of Environment (182-C-MDE-91) and the U.S. Environmental Protection Agency (X-003554-01).

ABSTRACT

Contaminants and adverse water quality conditions (pollution) in the Chesapeake Bay watershed have been postulated as factors responsible for declining resources. Conclusive research designed to establish a link between pollution in the ambient Bay environment and declining resources can only be accomplished by direct measurement of biological responses. In 1990, we initiated a pilot study to broadly assess ambient toxicity of living resource habitats for the purpose of identifying defined regions where ambient toxicity levels warrant further investigations (Hall et al., 1991). The primary goal of the second year (1991) of this ambient toxicity testing pilot study was to identify toxic areas in living resource habitats of the Chesapeake Bay watershed by using a battery of standardized, directly modified or recently developed water column, sediment and suborganismal toxicity tests. Tests were conducted twice at the following stations: Potomac River-Morgantown, Potomac River-Dahlgren, Patapsco River and Wye River. A suite of inorganic and organic contaminants was evaluated in the water column and sediment during these tests. Standard water quality conditions were also evaluated in water and sediment from all stations.

The following water column tests were conducted in saltwater: 8-d sheepshead minnow, Cyprinodon variegatus, survival and growth test; 8-d larval grass shrimp, Palaemonetes pugio, survival and growth test; 8-d Eurytemora affinis life cycle test and 8-d mysid

shrimp, Mysidopsis bahia, survival, growth and reproduction test. Sediment toxicity tests conducted in saltwater areas at the four stations were: 20-d amphipod, Lepidactylus dytiscus, survival, growth and reburial test; 20-d grass shrimp, Palaemonetes pugio, survival and growth test; 20-d polychaete worm, Streblospio benedicti, survival and growth test and 20-d amphipod, Hyalella azteca, survival and growth test. Suborganismal tests were conducted to evaluate EROD activity in feral fish collected adjacent to the four sample sites; determine the holding time required for Fundulus heteroclitus from St. Johns Creek and the Wye River to reach baseline EROD levels and measure EROD responses in Fundulus from different geographic areas following treatment with a known EROD inducer.

Significant biological effects (statistically different from controls) were demonstrated from water column tests during at least one sampling period for all stations except the Patapsco River. The most persistent biological effects in the water column were reported from the Wye River station as significant mortality from two different test species was reported from both the first and second test. Sediment tests demonstrated significant biological effects for both tests at the Dahlgren, Morgantown, and Patapsco River stations. Significant biological effects were reported in sediment during the first Wye River test but not the second.

Results from suborganismal evaluations of EROD activity in feral fish (Fundulus and spot) suggested the presence of planar aromatic hydrocarbons in St. John's Creek and the Patapsco River.

Fundulus collected from St. John's Creek required three to fo· weeks to reach baseline EROD levels. EROD levels in Fundulus collected from the Wye River showed initial decreases, followed by a rapid increase (≈6 fold) from week 1 to week 2 and then returned to collection levels during week 3. After 10 weeks, EROD activity declined to levels similar to those in the St. John's Creek fish. EROD activity at collection was similar in Fundulus collected from the Wye River and St. John's Creek.

A general ranking of sensitivity among water column test species demonstrated that the sheepshead minnow larval test was the most sensitive test. However, a specific example (Wye River Test 2) was reported where the sheepshead test did not show biological effects but the E. affinis test did demonstrate effects. These data emphasize the importance of multispecies testing. The amphipod L. dytiscus was the most sensitive test species fo. sediment. However, there were still cases where this amphipod did not demonstrate effects while other species did show significant effects. As with the water column tests, sediment toxicity data also demonstrated the importance of multispecies testing.

Water quality and contaminant evaluations conducted in the water and sediment during this pilot study provided supportive information on possible causes of biological effects but these data were not intended to provide conclusive data on specific "cause and effect" relationships. Comparisons of biological effects data with the limited contaminants data for water column tests in 1991 demonstrated both good and poor linkages between these types of

data. A good correlation was reported for the first Wye River test as significant mortality was observed for sheepshead minnow larvae concurrently with the presence of two potentially toxic metals (copper and nickel). The presence of a potentially toxic Gymnodinium dinoflagellate was also suspected as a factor contributing to the mortality of this larval fish species. The second Wye River test is an example of a poor correlation between biological effects and the presence of those toxic contaminants which were measured. Significant mortality of E. affinis was reported but concentrations of the toxic substances analyzed for were not detected.

Data collected from the Patapsco River provided a strong case for the link between biological effects in sediment and the presence of potentially toxic concentrations of chromium, lead and zinc. Three of the four sediment test species demonstrated biological effects concurrently with the presence of the metals mentioned above. Biological effects from two tests were also reported in sediment at the Morgantown station concurrently with the presence of potentially toxic concentrations of 4,4' - DDT. The Dahlgren station (Test 1 and 2) and Wye River (Test 1) provided examples of biological effects without the detection of potentially toxic contaminant conditions in sediment.

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SECTION 1
INTRODUCTION

The Chesapeake Bay watershed is a large dynamic aquatic ecosystem that provides habitat for numerous aquatic species. In recent years, there has been concern for this estuary due to the decline of various living resources such as submerged aquatic vegetation, anadromous fish and the American oyster (Majumdar et al., 1987). Various factors such as nutrient enrichment, fishing pressure, pollution and diseases are often postulated as possible causes of these declining resources. The link between contaminants and biological effects has been of concern in critical Chesapeake Bay habitat areas in recent years. Information derived from the loading of toxic chemicals and/or chemical monitoring studies are not adequate for assessing the biological effects resulting from numerous sources such as multiple point source effluents, nonpoint source runoff from agriculture, silviculture and urban sites, atmospheric deposition, groundwater contamination, and release of toxic chemicals from sediments. The most realistic approach for evaluating the adverse effects of toxic conditions on living resources is by direct measurement of biological responses in the ambient environment (aquatic areas located outside of mixing zones of point source discharges).

Various state and federal agencies in the Chesapeake Bay watershed have made strong commitments to support research efforts designed to address the link between contaminants and adverse effects on living aquatic resources. For example, the Chesapeake

Bay Basinwide Toxics Reduction Strategy has a commitment to develc and 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 (CEC, 1988a). This commitment is consistent with the recommendations of the Chesapeake Bay Living Resource Monitoring Plan (CEC, 1988b).

An Ambient Toxicity Assessment Workshop was held in Annapolis, Maryland in July of 1989 to provide a forum on how to use biological indicators to monitor the effects of toxic contaminants in Chesapeake Bay on living resources. Recommendations from this workshop were used to develop a two year pilot study in 1990 and 1991. Objectives from the first year of this effort in 1990 have been completed and a report was submitted to the sponsors (Hall et al., 1991). Results from our first year of this two year study demonstrated that toxic ambient conditions were present in the Elizabeth River and Patapsco River based on water column, sediment and suborganismal tests. Data from sediment and suborganismal tests also suggested that toxic conditions were present at the proposed reference site in the Wye River; water column tests did not demonstrate the presence of toxic conditions at this reference site. Several ambient stations in the Potomac River also had toxic conditions based on water column and sediment tests. The need for multispecies testing was supported by the water column tests as no significant ranking of sensitivity among species was reported. Results from the sediment tests showed that the amphipod test was

most sensitive, followed by the polychaete worm test and the grass shrimp test. The need for integrated water column, sediment and suborganismal testing was confirmed during our first year of testing. A spectrum of tests was needed to maximize our ability to identify toxic conditions in the ambient environment of the Chesapeake Bay watershed.

The purpose of this report is to present data from the second year of testing and summarize all information collected over the two year period. Many of the test procedures described in the first year report were used for the second year of testing; therefore, the first year report by Hall et al. (1991) should be used to provide details on specific procedures. Objectives for the second year of testing were as follows: (1) develop a survey program to broadly assess ambient toxicity of living resource habitats for the purpose of identifying defined regions where ambient toxicity levels warrant further investigation of effects on living resources; (2) assess the feasibility of such a program through a pilot study; (3) field test existing standardized, directly modified or recently developed water column, sediment and suborganismal test methods for use in ambient toxicity testing and determine the relative sensitivity of these three test methods and (4) identify any additional test methods development or follow up survey design testing needs (if any) to support Baywide assessment of ambient toxicity. Data from both the first and second year of ambient toxicity testing will be discussed in this report.

SECTION 2

OBJECTIVES

The broad objective of this pilot study was to identify toxic ambient areas in the Chesapeake Bay watershed by using a battery of standardized, directly modified or recently developed water column, sediment and suborganismal tests. Results from this study were synthesized to identify additional test methods development or follow up survey design needs to support additional Baywide assessment of ambient toxicity. A battery of ambient tests were conducted twice during 1991 in the Potomac River - Morgantown, Potomac River - Dahlgren, Patapsco River and Wye River stations to identify "short term" variability in results. The specific objectives of the water column, sediment and suborganismal tests are presented below.

2.1 Water Column

Objectives of the water column tests were as follows:

- determine the toxicity of ambient estuarine water twice at the four sites by using the following estuarine tests: 8-d sheepshead minnow, Cyprinodon variegatus, survival and growth test; 8-d larval grass shrimp, Palaemonetes pugio, survival and growth test; 8-d Eurytemora affinis, life cycle test and 8-d mysid shrimp, Mysidopsis bahia, survival, growth and reproduction test.
- measure inorganic contaminants, organic contaminants and

water quality conditions in ambient water concurrently during toxicity tests to determine "possible causes" of toxicity.

- determine the relative sensitivity of the four test species and compare these data with the sediment and suborganismal data.

2.2 Sediment Tests

Objectives of the sediment toxicity tests were as follows:

- evaluate the toxicity of ambient sediment twice at the four sites by using the following estuarine tests: 20-d amphipod, Lepidactylus dytiscus, survival, growth, and reburial test; 20-d grass shrimp, Palaemonetes pugio, survival and growth test; 20-d polychaete worm, Streblospio benedicti, survival and growth test and 20-d amphipod, Hyalella azteca, survival and growth test.
- measure inorganic contaminants, organic contaminants and pore water quality conditions in sediment concurrently with toxicity testing to determine "possible causes" of toxicity.
- determine the relative sensitivity of the four test species and compare these data with water column and suborganismal data.

2.3 Suborganismal Tests

Objectives of the suborganismal tests were as follows:

- evaluate EROD activity in feral fish collected adjac to the four sample sites.
- determine the holding time required for Fundulus from St. Johns Creek and the Wye River to reach baseline EROD levels.
- measure EROD response in Fundulus from different geographic areas (Wye River and St. Johns Creek).

SECTION 3

METHODS

3.1 Study Areas

Ambient toxicity tests were conducted during August and September-October in 1991 at the Potomac River - Morgantown, Potomac River - Dahlgren, Patapsco River and Wye River sites (Figure 3.1). The Potomac River stations were selected because they are located in a critical habitat area with potentially toxic conditions. The Potomac River, a lotic system shared by three states, also provides gradients of salinity, contamination and habitat similar to other areas of the Chesapeake Bay watershed. The Patapsco River was selected as a polluted area to allow field testing of existing test methods for ambient toxicity testing. Adverse biological effects expected at this site provided a means to "ground truth" the test methods. The Wye River was selected to represent a relatively "clean" reference area with no point source pollution. However, non-point source inputs exist in this area. Biological effects were reported from all four of these sites with at least one test during the 1990 study (Hall et al., 1991). A brief description of each site and rationale for selection are included below.

3.1.1 Potomac River - Morgantown

This station was located on the Maryland side of the Potomac River near the Harry Nice Memorial Bridge (38° 21' 48" N x 77°

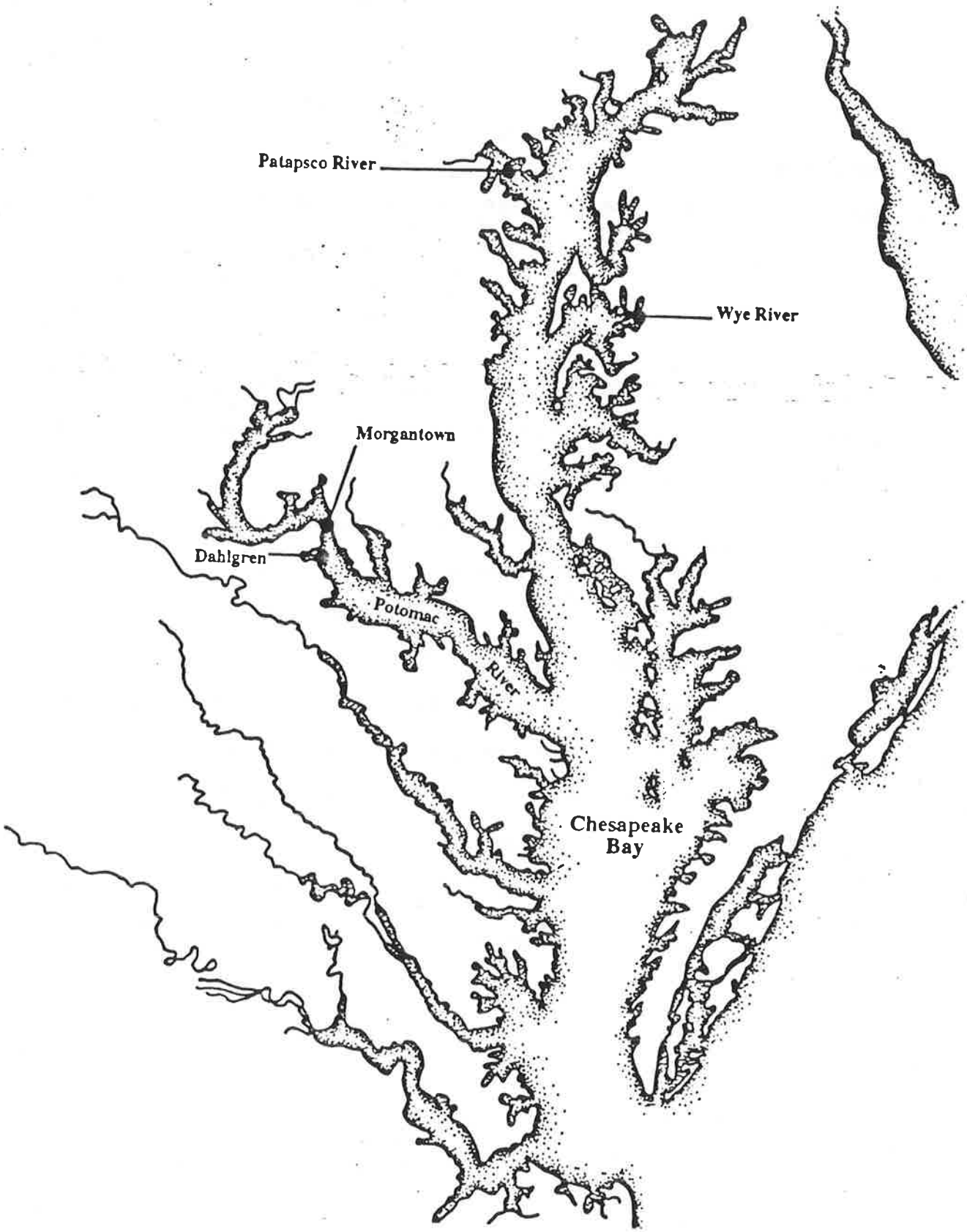


Fig. 3.1 Four stations sampled for ambient toxicity testing.

59' 30" W). Samples were collected approximately 200 m from shore; depth of the site was 3 m. Tributyltin (TBT) concentrations of 20 and 24 ng/L were detected at this station during a monitoring study in 1985 and 1986 (Hall et al., 1987b). These TBT concentrations exceeded the U.S. EPA provisional water quality criteria of 10 ng/L (U. S. EPA, 1987b). There are no other background contaminants data for this station. During our 1990 ambient testing, we reported significant biological effects at this site with both water column and sediment tests; suborganismal tests suggested liver abnormalities from white perch collected from this site (Hall et al., 1991).

3.1.2 Potomac River - Dahlgren

The Dahlgren station was located in a typical ambient mesohaline area on the Virginia side of the Potomac River at the mouth of Machodoc Creek near the Naval Surface Warfare Center at Dahlgren, Virginia (38° 19' 00" N x 77° 2' 00" W). Samples were collected approximately 300 m from shore; depth of the site was 2 m. The Dahlgren facility has six discharge points in this area. Results from our 1990 ambient tests at this site showed biological effects from one water column and one sediment test (Hall et al., 1991).

3.1.3 Patapsco River

This station was selected to allow field testing of our methods in an area where toxic conditions were suspected. The

station was located adjacent to the Bethlehem Steel Corporation at Sparrows Point on the Patapsco River near Bear Creek (39° 14' 00 N x 76° 29' 42" W). Samples were collected approximately 75 m from shore; depth of the site was 2 m. This site was located in a mesohaline area outside the mixing zone of Bethlehem Steel's largest discharge. Effluent toxicity tests conducted by the University of Maryland's Agricultural Experiment Station have reported chronic effects of Bethlehem Steel's effluent from both Ceriodaphnia and fathead minnow tests (Dan Fisher, personal communication). Various potentially toxic contaminants such as zinc, chromium, lead-free cyanide and tetrachloroethane have also been reported in effluents from the Bethlehem Steel Facility. Biological effects were reported at this site from all three types of tests conducted in 1990 (Hall et al., 1991).

3.1.4 Wye River

The Wye River was selected as a reference area where toxic conditions were not suspected. This station was located in a mesohaline area at Wye Narrows above the Manor house (38° 53' 12" N x 76° 01' 54" W). Samples were collected approximately 75 m from shore; depth of the site was 2 m. There are no known point sources of contaminants in this area although agricultural activity, a potential non-point source, exists. Results from ambient toxicity testing in 1990 demonstrated the presence of toxic conditions from both sediment and suborganismal tests (Hall et al., 1991).

3.2 Water Column Tests

3.2.1 General Description

The following estuarine tests were conducted with water collected at the Potomac River - Morgantown, Potomac River - Dahlgren, Patapsco River and Wye River sites: 8-d sheepshead minnow, Cyprinodon variegatus, survival, and growth test; 8-d larval grass shrimp, Palaemonetes pugio, survival and growth test; 8-d Eurytemora affinis, life cycle test and 8-d mysid, Mysidopsis bahia, survival, growth and reproduction test. Experiments were conducted at each station twice during 1991. Testing periods were August 13 - 21 and September 24 - October 2. Reference toxicant tests described later in this methods section were conducted at least once for each test species.

3.2.2 Culture and Maintenance for Test Species

Culture and maintenance procedures for grass shrimp, Eurytemora and sheepshead minnows described in Hall et al. (1991) were used. The mysid was not tested in year 1 of this study; therefore, culture and maintenance procedures for this species are described below.

3.2.2.1 Mysid

Mysids used for experiment 1 were obtained from Sea Plantations Inc. in Salem, Massachusetts. The test procedures for this mysid shrimp test requires that tests are initiated with 7-d old mysids. A larger than expected size range was reported in the

shipment we received, thus suggesting that some of these shri- were older than 7 days. Due to these size and possible age differences, we did not use growth or reproduction as endpoints in this experiment. Survival was the only endpoint used and all test organisms were randomly selected for the various test conditions.

Mysids used for experiment 2 were obtained from Chesapeake Cultures in Hayes, Virginia. The size range for these mysids was small suggesting that the age for all test species was 7 days. Survival, growth and reproduction were evaluated.

3.2.3 Reference Toxicant Tests

A 48-h static reference toxicant test was conducted with each test species in 1991. A reference toxicant is used to establish the relative health and sensitivity of the test organisms. Cadmium chloride was selected as the reference toxicant because we have 3 year data base with this chemical for all of the test species except Eurytemora. A complete description of the rationale and use of reference toxicant tests is described in Fisher et al. 1988 and Hall et al. 1991. Cadmium concentrations were made from a common stock solution of cadmium chloride (Sigma Chemical). A 1.0 mg/L cadmium stock solution was created by dissolving 407.7 mg $CdCl_2$ in 250 mls deionized water. The stock solution was diluted to final concentrations by pipetting stock solutions into 2 L glass beakers containing filtered artificial seawater (15 ppt).

Survival of test organisms was monitored at each test condition at both 24 and 48 hours (except E. affinis at 24 h). An

LC50 value and 95% confidence intervals were calculated at 24 and 48 h using the moving-average angle and probit methods (Stephan, 1978). Temperature, salinity, dissolved oxygen and pH were measured in each beaker at both 24 and 48 hours (except E. affinis).

3.2.4 Sample Collection, Handling and Storage

The procedures for sample collection, handling and storage described in Hall et al. (1991) for the first year of this study were used. Ambient water was collected from the four sites and transported back to our laboratory for testing at the Wye Research and Education Center. Grab samples collected from each station were a composite of two water column depths (~ 1 m for the top and ~ 1 m from the bottom). A mid-depth sample was taken at stations with a 2 m depth. A metering pump (12 V DC Little Giant Utility Pump, Model PPS-12) with polyethylene line was used to collect samples in 11.25 L glass containers.

Samples were collected on day 0, 3 and 6 during the 8-day tests. The time lapsed from the collection of a grab sample and the initiation or renewal of the test did not exceed 72 h. All samples were chilled after collection and maintained at 4 C until used. The temperature of the ambient water used for testing was 25 C. Salinity adjustments (increases) were performed on all aqueous samples to obtain a standard test salinity of 15 ppt. These temperature and salinity conditions were used during the first year of testing.

3.2.5 Test Procedures

The test procedures for grass shrimp, Eurytemora ar sheepshead minnows described in detail in Hall et al., (1991) were used. The mysid shrimp was not tested in the first year of this study; therefore, testing procedures are described below.

3.2.5.1 Mysid

The test was initiated with 7-d old mysids exposed to the following test conditions: Potomac River - Morgantown, Potomac River - Dahlgren, Patapsco River, Wye River and a contaminant-free seawater water control (15 ppt). Eight replicate 250 ml beakers, each containing five mysids, were used for each condition. Due to insufficient numbers of test organisms for experiment 1, we could not use equal numbers of test species for all test conditions. The Morgantown and Wye conditions had eight replicates while the control and Dahlgren conditions had five replicates. Test beakers were held in biological incubators to control temperature (25 C) and photoperiod (16 h L: 8 h D). A one liter sample was taken daily from each of the field samples being stored at 4 C. Field samples were warmed to 25 C and one-half the water volume in each test beaker was removed and replenished with the fresh sample. Temperature, pH, salinity and dissolved oxygen were monitored daily in selected beakers from all test conditions. Survival of mysids was recorded daily for eight days.

Mysids were fed twice daily (before and after water renewals) with newly hatched Artemia nauplii. Each beaker received 0.05 ml Artemia suspension per feeding (≈150 Artemia per mysid per day) for

day 0 through day 2. On day 3 through 8, each beaker received 0.075 ml Artemia per feeding. The proportion of male, female and immature mysids was determined in each test beaker after the test was terminated on day 8. Twenty mysids from each test condition were sampled for growth determination. Dry weights for pooled samples were recorded following 24-h drying at 78 C. Mean individual weights were calculated from the pooled samples.

Survival proportions were transformed using the arc sine (square root (Y)) transformation. Steeles many-one rank test and Wilcoxon rank sum test were used to compare results. Growth parameters were compared by using ANOVA and Dunnetts tests. Differences between means were considered significant at the $p < 0.05$ level.

3.2.6 Statistical Analysis

Statistical tests described in Fisher et al. (1988), Hall et al., (1988a) and Hall et al., (1991) were used for each test species when appropriate. The goal of this study was not to generate typical LC50 data with various dilutions of ambient water. For each test species response (survival, growth etc), a control was compared with one test condition (100% ambient water). A statistical difference between the response of a species exposed to a control condition and an ambient condition was used to determine toxicity. Analysis of Variance or Dunnetts Procedures were used in cases where comparisons of a species response on a spatial or temporal scale was needed.

3.2.7 Contaminant and Water Quality Evaluations

A limited number of contaminants were evaluated concurrently with toxicity tests. It was not our intention to suggest that the analyses for inorganic and organic contaminants would provide an absolute "cause and effect" relationship between contaminants and biological effects if effects were reported. Information on suspected contaminants in the study areas does, however, provide valuable insights if high potentially toxic concentrations of contaminants were reported in conjunction with biological effects.

Aqueous samples for analysis of organic and inorganic contaminants listed in Table 3.1 were collected during the ambient toxicity tests. Analytical procedures and references for analysis of these samples are presented in Table 3.2. Analysis for triazine herbicides (simazine, atrazine, alachlor, metachlor and cyanazine) was conducted during this study but not in 1990. Total inorganic contaminant analysis was conducted on filtered samples using 0.40 um polycarbonate filters.

Four liter whole water samples were collected for organic contaminants analysis (Table 3.1). Organic contaminants other than those identified in Table 3.1 (non-target organics) were measured if GC/MS peaks were identified. Detailed procedures for preparing samples for inorganic and organic analyses are described in Hall et al. (1988a). Analysis for both organic and inorganic contaminants was conducted at least one time on aqueous samples collected from each station. If toxicity was found during the experiments, then the suite of contaminants was analyzed on at least 2 separate

Table 3.1 Concentrations of the following organic and inorganic contaminants were evaluated in the water column.

Contaminant	Detection Limit (ug/L)
Simazine	<0.26
Atrazine	<0.32
Alachlor	<2.5
Metolachlor	<3.35
Cyanazine	<0.55
Aroclor 1248	0.050
Aroclor 1254	0.050
Aroclor 1260	0.050
DDE	0.02
Toxaphene	0.2
Chlordane	0.02
Perylene	0.70
Fluorene	0.90
Phenanthene	0.70
Anthracene	0.70
Fluoranthrene	1.1
Pyrene	1.0
Benz(a)anthracene	1.7
Chrysene	0.7
Aluminum	3.0
Arsenic	3.0
Cadmium	2.0
Chromium, total	3.0
Copper	2.0
Lead	2.0
Mercury	0.2
Nickel	5.0
Selenium	3.0
Tin	5.0
Zinc	5.0

Table 3.2 Analytical methods used for organic and inorganic analysis. The following abbreviations were used: GC-EC (Gas Chromatography - Electron Capture), GC -MS (Gas Chromatography - Mass Spectrometry), GC-NP (Gas Chromatography - Nitrogen Phosphorous Detector), Atomic Emission - ICP (AE-ICP), AA-H (Atomic Absorption - Hydride), AA- F (Atomic Absorption - Furnace) and AA-DA (Atomic Absorption - Direct Aspiration) and AA-CV (Atomic Absorption - Cold Vapor).

Contaminant	Method	Method #	Reference
Halogenated Hydrocarbon Pesticides	GC-EC	608	U. S. EPA, 1984
Polychlorinated Biphenyls	GC-EC	608	U. S. EPA, 1984
Base-Neutral Extractable Organic Compounds	GC-MS	625	U. S. EPA, 1984
Triazine Herbicides (Simazine, Atrazine, Alachlor, Metachlor, Cyanazine)	GC-NP	507	U. S. EPA, 1989
Aluminum	AE-ICP	200.7	U. S. EPA, 1984
Arsenic	AA-H	206.3	U. S. EPA, 1979
Cadmium	AA-F	213.2	U. S. EPA, 1979
Chromium, Total	AA-F	218.2	U. S. EPA 1979
Copper	AA-F	220.2	U. S. EPA 1979
Lead	AA-F	239.2	U. S. EPA 1979
Mercury	AA-CV	245.1	U. S. EPA 1979
Nickel	AA-F	249.2	U. S. EPA, 1979
Selenium	AA-H	270.3	U. S. EPA, 1979
Tin	AA-F	282.2	U. S. EPA, 1979
Zinc	AA-DA	289.1	U. S. EPA, 1979

samples taken during these tests. For example, if mortality was observed on day 5, the samples taken from day 0 and 3 were analyzed. Versar, Inc. was responsible for all organic and inorganic analyses except triazine herbicides, which were analyzed by Maryland's Department of Health and Mental Hygiene.

Standard water quality conditions of temperature, salinity, dissolved oxygen, pH and conductivity were evaluated at each site after sample collection. These parameters were also evaluated every 24 h at all test conditions.

3.3 Sediment Tests

3.3.1 General Description

All tests and analyses were conducted according to the Standard Operating Procedures and Quality Assurance plans previously submitted to the sponsors. The methods described in this report are general summaries of those protocols. Sediment samples (100% ambient sediment samples) from four stations were tested using three field collected species: the grass shrimp Palaemonetes pugio, the amphipod Lepidactylus dytiscus, the polychaete worm Streblospio benedicti (hereafter referred to as worm), and one laboratory cultured species, the amphipod Hyallela azteca. All tests were conducted for 10 days at 25C and monitored daily. At the end of 10 days, mortalities were recorded, and the animals were returned to the original test containers. The organisms were then monitored daily for an additional 10 days. Numbers of live animals were recorded on day 20, and any living

organisms were preserved for length/ weight measurements. The sediment samples were collected from one site in the Patapsc River, one site in the Wye River and two sites in the Potomac River (Morgantown and Dahlgren). The salinity at all sites was 10-12 parts per thousand (ppt) at sampling, except for the Patapsco River site, which was 9 ppt at sampling. All samples were adjusted to 15 ppt prior to testing by sieving with 15 ppt control water made from deionized water and artificial sea salts. Control sediments for each species consisted of native sediments from the area in which the test organisms were collected or sediments in which they were cultured. Control and reference sediments (see below) were tested with each set of experiments.

Sediment for performing particle size analysis was collected from each of the test stations several weeks prior to initiation of the toxicity tests, in order to select a reference sediment for each set of test samples. Control sediment tests were performed to assess inherent species effects (mortality, growth, etc.) in native sediment. Uncontaminated reference sediment tests were performed to assess particle size effects. It was determined from the initial sediment collection and particle analysis that all test sites were greater than or equal to 72% sand (see Table 3.3). The sediment sample from Dahlgren was 72% sand; Wye River sample 79% sand, Morgantown 89% sand, and Patapsco 98.6% sand. The initial particle analysis for S. benedicti control sediment from Lynnhaven Inlet indicated that the sediment was 68% sand. The sediment used as the control for L. dytiscus from the Lynnhaven Beach site was

Table 3.3 Initial particle size analysis of sediments from four stations and controls used in toxicity tests. Morgantown and Dahlgren are Potomac River stations. Samples were collected 8/1/91.

Site	% Sand	% Silt	% Clay
Morgantown	89.0	6.3	4.7
Dahlgren	72.0	12.7	15.3
Patapsco River	98.6	0.9	0.5
Wye River	79.2	12.9	7.9
Lynnhaven Beach	100	0	0
Lynnhaven Inlet	67.8	23.8	8.4

100% sand. These two control sediments bracket the particle size of all test samples and were therefore considered suitable as reference sediments as well. Therefore, the Lynnhaven Inlet control sediment for S. benedicti and H. azteca was used as the reference sediment for the P. pugio and L. dytiscus and the Lynnhaven Beach control sediment for P. pugio and L. dytiscus was used as the reference sediment for the S. benedicti and H. azteca (see Tables 3.4 and 3.5). The actual test sediment samples were collected and again analyzed for sand, silt, and clay content. Although the particle size/composition of the test sediments were slightly different from the initial samples (see Table 3.4), all samples were still primarily sand, with the lowest concentration occurring at the Wye River site (82.2% sand).

3.3.2 Culture and Maintenance of Test Species

Culture and maintenance procedures used for grass shrimp, worms and the amphipod Lepidactylus dytiscus are as described in Hall et al. (1991). Hyalella azteca was not used in year 1 of this study, therefore, culture and maintenance procedures for this organism are described below.

3.3.2.1 Hyalella azteca

Hyalella azteca was cultured at the Applied Marine Research Laboratory for approximately a year and a half prior to the experiments. Animals are cultured in 20 gallon tanks filled with freshwater (calcium, magnesium, potassium and sodium salts added to deionized water (ASTM, 1990)) in a 16 Light:8 Dark cycle. The tanks

Table 3.4 Particle size analysis of sediments from four stations and controls used in toxicity tests. Morgantown and Dahlgren are Potomac River stations. Set #1 was collected 8/14/91. Set #2 was collected 9/25/91.

Site	% Sand	% Silt	% Clay
Set #1:			
Morgantown	94.0	2.9	3.1
Dahlgren	91.2	6.2	2.6
Patapsco River	98.8	0.8	0.4
Wye River	93.7	3.9	2.4
Lynnhaven Beach	100	0	0
Lynnhaven Inlet	68.6	25.4	6.0
Set #2:			
Morgantown	94.5	2.7	2.8
Dahlgren	88.5	4.8	6.7
Patapsco River	98.7	0.6	0.7
Wye River	82.2	9.0	8.8
Lynnhaven Beach	100	0	0
Lynnhaven Inlet	63.3	26.6	10.1

Table 3.5 Control and reference test sediment allocation by species.

	Sediment Site	Sediment Description	Sediment Function
SPECIES			
L. dytiscus	Lynnhaven Beach	100% Sand	Control
	Lynnhaven Inlet	65-68% Sand	Reference
P. pugio	Lynnhaven Beach	100% Sand	Control
	Lynnhaven Inlet	65-68% Sand	Reference
H. azteca	Lynnhaven Beach	100% Sand	Reference
	Lynnhaven Inlet	65-68% Sand	Control
S. benedicti	Lynnhaven Beach	100% Sand	Reference
	Lynnhaven Inlet	65-68% Sand	Control

are gently aerated, and approximately 30 percent of the overlying water is replaced weekly. Dried maple leaves, presoaked and rinsed in deionized water, are added weekly as the primary substrate and food. Ground rabbit chow is added twice a week as a supplementary food. Two Hyalella azteca culture tanks were acclimated to 15 ppt over a seven day period. Animals were held at 15 ppt for an additional seven days prior to initiation of the tests. Test organisms were obtained from the population tanks by sieving with a 710 micron sieve stacked on a 425 micron sieve. Those animals that passed through the 710 sieve, but were retained on the 425 micron sieve were used as test organisms. Juvenile Hyalella azteca were sieved from the population tank no more than 24 hours prior to initiation of the toxicity test.

3.3.3 Reference Toxicant Tests

The relative sensitivities of each set of test organisms were evaluated by performing reference toxicant tests. These tests were designed to assess changes in toxicity tolerances of the organisms that may have occurred due to disease, or stress resulting from handling and acclimation. The same reference toxicant test methods and procedures were employed in the 1991 study (see Hall et al. 1991 for details) that were used in the 1990 project.

Hyalella azteca was tested in a 96-hour, water only, cadmium chloride reference toxicant test using 200 ml of solution with 10 animals per container. Five concentrations and a control (artificial seawater only) were prepared by serial dilution. Tests

were conducted at 15 ppt salinity with two replicates for each concentration. A data base of Hyalella azteca reference toxica... data exists at the AMRL laboratory for comparison.

Survival of test organisms was monitored every 24 hours. A 96-h LC50 value and 95% confidence intervals were calculated using Probit Analysis or the Moving Average Angle Method, as was appropriate for each set of data.

3.3.4 Sample Collection, Handling and Storage

Sediment sample collection, handling, and storage procedures described in Hall et al. 1991 were used in this study. Sediment samples were collected at each site by AMRL personnel and returned to the laboratory for testing. The first set of sediments was collected August 14, 1991, by petite ponar grab. The second set of sediment samples was collected by petite ponar grab on Septemb 25, 1991 at the same sites.

Approximately 8 gallons of sediment were collected at each site, homogenized and distributed to sample containers. All samples were transported on ice, out of direct sunlight. Samples were held in refrigerators at 4C until initiation of toxicity tests. Samples for chemical analysis were frozen and stored until tested. All samples were analyzed within EPA recommended holding times.

3.3.5 Test Procedures

The test procedures for Palaemonetes pugio, Streblospio benedicti, and Lepidactylus dytiscus described in detail in Hall et

al. (1991) were used. Hyalella azteca was not tested in the first year of this study, therefore, these procedures are described below.

3.3.5.1 Hyalella azteca

A series of test containers was set up according to the methods outlined in the ASTM "New Standard Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates" (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. Control sediment consisted of Lynnhaven Inlet muddy sand. A subset of the test animal population was selected for initial length/weight measurements. All length measurements were conducted using the Optimas Image Analysis system. Test containers were monitored daily for oxygen, temperature, and pH. Number of animals emerged from the sediment was also recorded. The amphipods were fed 14 mg of ground rabbit chow per test container every three days throughout the duration of the test. At the end of ten days, animals were sieved from test containers and mortality was recorded. Surviving animals were then returned to the test containers for an additional 10-day period. On day twenty, animals were again sieved from the containers and mortality recorded. Live animals were preserved for growth measurements.

3.3.6 Statistical Analysis

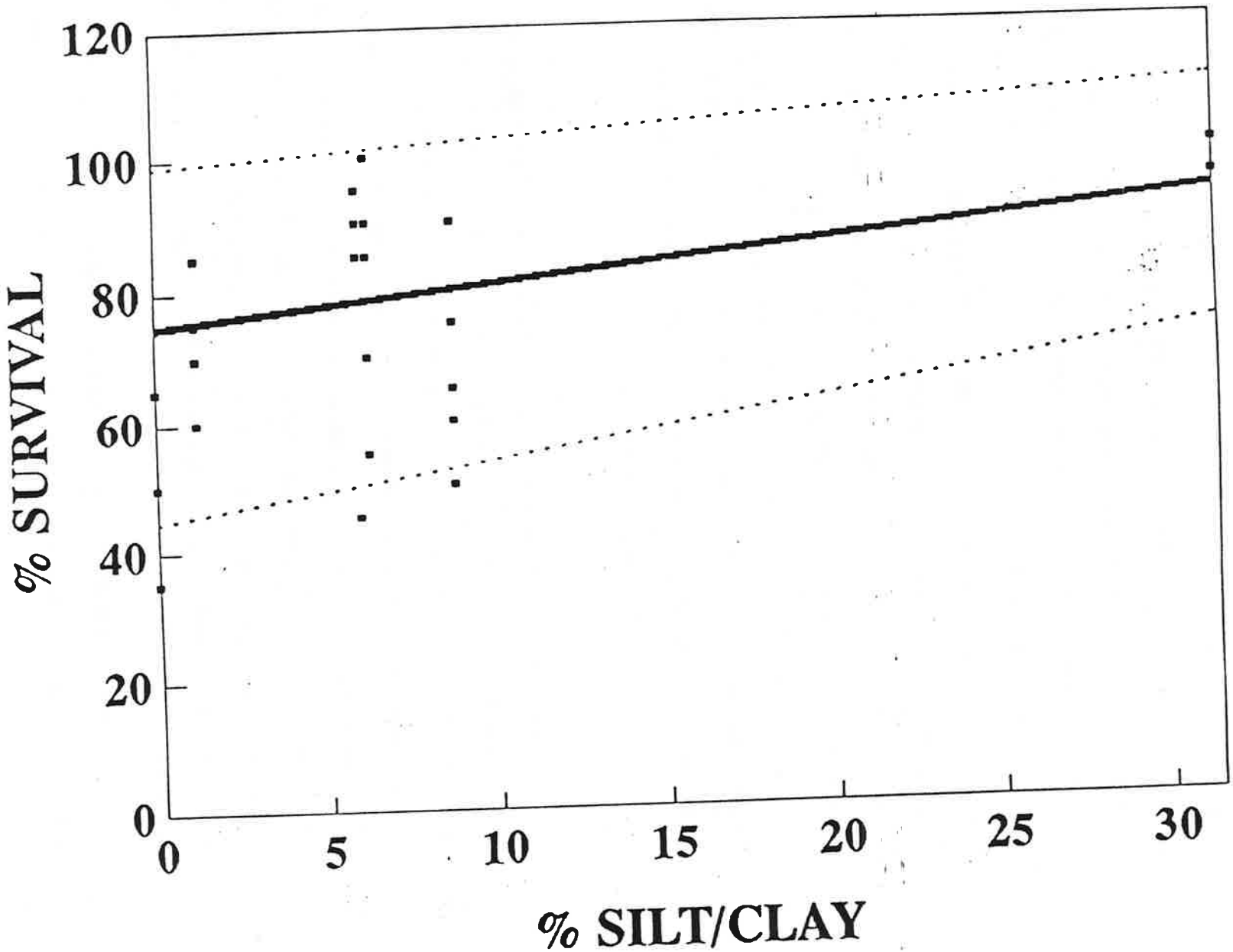
The goal of this study was not to generate LC50 data from

dilution series tests. The main objective was to evaluate for each test species, the response (mortality, growth, etc) when tested i' 100% ambient sediment, as compared to a control. Statistical differences between the responses of species exposed to control and ambient sediments were used to determine the toxicity. Evaluations relative to particle size effects were made based on the response seen in the reference sediments.

The same statistical approaches that were employed in the first year of the study (Hall et al., 1991) were utilized in the second year. Basically, the analyses consisted of analysis of variance (ANOVA) models with a. priori tests of each treatment contrasted to the controls. Arcsine transformations were used for the percent mortality data. For growth data comparisons, the length and weight measurements were tested in a multivariate analysis of variance design (MANOVA) to remove the effect of inter-variance correlation on the statistical comparisons. The length measurements at day 20 were also compared to the initial (pre-experimental) lengths by the ANOVA/contrast tests.

During the Set 1 experiments, it became apparent that Streblospio benedicti displayed mortalities if placed in very sandy sediments (> 90% sand) without supplementary feeding (Figure 3.2). A feeding rate for Set 2 experiments of 0.25 mg/replicate offered every third day was employed to limit this mortality. This "worm food" consisted to dried ground Ulva spp. and finely ground (<500 um) Tetramin in a 50:50 ratio. Supplementary experiments with clean sediments of different particle sizes demonstrated a

Fig. 3.2 Survival of Streblospio benedicti versus percent silt/clay.



regression relationship between percent survival (at day 10) and percent sand:

$$\% \text{ survival} = 135.2 - 0.60627 \times \% \text{ sand}$$

This relationship was employed to determine the portion of mortalities observed during the Set 1 experiments which were associated with particle size (and starvation) effects. These predicted mortalities were taken into account mathematically before treatment effects were statistically evaluated. This sort of procedure has been used for "adjusting" for particle-size effects in the amphipod Rhepoxynius abronius (DeWitt, 1988).

Much of the particle-size mortalities observed during Set 1 were thus eliminated by supplementary feeding in Set 2. Additional studies with this species have suggested an optimal feeding regime which will be employed in the year 3 studies.

3.3.7 Contaminant and Sediment Quality Evaluations

Contaminants were evaluated concurrently with toxicity tests. It was not our intention to suggest that the presence of inorganic and organic contaminants demonstrate an absolute "cause and effect" relationship between contaminants and any observed biological effects. Information on suspected contaminants does however, provide valuable insights, if high concentrations of potentially toxic contaminants were reported in conjunction with biological effects.

Sediment samples for organic contaminants analysis were collected in conjunction with bioassay sediment samples. The

contaminants assayed are listed in Tables 3.6 and 3.7. Organic analytical procedures used were in accordance with USEPA methods 3550 and 8270 (USEPA, 1986) and are detailed in Hall et al. (1991). Samples were analyzed for organochlorine pesticides (OCP) as well as polychlorinated biphenyls (PCBs) in accordance with USEPA Methods 3550 and 8080 (Table 3.7). Atrazine was not analyzed in year 1, but was included in the sediment analysis for year 2. The methods for atrazine detection are the same as those listed above. Organic analysis was conducted at least once per site. The Wye River sediments from both samplings (Set 1 and Set 2) were analyzed for organic contaminants.

All sediment samples were analyzed for Acid Volatile Sulfides (AVS) and Total Organic Carbon (TOC). Samples were frozen until analysis, at which time they were thawed and homogenized by gently stirring. Sediment samples were analyzed in duplicate for AVS using the method of DiToro et al., (1990). Details of the analytical procedures for both AVS and TOC are described in Hall et al., 1991.

Pore water samples were removed from all sediment samples by squeezing with a nitrogen press. All pore water samples were filtered then frozen until analyses of ammonia, nitrite and sulfides were conducted. These analyses were conducted on all samples. Details of the methods are described in Hall et al., 1991.

All sediment samples were analyzed for the following bulk metals: aluminum, cadmium, chromium, copper, lead, nickel, tin and

Table 3.6 Semi-volatile organic compounds analyzed, utilizing user-created calibration library. Sediment method detection limits (MDL) are reported in µg/kg dry weight.

CAS NO.	COMPOUND	SEDIMENT MDL
65-53-3	Aniline	14.5
95-57-8	2-chlorophenol	13.2
111-44-4	Bis(2-chloroethyl)ether	11.2
108-95-2	Phenol	11.9
541-73-1	1,3-dichlorobenzene	11.9
106-46-7	1,4-dichlorobenzene	12.5
95-50-1	1,2-dichlorobenzene	11.9
100-51-6	Benzyl alcohol	27.1
39638-32-9	Bis(2-chloroisopropyl)ether	5.9
95-48-7	2-methylphenol	15.8
91-57-6	2-methylnaphthalene	9.2
67-72-1	Hexachloroethane	21.8
621-64-7	n-nitroso-di-n-propylamine	13.2
106-44-5	4-methylphenol	13.9
98-95-3	Nitrobenzene	11.2
78-59-1	Isophorone	6.6
88-75-7	2-nitrophenol	27.1
65-85-0	Benzoic acid	18.5
105-67-9	2,4-dimethylphenol	15.8
111-91-1	Bis(2-chloroethoxy)methane	9.9
120-83-2	2,4-dichlorophenol	21.8
120-82-1	1,2,4-trichlorobenzene	15.2
91-20-3	Naphthalene	4.6
106-47-8	4-chloroaniline	26.4
87-68-3	Hexachlorobutadiene	22.4
59-50-7	4-chloro-3-methylphenol	20.5
77-47-4	Hexachlorocyclopentadiene	25.7
88-06-2	2,4,6-trichlorophenol	37.0
95-95-4	2,4,5-trichlorophenol	44.9
88-74-4	2-nitroaniline	37.6
91-58-7	2-chloronaphthalene	9.9
208-96-8	Acenaphthalene	5.9
84-66-2	Dimethylphthalate	9.9
606-20-2	2,6-dinitrotoluene	48.2
99-09-2	3-nitroaniline	247
83-32-9	Acenaphthene	9.9
51-28-5	2,4-dinitrophenol	262
132-64-5	Dibenzofuran	7.9
100-02-7	4-nitrophenol	268
121-14-2	2,4-dinitrophenol	43.6
86-73-7	Fluorene	9.9
7005-72-3	4-chlorophenylphenylether	20.5
84-66-2	Diethylphthalate	9.9
100-01-6	4-nitroaniline	279
534-52-1	4,6,-dinitro-2-methylphenol	122

Table 3.6 (Continued)

CAS NO.	COMPOUND	SEDIMENT MDL
86-30-6	n-nitrosodiphenylamine	19.1
101-55-3	4-bromophenylphenylether	41.6
85-01-8	Phenanthrene	9.2
118-74-1	Hexachlorobenzene	37.6
87-86-5	Pentachlorophenol	136
120-12-7	Anthracene	9.9
84-74-2	Di-n-butylphthalate	5.9
206-44-0	Fluoranthene	10.6
129-00-0	Pyrene	10.6
85-68-7	Butylbenzylphthalate	17.8
56-55-3	Benzo(a)anthracene	17.8
218-01-9	Chrysene	14.5
91-94-1	3,3'-dichlorobenzidine	101
117-81-7	Bis(2-ethylhexyl)phthalate	12.5
117-84-0	Di-n-octylphthalate	7.3
205-99-2	Benzo(b)fluoranthene	13.9
207-08-9	Benzo(k)fluoranthene	13.9
50-32-8	Benzo(a)pyrene	15.2
193-39-5	Indeno(1,2,3-cd)pyrene	16.5
53-70-3	Dibenz(a,h)anthracene	17.8
191-24-2	Benzo(ghi)perylene	16.5
103-33-3	Azobenzene	7.3
92-87-5	Benzidine	24.4

Table 3.7 Method detection limits for organochlorine pesticides and PCBs. Detection limits for sediment are reported in µg/kg dry weight.

CAS NO.	COMPOUND	SEDIMENT MDL
391-84-6	α-BHC	0.714
301-85-7	β-BHC	0.559
391-86-8	δ-BHC	1.062
58-89-9	Lindane	0.616
76-44-8	Heptachlor	0.819
309-00-2	Aldrin	0.608
1024-57-3	Heptachlor epoxide	0.570
959-98-8	Endosulfan I	0.859
60-57-1	Dieldrin	0.898
72-55-9	4,4'-DDE	0.528
33213-65-9	Endosulfan II	0.745
72-20-8	Endrin	1.240
72-54-8	4,4'-DDD	0.469
1031-07-8	Endosulfan sulfate	1.500
50-29-3	4,4'-DDT	3.420
72-43-5	Methoxychlor	5.0
57-74-5	Chlordane	5.0
80001-35-2	Toxaphene	10.0
2385-85-5	Mirex	1.000
7421-93-4	Endrin aldehyde	2.410
12574-11-2	Aroclor 1016	16.6
11104-28-2	Aroclor 1221	16.6
11141-16-5	Aroclor 1232	16.6
53469-21-9	Aroclor 1242	16.6
12672-29-6	Aroclor 1248	16.6
11097-69-1	Aroclor 1254	16.6
11096-82-5	Aroclor 1260	16.6

zinc, using an inductively coupled plasma atomic emission spectrometer (ICP) following USEPA/SW-846, Method 6010 (see Hall et al., 1991). Detection limits are given in Table 4.18. Arsenic and selenium were predigested using EPA SW-846, Method 3050, and analyzed by hydride generation following Standard Methods for the Examination of Water and Wastewater (1989), while mercury was analyzed using EPA SW-846, Method 7471. In addition, a Simultaneously Extractable Metals (SEM) analysis was conducted on all samples to use with the AVS data 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 AVS method was detailed in Hall et al. 1991. The SEM sample was the sediment suspension remaining in the generation flask after the cold acid extraction was completed. The sediment suspension was filtered through a 0.2 micron membrane filter into a 250 ml volumetric flask. The sample was then diluted to volume with deionized water. The concentrations of the SEM were determined by EPA-600/4-79-020 Methods for Chemical Analysis of Water and Wastes. Cadmium, lead, copper, nickel, and zinc were determined by ICP following USEPA Method No. 200.7. Mercury was determined by cold vapor generation following USEPA Method No. 245.1. The concentrations were then converted to micromoles per gram dry sediment and were added together to provide a total SEM estimate.

3.4 Suborganismal Tests

3.4.1. General Description

Based on the experience from Year 1 testing, the scope of the Year 2 effort was refined to include a reduced subset of tasks which were consistent with data production rates of the other tasks for the pilot testing program. There were three broad goals for Year 2: (1) Continuation of the evaluation of Ethoxyresorufin-O-deethylase (EROD) activity in feral fish collected in proximity of the test stations; (2) Determination of the holding time necessary to reduce EROD activity in feral Fundulus heteroclitus collected from the Wye River station and from the St. John's Creek (Patuxent River tributary) reference population used for Year 1 exposures to test sediment and water; and (3) To determine differences (if any) in the induction of EROD activity in the two populations of Fundulus following exposure to a model EROD inducer (Benzo(a)pyrene).

3.4.2. Collection of Fish

Fundulus heteroclitus were collected from the Wye River and St. John's Creek (a tributary of the Patuxent River ca. 1 mile from Solomons, MD). Fish were collected using standard minnow traps. Collections of Wye River fish were conducted on August 8, 1991, while St. John's Creek fish were collected on July 25, 1991.

Spot, Leiostomus xanthurus, were collected from the vicinity of the Patapsco River Station via hook and line on August 15 and September 26, 1991. We surveyed the shoreline areas of Bear Creek up to the vicinity of the railway bridge looking for Fundulus and/or suitable Fundulus habitat. We set traps at locations we

felt may hold Fundulus, but were unable to collect any fish from any locations. We did not attempt to find fish from areas further upstream due to the lack of any proximity to the sampling station.

White perch, Morone americana, were collected from the Dahlgren and Morgantown, Potomac River stations on August 16 and September 27, 1991. Fish were collected via a standard beach seine. As in 1990, we seined widely along these beaches in an effort to collect Fundulus, but were unable to do so. We did not set minnow traps in these areas in 1991. As with the Patapsco River area, there was not suitable Fundulus habitat proximate to the sampling stations.

3.4.3 EROD Evaluations in Feral Fish

As fish were collected, they were placed in containers filled with site water and held until processing. During processing, livers from individual fish were removed and immediately frozen in liquid nitrogen. The remainder of the fish was packaged in whirl-pak bags and transported to the laboratory on ice for measurements of body size (wt), sex determination and evaluation of gonadosomatic indices (gonad wt./body weight).

In the laboratory, livers were thawed and microsomes were prepared using methods described by Stegeman et al. (1987). Briefly, these methods involve homogenizing the liver in buffer and using differential ultracentrifugation to isolate the microsomal (endoplasmic reticulum) fraction. Microsomes were then stored frozen in liquid nitrogen until biochemical assays were performed.

Ethoxyresorufin-O-deethylase (EROD) assays were conducted using the fluorometric technique described by Burke and Mayer (1974). This enzymatic activity is catalyzed by the cytochrome P450IA isoform in fishes (Stegeman, 1989) and is the activity induced by aromatic hydrocarbon exposure.

In Year Two, EROD evaluations were performed on Fundulus from the Wye River and St. John's Creek. EROD activity was also measured in white perch from the Dahlgren and Morgantown stations on the Potomac River. Finally, EROD was measured in spot from the Patapsco River station and from a population (presumed to be reference) sampled in the Patuxent River near Sheridan Point.

3.4.4 Holding Time for Fundulus to Reach Baseline EROD Levels

In Year 1 we discovered that Fundulus sampled from various locations had elevated EROD activity in comparison to fish that were used for sediment and water column exposures. The fish that had been used for the lab exposures were from a reference population of Fundulus that had been sampled from St. John's Creek in the Patuxent River and held in the lab for several months prior to their use in the sediment and water exposures. This holding time was used originally to allow for the possibility that these reference fish could in fact have elevated EROD activity caused by the presence of aromatic hydrocarbon inducing substances present, but undocumented, in the sampling area.

The results from Year 1, where the lab held fish had low EROD activity as compared to all freshly sampled fish (from the Wye

River as well as the more highly contaminated Elizabeth and Patapsco Rivers), indeed suggested that the lab holding time had reduced activity as compared to freshly sampled fish.

In order to more thoroughly document the decline in EROD activity following sampling, we sampled groups of Fundulus from the Wye River and from St. John's Creek. A sample of fish was dissected on the day of collection and livers were frozen and subsequently processed for EROD measurements. The rest of the fish were held in separate tanks at the Chesapeake Biological Laboratory in flowing ambient, unfiltered, Patuxent River water (temp = 28-31 C and salinity = 14-16 ppt). Fish were fed Tetramin® fish food flakes once a day.

Additional samplings of fish were conducted on weeks 1, 2 3, 10 (Wye River) and 12 (St. John's Creek) post collection. In all cases, approximately 12 fish were sacrificed and mircrosomes prepared from pooled (same sex) liver samples from 2-3 fish. Samples sizes (n) varied from 4 to 7. Though additional discussion will be found below, it is important to note that these experiments were offset by a two week period and thus the actual dates which correspond to the sampling weeks are not the same. Wye River fish experiments were initiated on August 8, 1991, while St. John's Creek experiments were initiated on July 25, 1991 (two weeks earlier).

3.4.5 Evaluation of EROD Induction in Fundulus from the Wye River Versus St. John's Creek.

Fundulus from the two separate populations were held throu the winter in flowing ambient Patuxent River water. Approximately one month prior to the initiation of the induction experiments, fish were slowly acclimated to flowing, filtered, 20C, Patuxent River water (salinity = 14-15 ppt)..

To test whether the two different populations would respond similarly to a known aromatic hydrocarbon inducer, fish were injected intraperitoneally with corn oil solutions of benzo(a)pyrene (>99% pure, Aldrich). Fish were given 2 ul injections/g fish, equivalent to doses of 0, 2 and 10 mg B(a)P/kg. Fish were held following treatment in 38 liter glass aquaria with recirculating, charcoal filtered, Patuxent River water (temp = 21 C and salinity = 14-15 ppt). Ten fish were injected in each treatment group. Liver samples were pooled (by sex) at sacrifice to yield 5 samples per treatment group (i.e., n = 5 for all groups). Fish were sacrificed 96 hours following treatment. This time has been shown in previous investigations (Kloepper-Sams, 1989) to produce strong induction (6-fold) in Fundulus heteroclitus. Fish were sacrificed and livers removed and frozen in liquid nitrogen in the same fashion as field samples.

3.4.6 Supplemental Studies on Copper Concentrations in Livers of White Perch from the Potomac River

As described subjectively in the Year 1 report, we observed that the liver in many of the white perch sampled from the Potomac River were exceptionally dark in color (like an old penny). We

suggested that this observation was consistent with previous studies on white perch which have described a propensity for white perch to store copper at high levels in their livers (Bunton et al., 1987; Bunton and Baksi, 1988).

In order to corroborate these findings, we subsampled livers from a selection of white perch from the Dahlgren and Morgantown stations. Samples of liver were preserved in formalin in the field. During sampling, we made no formal attempt at random sampling. In fact, we purposely preserved some livers which appeared to be less affected (i.e., not dark brown) in order to get a range of concentrations.

Small pieces of liver were digested using concentrated nitric acid and hydrogen peroxide. This method is essentially that described by Roesijadi (1980) with the addition of hydrogen peroxide for final digestion and color removal. Levels of copper in the digestates were determined by flame atomic absorption spectrophotometry using external standards. Formalin buffer blanks were also analyzed. All concentrations are reported as wet weight values.

SECTION 4

RESULTS

4.1 Water Column Tests

The following results from water column tests are presented below: toxicity data, contaminants data, water quality data and toxicity data from reference toxicant tests.

4.1.1 Toxicity Data

Survival, growth and reproduction data from the four estuarine tests conducted from 8/14/91 to 8/21/91 in the Wye River, Patapsco River, Potomac River - Morgantown and Potomac River - Dahlgren are presented in Tables 4.1 and 4.2. Survival and reproduction data from the Eurytemora tests did not suggest the presence of toxic conditions (statistical difference between control and tes condition) at any of the ambient stations although percent survival at the Wye River station was 23% lower than the controls. Mean brood size from the controls (ground water with sea salt) was significantly lower than all other stations except the Patapsco River. We encountered this problem periodically during the 1990 studies and attributed the low reproduction in the controls to reduced levels of phytoplankton and nutrients. Both of these constituents are found naturally in the ambient water in addition to the concentrations added during feeding. During the second test discussed later in this section, we used another source of control water from a pristine area in the Indian River in Delaware in

Table 4.1 Survival data from E. affinis, sheepshead minnow larvae, grass shrimp and mysid shrimp 8-d tests conducted in the Wye River, Patapsco River, Potomac River-Morgantown and Potomac River-Dahlgren from 8/13/91 - 8/21/91.

Species	Station	Cumulative % Survival Per Day							
		1	2	3	4	5	6	7	8
Eurytemora	Control								90
	Wye								67
	Patapsco								97
	Morgantown								87
	Dahlgren								83
Sheepshead minnows	Control	100	100	100	100	100	100	100	100
	Wye**	38	35	33	33	33	33	30	25*
	Patapsco	100	100	100	100	100	100	100	100
	Morgantown	100	98	98	98	98	98	95	88
	Dahlgren	100	100	100	100	100	100	100	100
Grass shrimp	Control	100	100	100	98	93	78	68	60
	Wye	100	100	90	88	88	88	85	85
	Patapsco	100	100	100	98	95	93	88	85
	Morgantown	100	100	100	100	95	83	75	70
	Dahlgren	100	98	93	93	90	85	78	78
Mysid shrimp	Control	96	93	93	89	89	85	85	82
	Wye	95	90	90	90	88	88	88	88
	Patapsco	100	100	93	85	85	85	85	85
	Morgantown	93	86	86	86	86	83	83	83
	Dahlgren	88	80	80	80	80	76	76	76

* Indicates value is significantly different than the control, $p < 0.05$. Kruskall-Wallis Rank Test.

** A second set of beakers were set up with 10 larvae each with water collected on 8-14-91. Survival was >95% after 8 d.

Table 4.2 Reproduction (brood size) and growth data from E. affinis, grass shrimp larvae and sheepshead minr larvae 8-d tests conducted at the four stations fr 8/14/91 - 8/21/91.

E.affinis brood size comparisions following 8-d exposures

Station	n	Mean Nauplii Produced	S.E.
Control	5	2.6	2.4
Wye	5	51.8*	12.7
Patapsco	5	27.4	7.2
Morgantown	5	47.8*	13.7
Dahlgren	5	54.2*	10.3

* Indicates value is significantly different than the controls using a Dunnett's Test, $p<.05$.

Grass shrimp and Sheepshead minnow growth comparisons

Grass Shrimp Dry Weight (Mean weight at day 0 = 0.45 ± 0.01 mg)

Station	n	Mean (mg)	S.E.
Control	20	0.70	0.02
Wye	20	0.82	0.05
Patapsco	20	0.74	0.03
Morgantown	20	0.81	0.03
Dahlgren	20	0.76	0.04

Sheepshead Larvae Dry Weight (Mean weight at day 0 = 0.20 ± 0.03 mg)

Station	n	Mean (mg)	S.E.
Control	25	0.82	0.07
Wye	10**	0.91	0.13
Patapsco	25	0.81	0.04
Morgantown	25	0.55*	0.06
Dahlgren	25	0.51*	0.05

* Indicates value is significantly different than control, Dunnett's Test or Bonferroni's T-test, $p<0.05$.
** Larvae from the 8/13/91 test were used.

addition to our initial control water. Brood size of this copepod increased significantly in the Indian River control water. The percent of females carrying eggs after 8-d exposures in the E. affinis tests are presented in Appendix A. There were no statistical differences between the controls and any test condition.

Survival of sheepshead minnow larvae in the Wye River was significantly lower (25% after 8 days) when compared with the controls or other stations. Most of the mortality occurred within 24 h thus indicating a toxic condition in water collected on 8-13-91. A second set of beakers with 10 larvae each was set up using Wye River water collected one day later (8-14-91) to determine if the toxic conditions were persistent. Survival was greater than 95% in these two beakers after 8 day exposures. The growth of surviving larvae at the Wye Station (8-13-91) was not lower than the controls or other test conditions. Growth of sheepshead minnow larvae at both the Morgantown and Dahlgren stations was significantly lower than the controls or the other stations.

Survival and growth of grass shrimp larvae and survival of mysids was not significantly reduced in ambient water from any station when compared with the controls. Survival for grass shrimp in the controls was lower than the ambient water conditions. Due to problems with different age mysids, we could not conduct growth and reproduction evaluations with this test species during the first experiment (see Section 3.2.2.1).

Survival, growth and reproduction data from the second set of

experiments conducted from 9/24/91 to 10/2/91 are presented Tables 4.3 and 4.4. A significant reduction in survival was reported for Eurytemora exposed to Wye River water when compared with the H-W controls. Survival of Eurytemora at the Patapsco River station was also 36% lower than controls; however, this difference was not reported to be significant. There was no statistical difference between the number of egg carrying females after 8 d of exposure in the controls (H-W and I-R controls) when compared with any test condition (Appendix A).

Survival and growth of both sheepshead minnow larvae and grass shrimp larvae were not significantly lower in any of the ambient test conditions when compared with the controls. Mysid shrimp survival was not significantly reduced at any of the ambient conditions when compared with the controls. However, growth of mysids in both the Wye and Patapsco River stations was significantly increased when compared with the controls. The reproductive endpoint used for the mysid shrimp test was the proportion of females without eggs in the control compared with the test conditions. All surviving females had eggs; therefore, we detected no effects using this endpoint. In recent years, various investigators have used the proportion of immature mysids as an endpoint to identify stressful conditions (Susan Lussier, personal communication, U.S. Environmental Protection Agency; Anwar et al., in press). Using this endpoint, we reported a significant reduction in the proportion of immature mysids (and increase in mature mysids) at the Patapsco River station when compared with the

Table 4.3 Survival data from E. affinis, sheepshead minnow larvae, grass shrimp and mysid shrimp 8-d tests conducted in the Wye River, Patapsco River, Potomac River-Morgantown and Potomac River-Dahlgren from 9/24/91 - 10/2/91. E. affinis control water was H-W (groundwater and seasalt) and I-R (Indian River, Delaware).

Species	Station	Cumlative % Survival Per Day							
		1	2	3	4	5	6	7	8
Eurytemora	H-W Control								93
	I-R Control								90
	Wye								32*
	Patapsco								57
	Morgantown								96
	Dahlgren								93
Sheepshead larvae	Control	100	100	100	100	100	100	100	98
	Wye	100	100	100	100	100	100	100	100
	Patapsco	100	100	100	100	100	100	100	93
	Morgantown	98	98	98	95	95	95	95	95
	Dahlgren	100	100	100	100	100	100	100	100
Grass shrimp	Control	100	100	100	100	98	93	90	88
	Wye	100	100	98	95	95	95	95	95
	Patapsco	100	98	98	98	98	85	85	83
	Morgantown	100	100	100	100	100	98	98	95
	Dahlgren	85	85	85	85	85	85	83	83
Mysid shrimp	Control	100	100	100	100	100	100	100	98
	Wye	100	100	100	100	100	100	100	100
	Patapsco	100	100	100	98	98	98	98	98
	Morgantown	100	100	100	100	100	100	100	100
	Dahlgren	100	98	98	98	98	98	98	98

* Indicates value is significantly different than the H-W control, Bonferroni T-Test, $p<0.05$. For the I-R control, p is not exactly <0.05 as the T-statistic = 2.67 and the critical value is 2.69. Therefore, p is just slightly greater than 0.05.

Table 4.4 Reproduction and growth data from E. affinis, grass shrimp larvae, sheepshead minnow larvae and mysid shrimp 8-d tests conducted at the four stations from 9/24/91 - 10/2/91. E. affinis control water was H-W (groundwater plus sea salts) and I-R (Indian River, Delaware).

E.affinis brood size comparisions following 8-d exposures

Station	n	Mean Nauplii Produced	S.E.
H-W Control	5	36.6	12.3
I-R Control	5	63.6	6.8
Wye	4	61.3	1.1
Patapsco	4	38.3	15.7
Morgantown	5	63.4	8.6
Dahlgren	5	77.6	9.8

Comparisons were made with H-W control and I-R control and no signnificant differences were reported using the Bonferroni T-test ($p<0.05$).

Grass Shrimp Dry Weight

Station	n	Mean (mg)	S.E.
Control	25	1.05	0.10
Wye	25	1.08	0.11
Patapsco	25	1.08	0.08
Morgantown	25	1.24	0.06
Dahlgren	25	1.28	0.09

Sheepshead Larvae Dry Weight

Station	n	Mean (mg)	S.E.
Control	23	1.25	0.17
Wye	20	1.68	0.22
Patapsco	24	1.16	0.08
Morgantown	25	1.20	0.06
Dahlgren	21	1.49	0.09

Mysid Shrimp Dry Weight

Control	25	0.23	0.01
Wye	20	0.32*	0.01
Patapsco	20	0.30*	0.01
Morgantown	20	0.26	0.01
Dahlgren	20	0.25	0.02

* Indicates value is significantly different than control, Dunnetts Test, $p<0.05$.

controls or other ambient conditions (Table 4.5). This result does not necessarily mean that an increase in the proportion of mature mysids suggests an adverse condition was present but it does reflect a difference from the control that is defined as normal.

4.1.2 Contaminants Data

Inorganic contaminants (trace metals) data from the four stations for both test 1 and 2 are presented in Table 4.6. Concentrations exceeding U. S. EPA marine water quality criteria are underlined in the table (U. S. EPA, 1987a). Total filtered aluminum concentrations were detected at all stations but the reported values were likely non-toxic. Arsenic, cadmium and chromium were below marine water quality criteria at all stations. Arsenic was not detected at any of the stations while cadmium was detected at all stations except the Wye River. Chromium concentrations were detected at all stations during at least one of the tests and this metal was detected during both tests at the Wye River (reference site). Copper concentrations exceeded marine water quality criteria at all stations during at least one test.

Lead was not detected at any of the stations except the Patapsco River during test 2 (2.1 ug/L). Concentrations of mercury were not detected at any of the stations during testing. Nickel concentrations of 9.3 ug/L exceeded chronic water quality criteria during the first test at the Wye River station. Concentrations of this trace metal were only 0.1 of a ug/L below the water quality criteria at the Patapsco Station during the second test. Selenium,

Table 4.5 Proportion of immature mysid shrimp after 8 d of exposure to ambient water from the four stations.

Site	n	Proportion of Immature	S.E.
Control	8	0.23	0.08
Wye	8	0.05	0.03
Patapsco	8	0.03*	0.03
Morgantown	8	0.20	0.05
Dahlgren	8	0.35	0.05

* Indicates value is significantly different than control, Dunnetts Test, $p<0.05$.

Table 4.6 Inorganic contaminants data for test 1 (8/14/91 to 8/21/91) and test 2 (9/24/91 to 10/2/91) for the following four stations, Dahlgren (DG), Morgantown (MT), Wye River and Patapsco River (PA). All underlined values exceed U.S. EPA chronic marine water quality criteria (WQC), (U.S. EPA, 1987a).

		Stations and Test							
Metals (ug/L)	WQC	DG-1	DG-2	MT-1	MT-2	WYE-1	WYE-2	PA-1	PA-2
Al	-	23	13.2 10.4	34.5	7.8 18.9	44.7 18.0	30.4 17.1	12.5	18.2 32.7
As	-	ND	ND ND	ND	ND ND	ND ND	ND ND	ND	ND ND
Cd	9.3	.68	1.8 .71	.82	.59 1.0	ND ND	ND ND	ND	.62 1.4
Cr	50	3.1	ND ND	8.2	ND ND	7.3 3.3	3.1 3.4	ND	ND 3.9
Cu	2.9	3.4	4.5 3.7	5.5	ND 4.2	ND 2.9	ND 2.6	3.7	3.7 2.7
Pb	5.6	ND	ND ND	ND	ND ND	ND ND	ND ND	ND	ND 2.1
Hg	0.025	ND	ND ND	ND	ND ND	ND ND	ND ND	ND	ND ND
Ni	8.3	ND	ND ND	7.4	ND ND	9.3 ND	ND ND	5.2	8.2 6.5
Se	54	ND	ND ND	ND	ND ND	ND ND	ND ND	ND	ND ND

Table 4.6 continued

Metals (ug/L)	WQC	Stations and Test							
		DG-1	DG-2	MT-1	MT-2	WYE-1	WYE-2	PA-1	PA-2
Sn	-	ND	ND ND	ND	ND ND	ND ND	ND ND	ND	ND ND
Zn	86	ND	ND ND	ND	ND ND	ND ND	ND ND	ND	ND ND

tin and zinc were below detection limits at all stations during both tests.

None of the organic contaminants listed in Table 3.1 were measured above detection limits in any of the samples collected from the four stations during either test. A minimum of one sample was analyzed from each station for each test.

4.1.3 Water Quality Data

Water quality parameters reported from grab samples collected during both tests are presented in Table 4.7. The temperature and salinity of all ambient water collected from all stations was adjusted to 25 C and 15 ppt before testing. Ambient water quality conditions appeared adequate for survival of test species with the possible exception of dissolved oxygen concentrations of 4.0 mg/L at the Morgantown station on 8/13/91. Water quality conditions reported in test containers during testing are reported in Appendix B. All parameters appeared adequate for survival of test species.

4.1.4 Reference Toxicant Data

Twenty-four and 48-h LC50 values for the test species exposed to cadmium chloride during reference toxicant tests are presented in Table 4.8. These LC50 values were compared with mean 48-h values for cadmium chloride collected during the first year of the study. Mysids were not tested in the first year. Forty-eight h LC50s for three of the test species in the present study were generally similar to previous values. These data, in conjunction

Table 4.7 Water quality parameters reported in the field durir collection of water samples from both tests during 199 Stations were Patapsco River (PR), Wye River (WR), Dahlgren (DG) and Morgantown (MT).

Date	Station	Temp (C)	Salinity (ppt)	Cond umhos/cm	DO (ppm)	pH
8/13	PR	26.0	9.9	16900	8.8	8.44
	WR	29.0	10.0	17000	10.0	8.60
	DG	26.0	10.0	16000	5.2	7.60
	MT	27.0	10.0	16500	4.0	7.40
8/16	PR	25.0	8.5	13900	5.7	8.15
	WR	27.3	10.6	18900	6.5	8.01
	DG	26.0	10.0	16500	5.8	7.48
	MT	26.5	9.5	16000	5.2	7.66
8/19	PR	26.0	9.8	17000	6.5	8.06
	WR	27.0	11.0	19000	5.7	7.86
	DG	27.0	10.5	17000	6.4	7.56
	MT	27.0	11.0	18000	5.4	7.77
9/24	PR	20.8	11.2	17100	7.6	7.78
	WR	21.6	12.2	19000	7.5	7.9
	DG	22.0	11.0	15500	7.6	7.8
	MT	23.0	11.5	17000	7.2	7.74
9/27	PR	20.0	9.7	15000	8.9	8.29
	WR	21.0	11.3	18100	11.5	8.30
	DG	20.0	10.0	14500	6.8	7.85
	MT	19.0	11.0	16100	6.2	7.79
9/30	PR	18.2	11.2	16100	10.0	8.27
	WR	19.1	12.3	18000	10.9	8.41
	DG	19.5	11.0	16000	7.2	7.91
	MT	21.0	11.0	16500	6.4	7.96

Table 4.8 Toxicity data (LC50s mg/L) from reference toxicant tests conducted with cadmium chloride for the four test species. Two reference toxicant tests were conducted with mysid shrimp because this species was obtained from two different sources. The mean 48-h values from Year 1 were included.

Date	Organism	24 h	48 h	48-h Values-Year 1
9/20/91	Grass Shrimp	0.932	0.231	0.502
9/12/91	Sheepshead Larvae		1.554	0.510
8/30/91	Eurytemora		0.095	0.021
9/12/91	Mysids[1]	0.122	0.009	-
10/22/91	Mysids[2]	0.026	0.005	-

[1] Mysids were obtained from S.P., Inc. (Salem, MA).

[2] Mysids were obtained from Chesapeake Cultures (Haynes, VA).

with acceptable control survival, demonstrate that these species were healthy and test results were valid. The 48-h LC50 values f mysids (0.005 and 0.009 ug/L) were lower than a 3 year mean value of 0.077 ug/L obtained from an effluent toxicity testing program conducted by our laboratory. These lower LC50 values may be related to the test water used. Our tests were conducted in reconstituted water with sea salts. The 3 year mean value from the effluent testing program was obtained using natural seawater where cadmium was likely less bioavailable. Survival and growth data with mysids indicated the test species were healthy.

Two reference toxicant tests were conducted with mysids because the sources of these test species were different. Both 48-h values were similar (0.009 and 0.005 mg/L).

4.2 Sediment Tests

The following results from sediment toxicity tests are presented below: toxicity data, contaminants data, and data from reference toxicant tests. A summary of the water quality parameters monitored daily in each treatment, and the range of each parameter is included in Appendix C.

4.2.1 Toxicity Data

Survival results from toxicity tests of the four estuarine sediments from the Wye, Patapsco, and Potomac River (Morgantown and Dahlgren) with grass shrimp, amphipods and worms are included in Tables 4.9 and 4.10. Those stations that were significantly

Table 4.9 Cumulative survival data from grass shrimp, amphipod and worm tests conducted for the four stations during Set 1 experiments. Tests were conducted from August 16, 1991 to September 5, 1991. (LB = Lynnhaven Beach; LI = Lynnhaven Inlet).

Species	Site[1]	% Survival		
		Day 10		Day 20
Grass shrimp	Control (LB)	99		99
	Morgantown	99		97
	Dahlgren	100		99
	Patapsco River	100		97
	Wye River	100		97
	Reference (LI)	100		97
Amphipod (*Lepidactylus*)	Control (LB)	96		72
	Morgantown	92		66
	Dahlgren	93		57
	Patapsco River	71*		32*
	Wye River	92		77
	Reference (LI)	91		63
Amphipod (*Hyalella*)	Control (LI)	92		76
	Morgantown	51*		37*
	Dahlgren	64*		18*
	Patapsco River	20*		2*
	Wye River	72		54
	Reference (LB)	90		73
			Adjusted[1]	
Worm	Control (LI)	99	100	90
	Morgantown	80*	92	55*
	Dahlgren	68*	82*	61*
	Patapsco River	80*	92	77
	Wye River	53*	67*	47*
	Reference (LB)	70*	91	17*

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

Notes:

1 Adjusted worm survival is percent survival adjusted for predicted particle size effects.

Table 4.10 Cumulative survival data from grass shrimp, amphipod and worm tests conducted for the four stations duri Set 2 experiments. Tests were conducted from Septem 27, 1991 to October 17, 1991. (LB = Lynnhaven Beach; LI = Lynnhaven Inlet).

Species	Site[1]	% Survival	
		Day 10	Day 20
Grass shrimp	Control (LB)	98	97
	Morgantown	96	93
	Dahlgren	97	96
	Patapsco River	98	98
	Wye River	100	97
	Reference (LI)	100	98
Amphipod (*Lepidactylus*)	Control (LB)	95	69
	Morgantown	84	50*
	Dahlgren	63*	22*
	Patapsco River	46*	8*
	Wye River	89	80
	Reference (LI)	95	68
Amphipod (*Hyalella*)	Control (LI)	86	62
	Morgantown	72	49
	Dahlgren	49*	36*
	Patapsco River	9*	2*
	Wye River	75	62
	Reference (LB)	53*	15*
Worm	Control (63%s)	98	87
	Morgantown	89	89
	Dahlgren	93	93
	Patapsco River	80	70
	Wye River	96	91
	Reference (100%s)	78*	72*

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

different (α=0.05) from the controls are indicated by an asterisk. Growth data (mean length and dry weight) for grass shrimp, amphipods and worms after 20-day exposure to sediments are included in Tables 4.11 and 4.12. Any length measurements significantly greater than the initial length are indicated by a + mark. The weight measurements were not statistically compared to the initial values, because the initial weight consisted of 40 organisms grouped on one weigh pan to obtain an average weight per organism. Amphipod reburial data are shown in Table 4.13. Emergence data for both amphipods, Hyalella azteca and Lepidactylus dytiscus, are presented in Figures 4.1 - 4.4. In Figures 4.1 - 4.4, the predicted number alive was determined by linear interpolation based upon the number of live individuals on day 0, 10 and 20.

Set 1:

Survival and growth data from toxicity tests conducted August 16, 1991 through September 5, 1991 on the first set of sediments from the four stations are presented in Tables 4.9 and 4.11. Survival and growth data from the grass shrimp test did not suggest the presence of toxic conditions (statistical difference between control and test conditions) in any of the test sediments, and yielded greater than 97% survival in sediment from all sites. Growth of animals in the Dahlgren, Wye River and Reference sediments was significantly different from the initial organisms. The shrimp in the controls, Morgantown and Patapsco increased in size over the test period, but the average increases were not

Table 4.11 Growth data (dry weight and length) for grass shrimp, amphipods and worms after 20-day exposure to Set 1 sediments. Initial weight and length represent the average of 40 animals for each species at the start of the test. Data for each replicate are the mean of the surviving animals. Tests were conducted August 16 through September 5, 1991 (LB = Lynnhaven Beach: LI = Lynnhaven Inlet).

Site	# of True Replicates	Weight (mg)	S.E.	Length (mm)	S.E.
Grass shrimp:					
Initial	NA	14.47	0.00	23.10	0.45
Control(LB)	5	20.18	0.73	24.78	0.23
Morgantown	5	19.27	0.96	24.16	0.36
Dahlgren	5	23.16	1.01	25.72+	0.48
Patapsco River	5	21.96	1.67	25.02	0.52
Wye River	5	23.09	0.96	25.47+	0.24
Reference (LI)	5	23.19	1.07	26.15+	0.48
Amphipod (*Lepidactylus*):					
Initial	NA	0.95	0.00	6.96	0.19
Control (LB)	5	0.97	0.09	7.41	0.29
Morgantown	5	1.00	0.05	7.77	0.40
Dahlgren	5	1.05	0.05	7.78	0.13
Patapsco River	5	1.01	0.03	7.78	0.31
Wye River	5	1.03	0.03	7.84	0.20
Reference (LI)	5	0.90	0.03	7.70	0.06
Amphipod (*Hyalella*):					
Initial	NA	0.14	0.00	3.35	0.41
Control (LI)	5	0.16	0.07	5.10+	0.07
Morgantown	5	0.14	0.01	4.75+	0.24
Dahlgren	5	0.14	0.01	4.95+	0.07
Patapsco River	5	0.16	0.00	4.90+	0.64
Wye River	5	0.17	0.01	4.71+	0.05
Reference (LB)	5	0.07	0.02	2.80	0.06
Worm:					
Initial	NA	0.014	0.000	2.92	0.12
Control(LI)	5	0.05	0.010	7.63+	0.32
Morgantown	5	0.035*	0.005	5.04*+	0.23
Dahlgren	5	0.032*	0.003	5.40*+	0.25
Patapsco River	5	0.056	0.006	6.02*+	0.12
Wye River	5	0.041*	0.008	5.53*+	0.17
Reference (LB)	5	0.047*	0.004	3.84*+	0.15

* Significantly less than the controls ($p<0.05$).
\+ Lengths significantly greater than the initial ($p< 0.05$).

Table 4.12 Growth data (dry weight and length) for grass shrimp, amphipods and worms after 20-day exposure to Set 2 sediments. Initial weight and length represent the average of 40 animals for each species at the start of the test. Data for each replicate are the mean of the surviving animals. Tests were conducted September 27 through October 17, 1991 (LB = Lynnhaven Beach; LI = Lynnhaven Inlet).

Site	# of True Replicates	Weight (mg)	S.E.	Length (mm)	S.E.
Grass shrimp:					
Initial	NA	14.45	0.00	-	-
Control (LB)	5	12.33	1.00	20.20	0.55
Morgantown	5	13.18	0.69	19.96	0.34
Dahlgren	5	13.99	0.72	20.29	0.30
Patapsco River	5	12.98	0.56	18.77	0.28
Wye River	5	14.51	0.72	20.90	0.34
Reference (LI)	5	12.89	0.26	20.45	0.12
Amphipod (Lepidactylus):					
Initial	NA	0.84	0.00	6.57	0.15
Control(LB)	5	1.02	0.09	8.13+	0.23
Morgantown	5	1.00	0.06	7.53+	0.24
Dahlgren	5	1.05	0.16	7.12*	0.42
Patapsco River	5	1.15	0.09	6.90*	0.28
Wye River	5	0.93	0.05	7.98+	0.16
Reference (LI)	5	0.96	0.07	8.06+	0.14
Amphipod (Hyalella):					
Initial	NA	0.02	0.00	2.73	0.06
Control (LI)	5	0.12	0.02	4.20+	0.24
Morgantown	5	0.12	0.01	4.16+	0.20
Dahlgren	5	0.14	0.04	3.80+	0.14
Patapsco River	5	0.13	0.10	3.21*+	0.26
Wye River	5	0.13	0.06	4.48+	0.08
Reference (LB)	5	0.07	0.02	2.80*	0.06
Worm:					
Initial	NA	0.03	0.00	5.53	0.25
Control (LI)	5	0.04	0.00	4.35	0.15
Morgantown	5	0.07	0.01	6.45	0.51
Dahlgren	5	0.10	0.01	7.74+	0.14
Patapsco River	5	0.05	0.01	5.24	0.14
Wye River	5	0.09	0.01	6.98+	0.34
Reference (LB)	5	0.07	0.02	4.22	0.06

* Significantly less than the controls ($p<0.05$).
\+ Lengths significantly greater than the initial ($p< 0.05$).

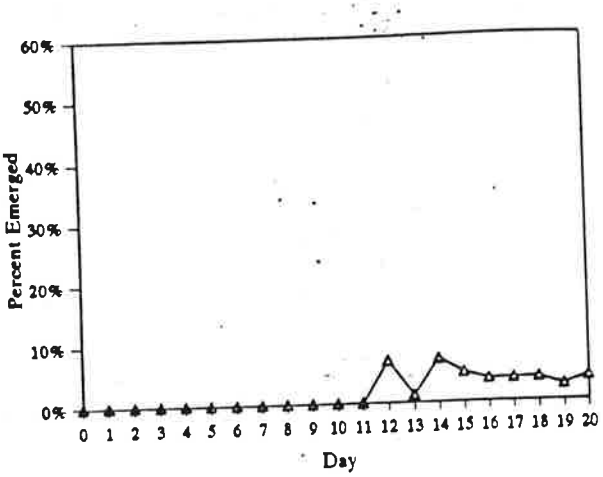
Table 4.13 Amphipod (Lepidactylus dytiscus) reburial data afte 10 day exposure to sediments. Table shows percent surviving animals able to rebury within one hour (LB = Lynnhaven Beach; LI = Lynnhaven Inlet).

Site	% Reburial	S.E.
Set 1:		
Control (LB)	100	0.0
Morgantown	99.0	1.0
Dahlgren	99.0	1.0
Patapsco River	89.7*	2.8
Wye River	100	0.0
Reference (LI)	100	0.0
Set 2:		
Control (LB)	100	0.0
Morgantown	98.7	1.3
Dahlgren	98.0	2.0
Patapsco River	82.9*	5.5
Wye River	100	0.0
Reference (LI)	99.0	1.0

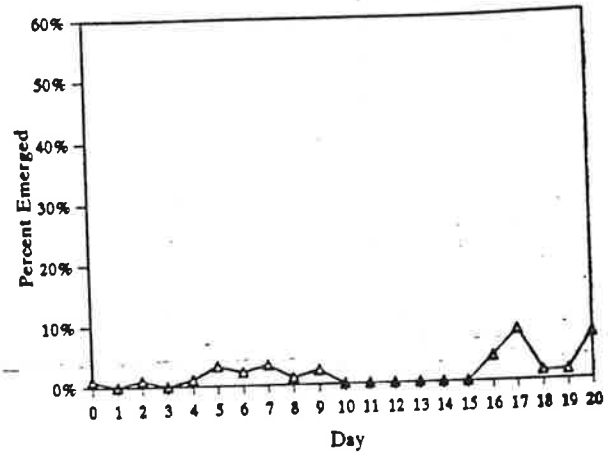
* Significantly less than control ($p<0.05$).

Fig. 4.1 Emergence of L. dytiscus Set 1 (Aug. '91). Number emerged expressed as a percent, corrected for predicted no. alive at each day.

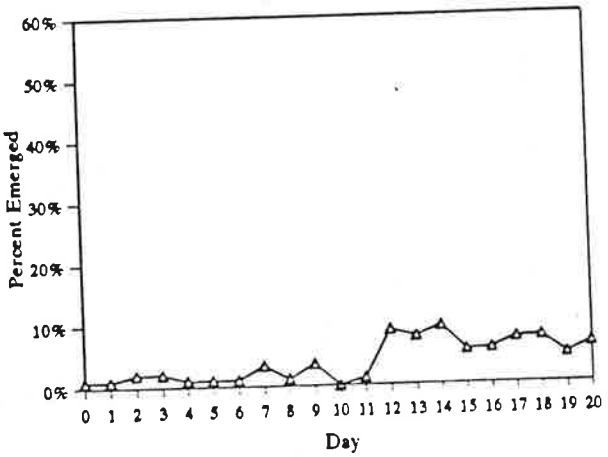
Control



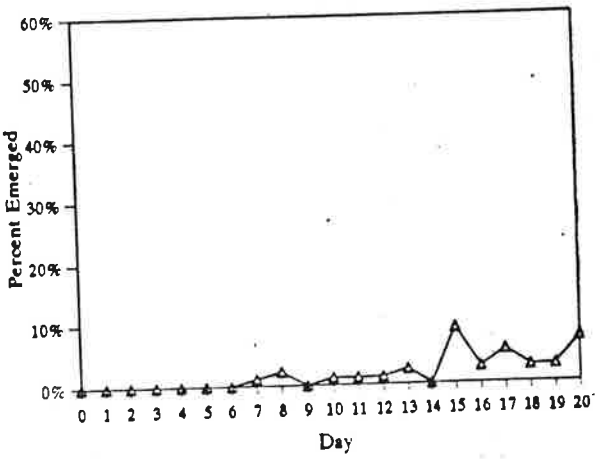
Reference



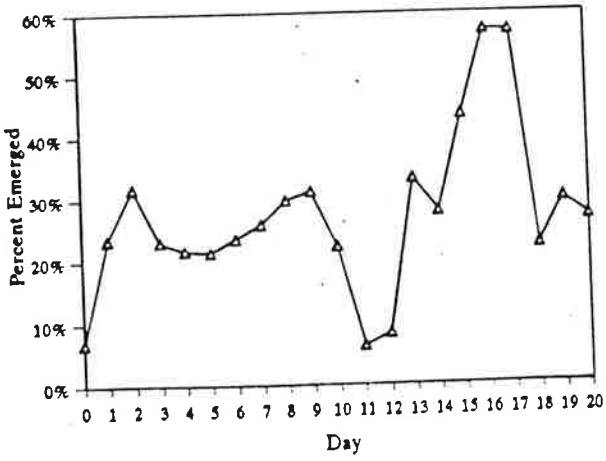
Dahlgren



Morgantown



Patapsco



Wye River

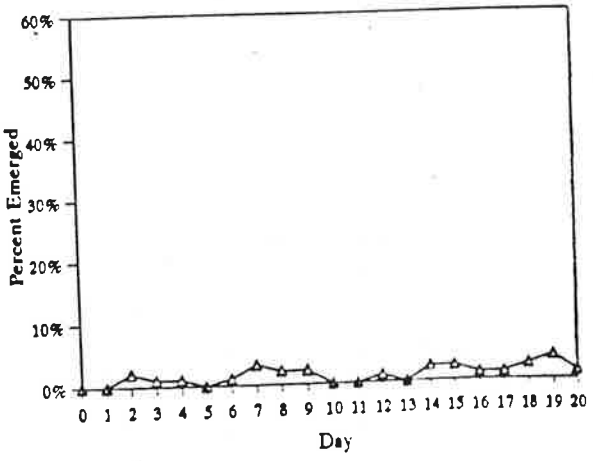
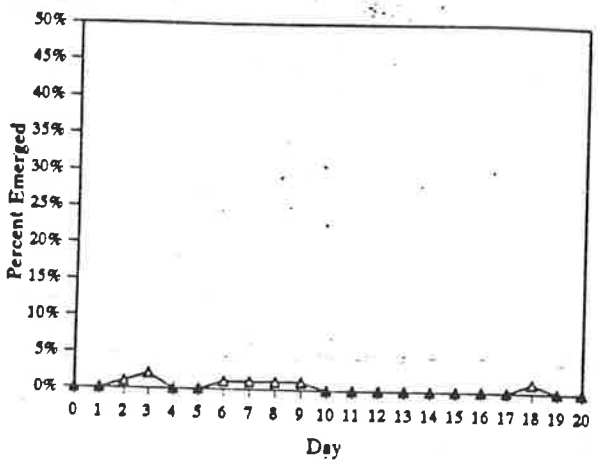
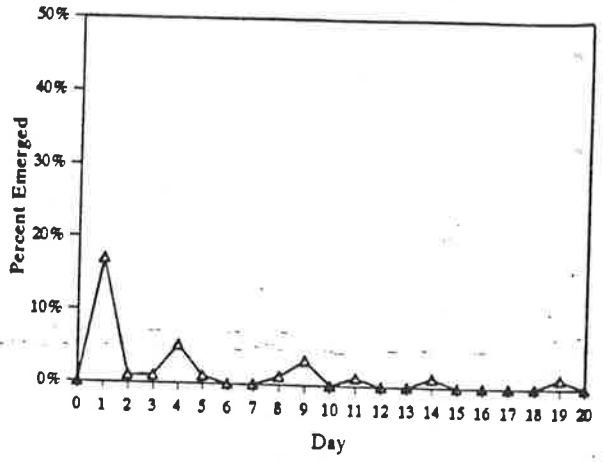


Fig. 4.2 Emergence of H. azteca Set 1 (Aug. '91). Number emerged expressed as a percent, corrected for predicted no. alive at each day.

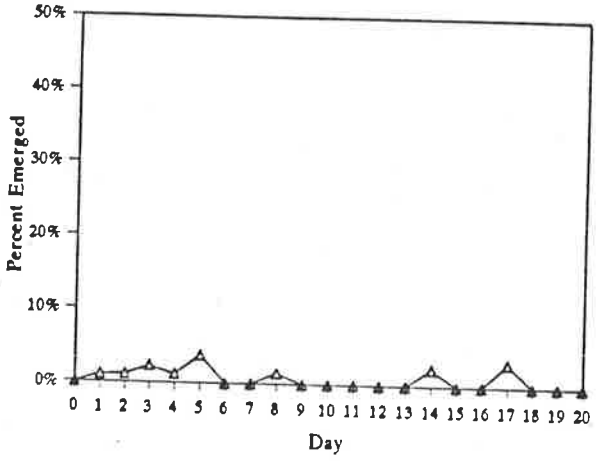
Control



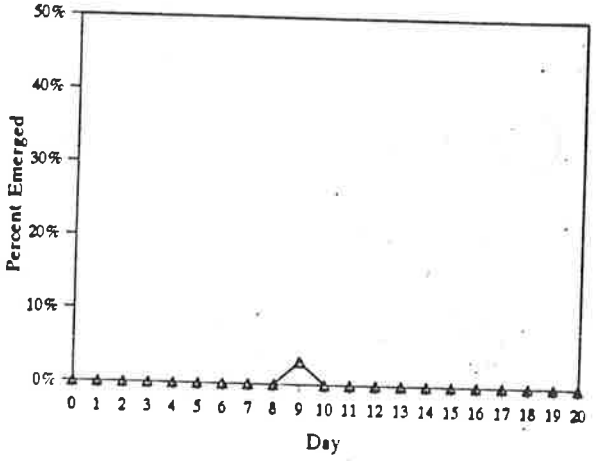
Reference



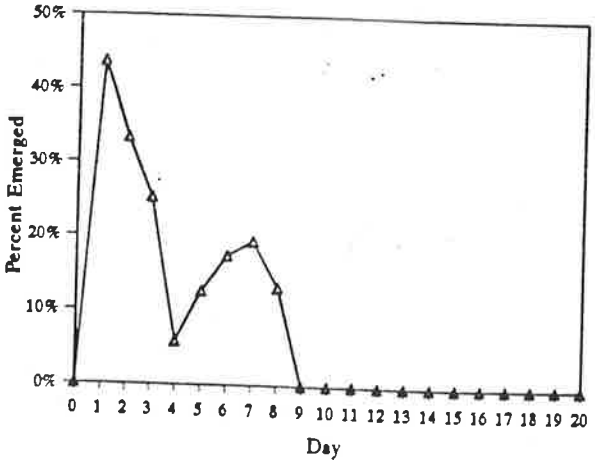
Dahlgren



Morgantown



Patapsco



Wye River

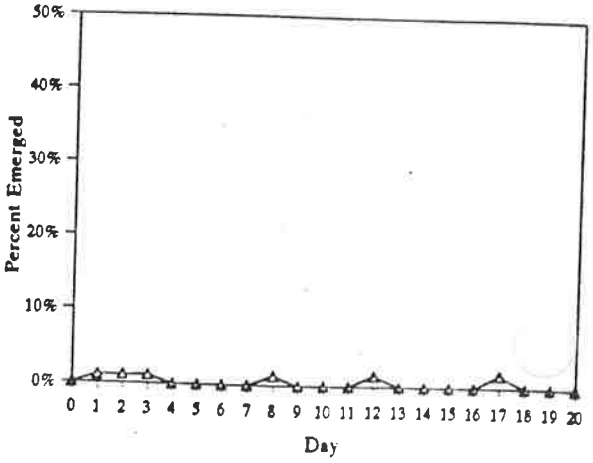


Fig. 4.3 Emergence of L. dytiscus Set 2 (Sep. '91). Number emerged expressed as a percent, corrected for predicted no. alive at each day.

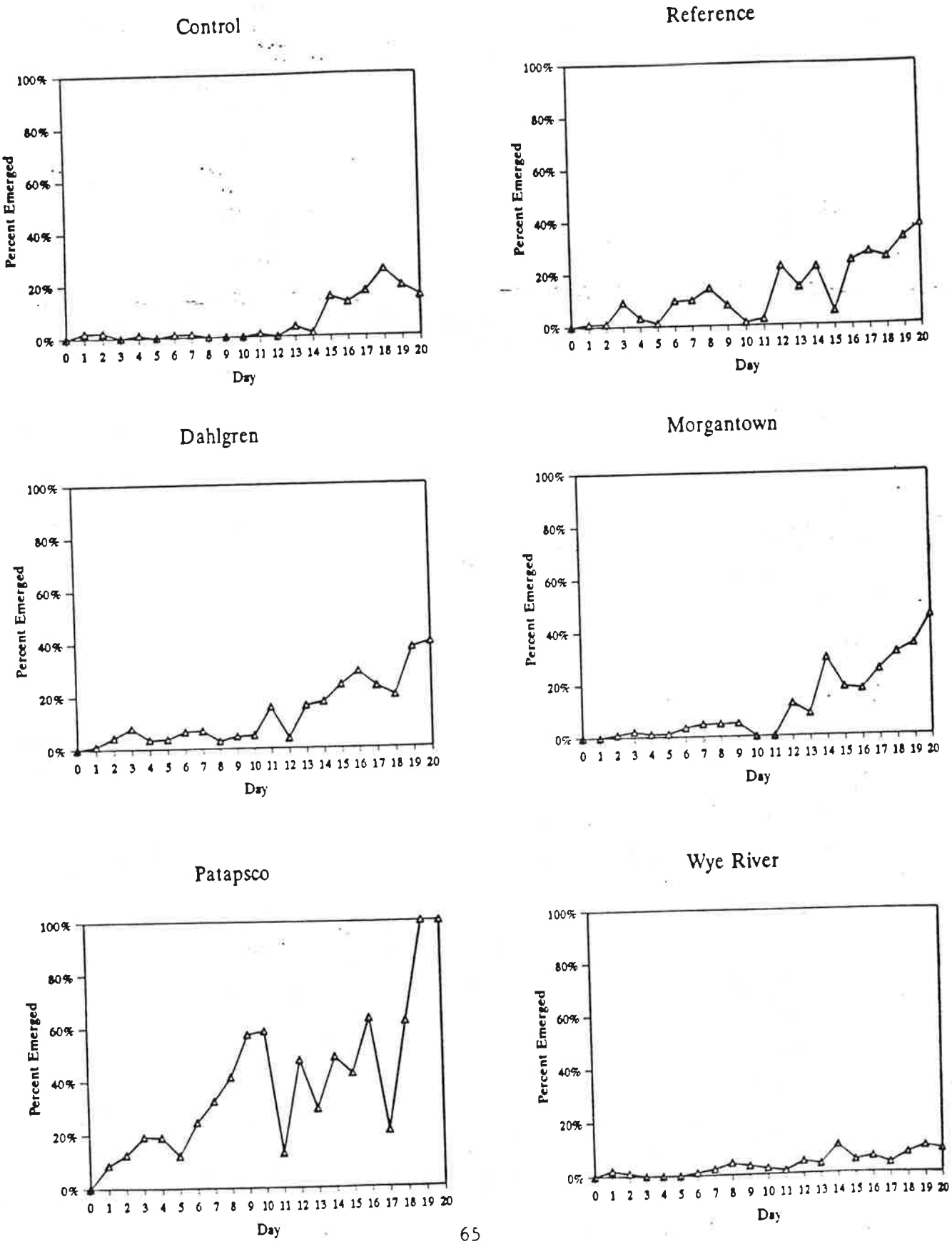
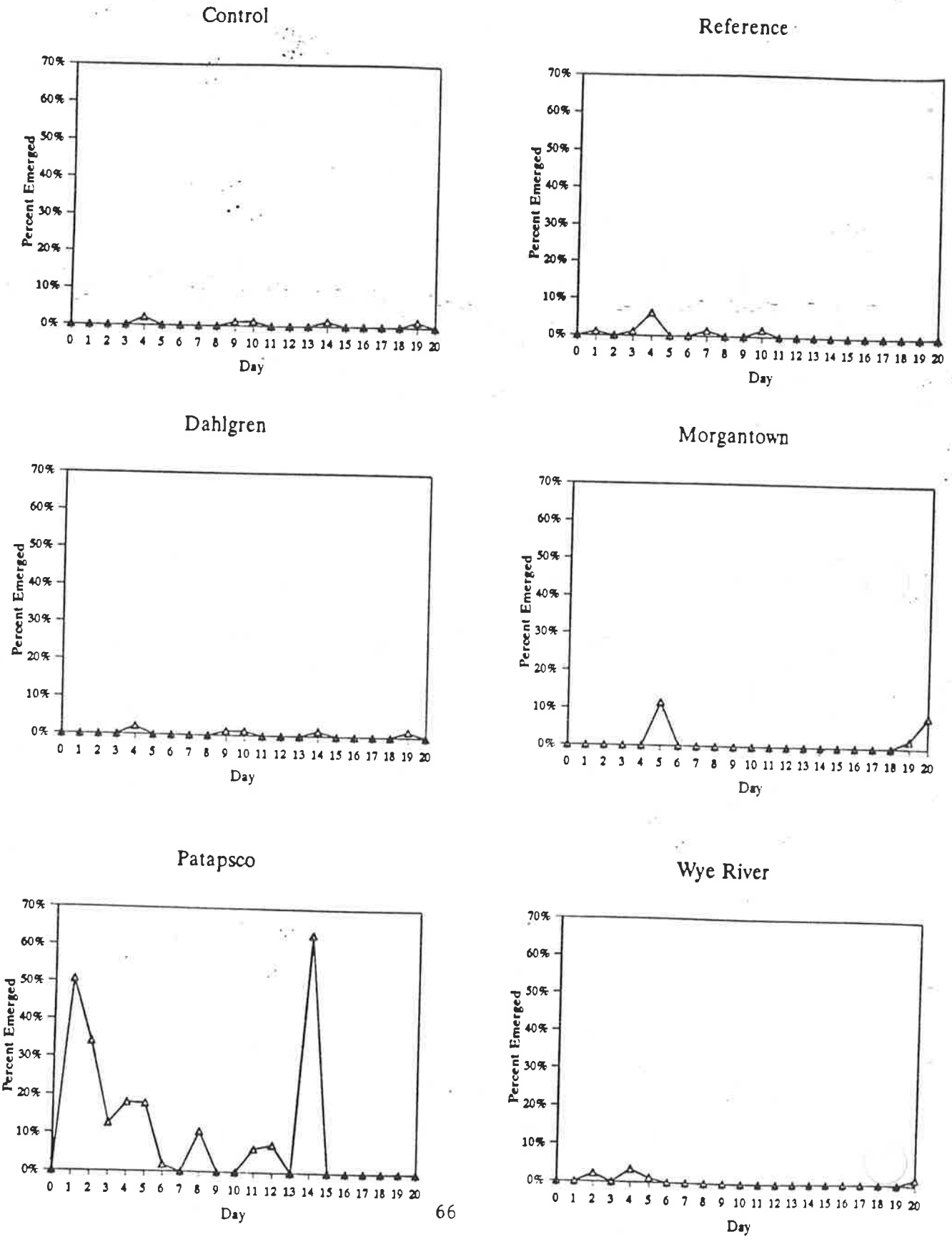


Fig. 4.4 Emergence of H. azteca Set 2 (Sep. '91). Number emerged expressed as a percent, corrected for predicted no. alive at each day.



significant, when compared to the initial measurements.

Lepidactylus dytiscus showed significantly reduced survival at days 10 and 20 in the Patapsco test sediments. Amphipod reburial data are presented in Table 4.13. Exposure to Patapsco sediments had a significant negative effect on amphipod reburial in clean sediment at the end of the test. Another indication of possible toxicity is the number of animals emerged throughout the test period. The normal behavior of the amphipod is to remain buried in the sediment, due to a negative phototactic response which also aids in avoidance of predators. Figure 4.1 illustrates the percent of Lepidactylus dytiscus emerged per day from each test sediment. The graphs are presented as the number of animals emerged per day adjusted by the predicted number of animals alive on that day. The predicted values were derived from regression analysis of the survival data at days 0, 10 and 20. The number of Lepidactylus dytiscus emerged in the Patapsco sediments was much greater than those in other test sediments (see Figure 4.1), with an average of approximately 25% emerged for the duration of the test. The increased emergence would have ecological significance, in that those animals swimming in the water column are exposed to increased predation pressure. If a high percentage of animals are exposed, due to this aberrant behavior, the increased predation may significantly reduce the amphipod population. No significant effects for survival or growth were seen with any other sediments in the first set of tests with Lepidactylus dytiscus. There were no significant changes in length in any of the treatments when

compared to the initial lengths.

Hyalella azteca showed significantly reduced survival at days 10 and 20 in the Patapsco, Morgantown and Dahlgren sediments. Patapsco was highly toxic, with only 20% of the amphipods surviving after 10 days, and only 2% survival at 20 days. The Wye River sediment test had reduced survival (72% and 54% at day 10 and 20, respectively) although the reduction was not statistically significant. No statistically significant growth effects were seen. Animals in all treatments except the reference (100% sand) grew significantly from the initial length. The emergence data (see Figure 4.2) show a high percentage of Hyalella azteca in the Patapsco treatments out of the sediment for the first 10 days of the test. The lack of emergence during the second 10 days is probably a function of the high mortality; only 2% of the organisms survived to 20 days.

Streblospio benedicti survival was significantly reduced in all test and reference sediments at day 10, and all sediments except Patapsco at day 20. All sediment tests also showed reduced growth (length and weight) as compared to the controls. The worms had very poor survival and growth in the reference sediment at 20 days. The reference sediment for the worms was 100% sand with very little organic matter. The worms are naturally found in somewhat muddy sediment (60-70% sand) and exhibit some particle size induced mortalities. A regression was run on data from tests conducted with clean sediments of varying particle size, to enable the worm survival data to be adjusted for sediment effects and re-evaluated

(see Section 3.3.6). The sediments from Dahlgren and the Wye produced significantly reduced survival as compared to the controls at day 10 of set 1 tests. The day 20 data were not included in the assessment of ambient toxicity, because it appears that the worms may have starved to death during the extended test period of 20 days. The worms in the reference sediment (100% sand) had only 17% survival at 20 days, and only a slight increase in size as compared to the lengths of individuals from the initial subset. Approximately 70-75% survival would be expected at day 10 for Streblospio benedicti in clean 100% sand. The reference sediment had a low organic content (1.38%) and, therefore, provided little or no food for the organisms. To alleviate this problem, all worm treatments for the second set of toxicity tests were fed every third day for the duration of the test.

Set 2:

Survival and growth data from toxicity tests conducted September 27, 1991 through October 17, 1991 on Set 2 sediments from the four stations are presented in Tables 4.10 and 4.12. Survival and growth data from the grass shrimp test did not suggest statistical differences between controls and any of the test sediments. The initial length measurement for the grass shrimp was lost due to a calibration error with the image analysis system.

The results of the Lepidactylus dytiscus sediment tests showed statistically reduced survival at 10 days in the Dahlgren and Patapsco River sediments. At day 20 there was significantly

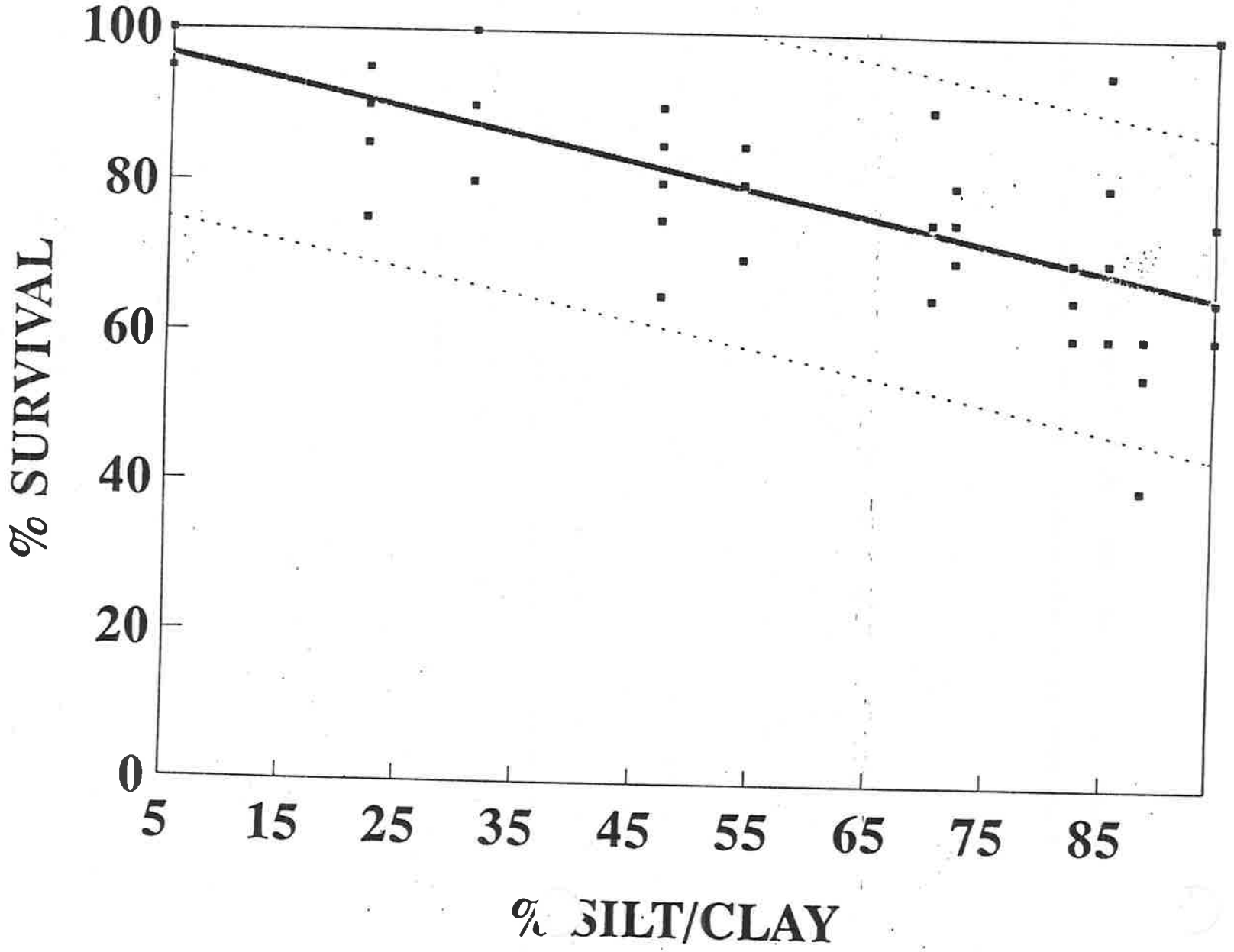
reduced survival in the Morgantown, Dahlgren and Patapsco sedim samples. The Patapsco appeared highly toxic, with only 8% of the amphipods surviving to day 20. Both the Patapsco and Dahlgren sediments produced statistically reduced growth of Lepidactylus dytiscus. The surviving amphipods in Patapsco and Dahlgren sediment tests did not grow significantly in length compared to the initial measurements, another indicator of a population under stress. All other test populations displayed significant increases in length from the initial values. Patapsco sediments also had a significantly negative effect on the amphipod during the reburial portion of the test. The emergence observations from set 2 sediments indicated that a large percentage of animals in the Patapsco sediments were swimming in the overlying water throughout the test. This is considered an avoidance response for th amphipod, indicating adverse sediment conditions, and has potentially important ecological implications. Animals exposed and swimming in the water column risk increased predation, thereby reducing local amphipod populations. Microcosm experiments have demonstrated that amphipods are capable of leaving contaminated sediments and preferentially seeking out clean sediments, even when it means migrating through the water column and circulating systems of microcosm tanks (Alden and Butt, 1988). Therefore, sediments contaminated enough to produce an avoidance response of this sort would be expected to display low benthic standing stocks and productivity. We are not aware of any direct experimental evidence from the field to support the idea that contaminated sediments are

avoided by field populations of amphipods. In fact, we believe that the experimental design for a study to provide such evidence would be very difficult and plagued with confounding effects. However, Dr. Dan Dauer at ODU has made field observations that amphipods routinely migrate out of the sediments, possibly to seek new substrates.

Lepidactylus dytiscus is naturally found in very sandy sediments, and exhibits some mortality due to particle size effects. Lepidactylus dytiscus exhibits increasing mortality with decreasing sediment sand content. Extensive testing has been conducted at the AMRL with Lepidactylus dytiscus in a range of sediment types. These data have been compiled to generate a regression that predicts expected mortality due to particle size (see Figure 4.5). In sediments greater than 80% sand, less than 5% of the mortalities would be due to particle size effects. The Reference sediment was the muddiest sediment tested (63% sand). From the Lepidactylus dytiscus regression, survival should be 85% or better in a 10-day test in this sediment. Lepidactylus dytiscus survival in the reference sediment was 91% (set 1) and 95% (set 2). Therefore, the mortalities seen in all test sediments can be attributed to toxicity, rather than particle size effects.

Hyalella azteca tests indicated statistically reduced survival in the Patapsco, Dahlgren and Reference sediments at days 10 and 20. The Patapsco sediment was highly toxic with only 9% of the test organisms surviving at day 10, and only 2% surviving at day 20. Both the Patapsco and the Reference sediment tests produced

Fig. 4.5 Survival of *L. dytiscus* versus percent silt/clay



statistically significant reductions in growth. All amphipods increased in size significantly from the initial subset, except the animals in the reference sediment. The emergence data for Hyalella azteca revealed that a large percentage of the organisms in the Patapsco sediment were emerged compared to emergence rates in other sediment samples. The low survival and growth observed in the reference sediment for the second set of tests was of concern. The first set of tests conducted with Hyalella azteca in the same reference sediment (100% sand) produced 90% survival at day 10 and 73% survival at day 20. The results from the second set of tests were radically different: 53% survival at day 10 and only 15% survival at day 20. Additional testing of Hyalella azteca in the reference sediment was conducted to investigate the unexpectedly high mortalities observed in the Set 2 reference sediments (August through September test). Samples of reference and control sediments were treated identically to the sediments used in the ambient toxicity tests. A series of ten day Hyalella azteca tests were conducted and the results evaluated (see Appendix D). The data from these tests indicated greater than or equal to 90% survival of Hyalella azteca in all of the 100% sand treatments. This is consistent with the results from the reference sediment used with Set 1 sediment samples. Therefore, it is probable that the results from the reference sediment used for the second set of the ambient sediment toxicity tests were aberrant. The population of Hyalella azteca used for the second set of tests may have been less healthy than those used for the first set, since the control

results were slightly lower for Set 2. The reference toxicant tests conducted on the Set 2 amphipods gave valid results, although the data did indicate a slightly more sensitive population than the first set of test organisms.

The Streblospio benedicti tests for Set 2 indicated no statistically reduced survival or growth. All treatments were fed 0.25 mg of "worm food", a combination of dried algae (Ulva) and tetramin ground to a fine powder (<500 um), every third day. Feeding the worms increased survival at day 20 in the reference sediment from 17% (Set 1) to 72% (Set 2). Worm survival in clean 100% sand is predicted by the particle size effects regression to be 70-75%. The survival in the reference sediment at day 20 was 72%. Therefore, the addition of a worm feeding protocol appears to have successfully eliminated the problem of starvation. The worm particle size regression was then used to estimate the predicted effects due to particle size and adjust the survival data. The reference sediment adjusted survival was not statistically different from the controls. In addition, no test sediments produced statistically significant effects with the worms in Set 2.

4.2.2 Contaminants Data

Toxicity of chemicals in sediments is influenced by the extent to which chemicals bind to the sediments. There are many factors that influence the binding capabilities of a particular sediment. The toxicity of non-ionic organic chemicals is related to the organic content of the sediments, and it appears that the

bioavailability of sediment-associated metals is related to the concentration of Acid Volatile Sulfides (AVS) present in the sediment (DiToro, 1990). Sediment samples from the four stations and the controls were analyzed for Total Organic Carbon (TOC) and Acid Volatile Sulfides (AVS). The results are shown in Tables 4.14 and 4.15. At present, there is no readily accessible data base for comparison of TOC normalized data, therefore the TOC analysis from this study was included to allow for future comparisons. The AVS approach to sediment contaminants evaluation is still developmental and has been published only recently (DiToro, 1990). To appropriately interpret the AVS data, simultaneously extractable metals (SEM) must also be analyzed. The data for SEM are presented in Table 4.16. In evaluating the AVS values, a ratio of the sum of the SEM to the total AVS is calculated. If the SEM:AVS ratio produces a value less than one, it is assumed that there is sufficient AVS present in the sediment to bind with the metals, rendering them biologically less available and therefore less toxic. If the ratio is greater than one (1), toxicity due to metals is less predictable, but generally expected to be more likely. An evaluation of the SEM to AVS ratio is included in Table 4.17. All stations had ratios much less than one, except the Patapsco River. The sediments collected in August had a ratio of 0.946, and the sediments collected in September produced an SEM:AVS ratio of 1.113. These ratios indicate the potential for toxicity due to metals. The SEM analysis (Table 4.16) indicated a very high concentration of zinc in both Patapsco samples.

Table 4.14 Chemical data (TOC) for sediment samples from the four stations and the controls. All data is on a dry weight basis. Set 1 was tested August 5 through September 5, 1991. Set 2 was tested September 27 through October 17, 1991.

Station	Total Organic Carbon (%)
Set 1:	
Lynnhaven Beach	<0.37
Morgantown	0.41
Dahlgren	0.42
Patapsco River	<0.37
Wye River	0.38
Lynnhaven Inlet	1.38
Set 2:	
Lynnhaven Beach	<0.37
Morgantown	<0.37
Dahlgren	0.58
Patapsco River	<0.37
Wye River	0.40
Lynnhaven Inlet	1.78

Table 4.15 Chemical data (AVS) for sediment samples from the four stations and the controls. All data is on a dry weight basis. Set 1 was tested August 16 through September 5, 1991. Set 2 was tested September 27 through October 17, 1991.

Site	AVS (μ mol/g)	Relative Error (%)
Set 1:		
Lynnhaven Beach	<1.0	-
Morgantown	<1.0	-
Dahlgren	1.78	27.61
Patapsco River	6.41	2.97
Wye River	1.48	40.00
Lynnhaven Inlet	3.25	16.95
Set 2:		
Lynnhaven Beach	<1.0	-
Morgantown	1.67	15.34
Dahlgren	1.64	58.10
Patapsco River	5.58	1.79
Wye River	<1.0	-
Lynnhaven Inlet	3.94	11.44

Table 4.16 SEM analysis for sediments for Set 1 (Aug. '91) and Set 2 (Sept. '91). Concentrations for each metal are expressed in umol per gram of sediment (LB = Lynnhaven Beach; LI = Lynnhaven Inlet).

Set 1

	Cadmium		Lead		Copper		Nickel		Zinc		Sum	
Site	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Morgantown	0.011	0.0005	0.031	0.0012	0.077	0.0005	0.059	0.0274	0.302	0.0035	0.479	0.0288
Dahlgren	0.018	0.0003	0.057	0.0015	0.066	0.0038	0.146	0.0473	0.643	0.0111	0.930	0.0378
Patapsco	0.022	0.0003	0.119	0.0012	0.161	0.0023	0.090	0.0225	5.669	0.0355	6.062	0.0394
Wye River	0.001	0.0002	0.007	0.0011	0.012	0.0034	0.020	0.0103	0.068	0.0039	0.109	0.0115
LB	0.000	0.0003	0.001	0.0013	0.003	0.0020	0.068	0.0301	0.025	0.0033	0.098	0.0297
LI	0.006	0.0010	0.037	0.0018	0.041	0.0023	0.029	0.0063	0.663	0.0073	0.776	0.0107

Set 2

	Cadmium		Lead		Copper		Nickel		Zinc		Sum	
Site	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Morgantown	0.010	0.0009	0.032	0.0015	0.060	0.0009	0.110	0.0381	0.256	0.0079	0.468	0.0297
Dahlgren	0.016	0.0005	0.049	0.0009	0.062	0.0025	0.105	0.0191	0.581	0.0057	0.812	0.0192
Patapsco	0.025	0.0013	0.123	0.0010	0.174	0.0047	0.081	0.0184	5.803	0.1074	6.206	0.1175
Wye River	0.003	0.0010	0.008	0.0013	0.021	0.0068	0.052	0.0252	0.080	0.0020	0.164	0.0306
LB	0.000	0.0000	0.002	0.0020	0.008	0.0025	0.133	0.0476	0.023	0.0012	0.166	0.0491
LI	0.005	0.0003	0.041	0.0023	0.033	0.0007	0.126	0.0607	0.621	0.0035	0.826	0.0555
Detection Limits	0.0003		0.005		0.0006		0.0004		0.0005			

NOTE: All Mercury values were below detection limits of 0.00005 in both sets for all sites.

Table 4.17 Average SEM and AVS values and the SEM:AVS ratio for sediment samples tested in 1991.

Site	Mean AVS	Mean SEM	Ratio
Set 1:			
Lynnhaven Beach	<1.0	0.098	-
Morgantown	<1.0	0.479	-
Dahlgren	1.78	0.930	0.524
Patapsco River	6.41	6.062	0.946
Wye River	1.48	0.109	0.074
Lynnhaven Inlet	3.25	0.776	0.239
Set 2:			
Lynnhaven Beach	<1.0	0.166	-
Morgantown	1.67	0.468	0.280
Dahlgren	1.64	0.812	0.497
Patapsco River	5.58	6.206	1.113
Wye River	0.73	0.164	0.226
Lynnhaven Inlet	3.94	0.826	0.2099

It should be noted that the zinc sulfide solubility parameter is greater than those of all the other metals measured except fo nickel (DiToro et al., 1990). Therefore, nickel and then zinc would be predicted to be the first two metals to be released to a dissolved free ion form (i.e., the toxic form of most metals) when the binding capacity of the AVS is exceeded by the total SEM concentrations. Therefore, toxicity may be associated with these two metals (particularly zinc, which is found in high concentrations in these sediments, see Table 4.18 below). On the other hand, the AVS-metals dynamics are not well understood and other metals not measured in the current study may play a role in the observed toxicity.

Inorganic contaminants data from the stations are presented in Table 4.18. All test sites had concentrations above the detection limits for ten of the eleven metals analyzed. The eleventh metal, tin, was below detection limit at all sites except the Patapsco River, which had 42 ug/g and 56 ug/g from the first and second sets, respectively. At the Wye River station, mercury concentrations were below detection limit in the sample collected on August 14th. The amphipod control sediment tested with the second set of sediments had concentrations of mercury, selenium, and tin below detection limits. The amphipod control sediment used in the first set of tests had concentrations of mercury, selenium, tin and nickel below detection limits. Sediment-sorbed contaminants have been extensively studied by Long and Morgan (1990). They have established a table of concentrations at which

Table 4.18 Inorganic contaminants for sediment samples from the four stations and the controls. (Note: single underlined values represent concentrations exceeding "Effects Range-Low", and double underlined values represent concentrations exceeding "Effects Range-Median" levels as defined in Long and Morgan, 1990 (LB = Lynnhaven Beach; LI = Lynnhaven Inlet).

	Contaminant (ug/g)										
Site	Al	As	Cd	Cr	Cu	Hg	Ni	Pb	Se	Sn	Zn
Set 1:											
LB	173	2.24	0.015	0.6	0.25	<0.020	<0.2	0.7	<0.01	< 2	2.0
Morgantown	7462	1.33	0.466	24.6	5.62	0.011	4.6	7.7	0.10	< 2	33.7
Dahlgren	6619	2.74	0.156	12.4	4.41	0.027	6.6	10.6	0.09	< 2	47.9
Patapsco	674	3.77	1.646	107.8	14.22	0.011	4.2	23.0	0.37	42	382.6
Wye	2918	0.52	0.074	4.6	0.57	<0.006	1.1	25.4	0.07	< 2	8.6
LI	10462	4.46	0.288	17.7	8.35	<0.006	8.8	11.7	0.25	< 2	56.4
Set 2:											
LB	274	0.35	0.017	0.8	0.70	<0.006	0.3	1.0	<0.01	< 2	3.2
Morgantown	7889	2.85	0.298	25.5	7.86	0.027	6.1	7.6	0.04	< 2	36.5
Dahlgren	7315	2.46	0.071	13.0	8.39	0.036	7.4	10.6	0.05	< 2	46.1
Patapsco	732	3.72	1.254	130.6	16.79	0.024	4.8	37.8	0.24	56	455.3
Wye	4443	0.95	0.053	6.4	1.77	0.008	2.1	3.8	0.05	< 2	12.6
LI	10309	3.95	0.085	18.3	9.13	0.036	9.5	13.4	0.01	< 2	55.7
Detection Limit	25	0.01	0.005	0.1	0.05	0.006	0.2	0.5	0.01	2	0.1
Effects range:											
LOW	--	33	5	80	70	0.15	30	35	--	NA	120
MEDIAN	--	85	9	145	390	1.3	50	110	--	NA	270

biological effects would be expected if these contaminants were present in the sediment. The lower ten percentile of their data was established as the "Effects Range-Low" (ER-L) and median concentrations were identified as the "Effects Range-Median" (ER-M). Comparisons can be made between sites with respect to the potential for adverse biological effects. This is accomplished by comparing the level of toxicants observed through chemical analysis with the ER-L or ER-M values. Those contaminants with levels exceeding the ER-L are in the "possible" effects range for toxic effects. The contaminant levels above the ER-M fall in the range of "probable" toxic effects.

The Patapsco River station had concentrations of zinc (382.6 ug/g for set 1 and 455.3 ug/g for set 2) that exceeded the ER-M of 270 ug/g as defined by Long and Morgan (1990). Therefore, evaluating the results according to these criteria leads to similar conclusions to that of the SEM/AVS evaluation: that zinc may be a problem in these sediments. The Patapsco River site also had concentrations of chromium and lead (Table 4.17) exceeding the ER-L for chromium (80.0 ug/g) and lead (35 ug/g). Thus, other contaminants, both measured and unmeasured may also be responsible for the observed toxicity.

The results of semi-volatile organic compounds and pesticides analyses in sediment samples are presented in Appendices E and F. The Morgantown sediment was the only sample with compounds listed in the Long and Morgan (1990) reference that exceeded the possible effects ranges. The concentration of 4,4'-DDT was above the ER-L

value (Appendix F).

4.2.3 Pore Water Data

Sediment pore water was analyzed for sulfide, ammonia and nitrite for all stations and the controls and references. High ammonia values were recorded for Lynnhaven Inlet (63% mud) and Lynnhaven Beach (100% sand) for Set 1 sediments and the reference for Set 2 sediments (Table 4.19). No relationship existed between test sediments that produced significantly reduced survivals and those sediments with high ammonia levels.

4.2.4 Reference Toxicant Data

The relative sensitivities of each set of test organisms was evaluated with reference toxicant tests. The results of each reference toxicant test conducted with each batch of grass shrimp, amphipods and worms are shown in Table 4.20. The grass shrimp was tested using SDS (sodium dodecyl sulfate); all other organisms were tested using cadmium chloride ($CdCl_2$). All test LC50s were within the range of the previous reference toxicant tests conducted, indicating healthy and sensitive populations of test organisms.

The grass shrimp reference toxicant tests results were similar to previous tests conducted at the AMRL, as well as results published in the literature. Tatem et al. (1976) found median lethal toxicity values ranging from 52 to 98 mg/L SDS for grass shrimp held in the laboratory less than a month. Reference toxicant tests from 1990 gave values ranging from 56.7 to 67.7 mg/L

Table 4.19 Chemical data for pore water samples from the four stations and the controls.

Site	Ammonia (mg/L)	Nitrite (mg/L)	Sulfide (mg/L)
Set 1:			
Lynnhaven Beach	13.209	0.0022	0.036
Morgantown	2.637	0.0451	0.031
Dahlgren	3.559	0.0303	0.010
Patapsco River	4.542	0.0069	0.115
Wye River	3.190	0.0041	0.011
Lynnhaven Inlet	19.639	0.0013	0.043
Set 2:			
Lynnhaven Beach	0.643	1.2117	0.036
Morgantown	1.996	0.0061	0.014
Dahlgren	3.989	0.0025	<0.008
Patapsco River	4.266	0.0041	0.040
Wye River	2.729	0.0016	0.019
Lynnhaven Inlet	20.407	0.0013	0.031

Table 4.20 Reference toxicant data results from 96-hr, water only, reference toxicant tests for the second year of the ambient toxicity project. Sodium dodecyl sulfate (SDS) was used with the grass shrimp and cadmium chloride ($CdCl_2$) was used for all other organisms.

Organism	Test Set #	Chemical	LC50 & CIs (mg/L)	Historical Mean
P. pugio	1	SDS*	53.04 (43.74 - 64.29)	53.01
	2	SDS	52.32 (38.70 - 70.44)	
L. dytiscus	1	CdCl2**	6.55 (4.54 - 9.45)	5.22
	2	CdCl2	7.13 (5.23 - 10.36)	
S. benedicti	1	CdCl2	4.12 (2.21 - 8.83)	5.79
	2	CdCl2	5.27 (4.19 - 6.56)	
azteca	1	CdCl2	0.045 (0.034 - 0.059)	0.028
	2	CdCl2	0.038 (0.029 - 0.050)	

SDS. The reference toxicant tests on grass shrimp used for this study produced comparable LC50s of 52 and 53 mg/L SDS.

Lepidactylus dytiscus and Streblospio benedicti reference toxicant responses were within the range of those from previous tests, and were similar to the 1990 reference toxicant data.

Since Hyalella azteca is a freshwater organism, most of the reference toxicant data available in the literature are from tests conducted at 0 ppt. However, multiple tests have been conducted at the AMRL with Hyalella azteca in 15 ppt water in a effort to build a data base for comparison of future tests. Previous reference toxicant tests conducted with Hyalella azteca in 15 ppt water produced LC50's ranging from 0.25 to 0.47 mg/L $CdCl_2$. The reference toxicant test results for this project fall within that range.

4.3 Suborganismal Tests

4.3.1 EROD Activity in Feral Fish

EROD activity was measured in feral Fundulus (Wye River and St. John's Creek), white perch (Morgantown and Dahlgren) and spot (Patapsco River and Patuxent River) on sampling dates in August and September (when water and sediment were also being sampled for bioassays) (Table 4.21).

4.3.1.1 Fundulus heteroclitis

EROD activity in Fundulus from the Wye River was variable and not significantly different in September versus August. EROD activity in Fundulus from St. John's Creek was greater than ($0.05 \leq$

Table 4.21 Hepatic ethoxyresorufin-O-deethylase (EROD) activity and in feral fish. August and September sampling dates correspond to dates for water column and sediment tests.

Species	N	EROD Activity (pmol/min/mg)	
Fundulus			
Wye River			
August	7	623.4	440.7
September	5	970.1	136.4
St. John's Creek			
July	5	1580.9	1051.6[A]
White Perch			
Dahlgren			
August	10	41.5	15.0
September	6	55.2	17.3
Morgantown			
August	10	41.2	11.4
September	6	49.5	9.6
Spot			
Patapsco River			
August	8	2706.0	1068.0
September	10	1214.5	337.2
Patuxent River*			
November	8	126.1	88.0

All data as mean std. ± deviation.

*Spot collected from Sheridan Pt. in the Patuxent River between November 19 and 25, 1991.

[A]Significantly different from August Wye River sampling ($0.10 \leq p \leq 0.05$).

$p \leq 0.10$) Fundulus sampled from the Wye River in August and similar to values found in the Wye River in Year 1. Values for ERO activity in Wye River Fundulus in Year 2 were less than those found in year 1 (ca. two-fold).

Elevations in EROD activity in Fundulus (Elskus and Stegeman, 1989), and other fish species (Stegeman and Kloepper-Sams, 1987) are associated with planar aromatic hydrocarbon exposure. Thus, where activities are high, it is inferred, in the absence of corroborating chemical information, that exposure to these compounds has occurred. Thus, the EROD activities suggest that Wye River Fundulus were less exposed to planar aromatic hydrocarbons than those from St. John's Creek. Since the area from St. John's Creek where these fish were collected is a relatively quiescent area with modest tidal exchange, and the Creek in general experiences a significant amount of motor boat activity (and attendant fuel pollution), this observation is perhaps not surprising.

However, EROD activity in feral Fundulus from the Wye River in Year 1 was much higher and similar to that seen in the St. John's Creek Fundulus and also similar to values seen in Fundulus from the heavily creosote contaminated areas of the Elizabeth River. In the absence of any corroborating chemical information, we cannot be certain as to the cause of these elevations in Wye River fish, though the data suggest that aromatic hydrocarbon exposure is occurring at levels high enough to elicit this cellular response. Alternately, other, uncharacterized, chemical (natural dietary) or

physiological factors could be influencing EROD activity. The large number of studies which have documented clear relationships between EROD activity and planar aromatic hydrocarbon exposure (See Stegeman and Kloepper-Sams, 1987 for review), however, argue against this alternate hypothesis. The lab studies which are described below also support this discussion.

4.3.1.2 White Perch

EROD activity in white perch from both Potomac River stations, at both sampling times, was low and similar among stations. The mean activity values are approximately 6-7 fold less than the values measured in Year 1.

The reason for the differences in mean activity values between years 1 and 2 are not immediately apparent. One explanation would be that fish in year 1 were actually experiencing a higher exposure level and the higher activity levels are a reflection of this. However, since laboratory studies have not documented the degree of responsiveness of white perch to PAH's, we cannot conclude with any certainty that this explanation is tenable.

One possible confounding factor could be related to the high concentration of copper that has been observed in livers from this species (See data below and Bunton and Baksi, 1988; Bunton et al., 1987). High concentrations of some trace metals are known to decrease the activity of the monooxygenase system in general (George, 1989) and the high levels of copper in livers of this species may obscure the ability to detect any environmental induction of EROD activity. Until laboratory studies are conducted

to examine this hypothesis, white perch (in the Chesapeake Bay, in particular) may not be a suitable species for field studies .. monooxygenase induction.

4.3.1.3 Spot

EROD activity in feral spot collected from the Patapsco River in August was approximately two-fold higher than spot collected in September. The values for EROD activity were 3-5 fold less than the levels found in spot collected from this area in year 1. In comparison, EROD activity in spot collected in the Patuxent River in November was 10-20 fold less than the values seen in spot from the Patapsco River.

However, there was a significant difference in the hepatosomatic index (liver wt./body wt.) in the Patuxent River spot. The livers of spot collected in the Patuxent River in November were nearly three-fold larger (mean HSI 3.3 versus 1.2 than those collected in the Patapsco. There is no immediate explanation for this observation and thus we cannot be certain if there is any relationship between factors causing this increase in liver size and changes in EROD activity, or responsiveness to aromatic hydrocarbon inducers. We suspect that the increased liver size may be due to changes in hepatic physiology (increased lipid storage) as the animals experience cooling water temperatures and approach the winter season (Hazel, 1979). Our casual observations at the time of dissection noted that the livers were friable and appeared to be carrying high levels of lipid. Previous experiments with Fundulus (Stegeman, 1979) have shown that cold acclimation can

inhibit the ability to induce benzo(a)pyrene hydroxylase (analogous to EROD) over short time frames. However, there was only a modest influence of temperature on basal monooxygenase activity.

We suggest, therefore, that the high levels of EROD activity in spot from the Patapsco are consistent with the aromatic hydrocarbon pollution in that region as compared to the Patuxent River. Van Veld et al. (1990) have recently provided convincing evidence that spot liver EROD activity can be used as a reliable reflection of the aromatic hydrocarbon contamination gradient present in the Elizabeth River, Virginia. The activities (high and low) we have detected are similar to those found in the Van Veld et al. (1990) study. Spot appear to be an excellent choice for further surveys of EROD activity in feral fishes around the Chesapeake Bay, though a further investigation of the increased liver size in late season spot is warranted.

4.3.2 Holding Time for Fundulus to Reach Baseline EROD Levels

Fundulus were collected from the Wye River and St. John's Creek during late July and early August. Experiments with the St. John's Creek Fundulus began on July 25, while experiments with Wye River fish began on August 8. Results are presented in Figure 4.6.

Fundulus from St. Johns' Creek exhibited a rapid decline in EROD activity from the time of collection through week 3. Also associated with this decline was a corresponding decrease in the variance (as shown by the narrowing error bars). Levels of EROD activity after three weeks were similar to those found after twelve

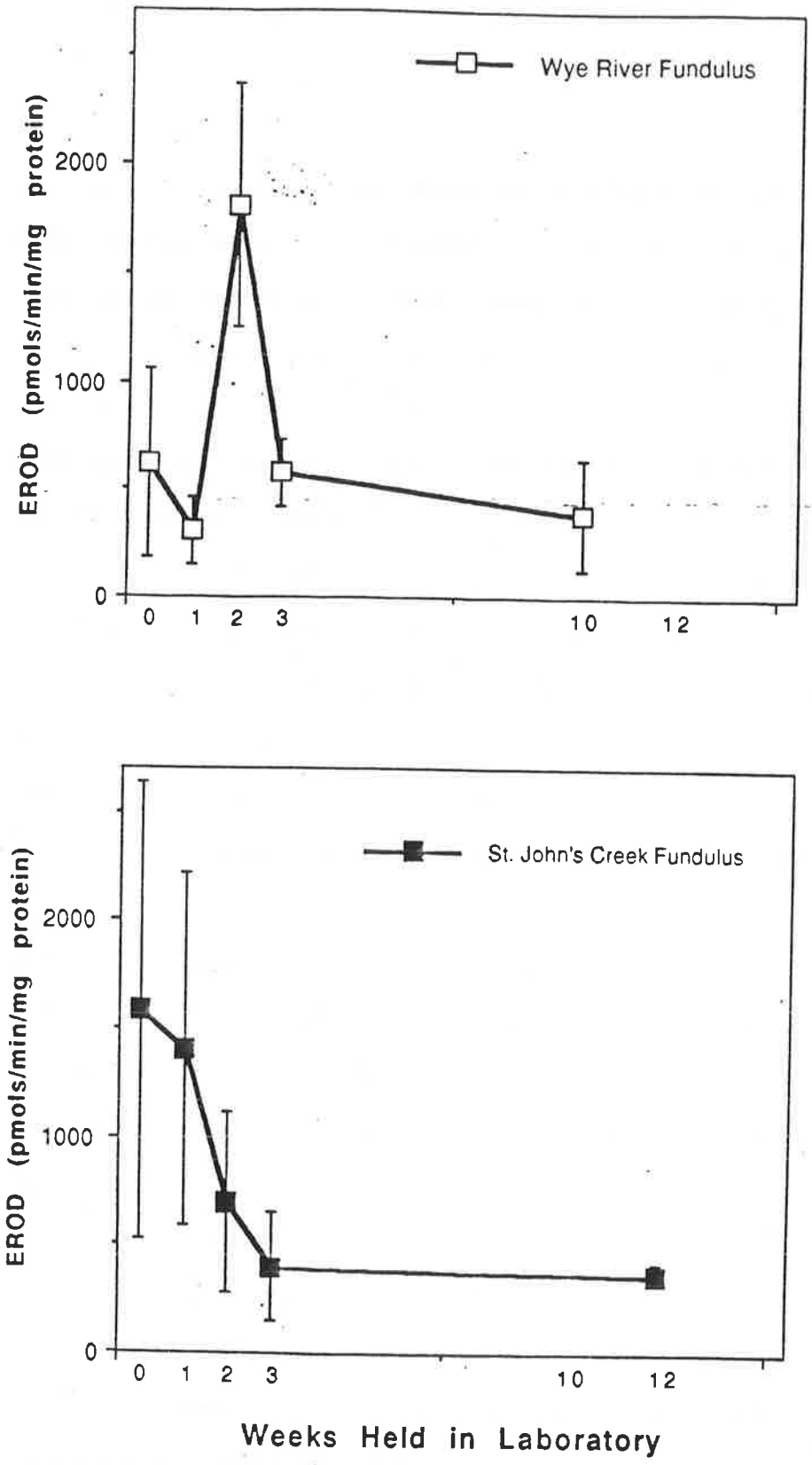


Fig. 4.6 EROD activity in Fundulus heteroclitus following collection in St. John's Creek and the Wye River.

weeks, indicating that 3 weeks to one month is the time necessary for EROD activity to reach baseline levels in St. John's Creek Fundulus.

EROD activity in Fundulus from the Wye River declined during the first week, increased (nearly 6-fold) from week 1 to 2 and then returned to collection levels at week 3. After 10 weeks, EROD activity had declined to levels similar to those in the St. John's Creek fish. There are several things to note from this data. First, Wye River fish in year 2 did not have the high levels of EROD activity at collection that we found in year 1. Thus the decline in activity at 10 weeks was not as great as with St. John's fish. In fact, the Wye River fish do not appear to have highly elevated EROD activity at all in comparison to control fish from treatments used in year 1. We will note below, however, that in the lab experiments using a model PAH inducer, the absolute activity levels seen in Fundulus from Wye River are similar to those seen in lab Fundulus treated with benzo(a)pyrene.

Secondly, we have no immediate explanation for the spike in activity between week 1 and 3 in Wye River fish as compared to the more idealized behavior of the St. John's Creek fish. As mentioned earlier, the weeks when these activities were measured do not correspond directly (i.e., the week 2 sample date for Wye River was August 22, while the fish from St. John's Creek were in between the 3 and 12 week sampling period on August 22). Since these fish were being held in ambient Patuxent River water, it is possible that a pulse of aromatic hydrocarbon contaminated water went through these

tanks and the Fundulus responded accordingly. We cannot be sure that the St. John's Fundulus did not also exhibit this spike activity because we did not sample them during this time. In this case, the EROD activity may have accurately reflected contamination.

An alternate hypothesis could be that the spike in activity was due to the mobilization of some stored contaminant in the Wye River Fundulus and subsequent induction of EROD. We suggest that this is unlikely since there do not appear to be substantial sources of persistent contaminants, such as PCBs, Dioxins, etc., near this site which could have caused this.

It is important to note that these fish ultimately reached similar baseline levels of EROD as the St. John's Creek fish. It should also be noted from the following section that EROD activity in all of these fish did continue to decline to low levels over th next six months, prior to the initiation of the laboratory induction studies.

4.3.3 Evaluations of EROD Induction in Fundulus from the Wye River Versus St. John's Creek

Fundulus from the Wye River and St. John's Creek were treated with 0 (corn oil), 2 and 10 mg/kg of benzo(a)pyrene and sacrificed after 96 hours. EROD activity from these fish is shown in Figure 4.7.

EROD activity was induced to a similar extent in Fundulus from the Wye River and St. John's Creek. That is, there was no

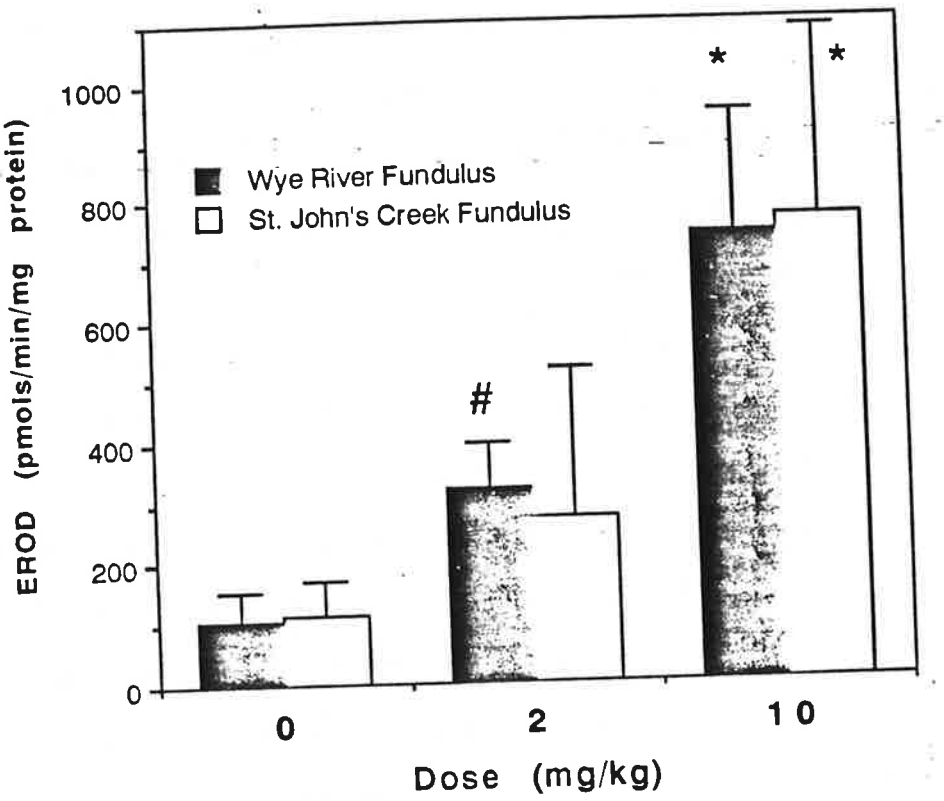


Fig. 4.7 Induction of EROD activity in Fundulus heteroclitus from two different populations. # = significantly different from control at $p \leq 0.10$. * = significantly different from control at $p \leq 0.05$.

significant difference in the magnitude of the response between the populations. Mean EROD activity was induced approximately two-fold at the 2 mg/kg dose in both populations, though the variability of the response of the St. John's fish prevented a statistical difference. The mean EROD activity in fish treated with 10 mg/kg was similar and both populations were statistically elevated (approximately 5-fold) over the controls.

We note here that the absolute levels of EROD activity were low in the controls, the lowest levels we have seen in the two years of study. The activity in the fish induced with 10 mg/kg, while 5-fold higher than the control fish, was similar to the levels seen in freshly collected fish from the Wye River in year 2, but still less than half that seen in fish from the Wye in year 1 and from the Elizabeth River in year 1.

Since the submission of the year 1 report, we have also conducted an additional dose-response experiment using ip injections of benzo(a)pyrene and fish from St. John's Creek (Figure 4.8). In this experiment, we used a broader range of treatment levels and detected significant induction only with 10 mg/kg of B(a)P. The response seen with the 10 mg/kg treatment was substantially higher than observed in the experiment described above. The data also suggest a rather steep dose-response between 1 mg/kg and 10 mg/kg, which is why the 2 mg/kg treatment was used in the more recent experiment.

We also reported data on the sex of the fish, since the fish were developing gonads at the time the experiment was conducted

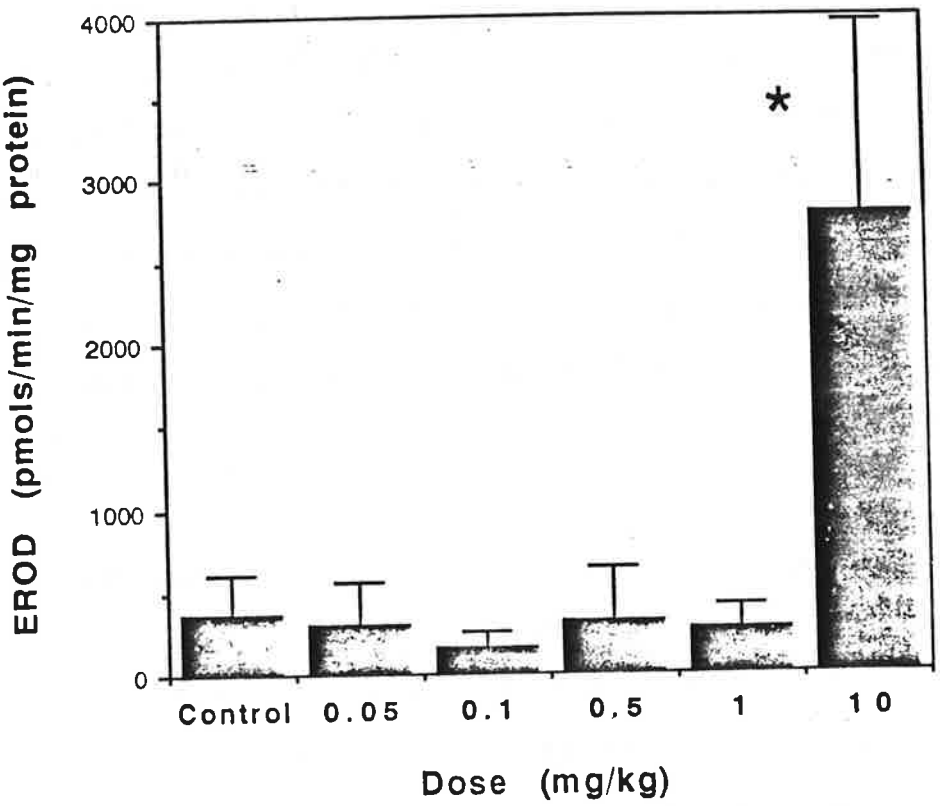


Fig. 4.8 Dose-response EROD activity in Fundulus heteroclitus treated with benzo(a)pyrene. * = significantly different from control at $p \leq 0.05$.

(some females actively extruded eggs with gentle abdominal pressure) and sexual maturity has been shown to effect EROD inducibility in female fish (Gray et al., 1991). As shown in Figure 4.9, induction was strong in both males and females, with the magnitude of induction similar among males and females, but the absolute rates less in the female controls and 10 mg/kg treatment group. While this observation is different from that seen with some female teleosts (little or no induction in gravid females), it is consistent with other recent experiments with Fundulus heteroclitus (Kloepper-Sams, 1989). This suggests that Fundulus will show environmental induction in the absence of an experimental design which includes segregation of animals by sex during active gonadal development.

4.3.4 Supplemental Studies on Copper Concentrations in Livers of White Perch from the Potomac River

In Year 1 we observed, during dissection, that the livers of many of the white perch sampled from the Dahlgren and Morgantown stations were abnormally dark brown in color. Data in the literature on other white perch from around the Chesapeake Bay (Bunton and Baksi, 1988; Bunton et al., 1987) suggested that this was due to high levels of copper. A phone conversation with Dr. Tracy Bunton of Johns Hopkins University confirmed this observation qualitatively.

In Year 2, we examined a set of livers from white perch from our August sampling at these stations for copper to confirm and

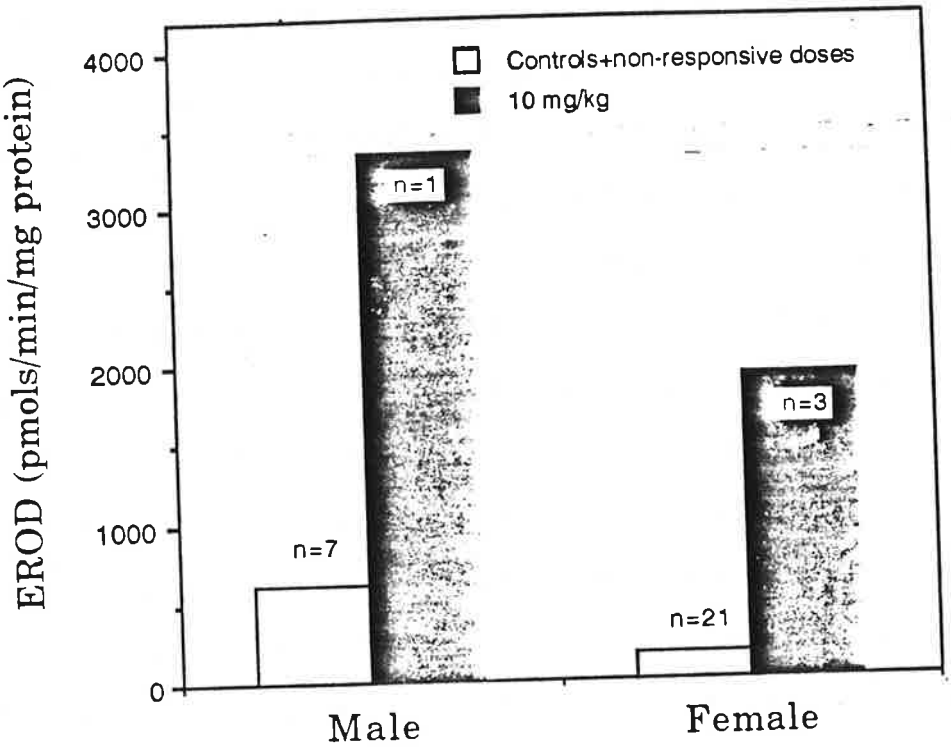


Fig. 4.9 Sex differences in EROD activity in Fundulus heteroclitus treated with benzo(a)pyrene.

quantitate this observation (Table 4.22). The concentrations found in the livers of these white perch are similar to those found by Bunton previously. The mean concentrations were not significantly different between the two stations. In general, Bunton et al. (1987) reported that concentrations increased with increasing size of the fish, and that general observation is apparent here. However, in some of the largest fish, copper concentrations are not necessarily as high as in some smaller fish. At the time we collected these samples we noted that some livers were not as dark as others and selected a few fish specifically for this reason. Therefore, these data may contain some bias and no special significance should be attached to the fact that some large fish have low hepatic copper concentrations. This appears to be part of the normal variability that is encountered in these fish.

Bunton et al. (1987) have also suggested that this observation does not appear to be related to generally high levels of copper in Chesapeake Bay waters, as this observation has been made on fish from many of the tributaries of the Chesapeake. In addition, hepatic copper concentrations in the closely related striped bass (Morone saxatilis), and other fish sampled from the same locations as white perch, do not show this anomaly. White perch from the Chesapeake appear to accumulate copper via some novel biochemical mechanism which has yet to be described. We do not know if this observation extends to white perch from other locations. It is also unknown what effect, if any, this high hepatic copper has on the ability to use white perch for monooxygenase monitoring or on

Table 4.22 Copper concentrations in livers from white perch sampled from the Morgantown and Dahlgren stations.

Station	Fish Wt. (g)	Copper Concentration (ug/g wet weight)
Dahlgren	55	1148.3
	59	915.7
	65	1602.0
	54	2162.7
	57	1570.3
	52	671.3
	63	2072.3
	51	514.1
Mean ± s.d.	**57 ± 5**	**1332.1 ± 618.8**
Morgantown	121	552.0
	101	3366.3
	55	1281.3
	77	2705.0
	142	290.9
	55	1096.2
	56	1249.0
	52	1822.1
	44	1021.2
Mean ± s.d.	**78 ± 35**	**1487.1 ± 993.8**

Copper concentrations were determined by nitric acid digestion of tissue samples and flame atomic absorption spectrophotometry.

other physiological processes in white perch such as growth reproduction.

SECTION 5

DISCUSSION

5.1 Potomac River - Morgantown

The Morgantown station is located in a critical mesohaline habitat of the Potomac River. Previous studies have suggested that toxic concentrations of tributyltin (TBT) (20 to 24 ng/L) were present at this station in 1985 and 1986 (Hall et al., 1987b). Results from our first year of water column testing at this station suggested the presence of toxic conditions as survival of sheepshead minnows was significantly reduced (Hall et al., 1991). Results from the first test conducted in 1991 demonstrated biological effects using a different endpoint with the same fish species. Growth of larval sheepshead minnows was significantly reduced after 8 d of exposure.

Contaminants data from this station during the first test did not provide absolute data for establishing "cause and effect" relationships. Copper was reported at potentially stressful concentrations of 5.5 ug/L during the first test and 4.2 ug/L during the second test. Both values exceeded the U.S. EPA marine water quality criteria for this metal (U. S. EPA, 1987a). These copper concentrations were higher than values reported from this station in 1990 (Hall et al., 1991). A nickel concentration of 7.4 ug/L was also reported during the first test in 1991. This concentration was similar to the 8.4 ug/L concentration reported at this station during the 1990 study. Although the value reported in

1991 was approximately 1 ug/L lower than the chronic water quality criteria of 8.3 ug/L, it may have been stressful. Comparison o. other contaminants data between the two years showed the absence of mercury in 1991 in contrast to the reported value of 0.29 ug/L in 1990. Neither organic contaminants or water quality conditions appeared to be stressful in either year for species used for water column testing.

Data from the 1990 sediment toxicity tests indicated low levels of toxicity (Hall et al., 1991). Grass shrimp and worms showed no significant difference in survival or growth compared to their control groups. However, amphipods exhibited a statistically significant reduction in survival (70% survival at day 10 and 20% survival at day 20). The Morgantown sediment was approximately 92% sand, and was therefore very similar to the native amphipod sediment, so differences in mortality cannot be attributed to particle size effects (see Figure 4.5). No toxic levels of inorganic contaminants or unionized ammonia were found in this sediment. Organic analysis was not conducted in 1990 due to funding limitations.

The results of the 1991 sediment toxicity tests again gave an indication of low ambient toxicity levels. Although there were no significant effects with the grass shrimp, worm or Lepidactylus dytiscus tests, Hyalella azteca showed significantly reduced survival in Set 1 tests in the Morgantown sediments. The organic contaminant analysis indicated levels of the pesticide 4,4'-DDT exceeding the Long and Morgan ER-L for expected toxic effects. No

significant concentrations of inorganic contaminants were identified.

The suborganismal (biomarker) component of the two year ambient toxicity pilot program has not shed additional light on the condition of the Potomac River Morgantown Station, other than the high hepatic copper concentrations documented in white perch from the 1991 sampling program. As stated previously, the copper observation in white perch is not unique to Morgantown or the Potomac River in general. Fundulus heteroclitus were not available at this station due to unsuitable habitat, and therefore monooxygenase monitoring with Fundulus while useful at other stations, was not fruitful here. We also did not encounter any spot during our trawling or beach seining activities at this station.

5.2 Potomac River - Dahlgren

This station is located downstream from the Morgantown station and represents a typical mesohaline area in the Potomac River. Data from our 1990 water column testing demonstrated reduced survival of larval sheepshead minnows after 8 day exposures. Results from the 1991 study also demonstrated effects with this larval fish; a significant reduction in growth was reported during the first test. Contaminants data provided minimal insight as to possible causes of biological effects during the first test. Copper concentrations of 3.4 ug/L exceeded the marine water quality criteria for this metal. Low concentrations of both cadmium (0.68

cadmium (0.68 ug/L) and chromium (3.1 ug/L) were also reported but values were well below the water quality criteria for each metal. Metals data from 1990 showed the presence of lead (3.6 and 5.6 ug/L) at this station; concentrations of this metal were not detected in the 1991 study. Neither organic contaminants or water quality parameters were reported at potentially toxic concentrations during the 1990 or 1991 water column testing.

Results of the 1990 sediment toxicity tests indicate a significant reduction in amphipod survival (22% survival), but no significant differences in shrimp and worm survival or growth (Hall et al., 1991). Contaminant analysis showed no evidence of toxic inorganic contaminants. Pore water analysis did show relatively high levels of unionized ammonia (0.159 mg/L), just below the EPA acute criteria of 0.233 mg/L for a one hour average of unionized ammonia. Organic analysis identified one pesticide, two semi-volatiles, and 11 tentatively identified semi-volatile compounds present in the sample.

The 1991 sediment toxicity tests resulted in reduced survival of Hyalella azteca in Set 1 tests at 10 and 20 days, and significantly reduced survival of the worms in the set 1 sediments after a 10 day exposure. Since the worms are sensitive to particle size composition, the percent survival was adjusted to account for sediment particle size effects. The worm survival in Dahlgren sediment was still significantly lower (82% adjusted survival) than survival in the controls (100% adjusted survival). In Set 2 sediment tests, both Hyalella azteca and Lepidactylus dytiscus

showed significant mortalities, and Lepidactylus dytiscus growth was significantly reduced. There were no significant differences in the grass shrimp survival or growth in either set of sediment tests. No high concentrations were observed for the sets of organic or inorganic chemical contaminants which were analyzed.

The same general observations made above for the Morgantown station also applied to suborganismal data at the Dahlgren station. White perch was the most abundant species available and did not show any apparent contaminant related effects on EROD activity.

5.3 Patapsco River

The Patapsco River station was selected to represent an area where toxic conditions were suspected based on effluent toxicity data from a local industrial facility. Selection of this station prior to the 1990 study was not based on previous data demonstrating biological effects in this ambient area. Results from our first year of water column testing showed biological effects from our larval grass shrimp test. Biological effects were not reported for the other two species (E. affinis and sheepshead minnows). Toxicity data from the 1991 study were similar to the data from these two tests. Results from four water column tests did not suggest the presence of toxic conditions at this station.

Concentrations of metals measured from this station do not suggest the presence of potentially toxic conditions with the possible exception of copper and nickel. Copper concentrations of 3.7 ug/L reported from both 1991 tests exceeded the marine water

criteria quality (U.S. EPA, 1987a). The value was also higher than concentrations (< 2.0 ug/L) reported in 1990. Nickel concentrations ranging from 5.2 to 8.2 ug/L were detected in all three samples collected during the 1991 study. The value of 8.2 ug/L is only slightly less than the water quality criteria of 8.3 ug/L (U. S. EPA, 1987a). This value is also similar to the concentration of 10.8 ug/L reported in 1990 (Hall et al., 1991). These data suggest that nickel was consistently present at this station in 1990 and 1991. Organic contaminants and water quality parameters were not reported at potentially toxic levels for species in water column tests during the 1991 study. A similar finding was also reported in 1990.

The 1990 sediment toxicity tests demonstrated toxic conditions at this site (Hall et al., 1991). Survival of two of the sediment test species, Lepidactylus dytiscus and the worm, was significantly reduced after 10 and 20 days of exposure to sediments collected from this site. Survival of the grass shrimp was not reduced compared to the controls. Test organisms exposed to Patapsco River sediments exhibited no significant reduction in growth. Although the Lepidactylus dytiscus showed no significant difference in growth at this station, they did show a significant difference in reburial in clean sediments at the end of the 20-day test. Ninety-nine percent of the control animals reburied, while only 75% of the surviving amphipods from the Patapsco River sediments reburied when placed into clean sediment, indicating a level of toxicity capable of producing behavioral effects. In addition, observations during

the test indicated that a high percentage of the organisms were emerged from the sediments or swimming in the overlying water throughout the test period. Elevated levels of zinc, chromium, and lead were observed in the Patapsco River sediments. Chromium (157.0 ug/g) and zinc (603.1 ug/g) were found in levels exceeding the ER-M, while lead (55.8 ug/g) was observed at concentrations which exceeded the ER-L level. The high levels of these metals is consistent with the various toxic responses exhibited at this site.

Results of the 1991 sediment tests confirm the findings of the 1990 study. Significantly reduced survival was seen with both Hyalella azteca and Lepidactylus dytiscus at 10 and 20 days for both Set 1 and Set 2 tests. In addition, both amphipod species tested in Set 2 exhibited significantly reduced growth in those animals that survived. There were no significant effects seen with the grass shrimp exposed to Patapsco River sediments in set 1 or set 2 tests. Lepidactylus dytiscus demonstrated significantly reduced reburial for both Set 1 and Set 2 tests. One hundred percent of the control animals reburied, while only 89.7% (Set 1) and 82.9% (Set 2) of the surviving amphipods from the Patapsco River sediments reburied when placed into clean sediment, indicating behavioral effects on the surviving organisms. In addition, observations during the test indicated that a high percentage of Lepidactylus dytiscus and Hyalella azteca were emerged from the sediments or swimming in the overlying water throughout the test period. This high percentage of emergence is abnormal for both species, and indicates an avoidance response. It

is possible that fewer mortalities may have occurred because the animals were swimming in the water column and not actually in contact with the sediment throughout much of the test period.

Elevated levels of zinc, chromium, and lead were again observed in the Patapsco River sediments during 1991. Chromium (107.8 ug/g Set 1 and 130.6 ug/g Set 2), while lead (37.8 ug/g) were found in levels exceeding the ER-L, and zinc (328.6 and 455.3 ug/g) concentrations exceeded the ER-M level. The high levels of these metals are consistent with the various toxic responses exhibited at this site. The high percentage of sand in the sediments found at this and several other sites (Table 3.4), generally coincides with lower AVS and TOC values for that sediment. Lower AVS and TOC tend to increase toxic responses to metals over sites with similar metal contamination, but lesser sediment sand fractions. The bioavailability of the metals frequently becomes diminished with increasing AVS and TOC. The SEM:AVS calculations indicated a ratio greater than 1.0, which predicts the potential for metal toxicity. The high level of zinc (5.7 umoles/g and 5.8 umoles/g, Sets 1 and 2 respectively) in the extractable metal phase and its rather high sulfide solubility parameter (DiToro et al., 1991) corroborates the speculation that zinc may be responsible for a large percentage of the toxicity exhibited. No toxic levels of organic contaminants were identified.

Suborganismal data collected from spot at the Patapsco station during 1991 continued to show evidence of induced EROD activity,

particularly in comparison to a reference sample taken in the Patuxent River in November. Hepatic EROD activities, however, were not as high as levels found in 1990. Mean EROD activities were approximately two-fold higher in August than in September. These activities are presumed to reflect the planar aromatic hydrocarbon contamination in the Patapsco (Helz and Huggett, 1987). However, we do not have data to ascribe differences between August and September to differences in environmental PAH concentrations.

There has been considerable debate as to whether elevations in EROD activity reflect a "toxic" response. This is due, in part, to the fact that measures at the cellular level are often made outside the context of toxic effects measured as depressed growth and/or increased mortality. What can be stated with confidence is that increases in cellular responses which can mechanistically be related to specific classes of contaminants (such as EROD and planar aromatic hydrocarbons), reflect an exposure which is high enough for cell or organ system response. That is, if you consider the cell, or in this case hepatic EROD activity, as a biosensor which will only respond if exposure exceeds some threshold level, then these fish have been exposed to concentrations which exceed this threshold. Furthermore, if cellular responses in fact precede overt toxic effects, along some dose response curve, then elevations can be considered a "toxic" effect. Clearly, whether this toxic effect has any relationship to effects which may be propagated as compromises in population vitality (presumably through effects on growth and reproduction) requires further

research.

5.4 Wye River

The Wye River was selected at the beginning of this two year study to represent a "relatively clean" pristine reference area without point source discharges. This station was located in a rural area where the major land-use is dominated by agricultural activity. Results from our water column testing in 1990 did not demonstrate the presence of toxic conditions in this area although both sediment and suborganismal testing did suggest adverse conditions (Hall et al., 1991). In contrast to our 1990 study, water column data from the present study demonstrated the presence of toxic conditions during both tests using two different species. A significant reduction in survival (25%) was reported for sheepshead minnow larvae during the first test. This finding was surprising since the sheepshead minnow is considered to be a moderately resistant species. The ambient water collected on the first day of Test 1 (8/13/91) had a pungent foul odor and a darker hue when compared with samples collected from other ambient areas. Survival of sheepshead minnow larvae was reduced to 38% after 24 h of exposure in this water. We examined the ambient water sample used during the first day of testing under a microscope and found dominant presence of a Gymnodinium dinoflagellate when compared with other plankton. Another water sample was collected from the Wye River the next day (8/14/91) and another test was initiated. This sample did not have a pungent odor and dominant presence of

Gymnodinium were not reported. Less than 5% mortality was reported from this test thus suggesting that some constituent in the Wye River water sample collected on 8/13/91 was responsible for the significant reduction in survival of sheepshead minnow larvae. One possible explanation was the presence of the Gymnodinium dinoflagellate. Other investigators have reported that small Gymnodinium-like species are responsible for mortality of various fish species (striped bass, spot and summer flounder, among others) held in the laboratory (Jo Ann Burkholder, personal communication, North Carolina State University). This dinoflagellate causes adverse effects in fish more readily than invertebrates. Based on conversations with Dr. Burkholder, it appeared that the Gymnodinium species in the Wye River sample was similar to the species she has reported. Current research by Dr. Burkholder is designed to identify the trigger for Gymnodinium blooms in the environment. She suspects nutrient loading (likely phosphorous) as the primary factor responsible for the blooms. These data have important implications for ambient testing in the Wye River which is heavily influenced by nutrient loading.

Results from the second test at this station in 1991 also demonstrated significant biological effects with a different test species. Significant reductions in survival were reported for E. affinis after 8 d of exposure in Wye River water. Gymnodinium was not suspected as a cause of mortality with this species as dominant numbers of this dinoflagellate were not observed in water collected during this test.

Copper (2.9 ug/L) and nickel (9.3 ug/L) concentrations exceeding U. S. EPA marine water quality criteria during the first test in 1991 may have been stressful to sheepshead minnow larvae and produced a "joint toxicity" response concurrently with the Gymnodinium species (U.S. EPA, 1987a). Both of these metals also exceeded water quality criteria during our 1990 testing at this station thus suggesting their continued presence over a one year period. None of the metals measured during the second test exceeded the U.S. EPA water quality criteria although detectable concentrations of both chromium (2.9 ug/L) and copper (2.6 ug/L) were reported. Potentially toxic organic contaminants and water quality conditions were not reported at stressful concentrations in the water column for tests conducted during the 1990 or 1991 study. However, it is important to note that the semi-volatile organic contaminant 4 methyl phenol was reported in the sediment at this station during the first test in 1991. Concentrations of this organic contaminant were below values expected to be toxic but it may have been an indicator of other potentially toxic conditions. Due to the polar nature of this soluble organic, it would be expected to be more of a potential toxicant in water and not sediment (see discussion below).

In the 1990 sediment toxicity tests, survival of the grass shrimp was $\geq$ 99% for both the 10- and 20-day tests, and showed no significant differences in growth as compared to the controls (Hall et al., 1991). The worm and Lepidactylus dytiscus tests, however, exhibited significantly lower survival. The amphipod had only 57%

survival at the Wye Station after 10 days of exposure and 24% survival after 20 days. Amphipod growth (mean length) was significantly lower than the controls. The results of the worm bioassays were similar to those of the amphipod tests. There was statistically reduced survival of the worms in the Wye River sediments after 10 and 20-day exposures. The worm showed no significant growth effects. Since two of the three species tested (the amphipod and the worm) showed significant reduction in survival at the Wye River station, it is possible that there was some undetected contaminant present that contributed to the toxic response. Organic contaminants were not analyzed in the Wye sediments in 1990 due to funding limitations.

The sediment samples collected at the Wye River in 1991 were very different sediment from those tested in 1990. The sample tested in 1990 was 80% silt/clay. The sediment samples tested in 1991 were 93.7% sand (6.3% silt/clay) and 82.2% sand (17.8% silt/clay) for sets 1 and 2, respectively. In addition, the 1991 samples had only about 0.4% organic carbon as compared to 7.8% TOC in 1990. No significant effects were observed in the grass shrimp or amphipod tests in the Wye River sediments. The 1991 sediment did produce a significant reduction in survival of the worms for set 1 test sediments. It is suggested that this increased mortality may have been due to starvation rather than toxicity of the sediment. After adjustment of the data to compensate for particle size induced mortalities, there was still a significant reduction in survival (67%) as compared to the controls (100%). No

other significant responses of sediment test organisms were recorded.

Very low levels of inorganic contaminants were found in the sediment in 1991. Organic analysis indicated the presence of 4-methylphenol, although not in significant concentrations. It should be noted, however, that this contaminant is considerably more polar and soluble than most of the semi-volatile organic contaminants analyzed, so would not be expected in high concentrations in the sediments. However, its presence may be indicative of organic contaminants in the water, which may have been associated with transient events. This organic compound is associated with wood treatment and preservatives, and may have been introduced during the construction of a new bridge slightly downstream from the tidally influenced sampling site. Field technicians noted the strong smell of creosote when passing under this structure in transit to the collection site.

Hepatic EROD activities in Fundulus from the Wye River in 1991 were less that those found in 1990 and less than levels found in the St. John's Creek on the Patuxent River. Levels of EROD activity tended to be higher in September than in August, though variability masked any statistical differences. EROD activities in Wye River Fundulus were also higher than EROD activities in Fundulus from the same location held in the laboratory over several months. Based on the two years of data, it appears as though the Wye River station may contain enough planar aromatic hydrocarbons (probably PAH) to cause elevations in hepatic EROD activities.

EROD activity is highly induced by components of creosote. The observations made above regarding 4-methylphenol and creosote suggest that these Fundulus may in fact be responding to these contaminants.

5.5 Laboratory Studies on Hepatic EROD Activity in Fundulus

In 1991, laboratory studies were conducted to determine the time necessary for EROD activities, which are elevated in field sampled fish, to decrease to baseline levels. In addition, laboratory experiments were conducted to determine if two different populations of Fundulus would respond similarly to challenge with a known planar aromatic hydrocarbon inducer. These experiments were conducted with Fundulus from the Wye River and from St. John's Creek in the Patuxent River.

It took approximately one month for hepatic EROD activities to fall approximately six-fold in Fundulus from St. John's Creek. EROD activity in Fundulus from the Wye River decreased the first week, but then elevated 2-3 fold from week one to two. Levels returned to initial collection levels at week three and continued to decline thereafter. While there is no immediate explanation for this behavior, we have hypothesized that it may have been due to a contamination event which came in through the Chesapeake Biological Laboratory Patuxent River intake lines. Wye River Fundulus were not sampled on the same dates as those from St. John's Creek and did not show this spike in activity.

There was no significant difference in the response of the two

different Fundulus populations to challenge with benzo(a)pyrene. Both were modestly induced 96 hours after a 2 mg/kg treatment and significantly induced by 10 mg/kg. Additional studies demonstrated that gonadal maturity of female Fundulus did not prevent the hepatic EROD induction response following treatment with B(a)P. This indicates that studies using Fundulus EROD activity as a measure of planar aromatic hydrocarbon exposure will not be compromised by the use of females during the reproductive season.

SECTION 6

CONCLUSIONS AFTER TWO YEARS OF TESTING

This two year pilot study was designed to use a suite of water column, sediment and suborganismal tests on a limited spatial and temporal scale to identify toxic ambient areas in the Chesapeake Bay watershed. In 1990, the first year of testing, eight stations from the following areas were tested once: Elizabeth River, Patapsco River, Wye River, Potomac River-Indian Head, Potomac River-Freestone Point, Potomac River-Possum Point, Potomac River-Morgantown and Potomac River-Dahlgren. Results from these three types of tests demonstrated the presence of toxic conditions in various environmental media in suspected contaminated areas such as the Elizabeth River and the Patapsco River and also in critical habitat areas in the Potomac River (Table 6.1, Fig. 6.1) (Hall et al., 1991). One of the more surprising results from the first year of testing was the data reported from sediment and suborganismal tests in the suspected toxic-free area of the Wye River. Both sediment and suborganismal tests suggested the presence of toxic conditions in this area while water column tests failed to demonstrate any adverse biological effects during the 1990 testing (Table 6.1). Results from sediment tests at the Freestone Point and Possum Point Stations in the Potomac River also suggested the presence of toxic conditions. Neither water column or suborganismal tests showed the presence of toxic conditions at these stations. The data collected in 1990 demonstrate the

Table 6.1 Toxicity data from water column, sediment and suborganismal tests conducted in 1990. Effects were noted if significantly different than controls for water column and sediment tests. The following abbreviations were used for various effects: NE = No Effect; M = Mortality; RG = Reduced Growth; RR = Reduced Reproduction; RB = Reduced Reburial; IE = Induced EROD Activity; RE = Reduced EROD Activity and LA = Liver Abnormality. Station abbreviations were as follows: ER = Elizabeth River; PR = Patapsco River; WR = Wye River; IH = Indian Head: FP = Freestone Pt; PP = Possum Pt; MT = Morgantown and DA = Dahlgren.

	Station							
	ER	PR	WR	IH	FP	PP	MT	DA
Test Type								
Water Column								
E. affinis	M	NE*	NE*	NE*	NE*	NE*	NE*	NE*
P. pugio	M	M	NE	NE	NE	NE	NE	NE
C. variegatus	NE	NE	NE	-	-	-	M	M
Ceriodaphnia sp.	-	-	-	M,NE	NE	NE	-	-
P. promelas	-	-	-	NE	NE	NE	-	-
Sediment								
L. dytiscus	M	M,RB	M,RG	M	M	M	M	M
S. benedicti	M	M	M	NE	NE	NE	NE	NE
P. pugio	M	NE	NE	NE	NE	RG	NE	NE
Suborganismal**								
Fundulus - water	M	RE	NE	-	-	-	NE	NE
Fundulus - sediment	M	NE	NE	NE	NE	NE	NE	NE
Feral Fish	IE	IE	IE	NE	NE	NE	LA	NE

*Low control reproduction limits the use of this endpoint; therefore, NE is based on survival.

**Qualitative determinations.

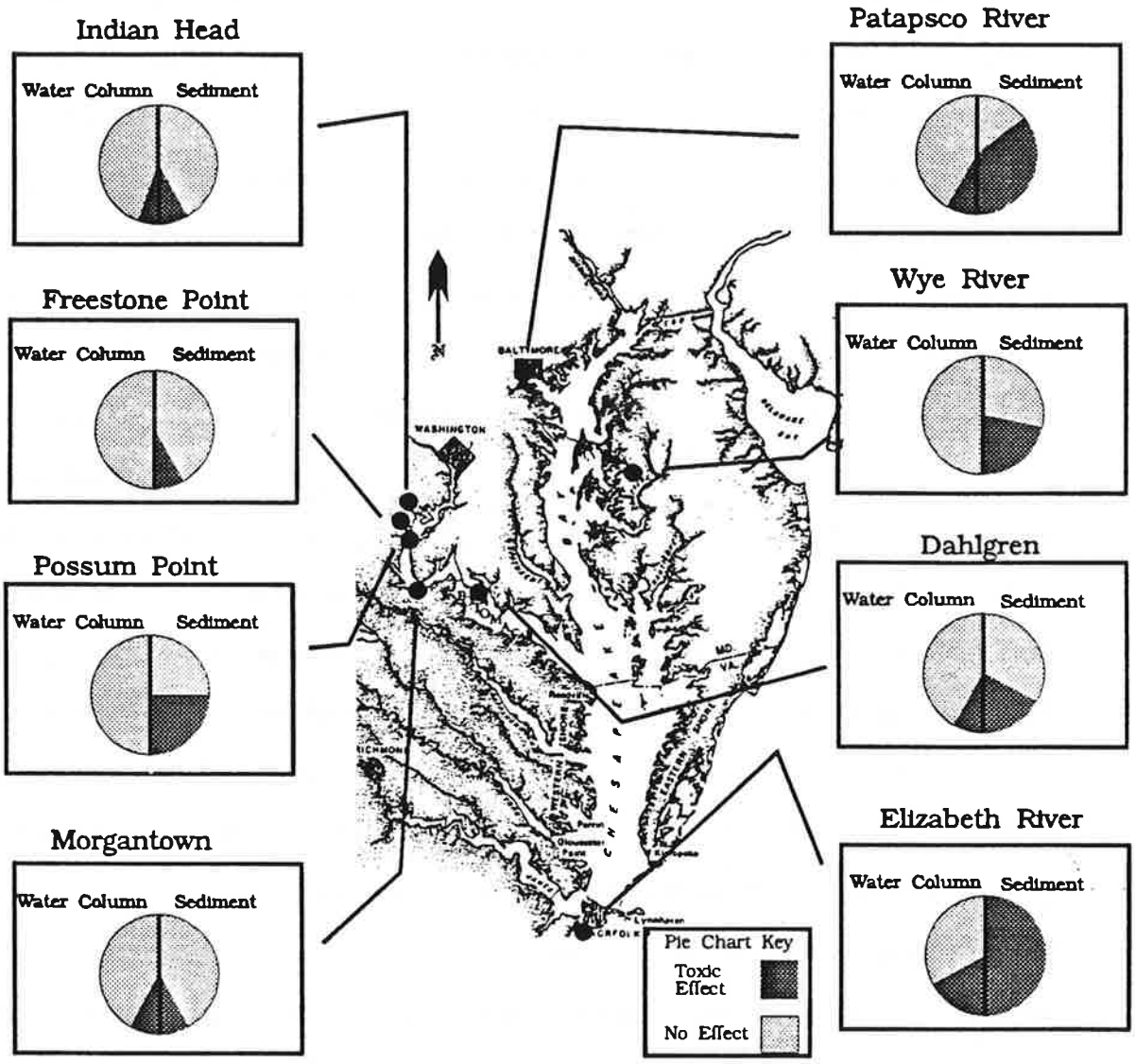


Fig. 6.1 Toxic effects reported from water column and sediment toxicity tests in 1990. The shaded portion of the pie-charts indicate the percent of the total tests in which the endpoints were significantly different from the controls ($p \leq 0.05$). There were at least 2 endpoints for each species.

importance of using a battery of tests when attempting to identify toxic conditions in the ambient environment.

Ambient toxicity testing in 1991 (year 2) was expanded on a temporal scale to include tests at two different time periods (summer and fall) but reduced on a spatial scale to only four of the eight stations tested in 1990 (Patapsco River, Wye River, Potomac River-Morgantown and Potomac River-Dahlgren). Biological effects were demonstrated from water column tests during at least one sampling period for all stations except the Patapsco River (Table 6.2, Fig. 6.2). The most persistent biological effects were reported from the Wye River station, as significant mortality from two different water column test species was reported from both the first (summer) and second (fall) tests. Sediment tests demonstrated significant biological effects for both summer and fall tests at the Dahlgren, Morgantown and Patapsco River stations (Table 6.2, Fig. 6.2). Significant biological effects were reported in sediment during the first test at the Wye River but not the second. Suborganismal tests suggested that the Wye River and the Patapsco River stations contained concentrations of planar aromatic hydrocarbon contaminants (e.g. PAH) sufficient to cause elevations of hepatic EROD activity in Fundulus and spot, respectively. Laboratory tests with Fundulus from the Wye River and St. John's Creek demonstrated that there were no population differences in the ability of Fundulus to respond to aromatic hydrocarbon EROD inducers. White perch from the Morgantown and Dahlgren stations contained high concentrations of copper in their

Table 6.2 Toxicity data from water column and sediment tests conducted in 1991. Test 1 and Test 2 were conducted August 13-21 and September 24-October 2, respectively. Effects were noted if significantly different than controls. The following abbreviations were used: NE = No Effect, M = Mortality, RG = Reduced Growth, RB = Reduced Reburial. Stations were DG = Dahlgren; MT = Morgantown; Wye = Wye, and PA = Patapsco.

Test Type	Station and Test							
	DG		MT		Wye		PA	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Water Column								
E. affinis	NE	NE	NE	NE	NE	M	NE	NE
Sheepshead	RG	NE	RG	NE	M	NE	NE	NE
Grass Shrimp	NE	NE	NE	NE	NE	NE	NE	NE
Mysid Shrimp	NE*	NE	NE*	NE	NE*	NE	NE*	NE
Sediment								
L. dytiscus	NE	M,RG	NE	M	NE	NE	M	M,RG,RB
P. pugio	NE	NE	NE	NE	NE	NE	NE	NE
H. azteca	M	M	M	NE	NE	NE	M	M,RG
S. benedicti	M,RG	NE	M,RG	NE	M,RG	NE	M,RG	M

*Mortality was the only parameter evaluated

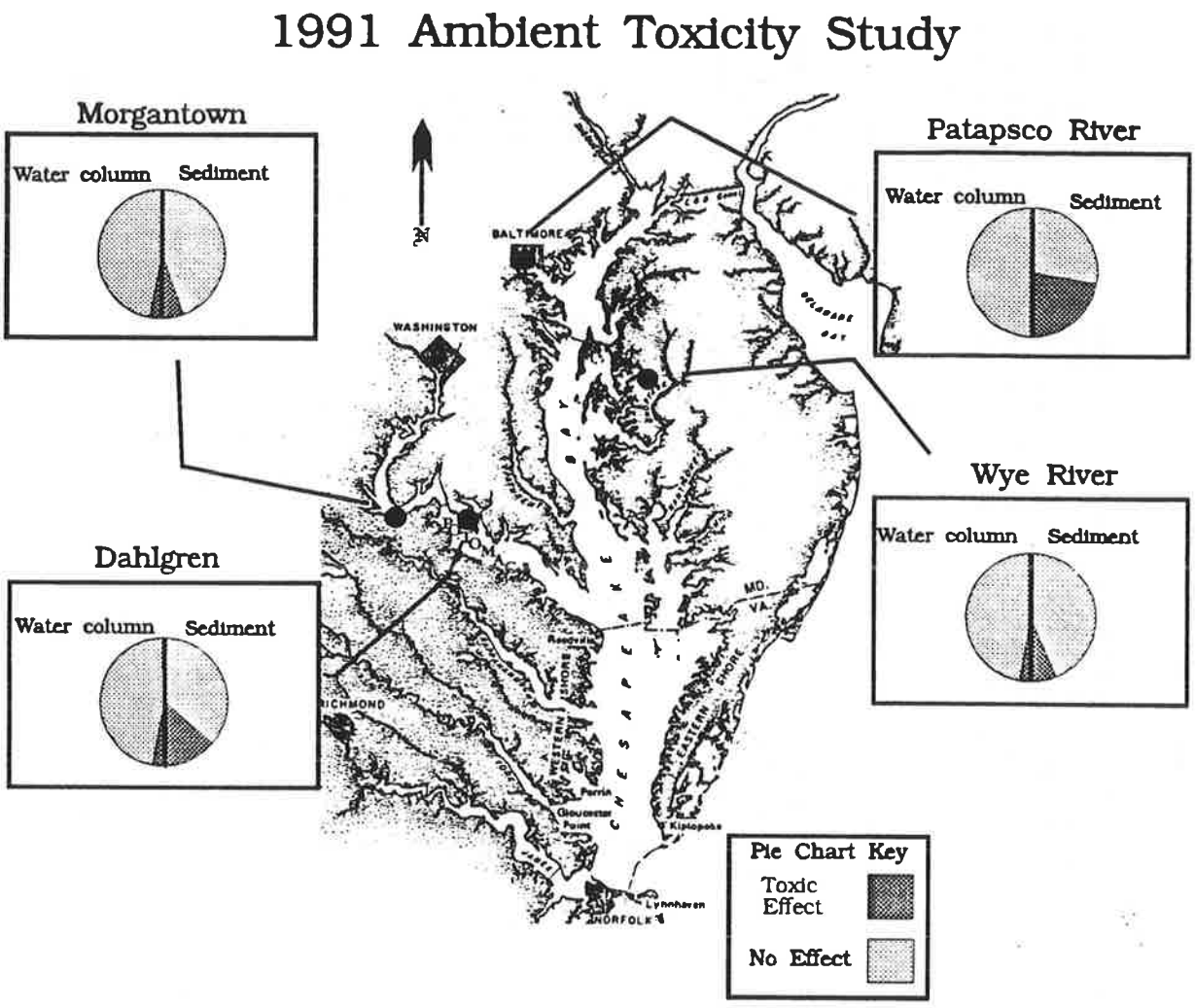


Fig. 6.2 Toxic effects reported from water column and sediment toxicity tests in 1991. The shaded portion of the pie-charts indicate the percent of the total number of tests in which the endpoints were significantly different from controls ($p \leq 0.05$). There were at least 2 endpoints for each species.

livers.

A comparison of water column and sediment data from 1991 testing shows general agreement at both Potomac River stations during test 1. One out of four test species showed a toxic effect with water column testing; two out of four tests showed a toxic effect with sediment. Water column and sediment data for test 2 at both Potomac River stations are less similar. No effects were reported at either station for water column tests. For sediment tests, significant toxic effects for two test species were reported at Dahlgren and one test species at Morgantown. Water column and sediment data at the Wye River station for test 1 showed a general agreement as a significant biological effect was reported from one test species for each test type. Water column tests showed significant biological effects from one test species for the second test while sediment tests showed no effects for any of the four test species. Water column and sediment tests demonstrated drastically different results at the Patapsco River station. No effects were reported for water column tests during either test; sediment tests (3 out of 4) demonstrated significant effects during both test 1 and 2.

The same result should not necessarily be expected with concurrent water column and sediment tests nor should samples collected at different times give the same result. Toxic conditions in water tend to be more transient and may be related to periodic discharges of toxic materials or episodic events (i.e., runoff of contaminants after precipitation events). Conversely,

toxic conditions in sediments are more persistent, as this media serves as a sink for contamination, while water column toxicity may represent current problems. Of course, sediments can be a non-point source of environmental contamination and can serve as a source for toxic conditions to over-lying water. Toxicity data generated from concurrent water column and sediment testing provide insight into areas that need additional investigation.

In general, the results from suborganismal hepatic EROD activity in feral fish from the Wye River and the Patapsco River agreed most closely with the sediment test results. The fish species we used are both benthos feeding fishes and would presumably receive most of their contaminant exposure through this route. Thus, while we are not suggesting that in all cases these two types of tests should concur, it is not unreasonable to suggest that sediment toxicity tests and sublethal measures in benthos feeding fishes might agree qualitatively, when the contaminants causing effects are primarily particle associated.

The need for multispecies testing within each type of test is clearly demonstrated for both years of testing. During the first year of testing, two water column tests (E. affinis and sheepshead minnow larvae) conducted at the Patapsco River station showed no significant effects. However, the third test species (larval grass shrimp) demonstrated reduced survival at this station (Table 6.1). The same scenario existed for sediment tests conducted at all Potomac River stations in 1990. The worm tests showed no effects at any of the 5 stations and the grass shrimp demonstrated no

effects at 4 of the stations. In contrast, reduced survival of amphipods was observed at all 5 stations.

Water column data from 1991 demonstrated significant effects (reduced survival) from the sheepshead minnow larval test during test 1 at both Dahlgren and Morgantown stations. The other three test species showed no effects. Two different test species (sheepshead minnows and E. affinis) showed effects at each of the two Wye River tests. Sediment tests generally demonstrated effects from more than one species when effects were reported. For example, adverse biological effects were reported from at least two test species during Dahlgren Test 1 and 2, Morgantown Test 1 and Patapsco River Test 1 and 2. A biological response from sediment tests was only reported from one test species during Morgantown Test 2 and Wye River Test 1.

A summary of species responses from water column tests in 1990 and 1991 is presented in Table 6.3. The sheepshead minnow larval test was the most sensitive estuarine water column test followed by the E. affinis and the larval grass shrimp test. The Ceriodaphnia test was more sensitive than the fathead minnow test based on a limited number of freshwate: water column tests. Although these data suggest a possible ranking of sensitivity among estuarine water column tests, it is important to note that if only the most sensitive species (sheepshead minnow) was used, biological effects would not have been reported at the Elizabeth River station during 1990 and the Wye River station (test 2) during 1991. These data strongly support the need for multispecies testing.

Table 6.3 Summary of water column test species responses from 1990 and 1991 ambient toxicity testing.

Test Species	Number of Tests	Number of Tests With Significant Effects	% of Tests with Significant Effects
E. affinis	16	2	12.5
Sheepshead Minnow	16	5	31
Grass Shrimp	16	2	12.5
Mysid Shrimp	8	0	0
Ceriodaphnia sp (freshwater)	4	1	25
P. promelas (freshwater)	3	0	0

A summary of species responses from sediment tests in 1990 and 1991 is presented in Table 6.4. Sediment tests were generally found to be more sensitive in detecting toxic conditions than water column tests (See Table 6.3). The amphipod (L. dytiscus) was the most sensitive species followed by H. azteca (freshwater), S. benedicti, and P. pugio (juvenile grass shrimp). The rationale for multispecies testing with sediment was not as obvious during the 1990 testing as significant effects were found at all eight stations with the most sensitive test species (L. dytiscus). However, 1991 data illustrate three examples (Dahlgren Test 1, Morgantown Test 1 and Wye River Test 1) where L dytiscus detected no biological effects while other test species did show significant effects. These data demonstrate the importance of multispecies testing with sediment.

Suborganismal testing provided several important pieces of information during 1990 and 1991. Hepatic monooxygenase activity (EROD) in feral Fundulus and spot provided a useful biological confirmation of what is already documented regarding PAH contamination of the Elizabeth and Patapsco River systems. Surprisingly, EROD activity in feral Fundulus from the Wye River suggested a level of contamination not previously suspected. These two species appear to be suitable for further surveys of this type.

EROD activity in feral white perch did not appear to provide the same type of information as spot and Fundulus. We caution, however, that we were not able to sample all three species at each station and therefore do not have adequate comparative information

Table 6.4 Summary of sediment test species responses from 1990 and 1991 ambient toxicity testing.

Test Species	Number of Tests	Number of Tests With Significant Effects	% of Tests with Significant Effects
L. dytiscus	16	12	75
S. benedicti	16	8	50
P. pugio	16	2	12.5
H. azteca (freshwater)	8	5	62.5

(one of the difficulties any survey of fishes from broad areas of the Chesapeake will encounter, is the lack of overlap in suitable habitat for different species). We did note that white perch had generally low hepatic EROD activities, hepatic concentrations of copper were extremely high. Until further laboratory studies are conducted with white perch to demonstrate their responsiveness to aromatic hydrocarbon monooxygenase inducers in the presence and absence of high hepatic copper, they are not suitable for surveys of the type attempted here.

Evaluation of hepatic monooxygenase activity in Fundulus exposed to test water and sediment in 1990 did not necessarily reflect what was seen in the feral fish. We therefore omitted these tests in 1991. Lack of concordance between measurements of EROD activity in feral Fundulus and of activity in Fundulus exposed to sediments from these same areas may not be surprising. If sediment contaminants are the main source of exposure, either through direct ingestion of sediments or through consumption of organisms that have been contaminated by these sediments, then the way these tests were conducted may have been inappropriate. Fundulus were exposed to test sediment in aquaria only after indigenous organisms had been removed. The fish were fed exogenously using a commercial feed. Therefore, the only exposure they would have received would have been via desorption of contaminants from the sediments into the overlying water column. These aqueous concentrations were apparently not high enough to cause induction to the levels seen in feral fish, though they were

high enough to cause mortality using Elizabeth River sediments. In this scenario, the use of feral fish is clearly supported.

Additional observations from laboratory studies suggest that Fundulus from different geographic areas should respond similarly to aromatic hydrocarbon inducers, regardless of stage of sexual maturity. Animals collected from the field should be held at least one month in order to reduce the influence of "background" elevation of hepatic EROD activity.

The "apparent temporal pattern" in ambient toxicity can be evaluated on a very limited basis using water column and sediment data from four stations tested once in 1990 and twice in 1991 (Tables 6.1 and 6.2). It is not possible to state conclusively that "apparent temporal patterns" are beyond the environmental "year to year" variability. Water column data from the Wye River showed a drastic change in the presence of toxic conditions between the two years. No biological effects were reported from any of the three test species in 1990 but significant effects were found during both tests in 1991. Water column data from the Patapsco River suggested contrasting results. Biological effects were reported from the grass shrimp test in 1990 but no effects were reported from either test 1 or test 2 in 1991 using any of the four test species. A comparison of data generated from water column tests at Morgantown and Dahlgren stations over a one year period demonstrated different results each year. A severe biological effect was reported in 1990 with sheepshead minnow larvae (significant mortality), a less severe sublethal effect was

reported with this species during test 1 of 1991 (reduced growth) and no effects with any of the test species were found during the second test in 1991.

Apparent temporal patterns in sediment toxicity data over the three testing periods during the two years (1990-1991) showed a decrease in toxicity at the Wye River station. Adverse biological effects from two species were reported in 1990, effects from one species were reported during the first test in 1991 and no effects were reported from the second test in 1991. Sediment toxicity data from the Patapsco River demonstrated persistent toxic conditions over the two year period. Two tests detected toxic conditions in 1990 while three tests suggested toxic conditions in 1991. Sediment toxicity data from both the Morgantown and Dahlgren stations also demonstrated persistent biological effects. Data from either one or two test species suggested the presence of toxic conditions from the three sampling periods in 1990 through 1991.

There were distinct temporal differences in EROD activity in feral fish. Fundulus from the Wye River had lower EROD activity in 1991 than 1990 and activity was similar during August and September of 1991. Spot from the Patapsco River had lower EROD activities in 1991 than 1990, with the lowest activity found in September of 1991. In general, this suggests that exposures to contaminants were less during 1991 at these two stations.

Water quality and contaminants evaluations conducted in water and sediment during this pilot study provided supportive information on possible causes of biological effects but these data

were not intended to provide conclusive data on specific "cause and effect" relationships. In 1990, the highest number of potentially toxic contaminants in both water and sediment were reported at the station (Elizabeth River) where biological effects from the greatest number of tests were reported. A similar scenario existed for the second most toxic area tested in 1990 (Patapsco River). The relationship between contaminants data and biological data at the Wye River station was not positively correlated. Both sediment and suborganismal tests suggested toxic conditions while water column tests did not. Toxic inorganic contaminants were not detected in the Wye River sediment and organic evaluations were not conducted in this media. In contrast, two potentially toxic metals were reported in the water column at the Wye River station.

Contaminants data from the Potomac River stations in 1990 provided various examples of biological effects with and without the identification of potentially toxic contaminants (Hall et al., 1991). One potentially toxic concentration of a metal (cadmium) was reported in the water column at the Indian Head and Freestone Point station and four to five potentially toxic metals concentrations (cadmium, mercury, nickel, lead and zinc) were measured in the sediment at each of these stations (Hall et al., 1991). Results from one water column test and one sediment test demonstrated toxic conditions at Indian Head. However, only one sediment test showed toxic conditions at Freestone Point. Potentially toxic concentrations of metals were not found in the water column at Possum Point but five potentially toxic metals were

found in the sediment (cadmium, mercury, nickel, lead and zinc). The amphipod test (sediment exposure) demonstrated reduced survival while no significant effects were reported during water column or suborganismal tests. Potentially toxic contaminants were not reported in sediment from the Morgantown or Dahlgren station. Two metals exceeded U.S. EPA water quality criteria at Morgantown (mercury and nickel) and no potentially toxic contaminants were reported in the water column at Dahlgren. Significant effects were reported from water column and sediment tests at both Morgantown and Dahlgren.

A comparison of a biological effects data with the limited contaminant evaluations for water column tests in 1991 demonstrated examples of both good and poor linkages between these types of data. A good correlation was reported for the first Wye River test as significant mortality was observed for sheepshead minnow larvae (August 13 test) concurrently with the presence of two potentially toxic metals (copper and nickel). As previously discussed in Section 5.4, the presence of a potentially toxic Gymnodinium dinoflagellate may have also contributed to the mortality of the test species. The second Wye River test is an example of a poor correlation between biological effects and the presence of toxic contaminants. Significant mortality of E. affinis was reported but potentially toxic contaminant conditions were not identified.

Data collected from the Patapsco River station in 1991 provided a strong case for the link between biological effects in sediment and the presence of potentially toxic contaminants.

Chromium, lead and zinc were reported at potentially toxic concentrations in sediment concurrently with biological effects from three of the four sediment test species. Data from the Morgantown station also provided an example of biological effects in sediment (adverse effects for two tests) concurrently with the presence of potentially toxic concentrations of 4,4 '- DDT. The Dahlgren station (Test 1 and 2) and Wye River (Test 1) provided examples of biological effects without the identification of potentially toxic contaminant conditions.

One of the primary conclusions from this two year pilot study is that integrated water column and sediment toxicity testing is a feasible approach for assessing toxic conditions in ambient areas of the Chesapeake Bay watershed. Suborganismal testing provided additional important insights, though it did not reveal any toxic conditions where water column and/or sediment tests were negative. Technical constraints on the rate at which suborganismal testing can provide data per unit cost, compared to the water column and sediment tests, lead us to conclude that this testing framework may not be optimal for the inclusion of suborganismal testing. While sublethal effects measurements in indigenous organisms may clearly play a role in determining the conditions of a habitat with respect to certain forms of contamination, they require an experimental design separate from the design of water column and sediment tests.

The pilot phase has allowed us to determine the most feasible tests for accomplishing our goals and discard those tests that are less promising. Detecting toxic conditions in areas suspected to

be toxic such as the Elizabeth River was expected but this step was necessary to "ground truth" our test approach. One of our initial concerns using this test approach was the sensitivity of the test methods in detecting toxic conditions in areas that may exhibit low levels of contamination. Results from both the Wye River and Potomac River stations demonstrated that our test methods (species) were sensitive enough to detect low levels of contamination. The next step in the developmental process of this ambient toxicity test approach is to determine the variability of test results on both a spatial and temporal scale and assess the relative toxicity of ambient water and sediment using statistical methods. The third year of this research effort has been designed to address the question of spatial and temporal variability on a limited scale using a battery of sensitive water column and sediment toxicity tests. Statistical analysis will also be conducted to determine the relative toxicity of water and sediment from ambient areas and provide a degree of confidence to observed differences between ambient areas and reference areas (controls).

The final developmental phase of this test approach will be to consolidate and evaluate data from all three years of the pilot study and then assess the nature and magnitude of any remaining uncertainties in the methodology. The third year of the pilot study began in July 1992. If the ambient toxicity methodology is determined to be a useful approach, the Bay program or any interested state agency could use the methodology to design a multi-year Bay-wide or regional assessment of ambient toxicity in

important living resource habitats. The ambient toxicity methodology developed during the three year pilot study could also be added to an array of multi-metric assessment tools that the Chesapeake Bay Program is beginning to develop and use in targeting tributaries and watersheds for nonpoint source monitoring and remediation. The goals of this targeting effort will be to determine where management-based habitat improvement programs should be focused, based on the status of biological communities and other environmental indicators. Follow-up studies could then be designed for any areas of the Bay watershed where these multi-metric assessments (including the ambient toxicity approach) demonstrate evidence of toxic conditions to purse "cause and effect" relationships, identify sources of toxic conditions, assess the degree of impact to living resources communities and develop control strategies.

SECTION 7

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APPENDIX A

The Percent of Females Carrying Eggs After 8-d Exposures in the *Eurytemera affinis* Tests

TEST #1

(8/21/91)

H-W Control		%	$\bar{x}$ % ± S.E.
A	5/10	50	57.9 ± 4.8
B	8/12	66.7	
C	4/7	57.1	
Dahlgren			
A	4/11	36.4	
B	6/7	85.7	62.9 ± 14.4
C	6/9	66.7	
Patapsco			
A	8/13	61.5	
B	4/11	36.4	49.3 ± 7.3
C	5/10	50	
Wye			
A	5/8	62.5	
B	3/8	37.5	42.9 ± 10.1
C	2/7	28.6	
Morgantown			
A	3/11	27.3	
B	6/10	60	41.6 ± 9.7
C	3/8	37.5	

TEST #2

(10/2/91)

H-W Control		%	$\bar{x}$ % ± SE
A	4/8	50	
B	1/11	9.1	39.7 ± 15.6
C	6/10	60	
IR Control			
A	3/13	23.1	
B	4/7	57.1	46.7 ± 11.8
C	9/15	60	
Patapsco			
A	5/7	71.4	
B	4/13	30.8	34.1 ± 20.7
C	0/1	0	
Dahlgren			
A	9/16	56.3	
B	6/8	75	57.1 ± 10.1
C	4/10	40	
Wye			
A	2/5	50	
B	6/8	75	46.7 ± 3.3
C	4/10	40	
Morgantown			
A	6/11	54.5	59.1 ± 4.6
B	7/11	63.6	

APPENDIX B

Water Quality Conditions Reported in Test Beakers During all Water Column Tests

Experiments conducted with Palaemonetes pugio (pg), Cyprinodon variegatus (Cv), Eurytemora affinis (Ea) and Mysidopsis bahia (Mb) using water samples collected from the Wye River (WR), Patapsco River (PR), Morgantown (MT) and Dahlgren (DG) from 8/13/91 to 8/21/91 and 9/24/91 to 10/2/91.

Date	Test Species	Station	Temp	Sal (ppt)	DO (mg/L)	pH
8/14/91	Pp	WR	26.2	14	9.0	8.6
		PR	26.3	15	8.8	8.4
		MT	25.3	16	5.6	8.1
		DG	25.2	17	6.9	8.0
		Control	26.9	15	6.2	8.2
	Cv	WR	25.0	14	5.8	8.0
		PR	24.8	15	7.8	8.3
		MT	24.7	16	6.5	8.2
		DG	24.9	17	6.2	8.2
		Control	24.8	15	6.5	8.2
	Ea	WR	26.0	14	5.2	8.1
		PR	26.0	15	7.1	8.5
		MT	26.0	15	7.2	8.3
		DG	16.0	17	6.4	8.2
		Control	25.8	15	7.3	8.3
	Mb	WR	25.0	14	6.0	7.8
		PR	25.0	15	6.6	7.9
		MT	25.0	16	5.9	8.1
		DG	25.2	17	5.2	7.8
		Control	24.8	15	5.1	8.1
8/15/91	Pp	WR	24.9	15	6.1	8.0
		PR	24.9	20	8.9	8.4
		MT	25.1	17	6.7	8.0
		DG	25.1	19	6.6	8.0
		Control	24.5	18	7.3	8.1
	Cv	WR	25.4	14	5.2	7.9
		PR	25.3	16	7.7	8.2
		MT	25.2	16	6.6	7.9
		DG	25.3	18	6.6	7.9
		Control	25.7	15	6.2	8.0
	Ea	WR	25.3	14	5.5	7.8
		PR	25.3	16	7.5	8.2
		MT	25.6	16	6.9	8.1
		DG	25.4	18	7.4	8.2
		Control	25.8	15	7.9	8.3

Date	Test Species	Station	Temp	Sal (ppt)	DO (mg/L)	pH
8/15/91	Mb	WR	25.8	14	4.3	7.5
		PR	25.0	16	6.6	7.7
		MT	25.4	16	5.3	7.8
		DG	25.7	18	5.0	7.7
		Control	25.6	15	5.5	7.9
8/16/91	Pp	WR	24.9	15	4.4	7.7
		PR	25.6	18	6.9	8.2
		MT	24.7	18	6.2	7.7
		DG	25.0	19	5.2	7.7
		Control	25.4	16	5.4	8.0
	Cv	WR	25.0	14	4.7	7.7
		PR	25.0	16	6.7	8.2
		MT	24.7	16	5.1	7.8
		DG	25.0	18	5.3	7.7
		Control	25.1	15	4.8	8.1
	Ea	WR	25.8	14	4.6	7.6
		PR	25.1	16	6.7	8.1
		MT	25.2	17	7.4	7.9
		DG	25.0	18	7.4	8.0
		Control	25.2	15	6.4	8.2
	Mb	WR	24.9	14	4.3	7.6
		PR	24.9	16	5.0	7.8
		MT	24.6	16	4.8	7.5
		DG	24.7	18	4.4	7.6
		Control	25.1	15	4.8	8.0
8/17/91	Pp	WR	25.7	16	8.8	8.3
		PR	25.9	17	6.5	8.1
		MT	25.6	15	5.8	7.9
		DG	25.0	17	5.6	7.9
		Control	25.4	16	5.6	8.1
	Cv	WR	25.0	15	4.7	7.9
		PR	25.0	16	5.6	8.1
		MT	25.0	15	5.6	7.8
		DG	25.1	16	5.8	7.9
		Control	25.0	15	5.1	8.0
	Ea	WR	24.8	15	5.4	7.9
		PR	24.9	16	7.0	8.1
		MT	24.9	15	7.1	8.1
		DG	24.8	16	7.1	8.1
		Control	25.0	15	7.7	8.2

Date	Test Species	Station	Temp	Sal (ppt)	DO (mg/L)	pH
8/17/91	Mb	WR	25.1	15	4.1	7.7
		PR	25.0	15	4.7	7.7
		MT	25.0	15	4.4	8.0
		DG	25.1	16	4.3	7.6
		Control	25.1	15	5.2	7.9
8/18/91	Pp	WR	25.1	16	7.5	8.1
		PR	25.1	17	6.9	8.1
		MT	25.3	16	6.1	7.9
		DG	25.0	17	5.7	7.9
		Control	25.1	16	6.8	8.2
	Cv	WR	25.0	15	5.1	7
		PR	25.0	16	4.7	7.8
		MT	24.9	15	5.6	7.8
		DG	25.0	16	5.8	7.8
		Control	25.0	15	4.7	8.0
	Ea	WR	25.0	15	6.1	8.0
		PR	25.2	16	6.8	8.2
		MT	24.9	15	6.8	8.1
		DG	25.0	16	6.8	8.1
		Control	25.1	15	7.0	8.2
	Mb	WR	24.8	15	4.0	7.5
		PR	25.0	16	5.2	7.6
		MT	25.0	15	5.4	7.8
		DG	24.8	16	4.8	7.6
		Control	25.0	15	5.0	8.0
8/19/91	Pp	WR	25.0	15	6.6	7.9
		PR	26.0	16	8.4	8.3
		MT	25.0	15	7.7	6.3
		DG	25.0	15	6.6	7.7
		Control	25.5	15	9.7	8.7
	Cv	WR	25.0	14	6.0	7.9
		PR	25.2	15	6.5	8.1
		MT	25.1	14	6.4	8.0
		DG	25.0	15	5.9	7.9
		Control	25.3	15	5.8	8.2
	Ea	WR	25.3	14	6.2	7.9
		PR	-	-	-	-
		MT	26.0	14	6.5	8.0
		DG	25.4	15	6.8	8.0
		Control	25.6	15	-	8.5

Date	Test Species	Station	Temp	Sal (ppt)	DO (mg/L)	pH
8/19/91	Mb	WR	25.0	14	4.9	7.7
		PR	25.0	15	5.0	7.7
		MT	24.9	14	5.5	7.8
		DG	24.9	15	4.8	7.7
		Control	25.5	15	5.1	8.0
8/20/91	Pp	WR	25.3	15	6.7	8.2
		PR	25.4	15	9.0	8.6
		MT	26.8	14	6.3	7.9
		DG	25.2	14	6.7	8.1
		Control	25.2	15	9.7	8.7
	Cv	WR	25.0	14	6.6	8.0
		PR	24.9	15	6.4	8.0
		MT	25.0	14	7.0	8.0
		DG	24.9	14	6.9	8.0
		Control	25.0	15	5.5	7.9
	Ea	WR	24.9	14	6.6	8.0
		PR	25.0	14	6.9	8.3
		MT	24.9	14	6.6	8.0
		DG	24.8	14	6.9	8.0
		Control	25.3	15	7.5	8.4
	Mb	WR	25.1	14	5.3	7.8
		PR	25.1	15	4.9	7.7
		MT	25.1	14	6.1	8.0
		DG	25.2	14	5.8	7.7
		Control	25.2	15	6.0	8.1
8/21/91	Pp	WR	25.4	13	6.9	8.2
		PR	26.0	14	7.0	8.5
		MT	25.8	14	7.4	8.0
		DG	24.9	13	7.7	8.2
		Control	25.0	15	5.9	8.7
	Cv	WR	24.5	14	6.9	7.9
		PR	24.5	14	7.0	8.0
		MT	24.5	13	7.4	8.0
		DG	24.5	13	7.7	8.2
		Control	24.5	15	5.9	8.2
	Ea	WR	25.5	14	6.5	7.9
		PR	25.0	14	6.8	7.9
		MT	26.0	12	6.2	7.9
		DG	25.0	13	6.7	7.9
		Control	25.0	15	7.9	8.4

Date	Test Species	Station	Temp	Sal (ppt)	DO (mg/L)	pH
8/21/91	Mb	WR	26.0	14	10.8	8.5
		PR	25.8	14	6.9	8.0
		MT	24.9	13	7.1	8.2
		DG	25.4	13	8.2	8.2
		Control	25.0	15	11.0	8.7
9/25/91	Pp	WR	24.5	15	6.3	7.9
		PR	24.5	15	6.1	7.8
		MT	24.5	12	6.3	7.8
		DG	25.0	13	6.5	7.9
		Control	25.0	15	6.2	8.3
	Cv	WR	25.0	15	6.8	8.1
		PR	25.0	16	7.4	8.1
		MT	25.0	12	6.4	7.8
		DG	25.0	12	6.5	8.0
		Control	25.0	15	6.6	8.3
	Ea	WR	25.5	15	7.3	8.0
		PR	25.0	15	7.5	8.0
		MT	25.0	13	7.2	8.0
		DG	25.0	14	7.2	8.0
		H-W Control	25.0	15	7.1	8.3
		I-R Control	25.5	15	7.0	8.0
	Mb	WR	25.0	15	6.7	8.0
		PR	24.5	16	6.8	7.9
		MT	24.5	12	6.0	7.8
		DG	25.0	12	6.4	7.8
		Control	25.0	15	5.9	8.3
9/26/91	Pp	WR	24.5	15	6.2	7.9
		PR	24.0	16	6.3	7.8
		MT	24.0	14	5.9	7.8
		DG	24.0	12	6.0	7.9
		Control	24.0	15	5.8	8.3
	Cv	WR	25.0	15	6.0	8.0
		PR	25.0	15	7.5	8.2
		MT	25.0	14	5.8	7.8
		DG	25.0	13	5.6	7.9
		Control	25.0	15	5.5	8.3
	Ea	WR	25.0	15	5.8	7.9
		PR	25.5	16	6.7	8.0
		MT	24.0	14	6.3	8.0
		DG	25.0	13	6.1	8.0
		H-W Control	25.5	15	6.8	8.3
		I-R Control	25.0	15	6.8	7.9

Date	Test Species	Station	Temp	Sal (ppt)	DO (mg/L)	pH
9/26/91	Mb	WR	24.0	15	5.7	7.9
		PR	24.5	16	6.2	7.8
		MT	24.5	14	5.9	7.8
		DG	25.0	14	5.8	8.1
		Control	24.0	15	5.1	8.2
9/27/91	Pp	WR	23.0	15	6.7	8.0
		PR	23.5	17	6.4	7.9
		MT	23.5	15	6.8	7.9
		DG	24.0	15	6.8	7.9
		Control	24.0	16	6.8	8.4
	Cv	WR	24.0	15	6.5	7.8
		PR	24.5	15	8.8	8.1
		MT	24.5	15	5.4	7.7
		DG	25.0	14	5.6	7.8
		Control	24.0	15	6.1	8.2
	Ea	WR	24.0	15	7.2	7.8
		PR	24.0	15	7.8	7.8
		MT	25.0	15	7.2	7.8
		DG	25.0	14	7.4	8.0
		H-W Control	24.0	15	8.0	8.3
		I-R Control	24.0	15	7.7	7.8
	Mb	WR	24.0	15	6.1	7.6
		PR	24.0	15	6.5	7.7
		MT	24.0	14	5.7	7.7
		DG	24.0	14	6.1	7.8
		Control	24.0	15	5.8	8.1
9/28/91	Pp	WR	22.0	15	6.4	7.9
		PR	22.0	16	6.6	7.8
		MT	22.5	15	6.9	7.9
		DG	22.5	15	6.9	7.9
		Control	22.0	15	6.8	8.4
	Cv	WR	24.5	15	4.1	7.8
		PR	25.0	15	5.5	7.9
		MT	24.0	15	4.9	7.6
		DG	24.5	14	4.6	7.6
		Control	24.0	15	4.4	8.2
	Ea	WR	25.0	15	6.5	8.0
		PR	25.0	15	6.6	8.0
		MT	24.5	15	6.8	8.0
		DG	24.5	14	7.0	8.0
		H-W Control	24.5	15	7.5	8.5
		I-R Control	24.5	15	7.5	8.1

Date	Test Species	Station	Temp	Sal (ppt)	DO (mg/L)	pH
9/28/91	Mb	WR	24.0	15	4.4	7.6
		PR	24.0	16	5.3	7.6
		MT	25.0	15	4.2	7.5
		DG	24.0	14	5.0	7.7
		Control	24.0	15	4.7	8.2
9/29/91	Pp	WR	24.0	15	6.9	8.0
		PR	24.0	16	6.7	7.9
		MT	24.0	16	7.0	7.9
		DG	24.0	15	7.0	8.0
		Control	24.0	16	7.2	8.4
	Cv	WR	24.5	15	4.2	7.6
		PR	24.5	15	5.3	7.7
		MT	24.0	14	4.6	7.5
		DG	24.0	14	4.2	7.6
		Control	25.0	15	4.5	8.2
	Ea	WR	24.5	14	7.0	8.0
		PR	24.5	14	6.9	8.0
		MT	24.5	15	7.2	8.1
		DG	24.5	15	7.1	8.0
		H-W Control	24.0	15	7.5	8.4
		I-R Control	24.0	15	7.3	8.1
	Mb	WR	24.0	15	4.1	7.6
		PR	24.0	15	4.6	7.6
		MT	24.5	15	4.4	7.5
		DG	24.0	14	4.8	7.6
		Control	24.0	15	4.4	8.1
9/30/91	Pp	WR	25.0	16	6.9	8.1
		PR	25.0	17	7.2	8.1
		MT	26.5	16	7.1	8.0
		DG	26.0	15	7.5	8.1
		Control	25.0	17	6.8	8.4
	Cv	WR	24.5	14	4.3	7.6
		PR	24.0	14	6.1	7.7
		MT	24.5	14	4.4	7.5
		DG	24.5	14	5.9	7.7
		Control	24.0	15	5.2	8.2
	Ea	WR	24.0	15	7.3	8.1
		PR	24.0	15	7.5	8.1
		MT	24.5	14	7.4	8.0
		DG	24.5	15	7.3	8.0
		H-W Control	23.0	15	7.9	8.5
		I-R Control	23.5	15	7.9	8.0

Date	Test Species	Station	Temp	Sal (ppt)	DO (mg/L)	pH
9/30/91	Mb	WR	25.0	15	4.4	7.6
		PR	24.0	14	4.9	7.6
		MT	24.0	14	4.4	7.5
		DG	24.0	14	5.6	7.7
		Control	24.0	15	4.3	8.1
10/1/91	Pp	WR	25.0	16	5.4	7.9
		PR	25.1	16	5.9	7.9
		MT	25.8	16	6.0	7.9
		DG	25.6	16	6.1	8.0
		Control	25.0	16	6.2	8.4
	Cv	WR	25.8	15	7.3	8.3
		PR	25.5	15	5.4	7.9
		MT	25.8	15	4.1	7.6
		DG	25.6	15	4.9	7.7
		Control	25.7	15	4.4	8.2
	Ea	WR	26.0	15	8.2	6.2
		PR	25.9	15	6.2	8.1
		MT	26.0	15	6.2	8.1
		DG	25.7	15	6.4	8.1
		H-W Control	25.9	15	6.6	8.5
		I-R Control	25.9	15	6.9	8.0
	Mb	WR	26.0	15	4.1	7.6
		PR	25.6	15	3.9	7.5
		MT	25.1	15	3.7	7.5
		DG	25.2	15	4.2	7.5
		Control	25.8	15	4.0	8.1
10/2/91	Pp	WR	25.0	16	6.5	8.0
		PR	25.0	16	6.9	8.0
		MT	25.0	16	7.0	8.1
		DG	25.0	16	7.0	8.0
		Control	25.0	16	6.6	8.4
	Cv	WR	-	-	-	-
		PR	25.5	15	6.2	7.8
		MT	25.5	15	7.1	8.0
		DG	25.5	15	8.0	8.2
		Control	26.0	15	6.2	8.3
	Ea	WR	25.0	15	6.5	8.0
		PR	-	-	-	-
		MT	25.5	15	7.1	8.0
		DG	25.0	15	7.4	8.5
		H-W Control	25.0	15	7.4	8.5
		I-R Control	26.5	15	7.5	7.8

Date	Test Species	Station	Temp	Sal (ppt)	DO (mg/L)	pH
10/2/91	Mb	WR	25.0	15	6.0	7.8
		PR	25.5	15	4.7	7.7
		MT	25.0	15	5.9	7.7
		DG	25.5	15	7.1	7.8
		Control	25.0	15	6.2	8.3

APPENDIX C

Summary of Water Quality Monitored During Sediment Toxicity Tests

Summary of water chemistry monitored during sediment toxicity tests, Set 1.

Set #1

Species	Site	Water Chemistry Range (min-max)					
		Temp (°C)		O_2 (mg/l)		pH	
		Day 1-10	Day 10-20	Day 1-10	Day 10-20	Day 1-10	Day 10-20
Grass shrimp *P. pugio*	Control	24-25	23-24	5.8-7.4	4.4-7.2	7.8-8.1	7.5-8.2
	Morgantown	24-25	23-24	6.5-7.0	5.5-7.3	7.8-8.1	7.6-8.1
	Dahlgren	24-25	23-25	5.3-6.9	3.4-7.2	7.8-8.1	7.6-8.1
	Patapsco	24-25	23-24	6.2-6.8	2.6-6.9	7.7-7.9	7.3-8.0
	Wye	24-25	24-24	6.3-7.0	5.3-7.1	7.8-8.0	7.7-8.0
	Reference	24-25	24-24	6.3-7.0	4.9-7.2	7.9-8.1	7.6-8.1
Amphipod *L. dysticus*	Control	23-25	25	7.0-7.5	6.5-7.8	8.3-8.0	8.3-8.1
	Morgantown	23-25	24-25	7.0-7.6	6.7-7.7	8.2-8.0	8.3-8.1
	Dahlgren	22-25	24-25	6.8-7.6	6.7-7.7	8.3-8.0	8.4-8.1
	Patapsco	23-25	24-25	6.5-7.5	6.5-7.6	7.8-8.0	7.7-8.0
	Wye	22-25	24-25	7.0-7.6	5.3-7.6	7.8-8.2	7.7-8.1
	Reference	23-25	24-25	6.5-7.8	6.7-7.8	8.0-8.2	7.9-8.1
Amphipod *H. azteca*	Control	24-25	24-24	6.6-7.4	6.8-7.5	8.1-8.2	7.9-8.4
	Morgantown	24-25	24-25	6.9-7.4	5.4-7.8	8.0-8.3	8.0-8.3
	Dahlgren	24-25	24-24	6.7-7.4	6.8-7.9	8.0-8.3	8.0-8.3
	Patapsco	24-25	24-25	6.8-7.4	6.8-7.9	7.8-8.2	8.0-8.2
	Wye	24-25	24-25	5.8-7.4	7.0-7.9	7.9-8.1	8.0-8.3
	Reference	24-25	24-25	7.0-7.5	6.2-7.9	8.0-8.3	8.1-8.3
Worm *S. benedicti*	Control	24-25	24-24	6.6-7.4	6.8-7.5	8.1-8.2	7.9-8.4
	Morgantown	24-25	24-25	6.9-7.4	5.4-7.8	8.0-8.3	8.0-8.3
	Dahlgren	24-25	24-24	6.9-7.4	6.8-7.9	8.0-8.3	8.0-8.3
	Patapsco	24-25	24-25	6.8-7.4	6.8-7.9	7.8-8.2	8.0-8.2
	Wye	24-25	24-25	5.8-7.4	7.0-7.9	7.9-8.1	8.0-8.3
	Reference	24-25	24-25	7.0-7.5	6.2-7.9	8.0-8.3	8.1-8.3

Summary of water chemistry monitored during sediment toxicity tests, Set 2.

Set #2

Species	Site	Water Chemistry Range (min-max)					
		Temp (°C)		O_2 (mg/l)		pH	
		Day 1-10	Day 10-20	Day 1-10	Day 10-20	Day 1-10	Day 10-20
Grass shrimp *P. pugio*	Control	24-25	23-24	5.6-7.8	5.5-7.4	7.7-8.2	7.7-8.1
	Morgantown	24-25	24-25	6.2-7.2	6.2-6.9	7.7-8.2	7.7-8.0
	Dahlgren	24-25	24	6.2-7.0	4.9-6.8	7.9-8.2	7.7-8.1
	Patapsco	24-25	24	5.5-6.6	4.2-7.3	7.6-8.1	7.6-8.1
	Wye	24-25	24	6.3-7.0	6.3-7.1	7.8-8.2	7.7-8.0
	Reference	24-25	24-25	5.5-7.2	6.2-7.2	7.8-8.2	7.8-8.1
Amphipod *L. dysticus*	Control	25-27	25	6.9-7.3	6.9-7.8	7.9-8.3	7.8-8.3
	Morgantown	25-27	25	6.8-7.3	6.9-7.4	7.9-8.3	7.8-8.3
	Dahlgren	25-27	25	5.5-7.0	6.9-7.2	7.9-8.4	7.6-8.2
	Patapsco	25-27	25	6.7-7.2	6.5-7.3	7.7-8.1	7.6-8.1
	Wye	25-27	25	5.7-7.3	6.7-7.4	7.7-8.2	7.8-8.1
	Reference	25-27	25	6.7-7.1	6.5-7.3	7.9-8.2	7.7-8.2
Amphipod *H. azteca*	Control	24	24	6.4-7.6	6.6-7.6	7.9-8.4	7.8-8.2
	Morgantown	24	24	6.4-7.9	6.0-7.4	7.9-8.3	7.8-8.3
	Dahlgren	24	24	6.8-7.5	1.9-7.6	8.1-8.6	7.6-8.2
	Patapsco	24	24	6.7-7.4	6.3-7.3	7.9-8.2	7.7-8.1
	Wye	24	24	6.8-7.6	6.6-7.6	7.8-8.4	7.8-8.1
	Reference	24	24	6.7-7.5	5.3-7.8	7.9-8.3	7.7-8.2
Worm *S. benedicti*	Control	24	24	6.4-7.6	6.6-7.6	7.9-8.4	7.8-8.2
	Morgantown	24	24	6.4-7.9	6.0-7.4	8.0-8.3	7.8-8.3
	Dahlgren	24	24	6.8-7.5	1.9-7.6	8.0-8.3	7.6-8.2
	Patapsco	24	24	6.7-7.4	6.3-7.3	7.8-8.2	7.7-8.1
	Wye	24	24	6.8-7.6	6.6-7.6	7.9-8.1	7.8-8.1
	Reference	24	24	6.7-7.5	5.3-7.8	8.0-8.3	7.7-8.2

APPENDIX D

Hyalella azteca Reference

Sediment Tests

BIOASSAY DATA SUMMARY

Laboratory: AMRL
Sample No.: NA
Sample Type: Sediment
Collection Date: 10-21-91
Time: 1030
Dilution Water Used: D.I. Water & Sea Salts
Test Temperature: 25°C +/- 2° C
Test Mode: Static Acute Non-Renewal
Test Duration: 10 and 20 days

Contractor: AMRL/In house
Effluent/Sediment Source: Lynnhaven
Test Organism: Amphipod
Species: Hyalella azteca
Age: Juveniles
Start of Test: Date: 11-5-91 Time: 1400
End of Test: Date: 11-15-91 Time: 1400
Test Technicians: PB, RT
Data Released By: E. Deaver

RESULTS
Water Chemistry and Toxicity Analyses
(Range- max/min)

Concentration %	Temp. (°C)	Dissolved Oxygen (mg/L)	pH	S (ppt)	Percent Survival
Sand A	25/24	7.8/6.7	8.1/7.8	15	92.5
Sand B	25/24	7.7/6.4	8.0/7.7	15	86.5
Sand C	25/24	7.8/6.5	8.1/7.5	15	81.3
Sand D	25/24	7.6/6.4	8.1/7.6	15	92.5
Sand E	25/24	7.8/6.4	8.0/7.4	15	88.8
CTRL (mud)	25/24	7.5/6.4	8.2/7.8	15	91.0

KEY: All test treatments were 100% sand. "Sterilized" refers to heat sterilized and dried to kill predators. "Aged" means the sand was placed into a tank of 15 ppt water(DI and sea salts) & aerated for 2 weeks. Algae (isochrysis) and artemia were added 3x/week.

Sand A= Sterilized, then aged (All 100% sand for the Amb. Tox. study was treated this way).
Sand B= Sterilized, aged, sterilized
Sand C= Sterilized, no aging
Sand D= No sterilization. Field collected, then aged
Sand E= Aged, then sterilized
CTRL= Control sediment. Lynnhaven mud, 68% sand (same sediment used as Control for Amb Tox)

APPENDIX E

Organics

AMRL
ORGANIC ANALYSIS DATA SHEET
SEMI-VOLATILE COMPOUNDS

Laboratory:	Organics	Contractor:	MD DNR
Project ID:	Ambient Toxicity	Sample No.:	37400
Sample ID:	Morgantown		
Dates:			
Collected:	8/14/91	Extracted:	8/16/91
Received:	8/15/91	Analyzed:	9/4/91
Method:	3550/8270	Instrument:	INCOS 50
Analyst:	RJM	Data Released By:	M. Helmstetter
Matrix:	Sediment	Units:	µg/kg dry
Sample w/v:	30.04	% Moisture:	27%

CAS No.	Compound	Conc.	Tag	Detection Limit

No compounds detected.

AMRL
ORGANIC ANALYSIS DATA SHEET
SEMI-VOLATILE COMPOUNDS

Laboratory:	Organics	Contractor:	MD DNR
Project ID:	Ambient Toxicity	Sample No.:	37401
Sample ID:	Dahlgren		
Dates:			
Collected:	8/14/91	Extracted:	8/16/91
Received:	8/15/91	Analyzed:	9/4/91
Method:	3550/8270	Instrument:	INCOS 50
Analyst:	RJM	Data Released By:	M. Helmstetter
Matrix:	Sediment	Units:	µg/kg dry
Sample w/v:	30.02	% Moisture:	29%

CAS No.	Compound	Conc.	Tag	Detection Limit

No compounds detected.

AMRL
ORGANIC ANALYSIS DATA SHEET
SEMI-VOLATILE COMPOUNDS

Laboratory:	Organics	Contractor:	MD DNR
Project ID:	Ambient Toxicity	Sample No.:	37402
Sample ID:	Patapsco		
Dates:			
Collected:	8/14/91	Extracted:	8/16/91
Received:	8/15/91	Analyzed:	9/4/91
Method:	3550/8270	Instrument:	INCOS 50
Analyst:	RJM	Data Released By:	M. Helmstetter
Matrix:	Sediment	Units:	µg/kg dry
Sample w/v:	30.07	% Moisture:	26%

CAS No.	Compound	Conc.	Tag	Detection Limit

No compounds detected.

AMRL
ORGANIC ANALYSIS DATA SHEET
SEMI-VOLATILE COMPOUNDS

Laboratory:	Organics	Contractor:	MD DNR
Project ID:	Ambient Toxicity	Sample No.:	37403
Sample ID:	Wye		
Dates:			
Collected:	8/14/91	Extracted:	8/16/91
Received:	8/15/91	Analyzed:	9/4/91
Method:	3550/8270	Instrument:	INCOS 50
Analyst:	RJM	Data Released By:	M. Helmstetter
Matrix:	Sediment	Units:	µg/kg dry
Sample w/v:	30.05	% Moisture:	24%

CAS No.	Compound	Conc.	Tag	Detection Limit
106-44-5	4-methylphenol	9.9	J	13.9

J = Compound detected below the calculated method detection limit.

AMRL
ORGANIC ANALYSIS DATA SHEET
SEMI-VOLATILE COMPOUNDS

Laboratory:	Organics	Contractor:	MD DNR
Project ID:	Ambient Toxicity	Sample No.:	37522
Sample ID:	Reference		
Dates:			
Collected:	8/14/91	Extracted:	8/16/91
Received:	8/15/91	Analyzed:	9/4/91
Method:	3550/8270	Instrument:	INCOS 50
Analyst:	RJM	Data Released By:	M. Helmstetter
Matrix:	Sediment	Units:	µg/kg dry
Sample w/v:	29.98	% Moisture:	35%

CAS No.	Compound	Conc.	Tag	Detection Limit

No compounds detected.

AMRL
ORGANIC ANALYSIS DATA SHEET
SEMI-VOLATILE COMPOUNDS

Laboratory:	Organics	Contractor:	MD DNR
Project ID:	Ambient Toxicity	Sample No.:	37523
Sample ID:	Control		
Dates:			
Collected:	8/14/91	Extracted:	8/16/91
Received:	8/15/91	Analyzed:	9/4/91
Method:	3550/8270	Instrument:	INCOS 50
Analyst:	RJM	Data Released By:	M. Helmstetter
Matrix:	Sediment	Units:	µg/kg dry
Sample w/v:	30.00	% Moisture:	15%

CAS No.	Compound	Conc.	Tag	Detection Limit
117-81-7	bis(2-ethylhexyl)phthalate	63.9		12.5

AMRL
ORGANIC ANALYSIS DATA SHEET
SEMI-VOLATILE COMPOUNDS

Laboratory:	Organics	Contractor:	MD DNR
Project ID:	Ambient Toxicity	Sample No.:	37841
Sample ID:	Wye River		
Dates:			
Collected:	9/25/91	Extracted:	10/24/91
Received:	9/26/91	Analyzed:	11/11/91
Method:	3550/8270	Instrument:	INCOS 50
Analyst:	RJM	Data Released By:	M. Helmstetter
Matrix:	Sediment	Units:	µg/kg dry
Sample w/v:	30.12 g	% Moisture:	15%

CAS No.	Compound	Conc.	Tag	Detection Limit (µg/kg)
541-73-1	1,3-Dichlorobenzene	14.0	B	11.9
10544-50-0	Sulfur, Mol. (S8)	6.01×10^3*		10.0

B = Compound detected in the QC blank.
* = Tentative identification. Concentration based on 1:1 response with internal standard.

APPENDIX F

Pesticides

Table 1. Semi-volatile organics (GC/MS) and pesticide/Aroclor (GC/ECD) compounds evaluated in sediments employing a user-created calibration library. Sediment detection limits are reported in µg/kg dry weight.

CAS #	Compound	Sediment MDL
62-53-3	aniline	14.5
95-57-8	2-chlorophenol	13.2
111-44-4	bis(2-chloroethyl)ether	11.2
108-95-2	phenol	11.9
541-73-1	1,3-dichlorobenzene	11.9
106-46-7	1,4-dichlorobenzene	12.5
95-50-1	1,2-dichlorobenzene	11.9
100-51-6	benzyl alcohol	27.1
39638-32-9	bis(2-chloroisopropyl)ether	5.9
95-48-7	2-methylphenol	15.8
91-57-6	2-methylnaphthalene	9.2
67-72-1	hexachloroethane	21.8
621-64-7	n-nitroso-di-n-propylamine	13.2
106-44-5	4-methylphenol	13.9
98-95-3	nitrobenzene	11.2
78-59-1	isophorone	6.6
88-75-5	2-nitrophenol	27.1
65-85-0	benzoic acid	18.5
105-67-90	2,4-dimethylphenol	15.8
111-91-1	bis(2-chloroethoxy)methane	9.9
120-83-2	2,4-dichlorophenol	21.8
120-82-1	1,2,4-trichlorobenzene	15.2
91-20-3	naphthalene	4.6
106-47-8	4-chloroaniline	26.4
87-68-3	hexachlorobutadiene	22.4
59-50-7	4-chloro-3-methylphenol	20.5
77-47-4	hexachlorocyclopentadiene	25.7
88-06-2	2,4,6-trichlorophenol	37.0
95-95-4	2,4,5-trichlorophenol	44.9
91-58-7	2-chloronaphthalene	9.9
88-74-4	2-nitroaniline	37.6
208-96-8	acenaphthalene	5.9
84-66-2	dimethylphthalate	9.9
606-20-2	2,6-dinitrotoluene	48.2
99-09-2	3-nitroaniline	247
83-32-9	acenaphthene	9.9
51-28-5	2,4-dinitrophenol	262
132-64-5	dibenzofuran	7.9
100-02-7	4-nitrophenol	268
121-14-2	2,4-dinitrophenol	43.6
86-73-7	fluorene	9.9
7005-72-3	4-chlorophenylphenylether	20.5
84-66-2	diethylphthalate	9.9
100-01-6	4-nitroaniline	279
534-52-1	4,6-dinitro-2-methylphenol	122
86-30-6	n-nitrosodiphenylamine	19.1
101-55-3	4-bromophenylphenylether	41.6

Table 1 (Cont'd):

CAS #	Compound	MDL
85-01-8	phenanthrene	9.2
118-74-1	hexachlorobenzene	37.6
87-86-5	pentachlorophenol	136
120-12-7	anthracene	9.9
84-74-2	di-n-butylphthalate	5.9
206-44-0	fluoranthene	10.6
129-00-0	pyrene	10.6
85-68-7	butylbenzylphthalate	17.8
56-55-3	benzo(a)anthracene	17.8
218-01-9	chrysene	14.5
91-94-1	3,3'-dichlorobenzene	101
117-81-7	bis(2-ethylhexy)phthalate	12.5
117-84-0	di-n-octylphthalate	7.3
205-99-2	benzo(b)fluoranthene	13.9
207-08-9	benzo(k)fluoranthene	13.9
50-32-8	benzo(a)pyrene	15.2
193-39-5	indeno(1,2,3-cd)pyrene	16.5
53-70-3	dibenz(a,h)anthracene	17.8
191-24-2	benzo(g,h,i)perylene	16.5
103-33-3	azobenzene	7.3
92-87-5	benzidine	24.4
1912-24-9	atrazine	0.100
391-84-6	α-BHC	0.714
391-85-7	ß-BHC	0.559
391-86-8	δ-BHC	1.052
58-89-9	lindane	0.616
76-44-8	heptachlor	0.819
309-00-2	aldrin	0.608
1024-57-3	heptachlor epoxide	0.570
959-98-8	endosulfan I	0.859
60-57-1	dieldrin	0.898
72-55-9	4,4'-DDE	0.528
33213-65-9	endosulfan II	0.745
72-20-8	endrin	1.240
72-54-8	4,4'-DDD	0.469
1031-07-8	endosulfan sulfate	1.500
50-29-3	4,4'-DDT	3.420
72-43-5	methoxychlor	5.0
57-74-5	chlordane	5.0
80001-35-2	toxaphene	10.0
2385-85-5	mirex	1.000
7421-93-4	endrin aldehyde	2.410
12574-11-2	Aroclor 1016	16.6
11104-28-2	Aroclor 1221	16.6
11141-16-5	Aroclor 1232	16.6
53469-21-9	Aroclor 1242	16.6
12672-29-6	Aroclor 1248	16.6
11097-69-1	Aroclor 1254	16.6
11096-82-5	Aroclor 1260	16.6
37324-23-5	Aroclor 1262	16.6

AMRL
ORGANIC ANALYSIS DATA SHEET
PESTICIDE AND AROCLOR COMPOUNDS

Laboratory: Organics
Project ID: Ambient Toxicity
Sample ID: Morgantown

Contractor: MD DNR
Sample No.: 37400

Dates:
Collected: 8/14/91
Received: 8/20/91

Extracted: 8/15/91
Analyzed: 9/12/91

Method: 3550/8080
Analyst: RJM
Data Released By: M. Helmstetter

Instrument: PE 9000
Calib/MIDPT Date: 8/23/91

Matrix: Sediment

Units: µg/kg dry

CAS No.	Compound	Conc.	Tag	Detection Limit (µg/kg)
391-84-6	α-BHC	0.793		0.714
391-85-7	ß-BHC	1.33		0.559
58-89-9	Lindane	1.38		0.616
76-44-8	Heptachlor	1.18		0.819
60-57-1	Dieldrin	1.40		0.898
1031-07-8	Endosulfan sulfate	2.32		1.50
50-29-3	4,4' -DDT	7.01		3.420

AMRL
ORGANIC ANALYSIS DATA SHEET
PESTICIDE AND AROCLOR COMPOUNDS

Laboratory: Organics
Project ID: Ambient Toxicity
Sample ID: Dahlgren

Contractor: MD DNR
Sample No.: 37401

Dates:
Collected: 8/14/91
Received: 8/20/91

Extracted: 8/15/91
Analyzed: 9/12/91

Method: 3550/8080
Analyst: RJM
Data Released By: M. Helmstetter

Instrument: PE 9000
Calib/MIDPT Date: 8/23/91

Matrix: Sediment

Units: µg/kg dry

CAS No.	Compound	Conc.	Tag	Detection Limit (µg/kg)
391-84-6	α-BHC	1.00		0.714
391-85-7	ß-BHC	0.702		0.559

AMRL
ORGANIC ANALYSIS DATA SHEET
PESTICIDE AND AROCLOR COMPOUNDS

Laboratory: Organics
Project ID: Ambient Toxicity
Sample ID: Patapsco

Contractor: MD DNR
Sample No.: 37402

Dates:
Collected: 8/14/91
Received: 8/20/91

Extracted: 8/15/91
Analyzed: 9/12/91

Method: 3550/8080
Analyst: RJM
Data Released By: M. Helmstetter

Instrument: PE 9000
Calib/MIDPT Date: 8/23/91

Matrix: Sediment

Units: µg/kg dry

CAS No.	Compound	Conc.	Tag	Detection Limit (µg/kg)
	No compounds detected.			

AMRL
ORGANIC ANALYSIS DATA SHEET
PESTICIDE AND AROCLOR COMPOUNDS

Laboratory:	Organics	Contractor:	MD DNR
Project ID:	Ambient Toxicity	Sample No.:	37403
Sample ID:	Wye		
Dates:			
Collected:	8/14/91	Extracted:	8/15/91
Received:	8/20/91	Analyzed:	9/12/91
Method:	3550/8080	Instrument:	PE 9000
Analyst:	RJM	Calib/MIDPT Date:	8/23/91
Data Released By:	M. Helmstetter		
Matrix:	Sediment	Units:	µg/kg dry

CAS No.	Compound	Conc.	Tag	Detection Limit (µg/kg)
391-84-6	α-BHC	0.793		0.714
391-86-8	δ-BHC	33.5	B	1.05

B = Compound detected in the QC blank.

AMRL
ORGANIC ANALYSIS DATA SHEET
PESTICIDE AND AROCLOR COMPOUNDS

Laboratory: Organics
Project ID: Ambient Toxicity
Sample ID: Reference

Contractor: MD DNR
Sample No.: 37522

Dates:
Collected: 8/8/91
Received: 8/20/91

Extracted: 8/15/91
Analyzed: 9/12/91

Method: 3550/8080
Analyst: RJM
Data Released By: M. Helmstetter

Instrument: PE 9000
Calib/MIDPT Date: 8/23/91

Matrix: Sediment

Units: µg/kg dry

CAS No.	Compound	Conc.	Tag	Detection Limit (µg/kg)
391-84-6	α-BHC	0.876		0.714
391-86-8	δ-BHC	1.48	B	1.05
58-89-9	Lindane	0.817		0.616
76-44-8	Heptachlor	0.880		0.819

B = Compound detected in the QC blank.

AMRL
ORGANIC ANALYSIS DATA SHEET
PESTICIDE AND AROCLOR COMPOUNDS

Laboratory: Organics
Project ID: Ambient Toxicity
Sample ID: Control

Contractor: MD DNR
Sample No.: 37523

Dates:
Collected: 8/6/91
Received: 8/20/91

Extracted: 8/15/91
Analyzed: 9/12/91

Method: 3550/8080
Analyst: RJM
Data Released By: M. Helmstetter

Instrument: PE 9000
Calib/MIDPT Date: 8/23/91

Matrix: Sediment

Units: µg/kg dry

CAS No.	Compound	Conc.	Tag	Detection Limit (µg/kg)
72-54-8	4,4'-DDD	0.559		0.469

AMRL
ORGANIC ANALYSIS DATA SHEET
PESTICIDE AND AROCLOR COMPOUNDS

Laboratory: Organics
Project ID: Ambient Toxicity
Sample ID: Wye River

Contracctor: MD DNR
Sample No.: 37841

Dates:
Collected: 9/25/91
Received: 9/26/91

Extracted: 10/24/91
Analyzed: 11/11/91

Method: 3550/8080
Analyst: RJM
Data Released By: M. Helmstetter

Instrument: PE 9000
Calib/MIDPT Date: 11/1/91

Matrix: Sediment

Units: µg/kg dry

CAS No.	Compound	Conc.	Tag	Detection Limit (µg/kg)
33213-65-9	Endosulfan II	1.32		0.745