

RESPONSES OF BENTHIC MACROFAUNA TO ENVIRONMENTAL STRESSORS:  
A SYNTHESIS OF CHESAPEAKE BAY DATA

by

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## **DEDICATION**

This is dedicated to my parents, Lawrence and Rose Smith, and my grandparents, Thomas and Emma West and Lawrence Sr. and Robinette Smith, who sacrificed such that my dreams were never too small or unachievable.

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## LIST OF ABBREVIATIONS

Akaike Information Criterion .....	AIC
AZTI Marine Biotic Index.....	AMBI
Benthic Index of Biotic Integrity .....	B-IBI
Dissolved Oxygen .....	DO
Estimated degrees of freedom .....	e.d.f.
Environmental Monitoring and Assessment Program .....	EMAP
Generalized Additive Model .....	GAM
Generalized Cross Validation .....	GCV
Generalized Linear Model.....	GLM
Integrated Taxonomic Identification System .....	ITIS
Lowest Practical Identification Level .....	LPIL
Mid-Atlantic Integrated Assessment.....	MAIA
National Coastal Assessment.....	NCA
National Coastal Condition Assessment .....	NCCA
Restricted Maximum Likelihood.....	REML
Species Sensitivity Distributions .....	SSD
Total Organic Carbon.....	TOC
United States Environmental Protection Agency .....	USEPA or EPA
World Registry of Marine Species.....	WoRMS

## ABSTRACT

### RESPONSES OF BENTHIC MACROFAUNA TO ENVIRONMENTAL STRESSORS: A SYNTHESIS OF CHESAPEAKE BAY DATA

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Chesapeake Bay is the largest estuary in the United States, and is of ecological, historical, economic and cultural importance. The pressures of increased population density, land development, and agricultural practices have resulted in a threatened bay ecosystem that needs ongoing restoration and protection. There are a number of monitoring programs, research institutions, non-profit organizations, and local, state and federal agencies in the Chesapeake region that are dedicated to and focused on collecting, monitoring and analyzing data, as well as developing management practices and tools to preserve and protect the Bay. Physical, chemical and biological data from these various research and assessment programs were used to identify specific individual taxa responses to specific stressors, first with correlation analysis followed by Generalized Additive Models (GAMs) to build models for amphipods *Ampelisca abdita*, *Ampelisca verrilli*, and polychaete *Spiochaetopterus costarum*. These three taxa were shown to have significant

relationships to environmental pollutants and water quality variables based on analyses performed. The models were tested with Delaware Bay taxa and one model – *A. verilli* – performed well. Some model fine-tuning is necessary to make the models broadly applicable to systems outside of Chesapeake Bay. Once achieved, these models have the potential to complement benthic condition tools such as the Benthic Index of Biotic Integrity (B-IBI), to provide decision-makers with information to better manage and direct monitoring and assessment resources.



## **CHAPTER ONE: INTRODUCTION**

Estuaries are dynamic systems that are continuously subjected to stressors. Environmental (or naturally-occurring) abiotic stressors include floods, hurricanes, currents, hypoxia, and changes in climate, while biotic stressors include predation, competition, and the introduction of invasive species (Boesch & Rosenberg, 1981; Boesch, Wass, & Virnstein, 1976; Cairns, 2003; Sousa, 1984; Thrush, Hewitt, Hickey, & Kelly, 2008). Stress is defined as a “perturbation” applied to a system that is either foreign to that system or natural to that system, but applied at an excessive level (Barrett, van Dyne, & Odum, 1976). Similarly, Van Straalen (2003) defined organismal stress as a condition induced by one or more environmental factors that pushes the organism near or over the edges of its ecological niche. Stress is “usually transient, involves specific physiological response, and is accompanied by the induction of mechanisms that counteract its consequences” (Van Straalen, 2003).

Traditionally, benthic macroinvertebrates are accepted as reliable indicators of disturbance or stress in estuarine environments. Given their limited mobility and considerable abundances, they are relatively easy to sample and cannot avoid exposure to contaminants; thus they are integrators of the biological and physical events in the sediments and the overlying water column (Weisberg et al., 1997; Pelletier, Gold, Heltshe, & Buffum, 2010a). Communities of benthic macroinvertebrates are comprised

of a variety of taxa with a wide range of living positions, feeding types, and physical adaptations, many of which make them ideal to reliably predict the presence of natural and anthropogenic stressors (Pearson & Rosenberg, 1978; Lenihan et al., 2003) and the resulting condition, or health, of aquatic systems such as estuaries. Understanding the relationships between benthic macroinvertebrates and how they respond to stressors is essential, in order to develop evaluation tools that properly assess ecosystem condition.

### **Types of Stressors in Estuaries**

Benthic macroinvertebrates reside in complex systems and are subjected to a variety of environmental (abiotic and biotic) and anthropogenic stressors. At any given point in time, benthic macroinvertebrates are simultaneously exposed to stressors in estuaries at varying magnitudes and frequencies. Numerous studies have identified predictable responses to natural and anthropogenic stressors (Borja, Franco, & Perez, 2000; Engle, Summers, & Gaston, 1994; Pearson & Rosenberg, 1978; Weisberg et al., 1997). Previous work to describe benthic macroinvertebrate responses to general stressors, as well as information on responses to specific stressors, is critical in determining if responses characteristic of particular combinations of stressors in estuaries exist. The following is a summary of benthic macroinvertebrate responses to stressors in estuarine environments, which is the foundation of this dissertation research to identify characteristic patterns of benthic macroinvertebrate responses to multiple stressors.

## **Levels of Biological Organization and Stressor Response**

Biological responses to stressors can occur at all levels of biological organization, from cells all the way up to communities, ecosystems and landscapes (Adams, 2005; Cairns, 2003). Observed responses can manifest as changes in abundance, diversity and fitness as a result of both direct and indirect processes (Adams, 2005), and may vary markedly not only at different levels of organization (Breitburg, 1992; Odum, Finn, & Franz, 1979) but also between species of the same or different genus.

### ***Individual-level Stressor Responses***

For individuals, a variety of behavioral, physiological, and biochemical responses can be triggered in a direct response to stressors: changes in enzymatic and metabolic activity, alterations of protein and gene expression, avoidance behavior, decreased reproductive activity (Adams, 2005; Diaz & Rosenberg, 1995), reduced uptake, increased excretion, detoxification, sequestration, and repair (Maltby, 1999). Indirect responses to stressors include changes in the quantity and quality of available habitat, food sources, and competition and predation interactions (Adams, 2005; Preston & Shackelford, 2002).

Benthic macroinvertebrate species do not always respond the same way to stressors, unless there is a severe event such as prolonged hypoxia/anoxia or dredging that cause mortality among all species present. Additionally, species can respond differently to stressors and disturbances due to individual species differences in relation to the physical, chemical, or biological environment, or autecology (Newman & Clements, 2008). Diaz and Rosenberg (1995) described significant differences in

responses of benthic genera to hypoxia. The polychaete *Nereis diversicolor* has higher blood sulphide oxidation activity than *N. virens*, *N. succinea*, and *N. pelagica*, in order to survive and cope with high concentrations of sulphide associated with hypoxic events. The brittle star *Amphiura filiformis* migrates to the sediment surface for oxygen well before *A. chiajei* appears, as dissolved oxygen concentrations in the sediment decrease; *A. chiajei* has a lower respiration rate than *A. filiformis* and can therefore tolerate hypoxic conditions at depth. The bivalve *Abra alba* extends many more siphons out into the water column than *A. nitidia* in search of oxygen. Adaptations to stress within genera have provided species a competitive advantage for survival over others in disturbed environments.

Sensitivity or tolerance to a stressor, and the frequency of stress or disturbance often determines how an organism responds. Juveniles are a sensitive life stage and are subsequently more susceptible to stress than adults. This tolerance or intolerance to stressors can also be attributed to species' life history traits: short-lived (r-selected) species tend to regenerate rapidly after disturbance as opposed to long-lived (k-selected) species that take longer to re-establish after a disturbance (Diaz & Rosenberg, 1995; Holland, 1985; Holland, Shaughnessy, & Hiegel, 1987; Marsh & Tenore, 1990; Newmann & Unger, 2003). Amphipods, compared to polychaetes, have shorter life spans and possess far fewer physiological adaptations that decrease sensitivity to stressors; this helps to explain why amphipods are more suitable for toxicity tests than polychaetes.

### ***Population- and Community-level Responses***

Individual and species level responses to stressors are the nexus between ecology and toxicology (ecological and environmental), as ecology alone cannot explain relationships between organisms and stressors. Stressors can trigger physiological (direct) and physical (indirect) responses in individuals, which ultimately influence changes seen at the population and community levels (Chapman, 2002). Population-level responses to stressors and mortality-inducing disturbances are varied and can often be predicted from aforementioned individual-level responses that reduce susceptibility to stress. The major responses of populations are increased or reduced abundances, changes in age structure and alterations of the gene pool (Maltby, 1999; Marsh & Tenore, 1990). Individual- and population-level responses together are important in understanding how communities of benthic macroinvertebrates respond to stress, mainly expressed as changes in community structure. Various conceptual models exist that attempt to explain how communities respond to disturbance or stress, namely the Intermediate Disturbance Hypothesis (Connell 1978) and the Environmental Stress Model (Menge and Sutherland 1987).

### **Community-level Stressor Response Models**

The Intermediate Disturbance Hypothesis (Connell, 1978) was originally developed to describe diversity patterns in tropical forests and coral reefs. It predicts that the maximum diversity in a system occurs at an intermediate level of disturbance, where disturbance is sufficient to limit the growth of competitive dominants, releasing previously excluded species and allowing other species to move in and increase diversity.

At a low level of disturbance, competitive exclusion takes place due to competition for space and a few species outcompete others. Conversely, mortality decreases diversity due to disturbance at a high level of disturbance. This model is appropriate and often used to explain benthic macroinvertebrate stressor response in estuarine soft-bottom benthic communities (Pearson & Rosenberg, 1978).

The Environmental Stress Model (Menge & Sutherland, 1987) takes the Intermediate Disturbance Hypothesis model one step further by incorporating resource availability, driven by competitive exclusion and predation, to predict community structure. When environmental stress is low and food resources are high, the population of competitive dominants increases and excludes other species due to predation. Similar to the Intermediate Disturbance Hypothesis, populations are limited by high environmental stress. In the rocky intertidal, for which this model was developed, diversity increases as environmental stress increases, but diversity is then dampened due to competition for space. When this model is applied to soft-sediment communities, there is no competition for space, therefore the model follows the Intermediate Disturbance Hypothesis. There is an iteration of the Environmental Stress Model that focuses on both environmental and predator stress on prey (Prey Stress Model; (Menge & Olson, 1990). The Prey Stress Model is important in explaining observed benthic community responses to stressors, when environmental and predator stress dynamics are present in a system.

## **Single versus Multiple Stressors**

Generally, every level of organization in estuaries responds to multiple stressors at any given time (Adams, 2005; Hyland et al., 2005; Lenihan et al., 2003; Thrush et al., 2008). The types and intensity of multiple stressors can determine whether multiple stressors have additive effects on individuals and populations (Folt, Chen, Moore, & Burnaford, 1999), although it is difficult to predict how communities, populations of different species in a given area, respond to multiple stressors. It is implicitly assumed that this response is additive, rather than antagonistic or synergistic (Newman & Clements, 2008), however, Thrush et al. (2008) found that responses to a single stressor or stressors acting additively were less apparent than multiplicative effects in regression-based models.

There are a multitude of stressors occurring simultaneously in estuaries that make it difficult to identify if stressors are additive or multiplicative, hence magnifying the response observed. In addition, there is a need to tease out what effects are due to internal patchiness and heterogeneity of natural systems versus anthropogenic influences. Elliot and Quintino (2007) coined this problem the Estuarine Quality Paradox. They recommend that to break out of this circularity, natural variability and stress should be fully quantified, and anthropogenic stress subtracted out, or a set of methods must be developed to detect anthropogenic stressors against a backdrop of natural stress. Likewise, Lenihan et al. (2003) noted that the presence of stressors are often confounded in space and time, which causes difficulty in detecting the effects of individual and multiple stressors, especially at the population and community levels. The logical point at

which to start understanding how stressors in estuaries affect benthic community structure is to focus on ecotoxicological principles of stressor responses for individuals and populations of species.

Previous studies in the Chesapeake Bay provide information on macrobenthic community responses to single stressors: low dissolved oxygen events correlate with degraded benthic communities and lower benthic diversity (Breitburg, 1992; Daniel M. Dauer, Rodi, & Ranasinghe, 1992; Diaz & Rosenberg, 1995; Holland et al., 1987; Llansó, 1992; Seitz, Dauer, Llansó, & Long, 2009), benthic community condition declines with exposure to sediment contaminants as sediment contaminants correlate with urbanization (Dauer et al., 1992; Dauer, 1993; Dauer et al., 1993; Kiddon et al., 2003), benthic community condition generally degrades as land use changes along a gradient from forested to urban (Bilkovic, Roggero, Hershner, & Havens, 2006), and overall degraded benthic community condition is associated with eutrophication (Kiddon et al., 2003). Many of these studies focus on biological responses to single stressors, but it is important to note that most benthic stressors do not occur one at a time, but rather in combination with other stressors, especially in estuarine environments. Benthic macroinvertebrate responses to single stressors will aid in discerning the response patterns exhibited by multiple stressors.



## **Dissertation Overview**

Chesapeake Bay is the largest estuary in the United States, and is of ecological, historical, economic and cultural importance. The pressures of increased population density, land development, and agricultural practices have resulted in a threatened bay ecosystem that is in need of ongoing restoration and protection. There is a multitude of data available that can be analyzed to assess stressor impacts, and to better manage them. This research draws on decades of previously collected data in the Chesapeake Bay region and provides a sound mechanism to evaluate actual and potential stressors to benthic communities, using datasets at hand. This is especially of importance in times of dwindling funds for monitoring and research programs.

In Chapter 1, the current chapter, I presented a review of the relevant literature on stressor-response relationships at various levels of biological organization. In Chapter 2, I describe key relationships and benthic macroinvertebrate response patterns to specific stressors. In Chapter 3, I use previous and new species- and stressor-specific relationships to propose a model that assesses ecological condition and status in Chesapeake Bay. This new model also incorporates measures of stress tolerance and species autecology to further enhance the model's capabilities. In Chapter 4, I demonstrate the performance of the model by comparing its output to existing health indices in Chesapeake Bay, and test its performance in nearby Delaware Bay, which is exposed to similar stressors as Chesapeake Bay. The overall goal of my dissertation research is to combine general and stressor-specific response information with ecological theory to build a model that

complements current assessment tools and allows resource managers to better protect, maintain and restore Chesapeake Bay's aquatic ecosystem.

## **CHAPTER TWO: CHARACTERIZING RELATIONSHIPS BETWEEN BENTHIC MACROFAUNA AND SPECIFIC STRESSORS**

### **Introduction**

Estuaries, with the multitude of stressors that occur within, are ideal systems for the study of stressor and disturbance ecology. Over 40% of the global human population lives within 100 km of the coast, making estuarine environments among the most heavily impacted systems in the world (Kenworthy et al., 2004). It is in estuaries where natural stressors come together with human-induced stressors and affect physical and biological systems. Benthic macroinvertebrates are commonly used in stressor-response studies because they are integrators of biological and physical events in the sediments and the overlying water column. They have limited mobility, cannot avoid exposure to contaminants and are relatively easy to sample. Additionally, they possess a wide range of living positions and habitats, feeding types, and physical adaptations that make them sensitive to most stressors at the community level.

Commonly found benthic macroinvertebrates include, amphipods, mollusks (bivalves and gastropods), chironomids, and worms (oligochaetes and polychaetes). Amphipods are commonly found in freshwater and marine systems and are critical components of aquatic food webs as major sources of food for predators (Holland et al., 1987; Virnstein, 1979). Given their opportunistic life history, direct contact with sediment, tolerance for a range of salinities and documented correlation between their

distribution and sediment contamination, amphipods are sensitive indicators of environmental disturbance and the most used organism for sediment toxicity testing (Schlekat, Mcgee, & Reinharz, 1992). *Ampelisca abita*, *Ampelisca verrilli* (both tube-dwelling species) and *Leptocheirus plumulosus* (burrowing species) are dominant in the Chesapeake Bay. While these species are sensitive to various environmental stressors, their ability to move deeper into the sediment to avoid stressors at the sediment-water interface provide a mechanism by which they can tolerate exposure to stressors.

Bivalves and gastropods, like amphipods, are commonly found in aquatic systems and are important links between the benthos and higher trophic levels (Holland et al., 1987; Virnstein, 1979). Many species of bivalves are suspension feeders and they influence the nutrient and organic coupling of benthic pelagic systems, accomplished by their ability to filter particles and biodeposit organic wastes on the benthic surface. The influence of benthic suspension-feeding bivalves, such as *Mulinia lateralis* and *Macoma mitchelli*, on benthic-pelagic coupling, sediment processes, deposition, and nutrient remineralization has been well studied (Diaz & Schaffner, 1990; Ocean Sciences Board & National Sciences Council, 2010).

Chironomids or non-biting midges, are flies that are found all over the world. Nearly all of the larvae are aquatic (or sub-aquatic) and tend to be more dominate in freshwater systems although they can be found in tidal fresh to brackish portions of estuaries. Chironomids are one of just a few aquatic insects that can span the entire salinity range of an estuary from freshwater to marine.

Polychaetes are common in marine environments worldwide and oligochaetes are common in freshwater environments, though they are still found in all aquatic environments globally. Like other benthic macroinvertebrates mentioned, polychaetes and oligochaetes are important for nutrient remineralization and vertical sediment redistribution as a result of feeding and burrowing behaviors (Dauer et al., 1992; Diaz, 1984), and also key links to between primary producers and higher trophic levels. The feeding tubes and burrows they form increase the surface area over which substrate and nutrients exchange at the sediment-water interface (Diaz & Schaffner, 1990; Schaffner, 1990). Regardless of life history strategy, most estuarine polychaetes can survive in a wide range of environmental conditions until conditions degrade to the point that even juvenile stages of opportunistic species are affected by stress (Boesch, 1977; Boesch & Rosenberg, 1981). Some polychaetes have the ability to produce detoxifying secondary metabolites and proteins in vivo that provide a relatively high tolerance to some contaminants (Lenihan et al., 2003; Marciano, Nusetti, Rodriguez-Grau, & Vilas, 1996).

Previous studies have demonstrated predictable responses of macrobenthic organisms to both natural and anthropogenic stressors collectively (Pearson and Rosenberg, 1978; Bosch and Rosenberg, 1981; Lenihan et al., 2003), although stressor-specific responses remain poorly known (Weisberg et al., 1997; Metcalfe, 2005). Many of the stressor-specific studies that exist, often laboratory experiments, focus on responses of one or just a few taxa (Crain, Kroeker, & Halpern, 2008), as a means of extrapolating for larger scale application. In this study, I attempt to utilize field monitoring data to identify general individual taxa response patterns to specific-stressors,

namely dissolved oxygen, sediment contaminants and excess nutrients. I will then use the patterns detected to build a model that complements and enhances the performance of tools such as the Benthic Index of Biotic Integrity (B-IBI), which are designed to identify and assess the effects of anthropogenic stressors to aquatic systems.

## **Methods**

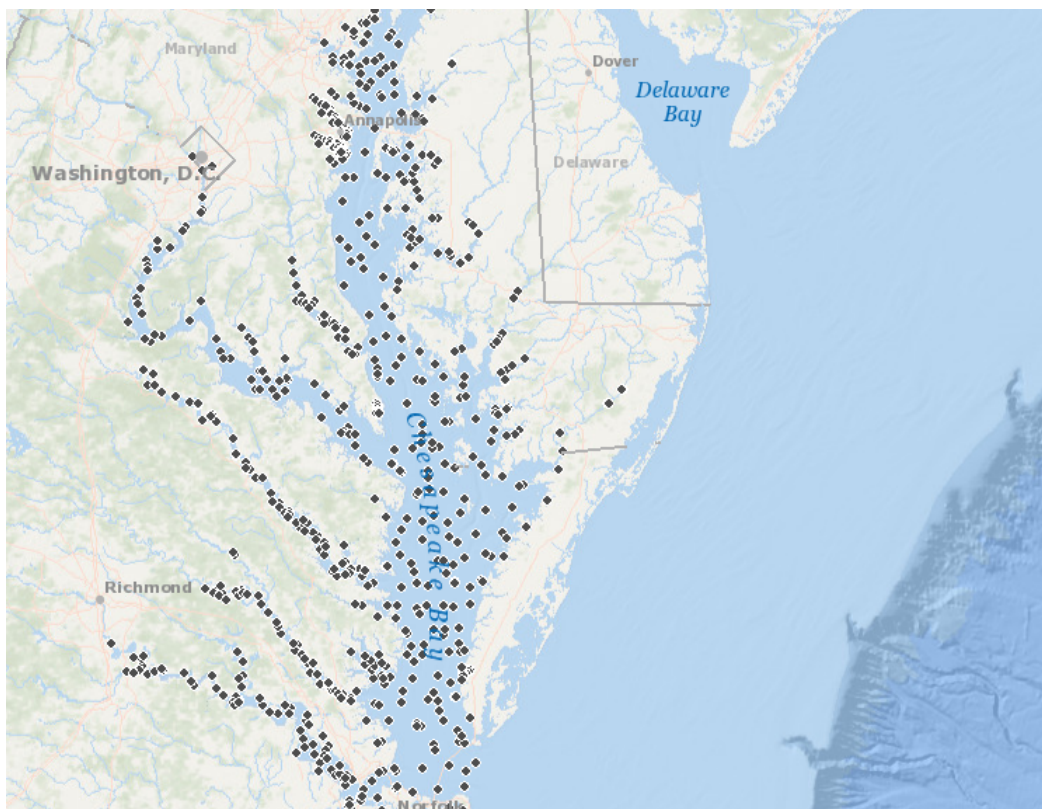
Data collected non-continuously in Chesapeake Bay from 1990 to 2010 were obtained from EPA monitoring programs for this study- the Environmental Monitoring and Assessment Program (EMAP), the Mid-Atlantic Integrated Assessment (MAIA), the National Coastal Assessment (NCA) and the National Coastal Condition Assessment (NCCA; Table 1). Monitoring data from these programs were collected by EPA staff, contractors and state monitoring crews during summer months (July – September). A probabilistic sampling design was utilized, and similar field, laboratory and rigorous quality assurance methods were used by all participants for sample collection and analysis. Sites were visited by boat or large research vessel and water column, sediment composition and quality, benthic macroinvertebrate and fish community and tissue data were collected at each site. Samples were analyzed by national and state laboratories.

**Table 1 Sources of Estuarine and Coastal Biological and Environmental Data from the U.S. Environmental Protection Agency**

<b>Monitoring Program</b>	<b>Years Covered</b>	<b>Data Location</b>
Environmental Monitoring and Assessment (EMAP)	1990 – 1993	<a href="https://archive.epa.gov/emap/archive-emap/web/html/index-21.html">https://archive.epa.gov/emap/archive-emap/web/html/index-21.html</a>
Mid-Atlantic Integrated Assessment (MAIA)	1997 – 1998	<a href="https://archive.epa.gov/emap/archive-emap/web/html/index-21.html">https://archive.epa.gov/emap/archive-emap/web/html/index-21.html</a>
National Coastal Assessment (NCA)	2001 – 2006	<a href="https://archive.epa.gov/emap/archive-emap/web/html/index-78.html">https://archive.epa.gov/emap/archive-emap/web/html/index-78.html</a>
National Coastal Condition Assessment (NCCA)	2010	<a href="https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys">https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys</a>

Chesapeake Bay data, located in the EMAP Virginian Province, were extracted and screened using the following criteria adapted from (D. M. Dauer, Lane, & Llansó, 2002) to arrive at a dataset appropriate for analysis: 1) samples collected must be within the geographic boundaries of Chesapeake Bay and its tributaries, 2) benthic macroinvertebrate samples must be collected using a Young-modified van Veen or Small van Veen grab with a sampling area of 0.0440 m<sup>2</sup>, 3) benthic macroinvertebrate samples must be collected during the index period of July 15 to September 30, 4) dissolved oxygen measurements must be collected at the same time as benthic macroinvertebrate samples, and 5) sediment contaminant data must be collected in the same year as the benthic macroinvertebrate samples. A subset of sediment contaminant data was used for this study, based on the widespread and local occurrence and extent of contaminants in Chesapeake Bay (US Environmental Protection Agency, US Geological Survey, & US

Fish and Wildlife Service, 2012). Toxicity data were generated from 10-day static acute amphipod toxicity tests in state and contracted laboratories. Sediment total organic carbon (TOC) concentrations were used as a surrogate indicator of eutrophication. In addition, only bottom salinity measurements were used as they are the closest measurements taken in proximity to the benthos. Data from the first of two site visits were used; triplicate benthic grabs were taken for MAIA and were averaged to represent one sample for analysis. Applying the above screening criteria resulted in 702 stations with complete records for analysis in this study.



**Figure 1 Map of sampling sites (n=702) in Chesapeake Bay and tributaries used in this study. The sites were sampled for US EPA monitoring programs from 1990 to 2010**



Benthic macroinvertebrate samples were identified to the lowest practical identification level (LIPL) and for this study, only organisms identified to genus or species were included. Taxonomic names were also checked and corrected according to the Integrated Taxonomic Information System (ITIS, <http://www.itis.gov>), the World Register of Marine Species (WoRMS, <http://www.marinespecies.org>), and the NCCA 2010 Corrected Taxa List (U.S. Environmental Protection Agency, 2016). The one exception was the inclusion of oligochaetes, which were identified to class. Epifaunal and pelagic organisms were removed as they are not considered to be benthic organisms (Pelletier, Gold, Heltshe, & Buffum, 2010b; Ranasinghe et al., 1993). Where information was available, taxa were classified as pollution tolerant or sensitive, as well as classified by life history traits (e.g., equilibrium or opportunistic). These characteristics are important because they can indicate the presence of stressors in the environment; stressed environments tend to be dominated by smaller, rapidly reproducing taxa that can withstand or repopulate stressed or polluted environments, while larger, longer-lived taxa are found in relatively unstressed or unpolluted environments.

### ***Data Manipulation for Analysis***

Prior to analysis, benthic macroinvertebrate raw abundances (or mean abundances for MAIA data) were converted to densities by dividing the abundances by the sediment grab area (0.0444 m<sup>2</sup>). Sediment toxicity data were binned into Good-Fair-Poor categories of 5, 3 or 1 based on NCCA sediment toxicity thresholds (Table 2). Total

DDT, PAH and PCB were calculated from individual class analytes for MAIA, NCA and NCCA sediment contaminant data.

**Table 2 Sediment toxicity thresholds from NCCA 2010 used to score toxicity results**

<b>Rank</b>	<b>Toxicity Threshold by Site</b>	<b>Score</b>
Good	Test results not significantly different from control ( $p > 0.05$ ) and $\geq 80\%$ control-corrected survival	5
Fair	Test results significantly different from control ( $p \leq 0.05$ ) and $\geq 80\%$ control-corrected survival or Test not significantly different from control ( $p > 0.05$ ) <u>and</u> $< 80\%$ control-corrected survival	3
Poor	Test results significantly different from control ( $p < 0.05$ ) <u>and</u> $< 80\%$ control-corrected survival	1

### *Data Reduction*

Data reduction techniques were used to make the dataset more manageable for subsequent interpretation, as well as to reduce collinearity and background noise in the data amongst the variables. The benthic taxa data were first sorted by site and taxa occurring in less than 10 sites were removed (Llansó, Scott, Dauer, Hyland, & Russell, 2002), reducing the number of taxa for analysis to 149. Next a correlation analysis was conducted in R (R Core Team, 2013) to identify whether any variables were correlated with each other that could subsequently be removed from the dataset, creating a set of

non-correlated variables to analyze. The Spearman rank test, appropriate for non-linear data, was used for the analysis. Figure 2 presents a hierarchical clustering of the environmental variables by correlation coefficients. The physical parameters, sand, silt-clay, total organic carbon (TOC), salinity and oxygen, are important variables that often define the life-history traits, habitat and feeding behaviors of benthic macroinvertebrates. Several chemical parameters were correlated (e.g., metals) and a subset of chemical parameters were selected that are listed as contaminants of localized (i.e., aldrin, DDT, dieldrin, heptachlor, iron, and lead) and widespread (i.e., PAHs, PCBs, and mercury) concern in Chesapeake Bay (US Environmental Protection Agency et al., 2012). Table 3 summarizes the parameters selected for analysis.

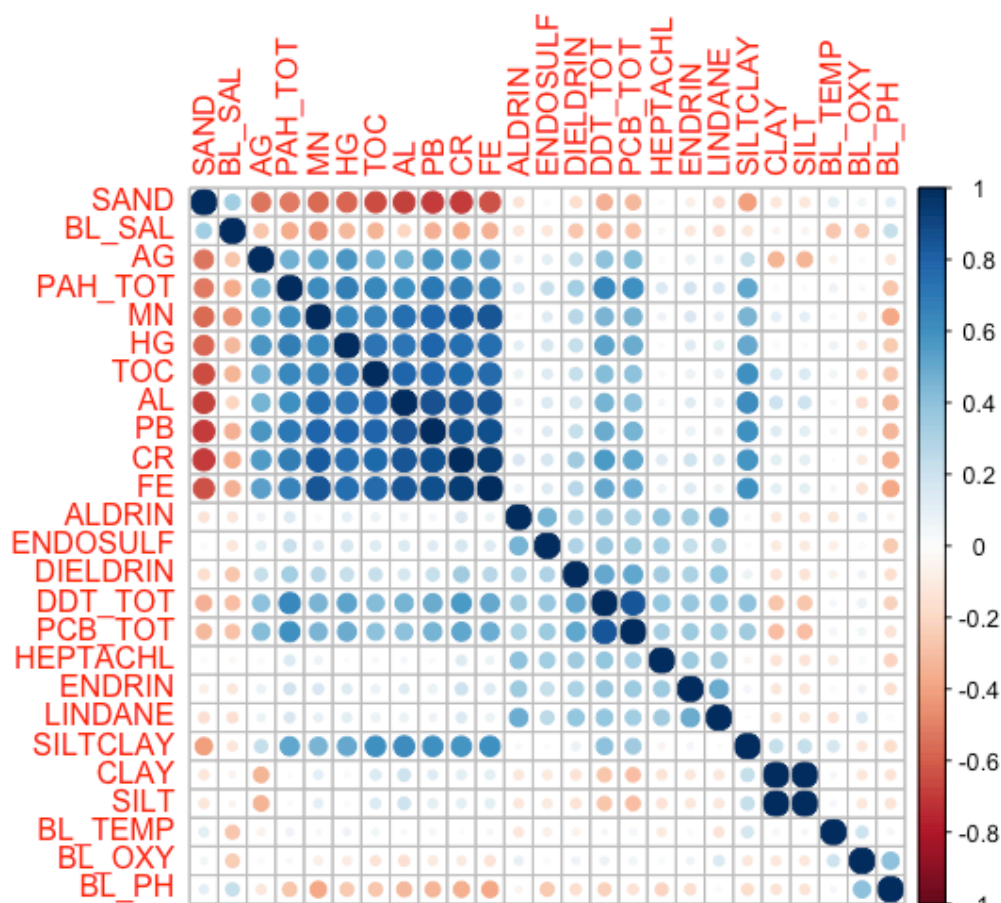


Figure 2 Correlation analysis of environmental variables. Blue circles indicate positive correlation and red indicate negative correlation. The size of circle indicates significance. Smaller circles are less significance.

**Table 3 Environmental variables selected by correlation analysis for further analysis in this study**

<b>Parameter Type</b>	<b>Variable</b>
Physical	Sand
	Silt-clay
	Total Organic Carbon (TOC)
	Salinity
	Dissolved Oxygen (DO)
	Toxicity
Sediment Contaminants	Polycyclic Aromatic Hydrocarbons (PAH)
	Polychlorinated Biphenyls (PCB)
	Aldrin
	Dichlorodiphenyltrichloroethane (DDT)
	Dieldrin
	Heptachlor
	Iron (Fe)
	Mercury (Hg)
	Manganese (Mn)
	Lead (Pb)

#### *Individual Taxa Response Patterns*

The relative abundances of each taxa were plotted against the selected environmental variables to examine response patterns in a series of scatterplot matrices (n = 298; 149 scatterplot matrices each for the suite of chemical and physical parameters). Every combination of taxa and stressor in the scatterplot matrices was reviewed by eye to discern what patterns emerged. Potential response patterns of individual taxa to one or more environmental variables were observed with 34 taxa. Correlation matrices were run for each taxa using the *corr.test* function in the R *psych* package (Revelle, 2018) and tested for significance ( $p < 0.05$ ). The Kendall tau-b test was used because it 1) is appropriate to test non-linear, non-parametric data, 2) allows for the presence of

concordant data, 3) is less sensitive to outliers in the data, and 4) is suitable for small sample sizes.

## Results

There were 34 significant relationships observed between taxa relative abundance and environmental variables. While the following relationships described were significant ( $\alpha = 0.05$ ), many of the relationships were weak to moderate, meaning the strength of the relationship was not strongly positive or negative but still passed the significance test. For example, *Mediomastus ambiseta* was negatively correlated with Dichlorodiphenyltrichloroethane (DDT) with a correlation coefficient of -0.09 ( $\alpha = 0.05$ ) compared to the correlation between *Owenia fusiformis* and Hg which was -0.47.

### *Sediment Contaminants*

Three taxa had significant correlations with DDT (Figure 3). Negative correlations were observed with *Glycinde solitaria* and *Mediomastus ambiseta*, while a positive correlation was observed with *Heteromastus filiformis*. Two taxa had significant positive correlations with dieldrin- *H. filiformis* and *Sayella chesapeakea* (Figure 4). Two taxa had significant positive correlations to iron (Fe)- *Leptocheirus plumulosus* and *Spiophanes bombyx* (Figure 5). One taxon had a significant positive correlation with heptachlor- *H. filiformis* (Figure 6). Three taxa had significant negative correlations with Mercury (Hg)- *Macoma mitchelli*, *Owenia fusiformis*, and *Spiochaetopterus costarum* (Figure 7). Three taxa had significant correlations with Manganese (Mn) (Figure 8). Negative correlations were observed with *G. solitaria* and *S. costarum*, and a positive

correlation was seen with *L. plumulosus*. One taxa had a significant correlation with Polycyclic Aromatic Hydrocarbons (PAH)- *L. plumulosus* (Figure 9). Three taxa had significant correlations with Lead (Pb). Positive correlations were observed with *Ampelisca verrilli* and *L. plumulosus*, and a negative correlation was observed with *M. mitchelli* (Figure 10). One taxon had a significant negative correlation with Polychlorinated Biphenyls (PCB)- *M. ambiseta* (Figure 11Figure 11). Table 4 provides a summary of these results.

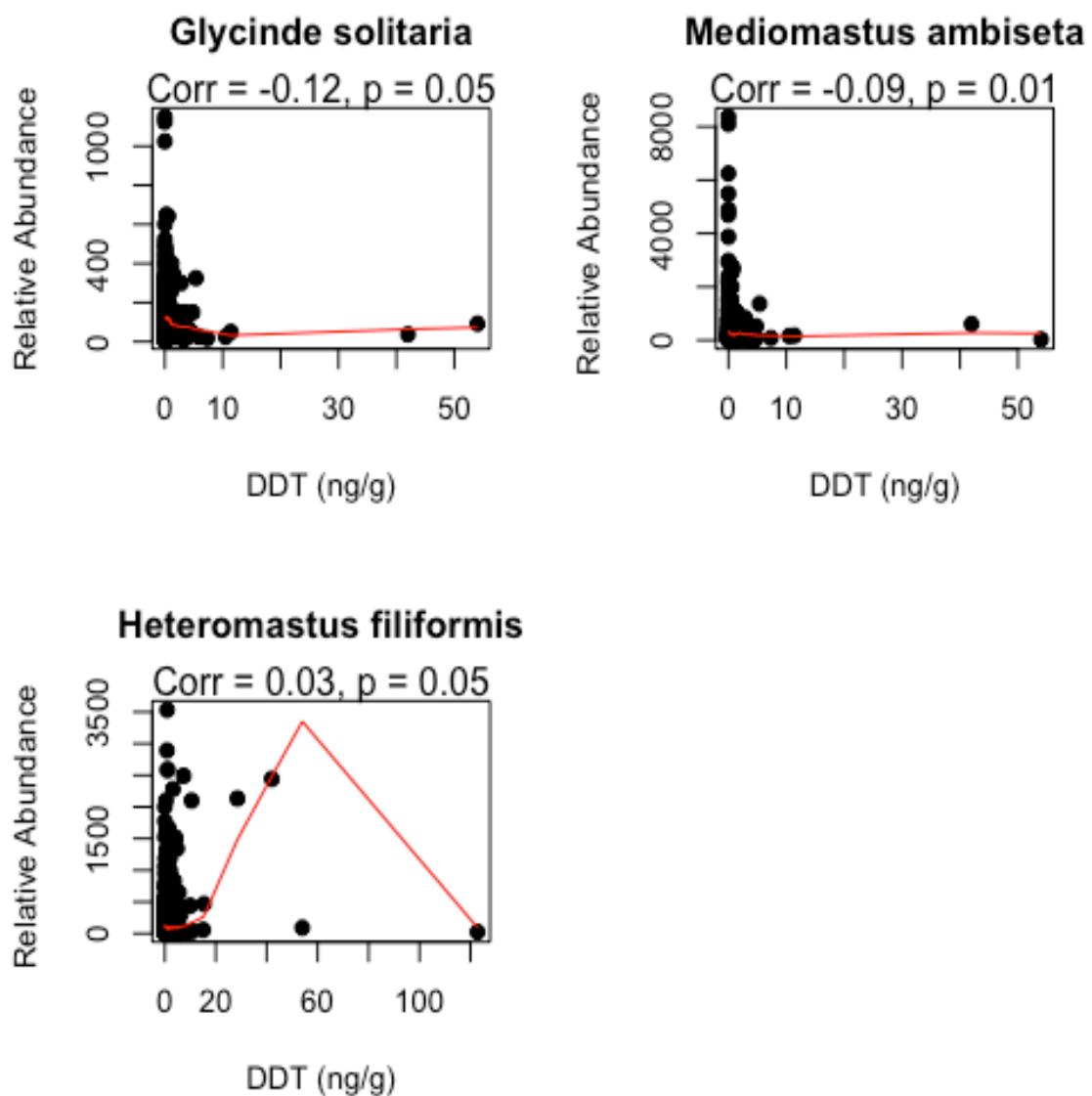


Figure 3 Significant correlation of DDT concentrations with the relative abundances of *G. solitaria*, *M. ambiseta* and *H. filiformis* ( $\alpha = 0.05$ )



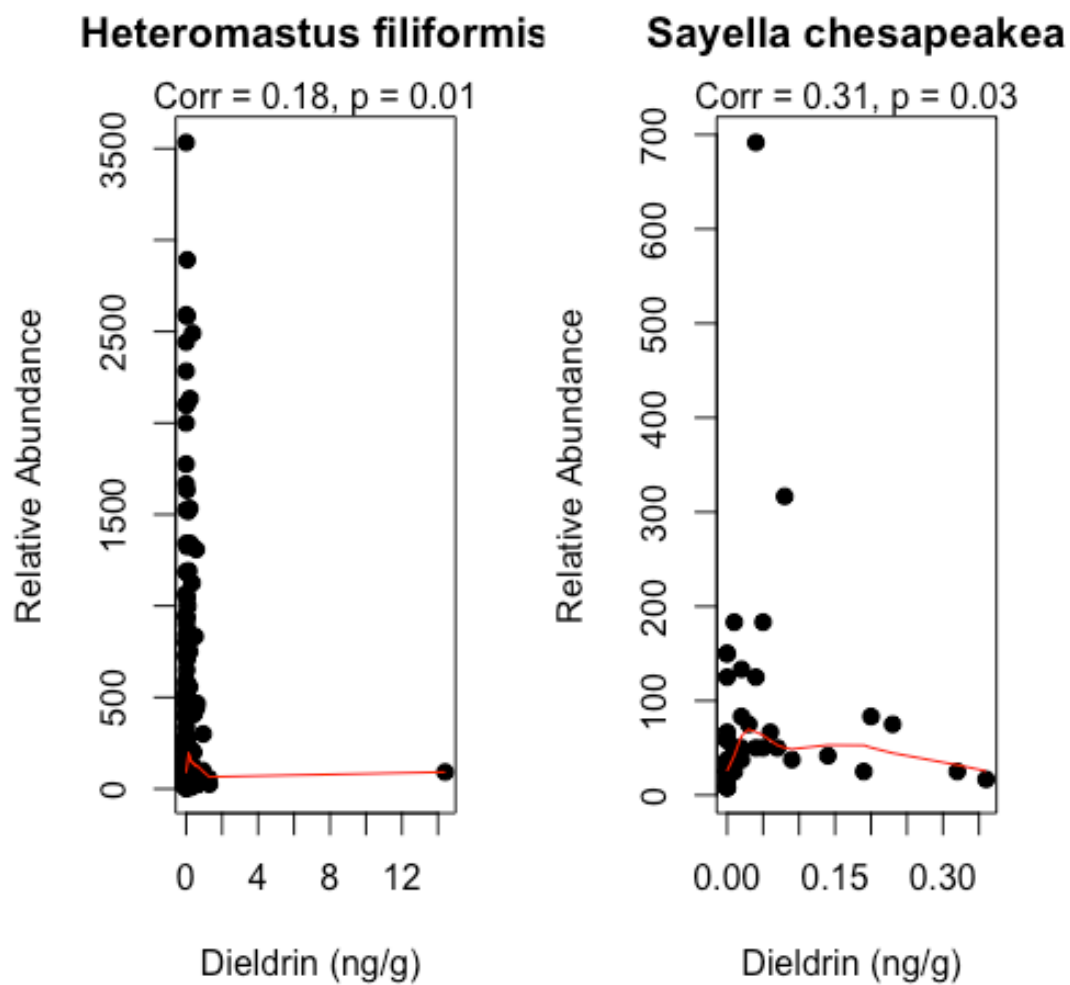


Figure 4 Significant correlation of Dieldrin concentrations with the relative abundances of *H. filiformis* and *S. chesapeakea* ( $\alpha = 0.05$ )

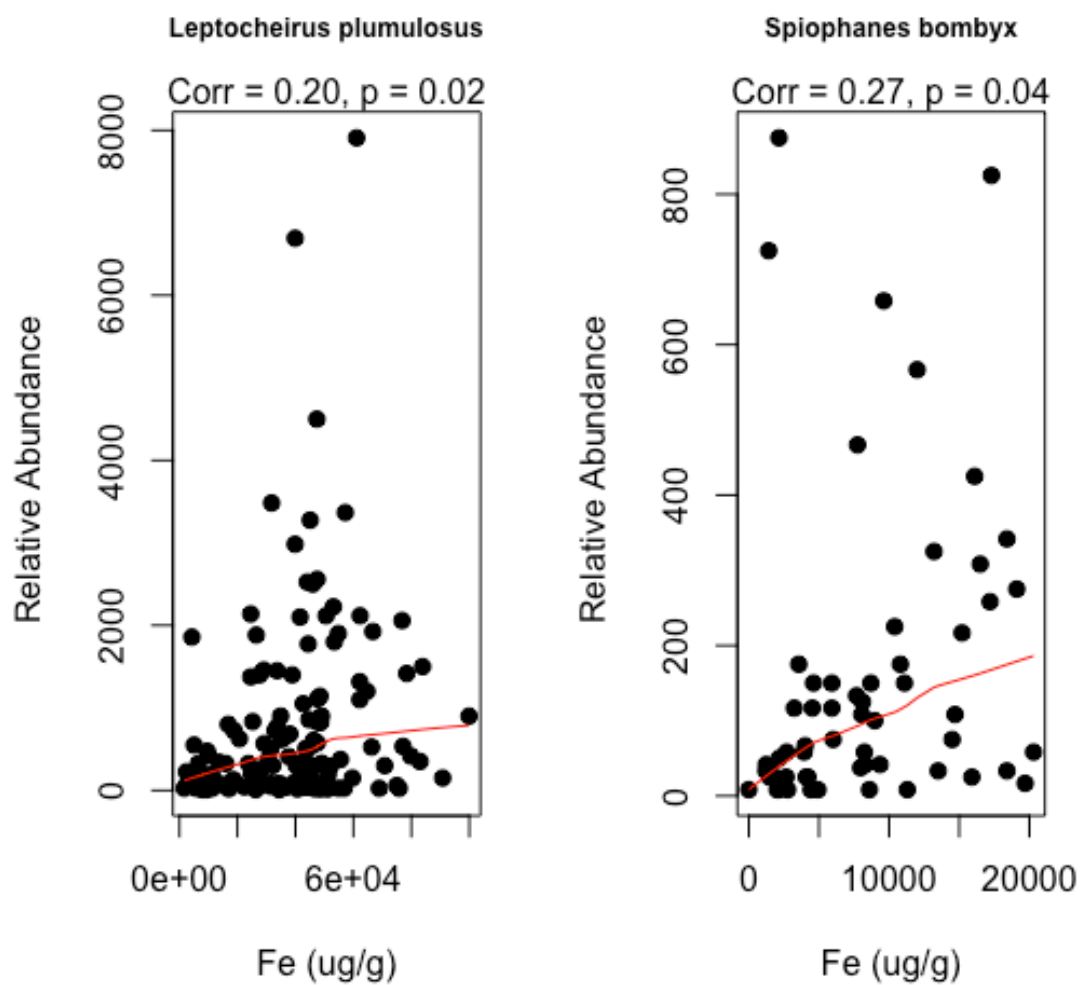


Figure 5 Significant correlation of Fe concentrations with the relative abundances of *L. plumulosus* and *S. bombyx* ( $\alpha = 0.05$ )

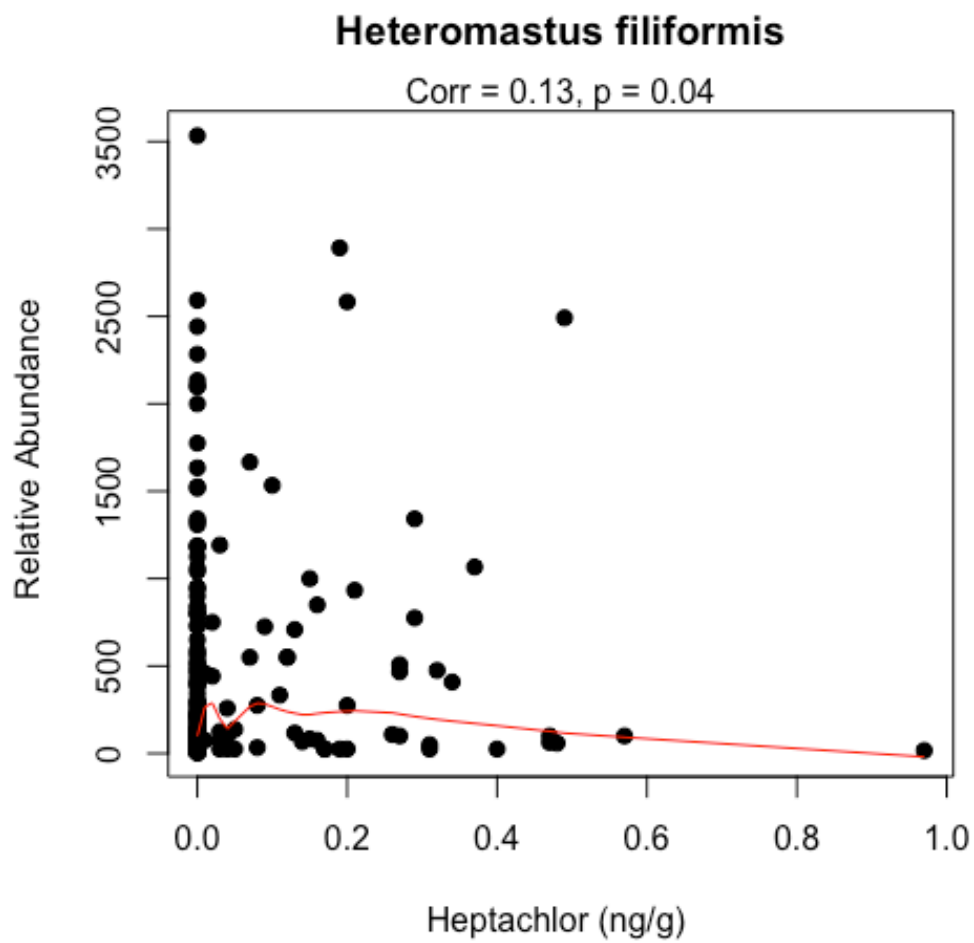


Figure 6 Significant correlation of Heptachlor with the relative abundance of *H. filiformis* ( $\alpha = 0.05$ )

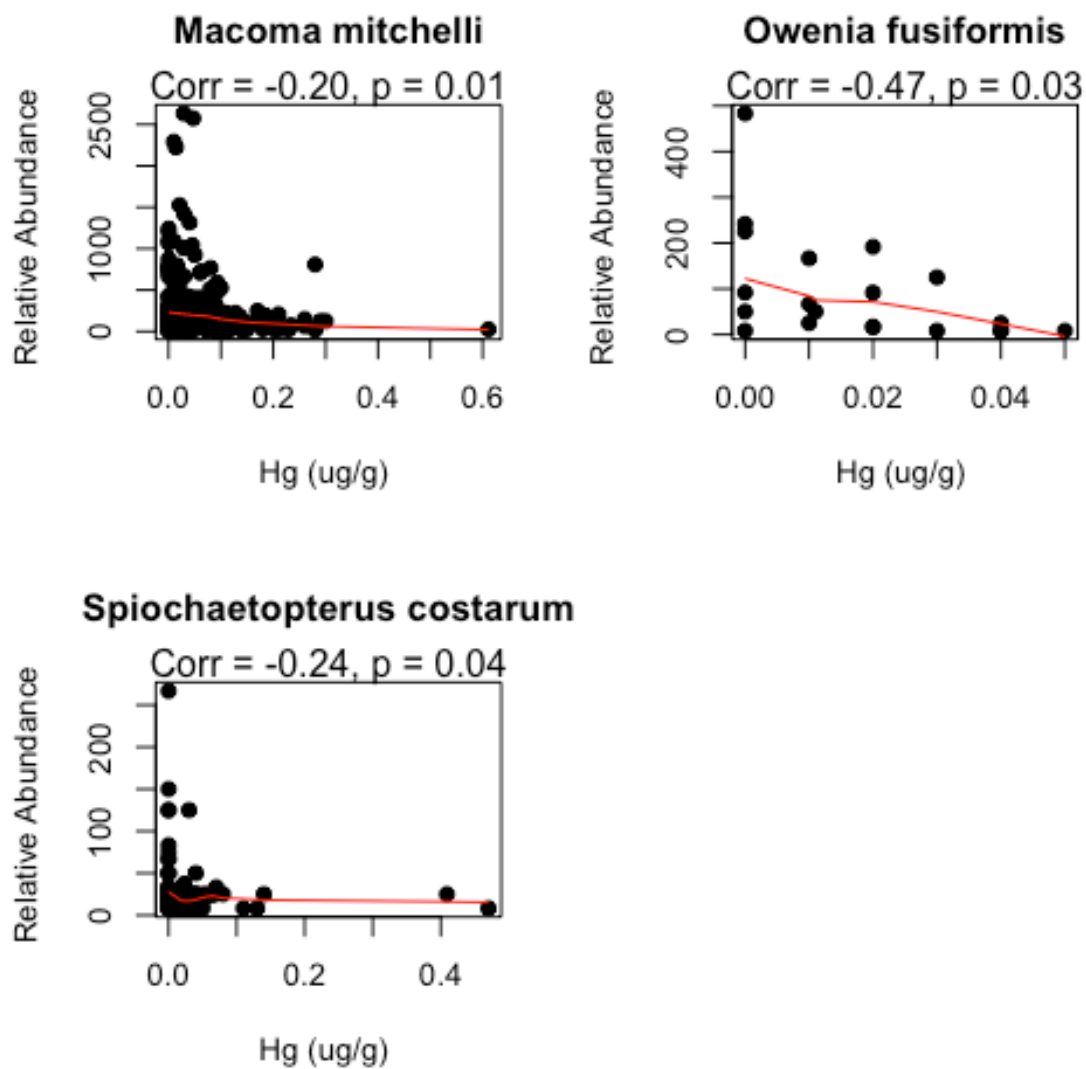


Figure 7 Significant correlation of Hg with the relative abundances of *M. mitchelli*, *O. fusiformis* and *S. costarum* ( $\alpha = 0.05$ )

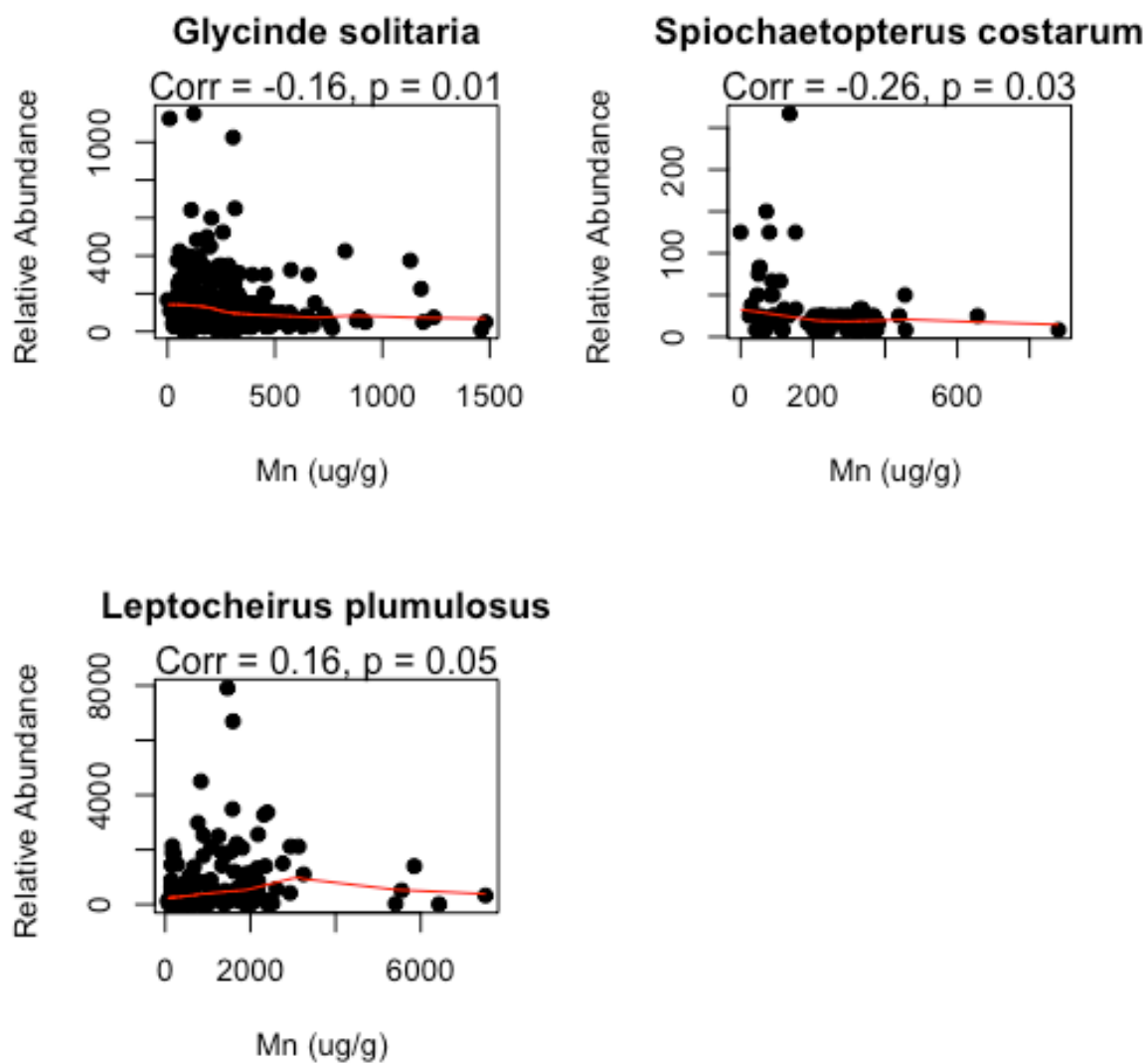


Figure 8 Significant correlation of Mn concentrations with the relative abundances of *G. solitaria*, *S. costarum* and *L. plumulosus* ( $\alpha = 0.05$ )

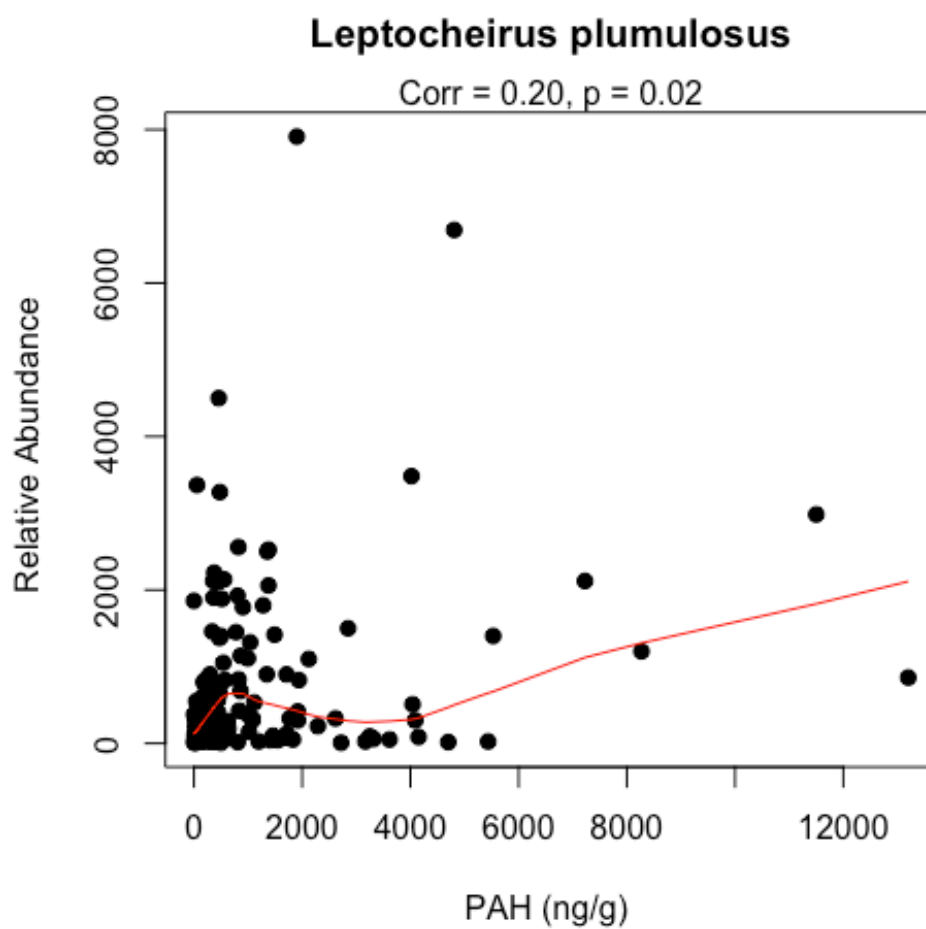


Figure 9 Significant correlation of PAH concentrations with the relative abundance of *L. plumulosus* ( $\alpha = 0.05$ )

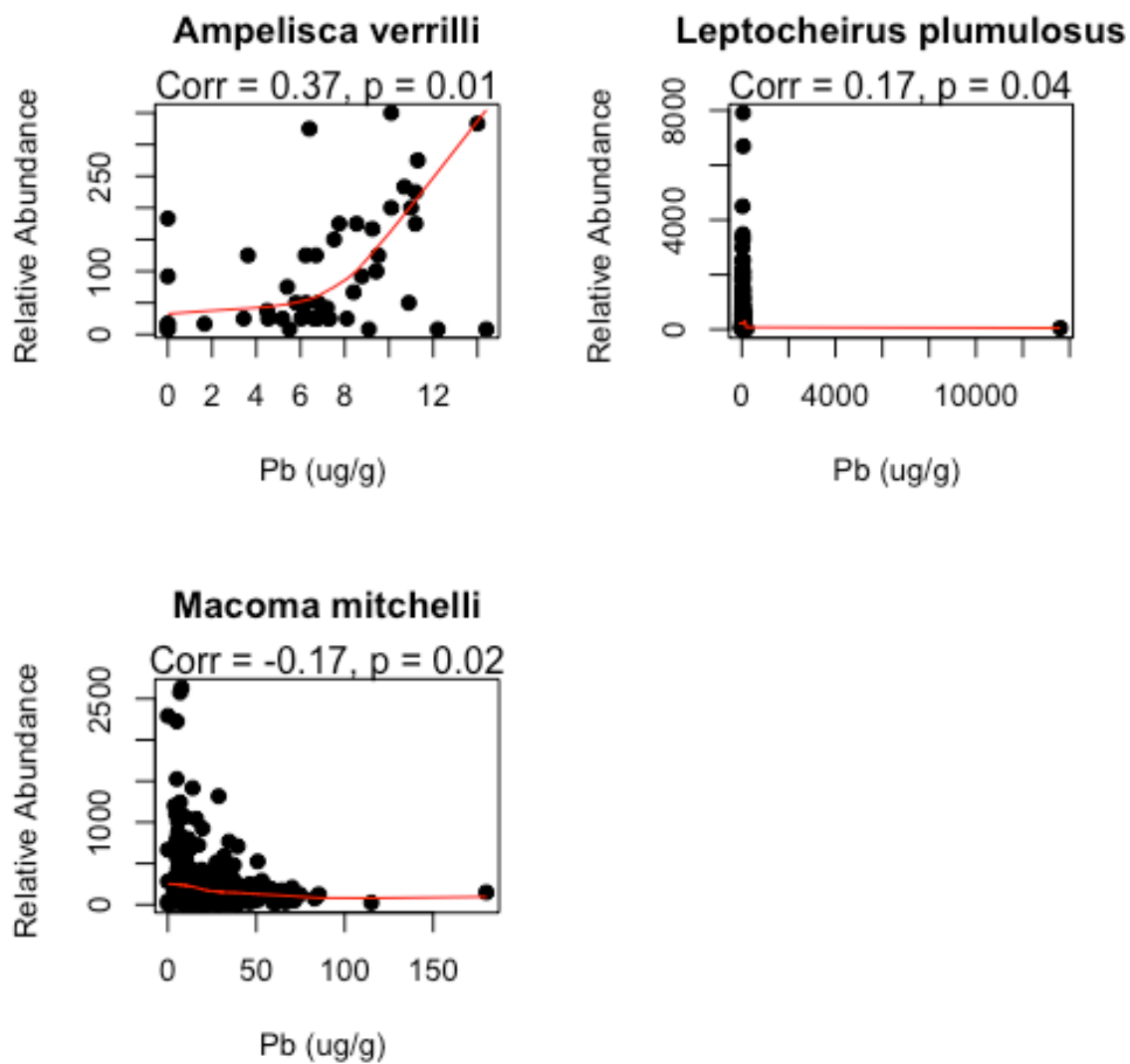


Figure 10 Significant correlation of Pb concentrations with the relative abundances of *A. verrilli*, *L. plumulosus* and *M. mitchelli* ( $\alpha = 0.05$ )

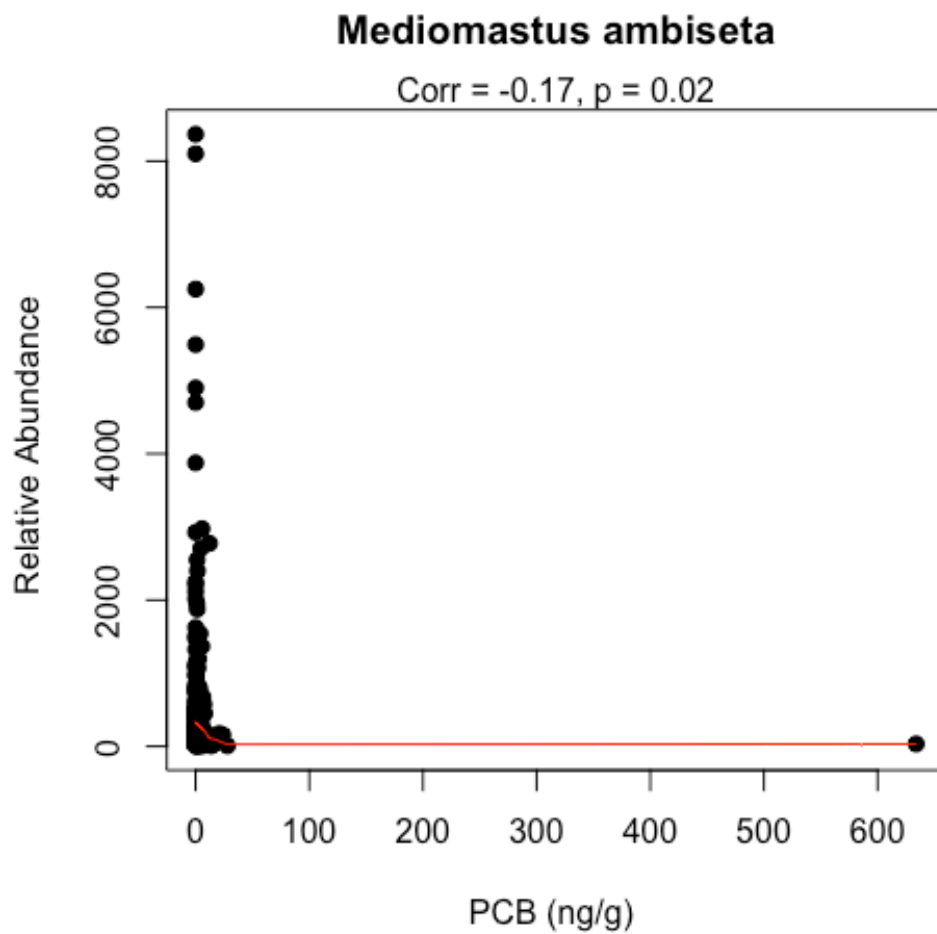


Figure 11 Significant correlation of PCB concentration with the relative abundance of *M. ambiseta* ( $\alpha = 0.05$ )



**Table 4 Summary of significant correlations between taxa and sediment contaminants**

<b>Contaminant</b>	<b>Taxa</b>	<b>Coefficient</b>	<b>p-value</b>
<b>DDT</b>	<i>Glycinde solitaria</i>	-0.12	0.05
	<i>Heteromastus filiformis</i>	0.03	0.05
	<i>Mediomastus ambiseta</i>	-0.19	0.01
<b>Dieldrin</b>	<i>Heteromastus filiformis</i>	0.18	0.01
	<i>Leptocheirus plumulosus</i>	0.20	0.02
	<i>Sayella chesapeakea</i>	0.31	0.03
	<i>Spiophanes bombyx</i>	0.27	0.04
<b>Heptachlor</b>	<i>Heteromastus filiformis</i>	0.13	0.04
<b>Hg</b>	<i>Macoma mitchelli</i>	-0.20	0.01
	<i>Owenia fusiformis</i>	-0.47	0.03
	<i>Spiochaetopterus costarum</i>	-0.24	0.04
<b>Mn</b>	<i>Glycinde solitaria</i>	-0.16	0.01
	<i>Leptocheirus plumulosus</i>	0.16	0.05
	<i>Spiochaetoterus costarum</i>	-0.26	0.03
<b>PAH</b>	<i>Leptocheirus plumulosus</i>	0.20	0.02
<b>Pb</b>	<i>Ampelisca verrilli</i>	0.37	0.01
	<i>Leptocheirus plumulosus</i>	0.17	0.04
	<i>Macoma mitchelli</i>	-0.17	0.02
<b>PCB</b>	<i>Mediomastus ambiseta</i>	-0.17	0.02

### ***Physical Variables***

Three taxa had significant correlations with dissolved oxygen (Figure 12). Negative correlations were observed with *Ampelisca abdita* and *Mulinia lateralis*. A significant positive correlation was observed with *Coelotanypus*. Nine taxa had significant correlations to bottom salinity (Figure 13 and Figure 14). Negative correlations were observed with *Hypereteone heteropoda*, *M. lateralis*, and *Polydora*

*cornuta*. Positive correlations were observed with *M. ambiseta*, *O. fusiformis*, *Prionospio perkinsi*, *Sigambra tentaculata*, *S. bombyx*), and *Tharyx*. One taxon had a significant positive correlation to sand- *G. solitaria* (Figure 15). Two taxa had significant negative correlations with silt-clay- *M. mitchelli* and *Marenzelleria viridis* (Figure 16). Two taxa had significant negative correlations with toxicity- *S. tentaculata* and *Tubificoides* (Figure 17). Table 5 provides a summary of these results.

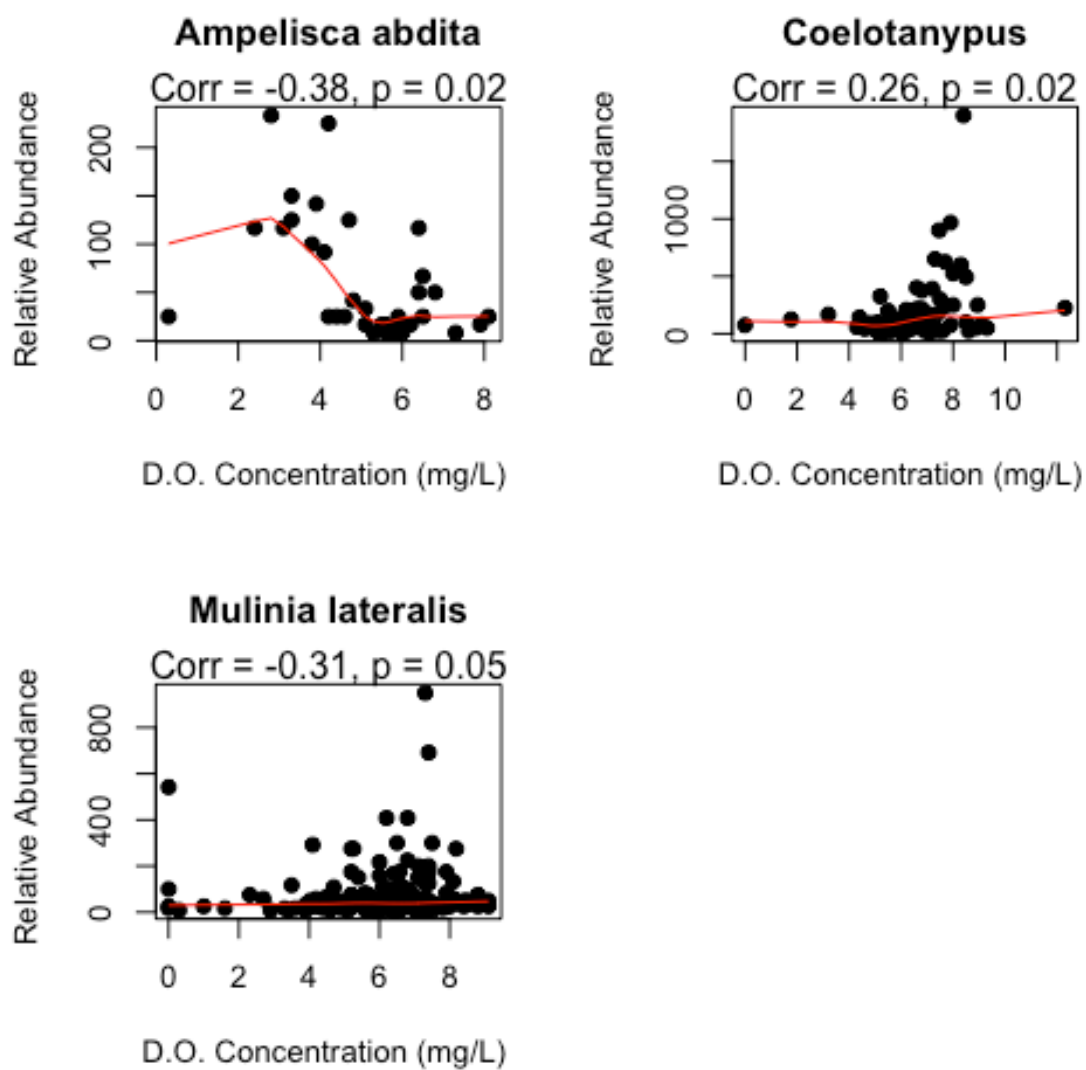


Figure 12 Correlation of dissolved oxygen (D.O) concentrations to the relative abundances of *A. abdita*, *Coelotanypus* and *M. lateralis* ( $p = 0.05$ )

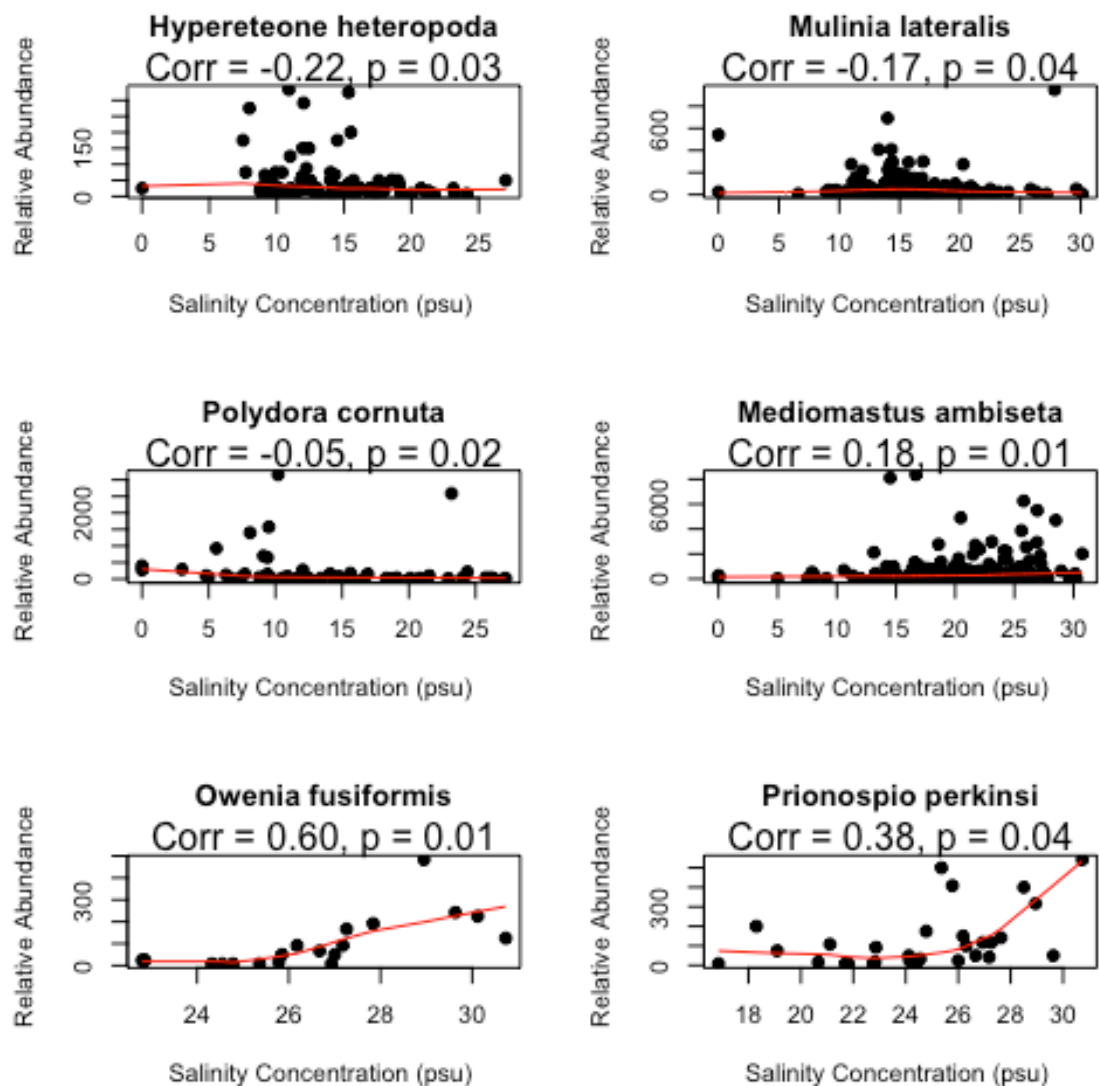


Figure 13 Correlation of salinity concentration to the relative abundances of *H. heteropoda*, *M. lateralis*, *P. cornuta*, *M. ambiseta*, *O. fusiformis* and *P. perkinsi* ( $p = 0.05$ )

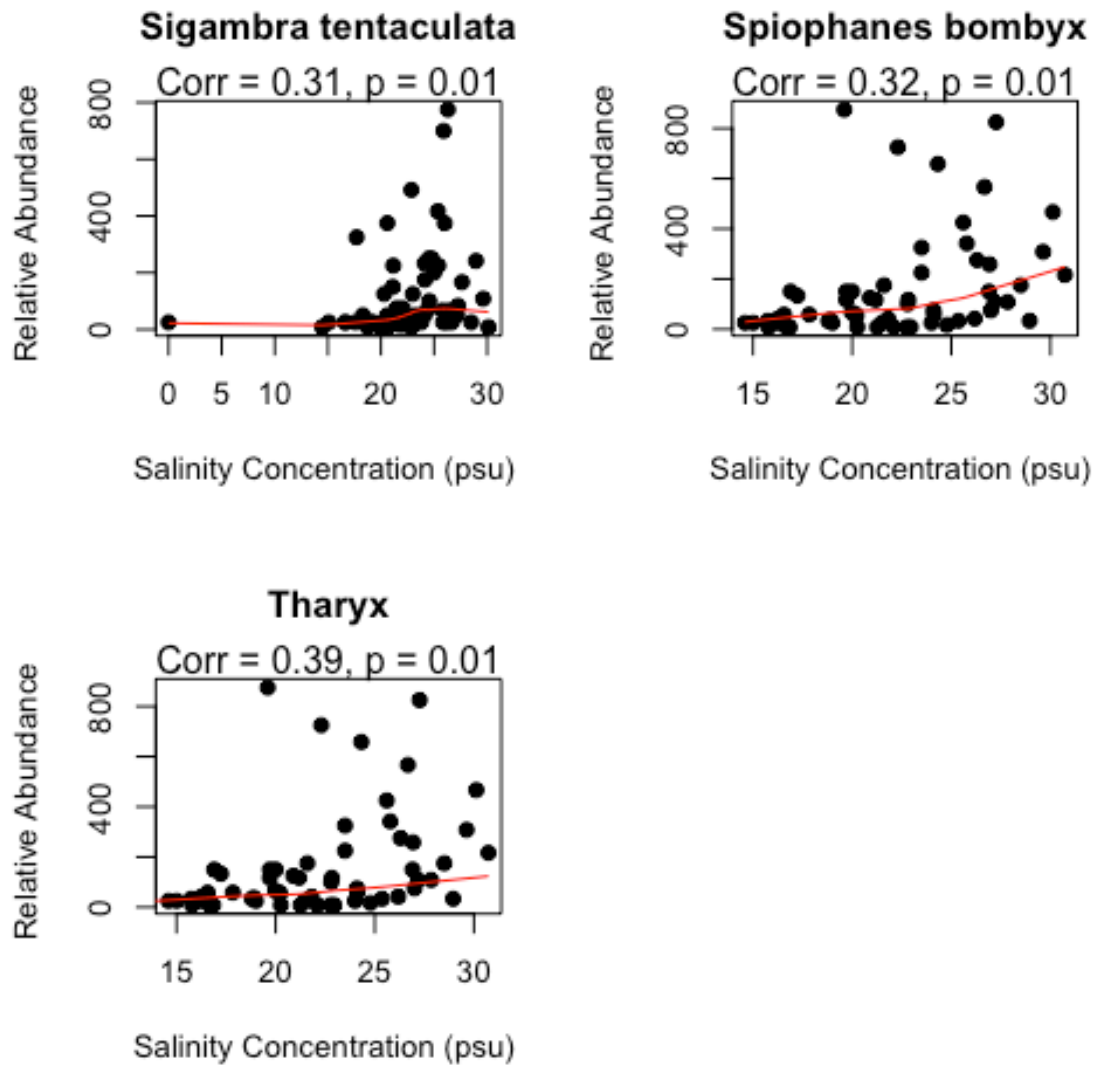


Figure 14 Correlation of salinity concentrations to the relative abundances of *S. tentaculata*, *S. bombyx* and *Tharyx* ( $p = 0.05$ )

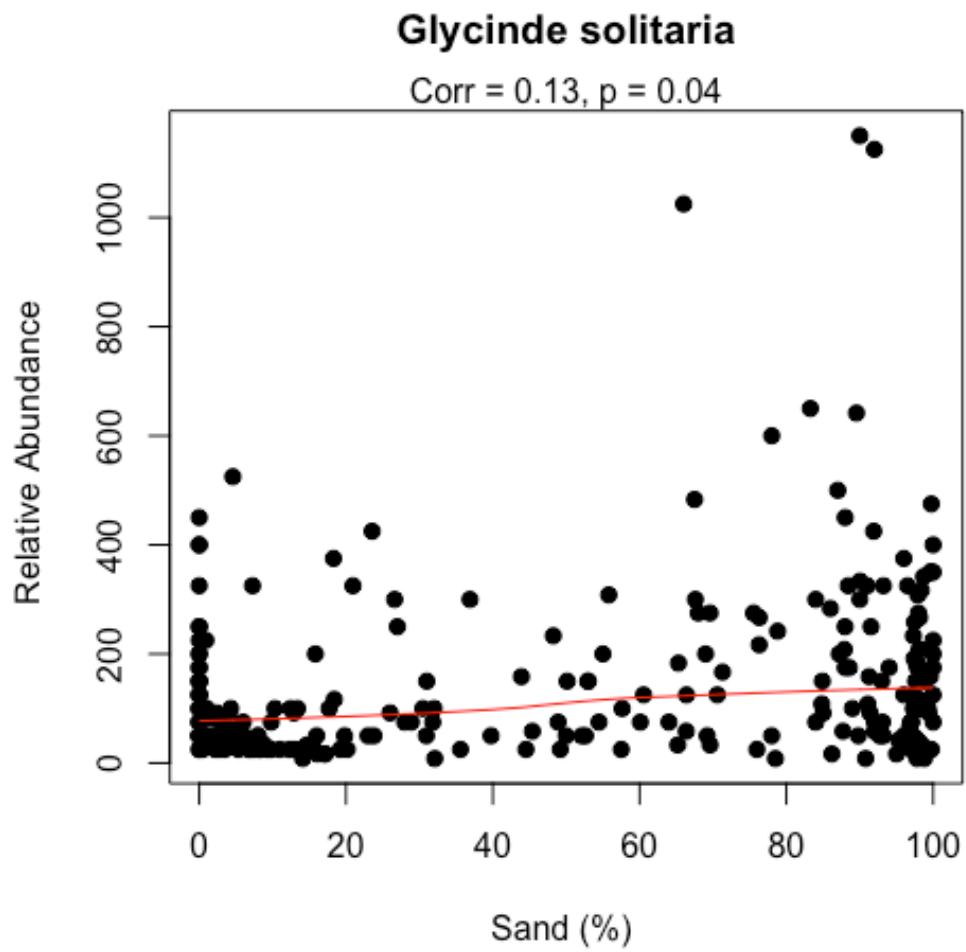


Figure 15 Correlation percent sand to the relative abundance of *G. solitaria* ( $p = 0.05$ )

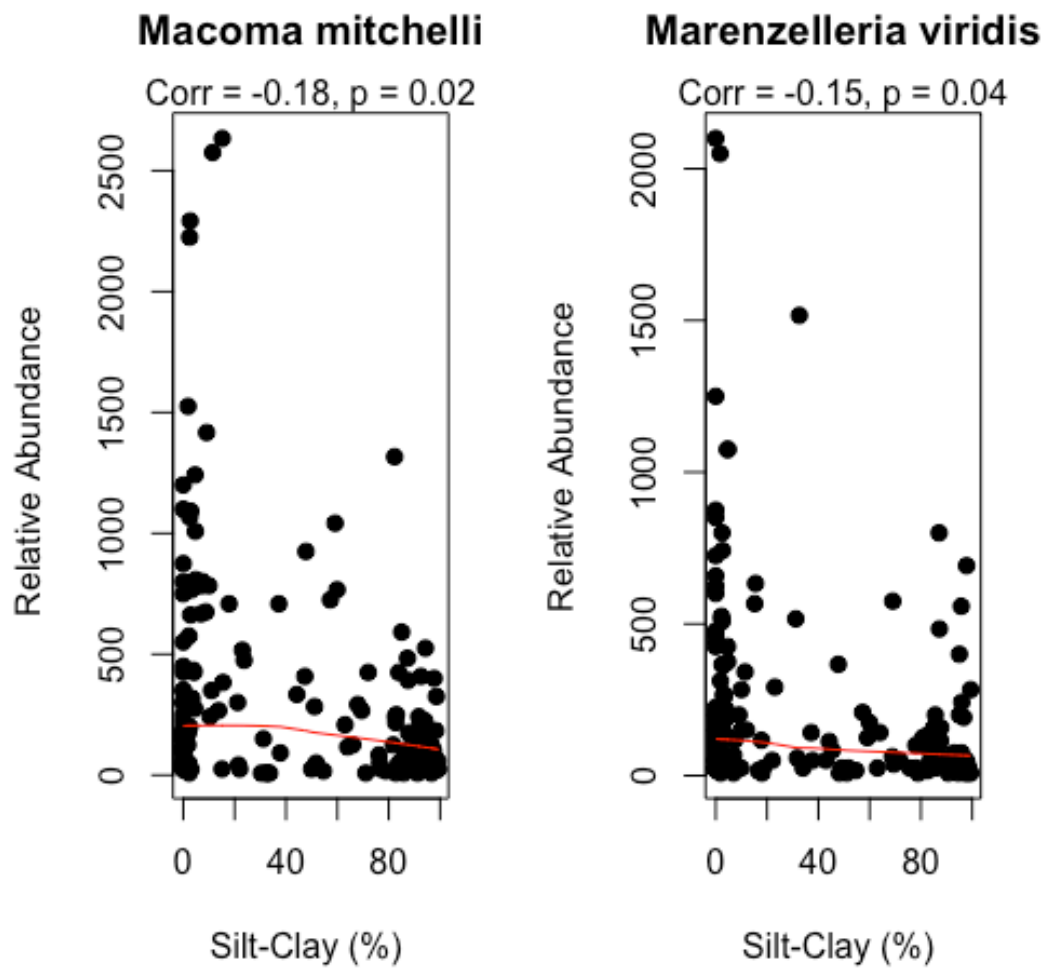


Figure 16 Correlation of percent silt-clay to the relative abundances of *M. mitchelli* and *M. viridis* ( $p = 0.05$ )

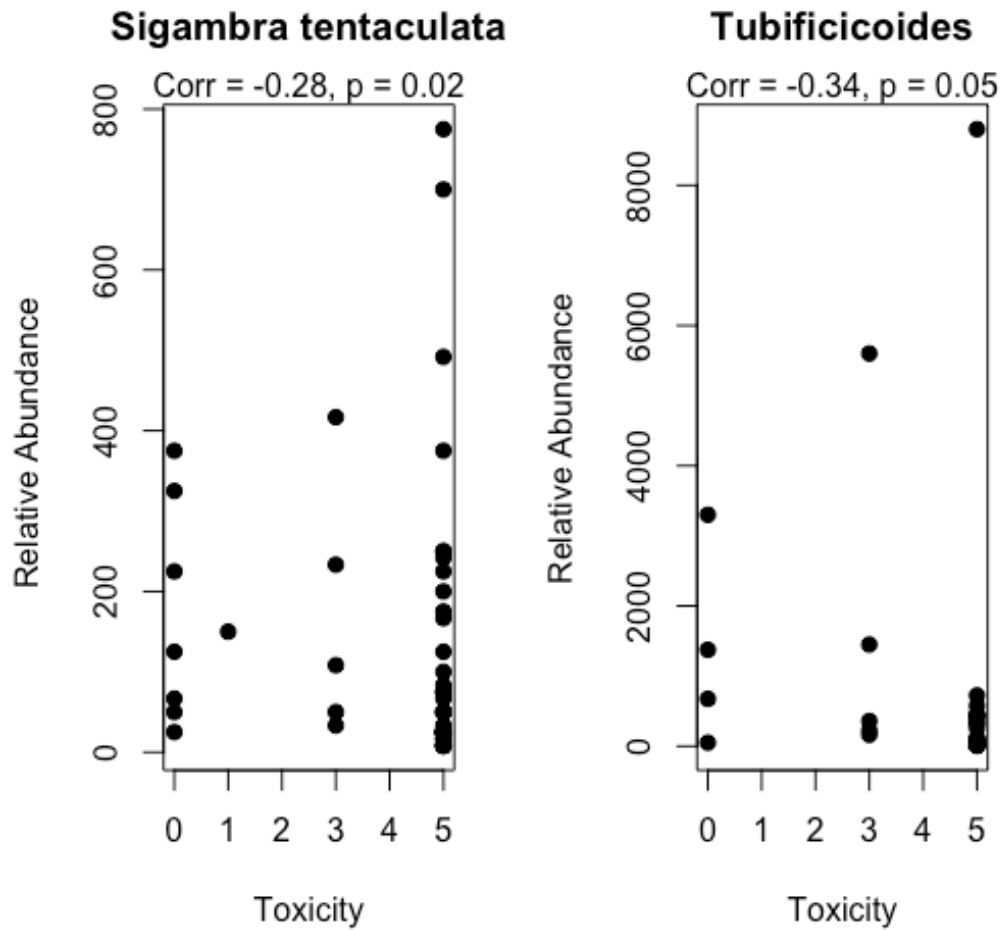


Figure 17 Correlation of Toxicity to the relative abundances of *S. tentaculata* and *Tubificoides* ( $p = 0.05$ ). Toxicity scale: 1.0 = Poor, 3.0 = Fair and 5.0 = Good.



**Table 5 Summary of significant correlations between taxa and physical variables**

<b>Physical Variable</b>	<b>Taxa</b>	<b>Coefficient</b>	<b>p-value</b>
<b>Dissolved Oxygen</b>	<i>Ampelisca abdita</i>	-0.38	0.02
	<i>Mulinia lateralis</i>	-0.31	0.05
	<i>Coelotanypus</i>	0.26	0.02
<b>Salinity</b>	<i>Hypereteone heteropoda</i>	-0.22	0.03
	<i>Mulinia lateralis</i>	-0.17	0.04
	<i>Polydora cornuta</i>	-0.05	0.02
	<i>Mediomastus ambiseta</i>	0.18	0.01
	<i>Owenia fusiformis</i>	0.60	0.01
	<i>Prionospio perkinsi</i>	0.38	0.04
	<i>Sigambra tentaculata</i>	0.31	0.01
	<i>Spiophanes bombyx</i>	0.32	0.01
	<i>Tharyx</i>	0.39	0.01
	<i>Glycinde solitaria</i>	0.13	0.04
<b>Sand</b>			
<b>Silt-clay</b>	<i>Macoma mitchelli</i>	-0.18	0.02
	<i>Marenzelleria viridis</i>	-0.15	0.04
<b>Toxicity</b>	<i>Spiophanes bombyx</i>	-0.28	0.02
	<i>Tubificicoides</i>	-0.34	0.05

## Discussion

A total of 34 of the 149 taxa met the selection criterion (i.e., occurred in at least 10 sites) and 20 showed significant relationships with the specific environmental variables identified. The taxa were three amphipods (*A. abdita*, *A. verrilli*, and *L. plumulosus*), one chironomid (*Coelotanypus*), twelve polychaetes (*G. solitaria*, *H. filiformis*, *H. heteropoda*, *M. viridis*, *M. ambiseta*, *O. fusiformis*, *P. cornuta*, *P. perkinsi*, *S. tentaculata*, *S. costarum*, *S. bombyx* and *Tharyx*), two bivalves (*M. mitchelli* and *M. lateralis*), one gastropod (*S. chesapeakea*) and one oligochaete (*Tubificicoides*). Based

on available life history trait information for benthic macroinvertebrates found in Chesapeake Bay, twelve taxa (*A. verrilli*, *A. abdita*, *Coelotanypus*, *G. solitaria*, *H. filiformis*, *H. heteropoda*, *L. plumulosus*, *M. ambiseta*, *M. lateralis*, *P. cornuta*, *S. bombyx*, and *Tubificoides*) are categorized as opportunistic (i.e., small, relatively short-lived and tolerant taxa with high reproductive and recruitment potential) while none are categorized as equilibrium (i.e., large, long-lived) (Dauer, 1993; Ranasinghe et al., 1993). Opportunistic taxa abundance and biomass tend to dominate disturbed and stressed areas therefore it is not surprising that many of the taxa with significant responses to stressors in this study are considered opportunistic, as I am seeking taxa that respond in some significant way to stress. The following discussion examines the individual taxa responses to stress observed in this study.

Three tube-building amphipods- *A. abdita*, *A. verrilli*, and *L. plumulosus*- had significant relationships in this study: *A. abdita* responded negatively to dissolved oxygen, *A. verrilli* positively to Pb, and *L. plumulosus* all positively to dieldrin, Mn, PAH, and Pb. All three species are considered as pollution-sensitive according to Weisburg et al., (1997). The response of *A. abdita* to dissolved oxygen is most likely attributed to the fact that *A. abdita* is a tube-dweller and it can withstand low oxygen concentrations by retreating into its tube. Also, their abundances could be higher because predators are affected by low oxygen and either move away or die, leaving *A. abdita* free from predator pressure. (Cicchetti et al., 2006; Holland, 1985). Similarly, *A. verrilli* has a positive relationship to Pb as its tube serves as a barrier to the surrounding sediments. Also, Pb could be bound to sediment or organic matter making it less bioavailable and

less toxic to the environment. Similar to suspension feeding *A. abdita*, *M. lateralis* also responded negatively to dissolved oxygen in this study. Again, this is most likely due to impacts to feeding behavior and physiology (Schaffner, 1990). *M. mitchelli* prefer muddy habitats and had significant negative responses to Hg, Pb and silt-clay in this study. In estuarine benthic environments, organic matter tends to be higher in silt-clay (muddy) environments with concomitant increased concentrations of heavy metals that bind to sediment particles (Weisburg et al., 1997; Pearson and Rosenberg, 1978).

The chironomid *Coelotanypus* is commonly found in Chesapeake Bay and is classified as pollution-indicative in all salinity ranges by Weisburg et al. (1997) and Llanso and Dauer (2002). In the current study, *Coelotanypus* exhibited a significant positive response to dissolved oxygen which could be due to the presence of elevated organic matter concentrations and potentially hypoxic events (Diaz & Schaffner, 1990).

Over half of the 20 taxa analyzed in this study were annelids- twelve polychaetes and one oligochaete- as they were the most abundant taxa amongst all sites. Four polychaetes- *G. solitaria*, *M. ambiseta*, *O. fusiformis* and *S. costarum*- are classified as pollution-sensitive taxa, and had significant response relationships to DDT, dieldrin, Hg, Mn, PCB, salinity and sand. *H. filiformis* and *S. bombyx*, as well as the oligochaete *Tubificoides*, are classified as pollution-indicative with significant responses to DDT, dieldrin heptachlor and salinity and toxicity. The remaining polychaetes- *H. heteropoda*, *P. cornuta*, *P. perkinsi*, *S. tentaculata*, *Tharyx* and *M. viridis* had significant relationships most often to salinity, indicating the influence that habitat factors also have on benthic taxa stress response.

The aim of this study was to elucidate whether specific benthic macroinvertebrate responses to environmental stressors could be extracted from long-term datasets. Because I focused on stressor responses, it made logical sense that the taxa with responses were all opportunistic species and that equilibrium species, or larger, long-living species, were not present in the subset of taxa analyzed. Opportunistic species thrive in disturbed environments where their life history traits result in high abundances, high reproductive rates and high mortality rates, all factors that allow them to exist in dynamic settings. In addition, just over half of the taxa were opportunistic annelids that are common to and rapidly colonize disturbed aquatic environments. The inclusion of chironomids and oligochaetes, well-documented indicators of stress in freshwater environments, demonstrates that individual taxa stressor responses were detected along the complete range of Chesapeake Bay habitats.

The stressors analyzed were a combination of anthropogenic (contaminants) and natural stressors (primarily salinity and grain size). As expected, several taxa responded to various contaminants, whether it was just one or a few. Many of these taxa also responded to grain size and salinity which have documented effects on the distribution of taxa in soft bottom sediments (Pearson and Rosenberg, 1978; Weisburg et al., 1997; Lenihan et al. 2003). For the purposes of this study, I was interested in general stressor response patterns and therefore did not constrain by habitat parameters prior to analyses. Analyses of the stressor response patterns in future chapters will involve classifying the taxa by habitat to remove confounding factors in the observed responses, as well as to allow comparison of results to related studies.

Absent from this analysis were significant taxa responses to aldrin and total organic carbon (TOC). Aldrin, along with dieldrin, heptachlor and DDT are considered to be of localized extent and severity in Chesapeake Bay (US Environmental Protection Agency et al., 2012), indicating that these pesticides are found in limited areas (primarily adjacent or in close proximity to areas of intense agricultural use) within the Bay. Significant taxa responses to dieldrin, heptachlor and DDT were observed in this study. It may be that the locations from which data were collected were not grossly affected by aldrin, the influence from the other pesticides were greater and masked the effects of aldrin, or aldrin had a greater effect on more sensitive taxa that were not captured in the sampling events.

Unexpectedly, there were no significant responses observed to TOC. Organic matter, usually expressed as TOC, in sediments serves as an important source of food for benthic animals. An oversupply of organic matter can lead to decreases in benthic abundance, species richness and biomass caused by oxygen depletion and the buildup of ammonia and sulphide from the breakdown organic matter (Diaz & Rosenberg, 1995). Frequently, increases in organic matter concentrations in the sediment are accompanied by increased concentrations of contaminants, which is especially true in finer-grained (i.e., silt-clay or muddy) sediments where there is more surface area for the contaminants to adhere to (Hyland et al., 2005; Pearson & Rosenberg, 1978). There were several instances where taxa had significant responses to both contaminants and silt-clay, and also instances where taxa had significant responses to dissolved oxygen. While a direct

significant relationship between individual taxa and TOC was not observed, the direct and indirect effects of increased concentrations of organic matter were observed.

The information provided by the individual taxa responses here will be useful in developing a tool that could enhance the Benthic Index of Biotic Integrity (B-IBI). The B-IBI incorporates metrics of assemblages rather than metrics of individual species, because assemblage metrics are less sensitive to salinity and grain size changes. Given my findings, I contend that there is value in utilizing individual taxa metrics. They may provide a way to obtain results that serve as either an early warning sign of the presence of anthropogenic stressors, or to confirm that the ecosystem is not functioning as expected at a finer scale and with less effort to evaluate. The relationships identified here will be used in the next chapter to build a model that incorporates the direction of the change (increasing or decreasing) in taxa abundance as a function of the stressor(s) in question.

## **Conclusion**

Benthic community condition is a critical element in understanding overall estuarine condition or health. Assessment tools often rely on various measures of benthic community structure to accomplish this, based on community-level metrics of stressor-responses. Here, I approached stressor-response from the bottom up to determine if there are characteristic patterns in how individual taxa respond to specific stressors. A set of taxa emerged with significant responses to both natural and anthropogenic stressors, and once the types of these response are determined, they can be incorporated into a model

that will aid in discerning the types of stressors that may exist in a given area, where an IBI suggests that there is a disturbance. The information that this model provides could be used to initiate a stressor identification process, target where more intense monitoring should be and help inform and guide management decisions on where limited resources should be directed for monitoring and assessment activities.

## **CHAPTER THREE: STRESSOR-RESPONSE MODEL DEVELOPMENT**

### **Introduction**

Benthic indices are important and useful tools to better understand aquatic system health, structure and function. Benthic systems are diverse and dynamic systems (Widdicombe & Spicer, 2008), making it difficult to clearly tease out benthic stressor response relationships as there are several present at any given time. Assessment indices and the indicators selected are pivotal in understanding the degree of stressors at hand with some degree of uncertainty (Hyland et al., 2005). There are a multitude of indices established with validated metrics that also consider diversity and biomass, as both relate to population and community level responses (e.g. Benthic Index of Biotic Integrity (B-IBI; Weisberg et al. 1993) and AZTI Marine Biotic Index (AMBI; Borja et al. 2000, 2003) and their component metrics). Many of these indices focus on assessing assemblages or communities, rather than individual species or taxa. To understand how organisms handle stress, explain the mechanistic basis of stress tolerance, and predict effects on populations and communities, we must understand the effects of stress on individuals (Maltby 1999, Newman 2001, Newman and Clements 2008).

There are various approaches to categorize species into tolerance groups, as a means of assessing biological condition. The B-IBI was developed to assess summer estuarine benthic ecological condition in the Chesapeake Bay. It is based on the multi-



metric index of biotic integrity developed for freshwater habitats to integrate biological responses to stress and account for natural habitat variation. Metrics selected included species diversity, abundance, biomass, or abundance of pollution sensitive species, trophic composition, and presence/absence of pollution-indicative species. The presence/absence of pollution indicative species metric classifies individual species into pollution tolerance groups of sensitive, tolerant, or unclassified, based on whether a species exhibits an opportunistic or equilibrium life history and, primarily, expert opinion. Borja et al. (2003) and Eaton (2001) employ similar approaches in classifying pollution tolerance based on life-history characteristics. An implicit assumption in this approach to classify species into pollution tolerance groups is that tolerance responses are not stressor-specific, and are often ambiguous or inconsistent to pollution (Weisburg et al., 1997). This can lead to potential misclassification of a given area due to limited availability of life history and pollution sensitivity data.

The misclassification of the capitellid polychaete *Mediomastus ambiseta* is a good example of this issue. Based on life history information of capitellids and expert knowledge, *Mediomastus* sp. was initially classified as a pollution-tolerant, opportunistic organism with a widespread distribution in estuaries. Distribution data obtained during B-IBI development showed that *M. ambiseta* had the highest abundances at healthy reference sites and was rare at degraded (primarily eutrophic and/or low dissolved oxygen) reference sites, which is completely opposite to how experts classified the pollution sensitivity of this polychaete. It is unknown what stressor(s) *M. ambiseta* was reacting to in the system to result in an unexpected sensitive, rather than tolerant

response. Certain polychaete genera such as *Mediomastus sp.* and *Capitella sp.* have traditionally been categorized as pollution-tolerant, due to high tolerance to organic enrichment, metal-contaminated sediments and low dissolved oxygen concentrations (Pearson and Rosenberg 1978). A review by Dean (2008) explored the fact that many polychaetes like *M. ambiseta* are indeed misclassified, as polychaetes exhibit variability in sensitivities to specific metals and between different species.

Baird and Van den Brink (2007) hypothesized that species biological traits, such as morphology, life history, physiology, and feeding ecology, could be used to predict an organism's sensitivity to stress (toxic substances in particular) and develop species sensitivity distributions (SSD). Taxonomic group, body size, life cycle duration, mode of respiration, and feeding type were the major traits analyzed within a group of 12 species exposed to 15 chemicals. It was concluded that skin respiration, gill respiration, insect/crustacean taxa, and life-cycle duration were traits that explained 71% of the variability in sensitivity to toxic chemicals.

Stressor-response relationships that are identified using species-specific biological traits and stressor sensitivity information, likelihood of exposure, and recovery potential are key determinants in identifying important stressor relationships to assess ecological condition. I hypothesize that utilizing some of this information from benthic macroinvertebrate and stressor data will allow me to build a model that characterizes specific taxa responses to specific stressors, as a means to help further explain the results of benthic condition assessments currently used. This model would be composed from the same data set that a B-IBI (or similar index) is built and would not require additional

time-consuming and costly data collection efforts develop. Additionally, this model would be simple to use and could indicate where resources for more sampling and data are needed to address the presence of stressors or disturbances in a system that it highlights as a potential concern.

## Methods

The taxa and the significant stressor relationships identified in Chapter 2 (Table 4 and Table 5) were analyzed with Generalized Additive Models (GAM) in R, using the *mgcv* package (Wood, 2017). GAM is a semi-parametric extension of Generalized Linear Models (GLM) that makes assumptions that relationships between individual predictors and the response variable are not restricted by any shape and is determined by the data. Unlike GLM, the linear predictor is not forced to be linear but rather is the sum of smoothing functions. Link (smoothing) functions are used to generalize data into smooth curves that are locally fit to data. The data are essentially divided into sections and low order polynomials are fit to each section, combined by “knots” at the ends each section to smooth the curve. Those sections are then added up resulting in an additive model with a goal to strike a balance between goodness-of-fit and parsimony. The relationships are estimated simultaneously, and the response variable is predicted by adding the relationships up using the formula:

$$g(E(Y)) = \alpha + s_1(x_1) + \dots + s_p(x_p)$$

where  $Y$  is the dependent variable,  $E(Y)$  is the expected value, and  $g(Y)$  is the link function that links the expected value to the predictor variables  $x_1, \dots, x_p$  (Hastie & Tibshirani, 1987).

A model was constructed for each taxa and the associated predictor variables (covariates,) with identity-link function for a Gaussian error distribution (default) or a log-link function for Poisson error distributions. The Poisson error distribution is appropriate for count data and therefore appropriate to use here. Restricted maximum likelihood (REML) was selected as the smoothing function for both sets of models instead of the default Generalized Cross Validation (GCV), as GCV tends to underfit models.

The default smoothing term  $s$  and default basis dimension  $k$ , which sets the upper limit of the degrees of freedom associated with a smooth  $s$ , was applied to each of the model covariates. If the estimated degrees of freedom (e.d.f) of the model terms approach  $k' (k-1)$ , then  $k$  is considered to be too low and should be adjusted for a better fit, as  $k$  should be chosen to be sufficiently large or small enough to have enough degrees of freedom to represent the underlying data. In this study, the default  $k$  was used ( $k = 10$ ) as to control factors of the models for comparison.

Models were plotted to observe the shape of the response curve and identify the number of knots that form the shape of the curve. Akaike Information Criterion (AIC), a log-likelihood measure, was used to select the best models amongst the two distribution types, and the shapes of the responses of the best models were identified. Salinity, sand and silt-clay covariates were removed from the models to tease out the natural variation

and remove confounding driving factors of benthic macroinvertebrate abundance. Taxa that only had significant relationships to salinity and/or silt clay were also removed, resulting in 11 taxa-stressor response relationships to analyze (Table 6).

**Table 6 Summary of the model covariates and number of knots to smooth the curves produced by the models**

<b>Taxa</b>	<b>Model Covariates</b>	<b># knots</b>	<b># knots</b>
		<b>Gaussian</b>	<b>Poisson</b>
<i>Ampelisca abdita</i>	Dissolved Oxygen	4.4	8.83
<i>Ampelisca verrilli</i>	Pb	1.43	8.8
<i>Coelotanypus spp.</i>	Dissolved Oxygen	3.23	8.98
<i>Glycinde solitara</i>	DDT	1.45	1.89
	Mn	8.93	8.86
<i>Heteromastus filiformis</i>	DDT	2	8
	Dieldrin	1	8
	Heptachlor	1	8
<i>Leptochirous plumulosus</i>	Dieldrin	1	8.99
	Mn	2.16	8.98
	PAH	1	9
	Pb	1	8.93
<i>Mediomastus ambiseta</i>	DDT	1.86	8.06
	PCB	1	8.99
<i>Mulinia lateralis</i>	Dissolved Oxygen	1	1
	Hg	1	8.78
<i>Macoma mitchelli</i>	Hg	1	2.37
	Pb	8.76	8.74
<i>Sayella chesapeakea</i>	Dieldrin	1	8.99
<i>Spiochaetopterus costarum</i>	Hg	1.58	1
	Mn	8.8	8.36

## Results

GAMs were calculated for each taxon. The covariates as well as their responses were different among the taxa. Three taxa produced models that explained the highest percent deviance- *A. abdita* (59.1% Gaussian, 71.5% Poisson), *S. costarum* (50.9% Poisson) and *A. verrilli* (31% Poisson). The three taxa with the lowest percent deviance were *S. chesapeakea* (0.11 % Gaussian), *M. lateralis* (1.31 % Gaussian) and *M. ambiseta* (2.45 % Gaussian). Of the models tested, nine models had estimated degrees of freedom (*e.d.f*) close to  $k'$ , indicating that  $k$  is too low and needed adjusting for better curve smoothing. All but one of these nine models had *e.d.f.* close to  $k'$  with the Poisson error distribution: *A.abdita*, *A verrilli*, *G. solitaria*, *H. filiformis*, *L. plumulosus*, *M. ambiseta*, *M. lateralis*, and *M. mitchelli*; the *e.d.f.* for *Coelotanypus spp.* was close to  $k'$  with the Gaussian error distribution.

Figures 18 to 28 present the associated plots for each model tested. Each plot includes the number of knots needed to connect and smooth the data sections into a curve and is summarized in Table 6. Almost all models with the default Gaussian error distribution required less than half the number of knots than models with the Poisson error distribution. Nearly every Poisson model had at least 8 knots fitted to the data, while most of the Gaussian models had 1 to 2 knots fitted to the data. All models were compared to determine the best models, based on AIC values for each model type (Table 7). The species with the lowest AIC values were *A. abdita*, *A. verrilli* and *S. costarum* for the Gaussian error distribution, and *A. abdita*, *S. costarum* and *A. verrilli* for the Poisson error distribution. It is not appropriate to mix distribution types and the AIC values for

the Poisson distribution models are higher, indicating that different combinations of smooths and basis functions for each model term are needed to improve the models fits. For these reasons and because the Gaussian models had the lowest AIC scores, only the Gaussian models will be used at this time to develop stressor-response models in this study.

### ***Stressor Response Models***

The shapes of the response curves for the top three models for each distribution type varied and none of the models contain the same covariates. A non-linear response curve was observed for *A. abdita* to dissolved oxygen, and based on the 4.4 knots to smooth the curve, the equation for this model is a 4<sup>th</sup> degree polynomial. The data points for dissolved oxygen range primarily from 2 to 8 mg/L, which is the linear portion of the response curve. Given this, the equation of the response for *A. abdita* to dissolved oxygen can be written as a linear equation for purposes here (Equation I). The *A. verrilli* model had a linear response curve to Pb, with 1.4 knots to smooth the curve (Equation II). Lastly, *S. costarum* had linear response curves to both Mn and Hg, with 1.58 and 1 knots, respectively to smooth the curves (Equation III).

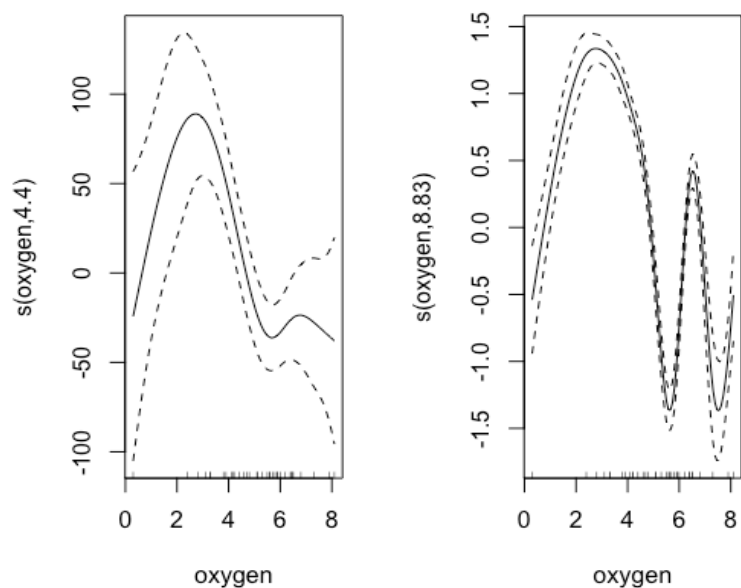


Figure 18 Modeled response of *A. abdita* to dissolved oxygen. Gaussian models on the left, Poisson on the right. The solid line is the predicted value of the predictive value as a function of  $x$ . The dotted lines are  $\pm$  two standard errors, and the tick marks on  $x$  axis indicate the location of sample data.

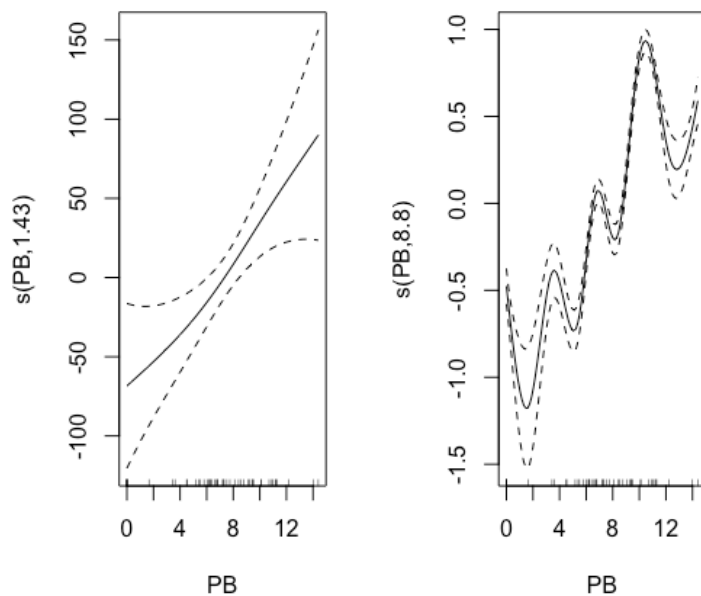


Figure 19 Modeled response of *A. verrilli* to Pb. Gaussian models on the left, Poisson on the right. The solid line is the predicted value of the predictive value as a function of  $x$ . The dotted lines are  $\pm$  two standard errors, and the tick marks on  $x$  axis indicate the location of sample data.



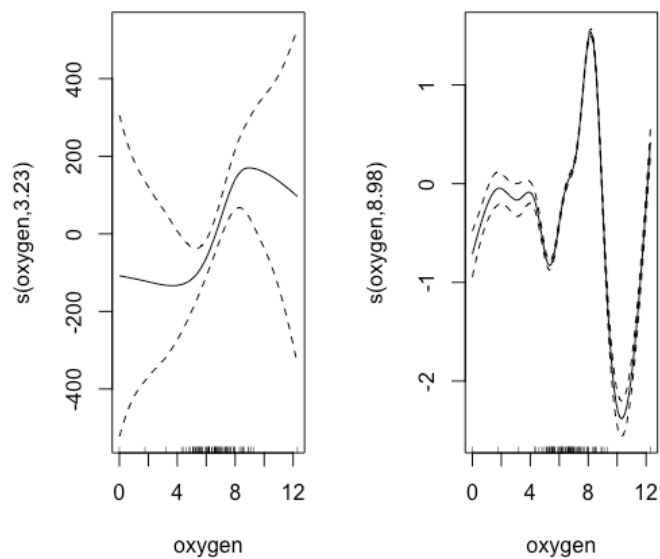


Figure 20 Modeled response of *Coelotanypus* to dissolved oxygen. Gaussian models on the left, Poisson on the right. The solid line is the predicted value of the predictive value as a function of  $x$ . The dotted lines are  $\pm$  two standard errors, and the tick marks on  $x$  axis indicate the location of sample data.

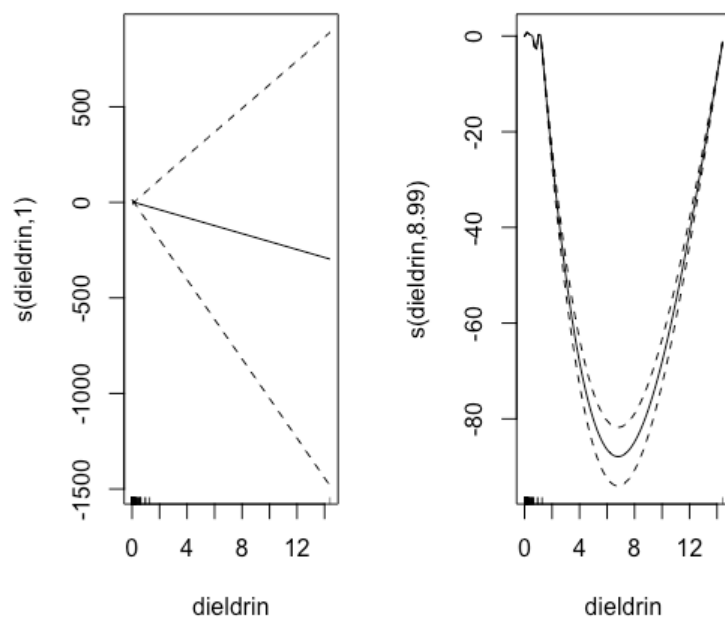


Figure 21 Modeled response of *S. chesapeakee* to dieldrin. Gaussian models on the left, Poisson on the right. The solid line is the predicted value of the predictive value as a function of  $x$ . The dotted lines are  $\pm$  two standard errors, and the tick marks on  $x$  axis indicate the location of sample data.

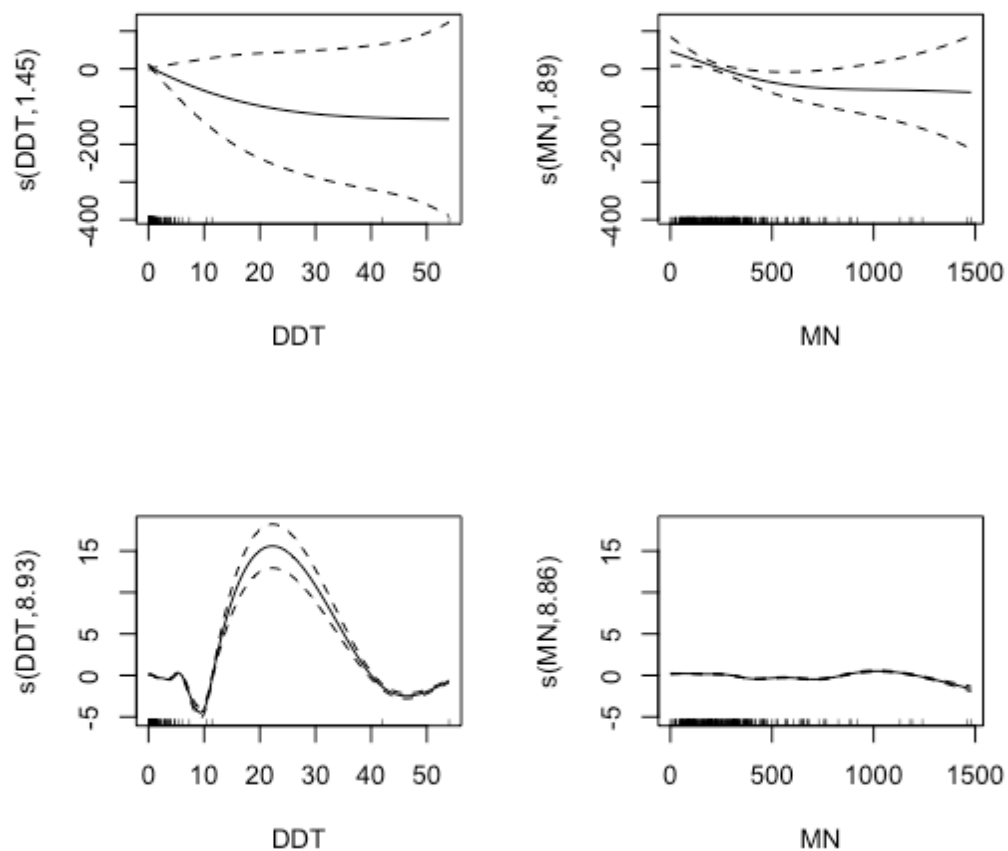


Figure 22 Modeled response of *G. solitaria* to DDT and Mn. Gaussian models on top, Poisson on bottom. The solid line is the predicted value of the predictive value as a function of x. The dotted lines are  $\pm$  two standard errors, and the tick marks on x axis indicate the location of sample data.

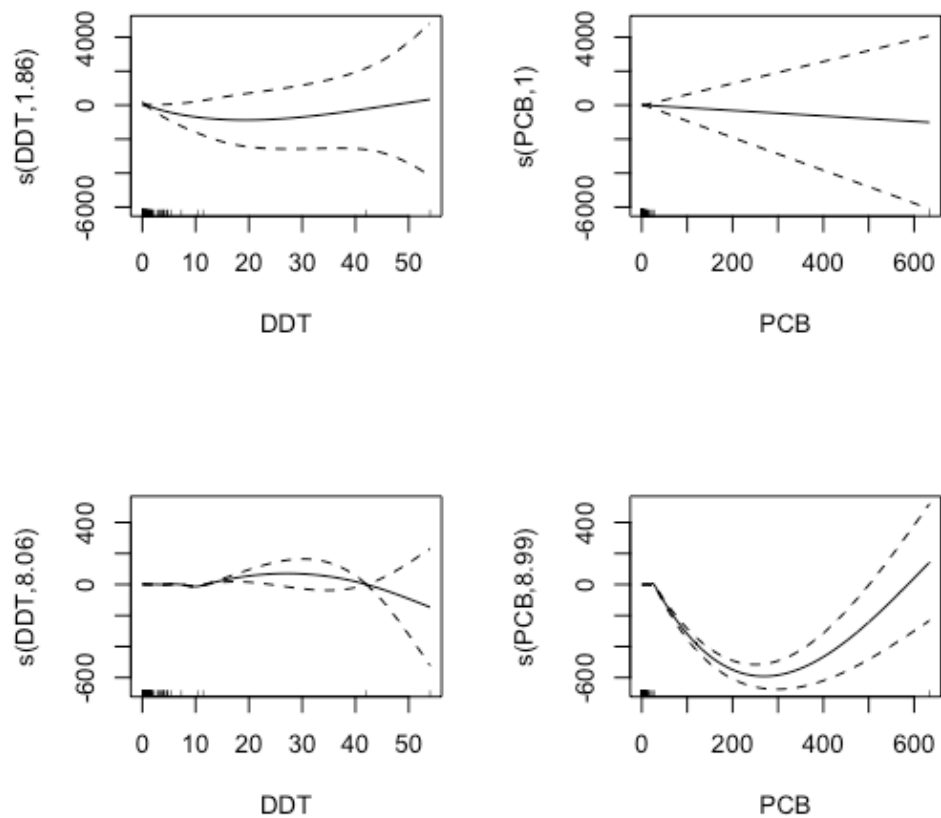


Figure 23 Modeled response of *M. ambiseta* to DDT and PCB. Gaussian models on top Poisson on bottom. The solid line is the predicted value of the predictive value as a function of  $x$ . The dotted lines are  $\pm$  two standard errors, and the tick marks on  $x$  axis indicate the location of sample data.

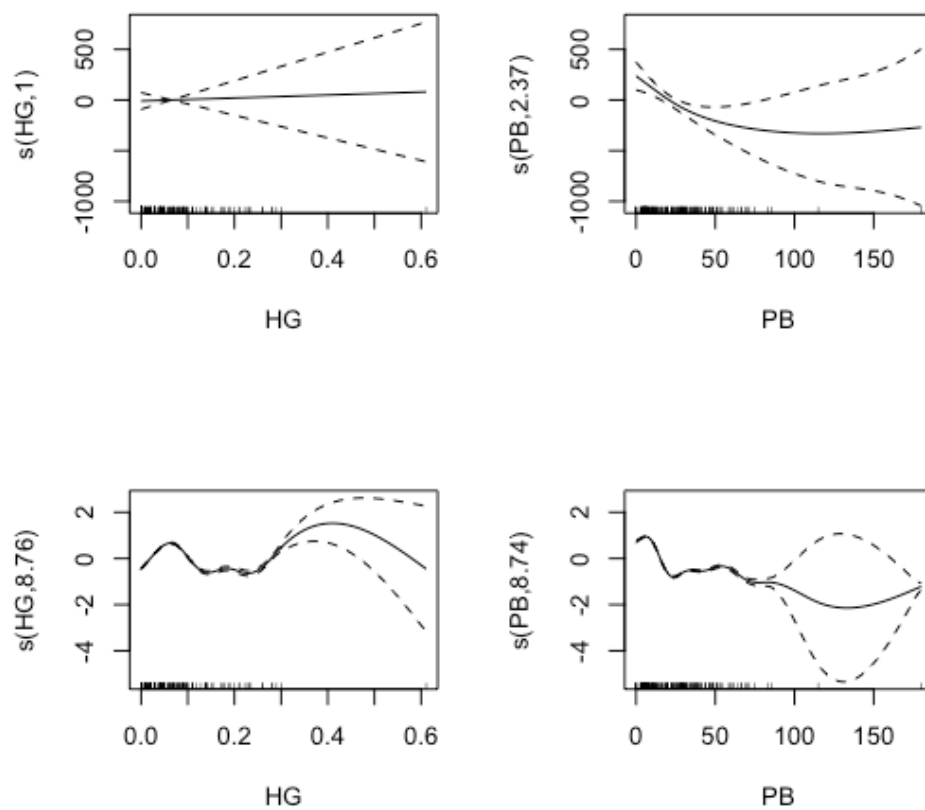


Figure 24 Modeled response of *M.mitchelli* to Hg an Pb. Gaussian models on top, Poisson on bottom. The solid line is the predicted value of the predictive value as a function of x. The dotted lines are  $\pm$  two standard errors, and the tick marks on x axis indicate the location of sample data.

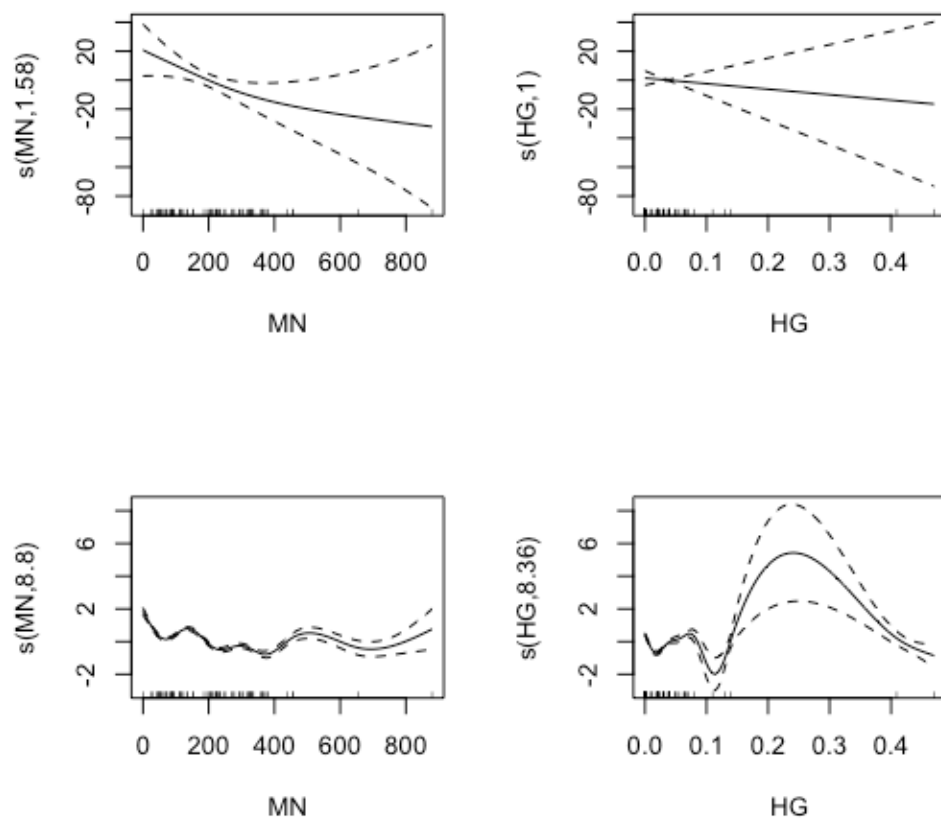


Figure 25 Modeled response of *S. costarum* to Mn and Hg. Gaussian models on the left, Poisson on the right. The solid line is the predicted value of the predictive value as a function of  $x$ . The dotted lines are  $\pm$  two standard errors, and the tick marks on  $x$  axis indicate the location of sample data.

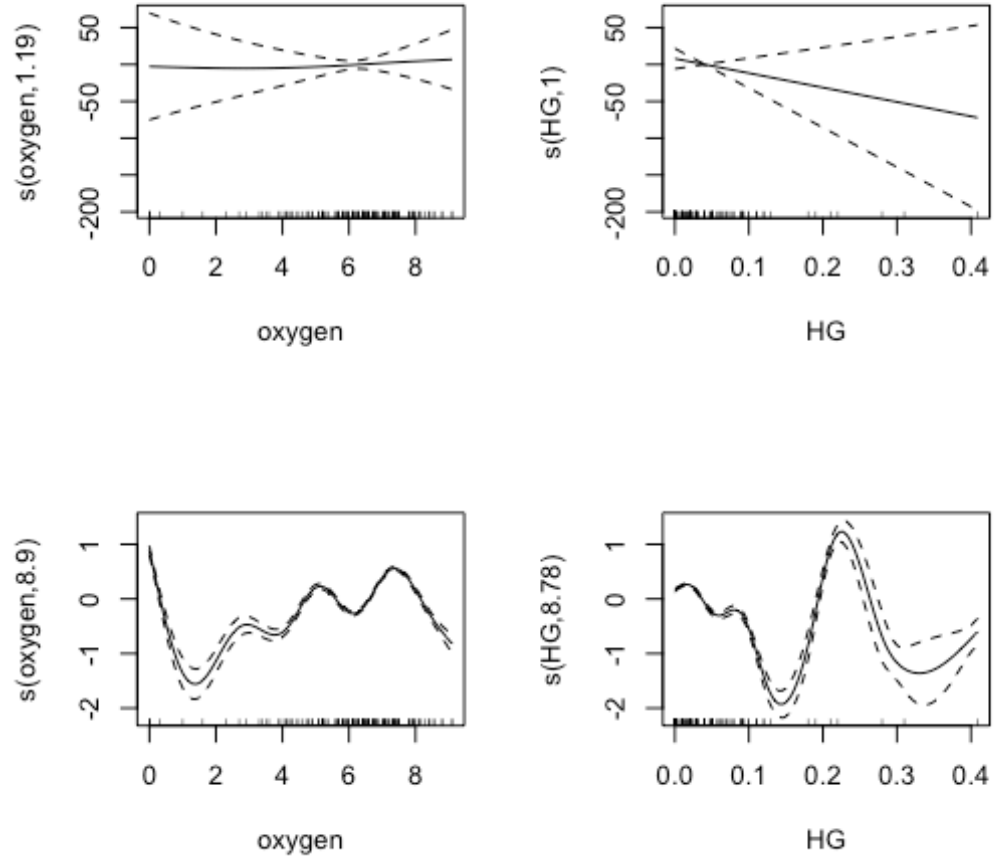


Figure 26 Modeled response of *M. lateralis* to dissolved oxygen and Hg. Gaussian models on the top, Poisson on the bottom. The solid line is the predicted value of the predictive value as a function of x. The dotted lines are +/- two standard errors, and the tick marks on x axis indicate the location of sample data.

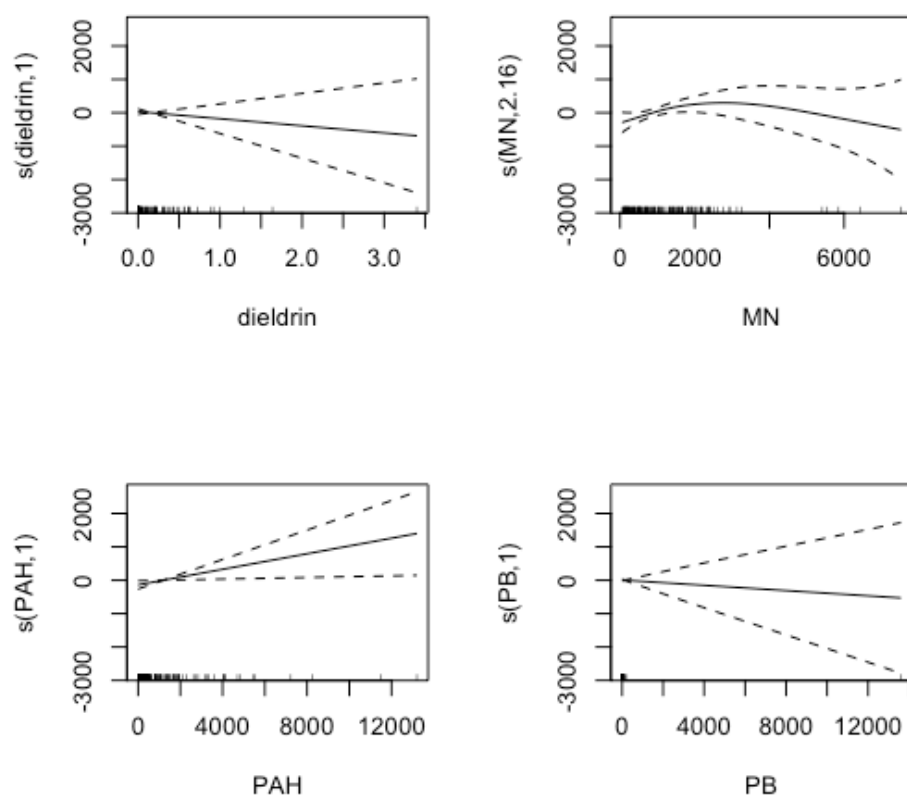


Figure 27 Modeled response of *L. plumulosus* to dieldrin, Mn, PAH and Pb, with Gaussian distribution. The solid line is the predicted value of the predictive value as a function of  $x$ . The dotted lines are  $\pm$  two standard errors, and the tick marks on  $x$  axis indicate the location of sample data.

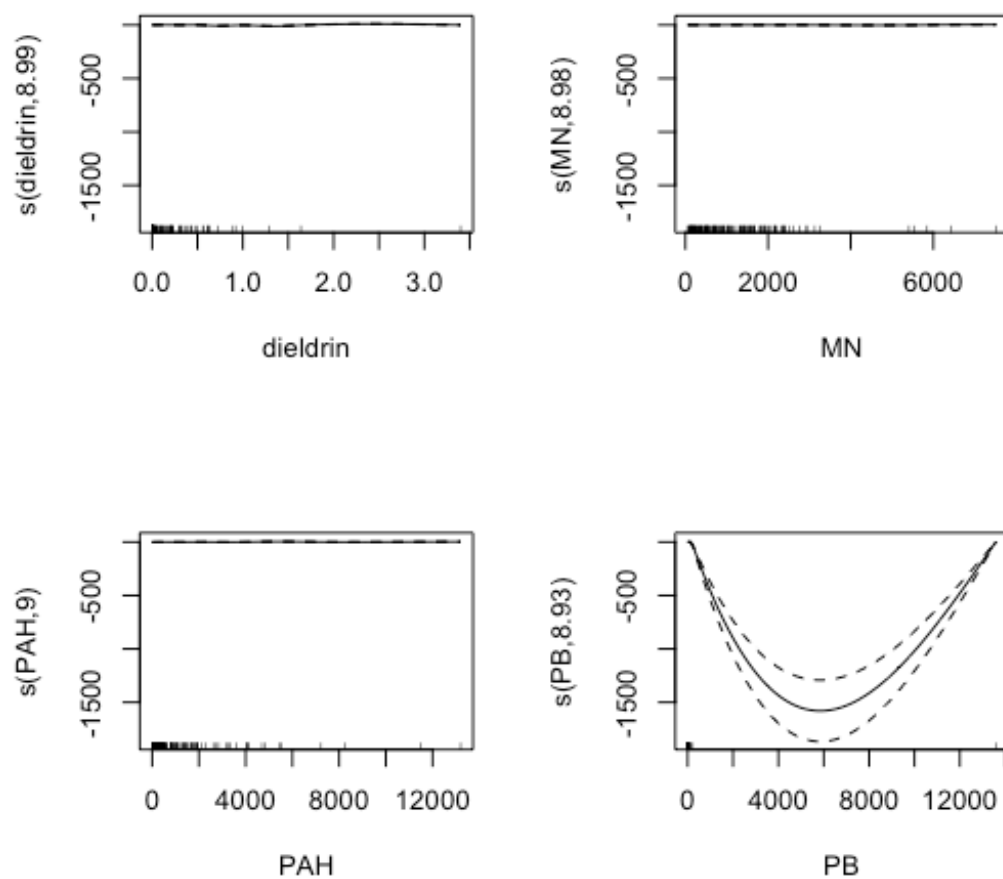


Figure 28 Modeled response of *L. plumulosus* to dieldrin, Mn, PAH and Pb, with Poisson distribution. The solid line is the predicted value of the predictive value as a function of  $x$ . The dotted lines are  $\pm$  two standard errors, and the tick marks on  $x$  axis indicate the location of sample data.



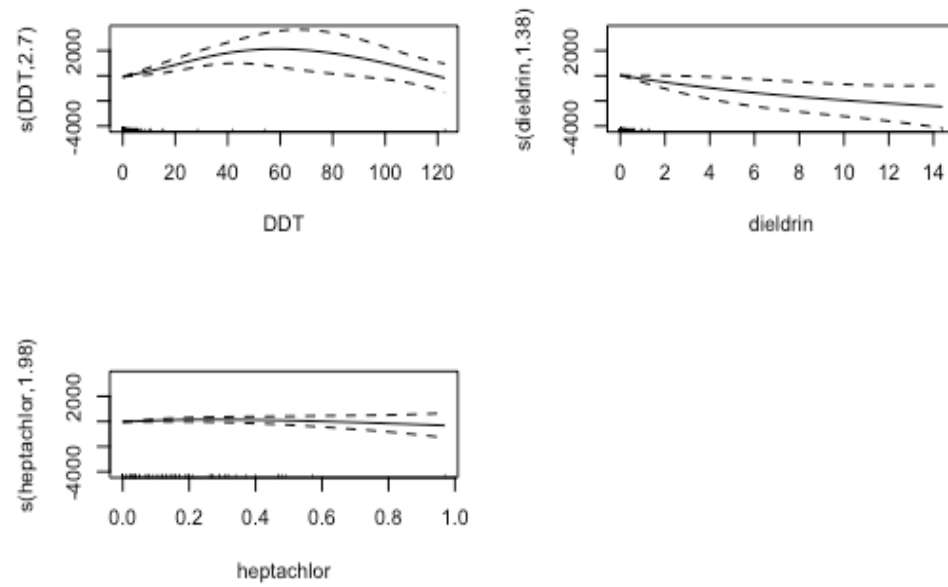


Figure 29 Modeled response of *H. filiformis* to DDT, dieldrin and heptachlor, with Gaussian distribution. The solid line is the predicted value of the predictive value as a function of  $x$ . The dotted lines are  $\pm$  two standard errors, and the tick marks on  $x$  axis indicate the location of sample data.

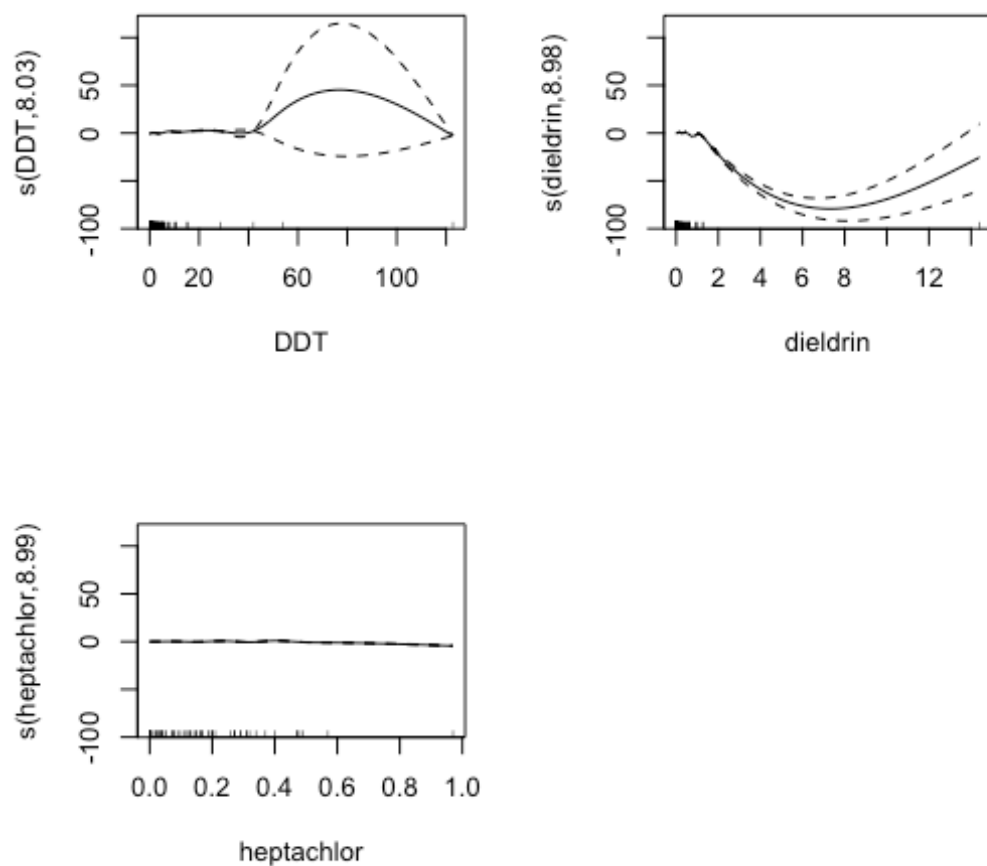


Figure 30 Modeled response of *H. filiformis* to DDT, dieldrin and heptachlor, with Poisson distribution. The solid line is the predicted value of the predictive value as a function of x. The dotted lines are +/- two standard errors, and the tick marks on x axis

**Table 7 Model selection based on AIC scores. The best models are those with the smallest AIC values for each distribution type in bold**

<b>Distribution</b>	<b>Taxa</b>	<b>Model</b>	<b>d.f.</b>	<b>AIC</b>
Gaussian	<i>A. abdita</i>	Dissolved Oxygen	7.094199	<b>369.8986</b>
	<i>A. verrilli</i>	Pb	3.73195	<b>580.4993</b>
	<i>Coelotanypus</i> <i>spp.</i>	Dissolved Oxygen	6.036909	1106.2511
	<i>G. solitaria</i>	DDT + Mn	6.120089	3321.4977
	<i>H. filiformis</i>	DDt + Dieldrin	9.173446	3699.1265
	<i>L. plumulosus</i>	Dieldrin + Mn + PAH + Pb	7.66053	2439.4015
	<i>M.ambiseta</i>	DDT + PCB	5.157773	3268.3849
	<i>M. mitchelli</i>	Hg + Pb	5.935504	2696.3279
	<i>S. chesapeakea</i>	Dieldrin	3.004085	3715.6797
	<i>S. costarum</i>	Hg+Mn	4.952518	<b>738.0584</b>
Poisson	<i>A. abdita</i>	Dissolved Oxygen	9.962571	<b>749.663</b>
	<i>A. verrilli</i>	Pb	9.934158	<b>3138.66</b>
	<i>Coelotanypus</i> <i>spp.</i>	Dissolved Oxygen	9.9882	10871.933
	<i>G. solitaria</i>	DDT + Mn	18.990131	31019.429
	<i>H. filiformis</i>	DDt + Dieldrin	27.070288	119232.542
	<i>L. plumulosus</i>	Dieldrin + Mn + PAH + Pb	36.995447	96190.533
	<i>M.ambiseta</i>	DDT + PCB	18.07861	207128.041
	<i>M. mitchelli</i>	Hg + Pb	18.559904	56300.613
	<i>S. chesapeakea</i>	Dieldrin	9.999941	143880.696
	<i>S. costarum</i>	Hg + Mn	18.720463	<b>1463.363</b>

**Equation I Model of *A. abdita* to dissolved oxygen, derived from Generalized Additive Models (GAMs) with Gaussian error distribution**

$$Ampelisca\ abdita = abundance + f(DO)$$

Equation II Model of *A. verrilli* to Pb, derived from Generalized Additive Models (GAMs)

$$\text{Ampelisca verrilli} = \text{abundance} + f(\text{Pb})$$

Equation III Model of *S. costarum* to Hg and Mn, derived from Generalized Additive Models (GAMs)

$$\text{Spiochaetopterus costarum} = \text{abundance} + f(\text{Hg}) + f(\text{Mn})$$

## Discussion

Biological or ecological indices are useful to evaluate ecosystem health and condition and provide information to decisionmakers. Many of these indices are based heavily on community-level metrics, while very few focus on species-level metrics. In this study, my goal was to identify if useful species-level responses exist that could be incorporated into a new or revised index. For example, Weisberg et al. (1997) chose assemblage measures over individual species metrics in the development of the Chesapeake Bay B-IBI because assemblages are less sensitive to small changes in habitat, even though assemblages and individual species are equally sensitive pollution effects.

The GAM results applied to my dataset resulted in the identification of three taxa with significant response patterns to environmental parameters- the amphipod *A. abdita* to dissolved oxygen, the amphipod *A. verrilli* to Pb, and the polychaete *S. costarum* to Hg and Mn. These three taxa are common in soft-bottom benthic habitats, making them

suitable indicators of stress (Llanos et al., 2002). The shape of the response of *A. abdita* to dissolved oxygen appears to be a non-linear curve and resembles the shape of the curve observed in the correlation analysis in Chapter 2. The data spread between 2 and 8 mg/L and abundance peaked at lower dissolved oxygen concentrations after which the abundances began to decline. *A. abdita* are tube-dwellers, rendering them more isolated from contaminants and able to withstand low dissolved oxygen concentrations. As dissolved oxygen concentrations increase, predators return to the system and *A. abdita* abundance declines due to predation (Connell, 1978).

The shape of the response of *A. verrilli* to Pb appears to be a positive linear curve. Like *A. abdita*, *A. verrilli* is a tube-dwelling amphipod that has much less exposure than burrowing organisms to sediment contaminants. Heavy metals such as Pb are complexed with organic ligands and sulfides in sediment, binding the metals as insoluble sulfide complexes that are no longer toxic nor bioavailable (Di Toro, 1989). This phenomenon, along with the protection that the tubes provide allow *A. verrilli* abundances to increase as Pb (and other metals) concentrations increase.

The shape of the response of *S. costarum* to both Hg and Mn appears to be a decreasing linear curve. These curves also resemble the shape of the curves observed in the correlation analysis in Chapter 2. *S. costarum* is a tube-dwelling, filter-feeding polychaete (Diaz, 1984) that feeds at the sediment-water interface. While its tube provides some protection from sediment contaminants, it must extend its gills into the water column to trap food, making it more susceptible to contaminants.

The GAM provided a reasonable number of covariates to incorporate into a model for stressor identification in estuaries. Explanatory models like this seek to provide insights into the ecological processes that produce the patterns observed from the model (Austin, 1987; Guisan, Edwards Jr, & Hastie, 2002). Given the number of covariates available to test, I expected to see more significant response patterns to other environmental variables emerge. There is no requirement that indices have a certain number of metrics to be useful. Indices such as the Organism-Sediment Index (OSI; Rhoads & Germano, 1987), the Infaunal Trophic Index (ITI; Word, 1980), and the Gulf of Mexico Benthic Index (Engle and Summers, 1994) are all examples of benthic indices that did not require an abundance of metrics to be useful. In fact, it is appropriate to complement indices to get a more holistic picture of the system in question (Dauer et al., 2002; Weisberg et al., 1997). Manipulation of GAM components (e.g., adjusting smoothing terms, basis dimensions, etc.) could result in more accurate models, and response patterns in future iterations. By using GAM default terms for this study, I was able to demonstrate that this method is viable for characterizing stressor response patterns, that corresponded well with my correlation analysis results.

## **Conclusion**

GAMs have proven to be a useful tool for identifying and understanding the patterns of benthic macroinvertebrate response to stressors, as data drive the shape of the patterns rather than forcing a preconceived fit on the data. Here I demonstrated that there are significant responses of three specific taxa to specific environmental stressors, with

which I proposed a model to predict the presence of specific stressors in Chesapeake Bay. I suspect that additional models can be developed by analyzing some of the contaminants that proved to be collinear with the ones I selected for analysis, and by applying the various model fitting functions that GAMs can provide. Individual stressor response data such as these can provide information that IBI tools do not necessarily provide. Combining my taxa-stressor specific model with B-IBI community-level results can provide a more holistic picture of what particular stressors are affecting the system and provide some insight to decisionmakers on what measures to take in addressing and managing ecosystem stressors, and in a way that is cost-effective. Use and application of this model does not require any additional data collection as this model starts with already collected data; the same data to develop a B-IBI can be used for this model, and can help to direct what additional data may be necessary to help explain the results of benthic condition assessment tools that indicate that fair or poor conditions are present in a system.

## **CHAPTER FOUR: TESTING STRESSOR-RESPONSE MODELS**

### **Introduction**

Both general and statistical ecological models are useful in comprehending what is going on in ecosystems. General models are usually based on some type of theoretical or conceptual model (Austin, 1987) with which a statistical model can provide the mathematical basis interpreting the results of conceptual models. In ecology, one of the most widely used aspects of statistical models is understanding the relationship between explanatory and predictive variables (Guisan et al., 2002). Exploratory models seek to define the relationship between response and explanatory variables, using statistical methods to test and validate the strength of the relationship or describe how well the predictor variables explain or fit the response (Austin, 1987). Conversely, predictive models provide a statistical relationship between predictor variables and the response that predict the probabilities of occurrence or abundance of an organism in other locations given the predictor variables.

Linear regression analyses are one of the most widely-used techniques to test the strength of a response to explanatory variables in ecology, though the predictive capability is low (Guisan et al., 2002; Pearce & Ferrier, 2000). Linear regressions are bound by 3 main assumptions: 1) the errors are assumed to be identical and independent, 2) the errors follow a normal or Gaussian distribution, and 3) the relationship between



predictor variables and response is linear. Most ecological data are not normal and follow a Poisson, rather than a Gaussian distribution, so it is not appropriate to use linear regression, unless the data are transformed to meet the assumptions. Interestingly, statistical validation is often not incorporated into the regression analysis methods. The same can be said about the lack of field validation exercises, resulting in a high level of unexplainable uncertainty (Guisan and Zimmermann, 2000).

Generalized Linear Models (GLM) provide an improvement to linear regressions. GLMs can manage a larger number of distributions beyond the Gaussian distribution and deal with more general qualitative response variables. Link functions between the response variable and linear predictor are forced into linearity and has some measure of likelihood to deal with over dispersion (Davison, 2001; Austin, 1987). Relatedly, Generalized Additive Models or GAMs are parameterized like GLMs, but GAMs can handle non-linear and polynomial terms for predictors. Also unique to GAMs are the selection of ‘smoother’ to fit a curve to the data, which is fitted for every variable and then added up. The assumptions of each of these tools must be met in order to be used in a broader context.

In Chapter 3, a GAM was used in an exploratory manner to identify specific benthic response patterns to a small number of variables. The response patterns from the GAM were then used to build a mathematical model to predict the presence of stressors based on the abundances of macroinvertebrates present in a particular environment. The model developed was done so with Chesapeake Bay data obtained from several EPA monitoring programs. Here, I will attempt to test the model I developed by applying it to

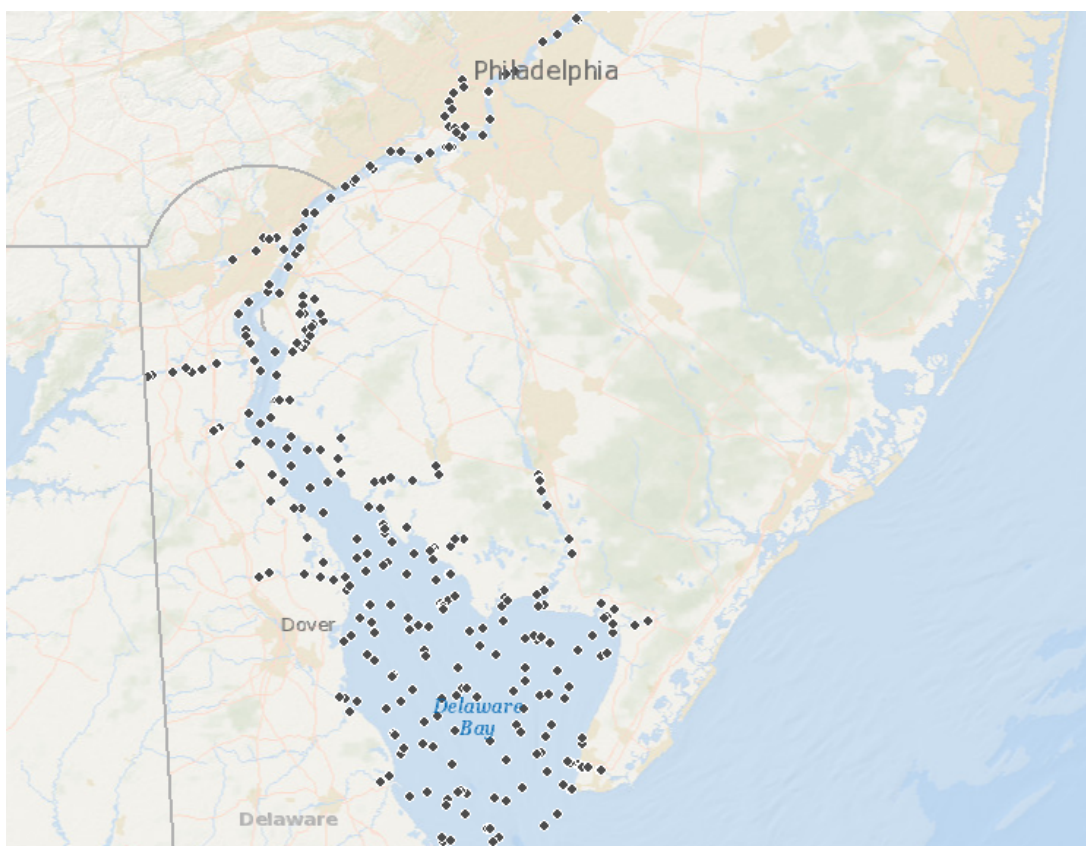
Delaware Bay data. I expect that the model will work in Delaware Bay, as both estuaries have many of the same taxa and are subject to many of the same stressors.

## **Methods**

The Delaware Bay is the tidal portion of the Delaware River Basin that spans over 784 square miles, bounded by Delaware and New Jersey. Like the Chesapeake Bay, it is subject to a multitude of anthropogenic stressors such as wastewater discharges, industrial pollution, and urban and rural non-point source pollution. Data from 1990 to 2010 were collected from the same EPA monitoring programs that Delaware Bay data were collected for in the previous chapters- the Environmental Monitoring and Assessment Program (EMAP), the Mid-Atlantic Integrated Assessment (MAIA), the National Coastal Assessment (NCA) and the National Coastal Condition Assessment. Monitoring data from these programs were collected by EPA staff, contractors and state monitoring crews during summer months (June – September). A probabilistic sampling design was utilized, and similar field, laboratory and rigorous quality assurance methods were used by all participants for sample collection and analysis. Sites were visited by boat or large research vessel and water column, sediment composition and quality, benthic macroinvertebrate and fish community and tissue data were collected at each site. Samples were analyzed by national and state laboratories.

Data from Delaware Bay, located in the EMAP Virginian Province, were extracted and screened following criteria adapted from (D. M. Dauer et al., 2002) to arrive at a dataset appropriate for analysis: 1) samples collected must be within the

geographic boundaries of Delaware Bay and its tributaries, 2) benthic macroinvertebrate samples must be collected using a Young-modified van Veen or Small van Veen grab with a sampling area of 0.0440 m<sup>2</sup>, 3) benthic macroinvertebrate samples must be collected during the index period of July 15 to September 30, 4) dissolved oxygen measurements must be collected at the same time as benthic macroinvertebrate samples, and 5) sediment contaminant data must be collected in the same year as the benthic macroinvertebrate samples. The same subset of sediment contaminant data was used for this study, based on the widespread and local occurrence and extent of contaminants in Delaware. Toxicity data were generated from 10-day static acute amphipod toxicity tests. Sediment total organic carbon (TOC) concentrations were used as a surrogate indicator of eutrophication. In addition, only bottom salinity measurements were used as they are the closest measurements taken in proximity to the benthos. Data from the first of two site visits were used; triplicate benthic grabs were taken for MAIA and were averaged to represent one sample for analysis. Applying the above screening criteria resulted in 284 stations with complete records for analysis in this study.



**Figure 31** Locations of the 284 sites sampled with complete datasets for analysis in the Delaware Bay from 1990 to 2010

The benthic data were first classified into seven bins by grain size and salinity (tidal fresh, oligohaline, low mesohaline, high mesohaline sand, high mesohaline mud, polyhaline sand, polyhaline mud), to account for and remove the influence of grain size and salinity on benthic macroinvertebrates. Next the three stressor response models created in Chapter 3 were evaluated in each habitat for *Ampelisca abdita*, *Ampelisca verrilli* and *Spiochaetopeterus costarum* by comparing respective abundances to the stressor of question

$$1. \text{ } Ampelisca \text{ } abdita = abundance + f(DO)$$

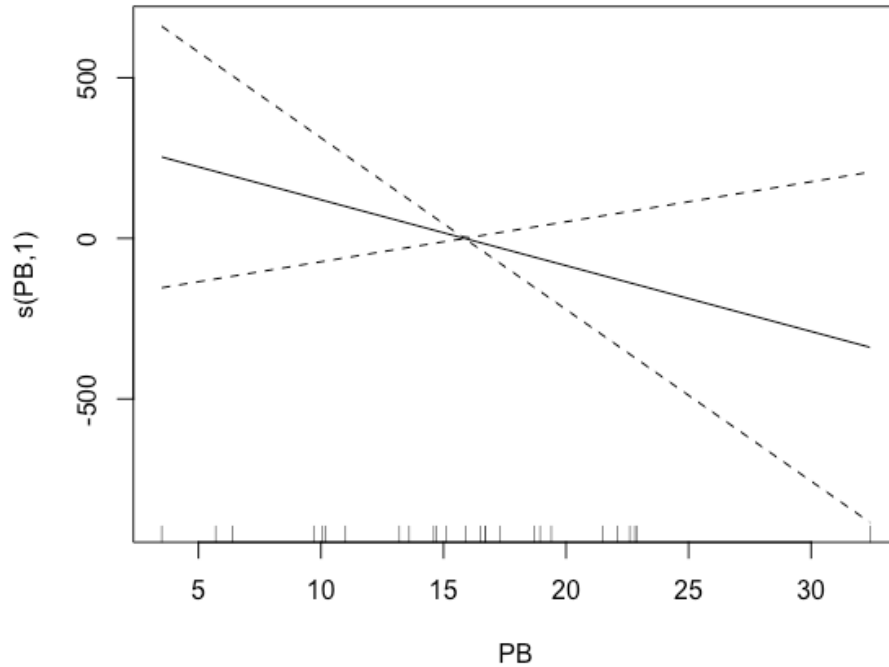
$$2. \textit{Ampelisca verrilli} = \textit{abundance} + f(\textit{Pb})$$

$$3. \textit{Spiochaetopterus costarum} = \textit{abundance} + f(\textit{Hg}) + f(\textit{Mn})$$

using the *mgcv* package in R, to see if the same taxa-stressor response curve patterns were similar to those in Chesapeake Bay.

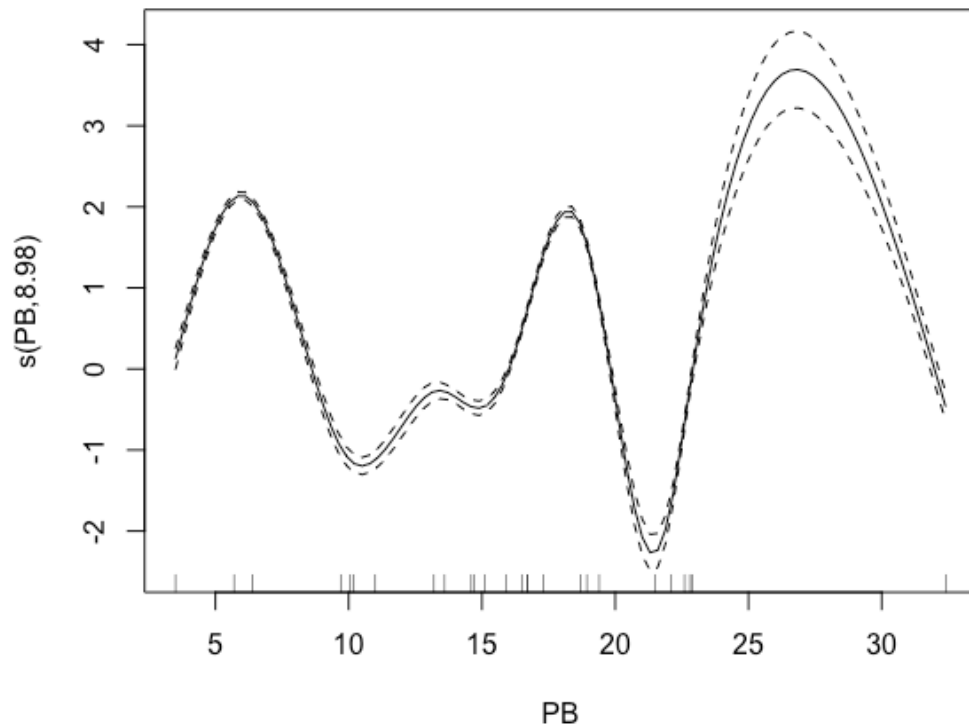
## Results

The Delaware Bay data contained the three species in the models identified in Chapter 3 and GAMs were run for each of the three models. The model runs for *A. abdita* and *S. costarum* were unsuccessful because there were too zero values in the contaminant data set, and were dropped from the analysis. The *A. verrilli* response model (n = 26) to Pb explained 6% of the deviance with an adjusted R<sup>2</sup> of 0.0214. The k-index was 0.758 and less than 1, indicating that k should be adjusted to better fit the data. The model presented just one knot, indicating that the response to Pb is linear, which aligns with the developed model (Figure 32).



**Figure 32 Modeled response of *A. verrilli* to Pb. The solid line is the predicted value of the predictive value as a function of x. The dotted lines are +/- two standard errors, and the tick marks**

While the models tested were created with the Gaussian error distribution, the *A. verrilli* model was re-run with the Poisson error distribution for comparison, which is appropriate for count data. This model explained 73.8% of the variance, with an  $R^2$  of 0.552. The k-index was 1.21 and the estimated degrees of freedom (e.d.f.) was approaching one at 8.98, indicating that k should be adjusted to better fit the data. The model presented 8 knots, indicating that the response to Pb was non-linear (Figure 33), which contradicts the original model.



**Figure 33 Modeled response of *A. verrilli* to Pb, with the Poisson error distribution. The solid line is the predicted value of the predictive value as a function of  $x$ . The dotted lines are  $\pm$  two standard errors, and the tick marks**

## Discussion and Conclusion

I hypothesized that the models that were created with Chesapeake Bay data were applicable to Delaware Bay data, given the many similarities that the two systems share, including many of the same benthic macroinvertebrate taxa. Instead I found that only one model was applicable- the response of *A. verrilli* to Pb. In building the models, the default values were applied across the board to control spurious effects from entering the

models. It is necessary to adjust various aspects of the models, such as the basis functions, error distributions and link functions in order to come up with better fitting models. This must be done in a methodical way to abstain from introducing or influencing the model outputs and thus making them more accurate.

Delaware Bay is shallower than Chesapeake Bay, with an abundance of creeks and tidal marshes right up to the water's edge, while Chesapeake Bay is deeper with marshes concentrated in the lower region (Fisher et al., 1988). These physical aspects alone help to explain some of the differences in taxa observed. Before conducting analysis, I reviewed the Delaware Bay dataset and noticed that while there are several taxa that are found in both Chesapeake and Delaware Bays, there were quite a few taxa that were distinct to each water body (Maurer et al., 1978; Kinner et al., 1974). The taxa that were analyzed in this study are found in both systems and there was a difference in dominant taxa. For example, *S. costarum* is found in both systems, but *S. oculatus* is more abundant in Delaware Bay. I applied the *S. costarum* test to *S. oculatus* and the model did not to perform well. This could be because my Chesapeake model works only for *S. costarum*, or this could be because *S. costarum* and *S. oculatus* are not affected by Pb concentrations in Delaware Bay. An in-depth review and analysis of my suite of contaminants may reveal that another suite of taxa and stressor may be identified and appropriate for both Chesapeake and Delaware Bays, and beyond.

GAMs have proven to be useful tools in identifying taxa response patterns to stressors in estuaries. Here, I tested a model developed from Chesapeake Bay data with Delaware Bay data. While only one of the three models worked, there is some promise



that with some fine-tuning, finding the best models that function well in both systems is possible. The contaminants should be reviewed and refined to determine a common suite among comparable estuaries, if possible.

## **CHAPTER FIVE: SUMMARY AND FUTURE WORK**

Estuaries such as the Chesapeake Bay are complex and dynamic systems that are subjected to a variety of environmental (abiotic and biotic) and anthropogenic stressors simultaneously. The pressures of increased population density, land development and agricultural practices have resulted in a threatened ecosystem in need of ongoing monitoring, restoration and protection. Over that past 30-plus years, there has been a concerted effort to monitor and assess the health of the Bay with monitoring and assessment programs as well as indicator tool development (e.g. IBIs). These tools often focus on the effects of disturbance on benthic macroinvertebrates, because they relatively sedentary, have short life-cycles and reflect the cumulative effect of stressors in the overlying water column. The majority of the tools developed focus primarily on effects at the community-level. Stressors can and do impact estuary inhabitants at different levels of organization, yet the focus remains on community level assessments. In this dissertation I chose to examine stressor response patterns at the individual, rather than the community or higher level, as there is much information to glean from understanding what is happening at lower levels of biological organization, which can be aggregated as necessary.

In Chapter 1, I presented a literature review of the mechanisms of benthic macroinvertebrates stressor responses from the molecular to the community and

ecosystems levels. I described some of the physiological, chemical and behavioral traits that allow benthic organisms to cope with environmental stressors. I also reviewed various tools that were designed to assess ecosystem health, based on benthic macroinvertebrates stressor response tactics, and provided evidence for the utility of a tool or model to conduct assessments at a finer scale, using existing long-term datasets.

In Chapter 2, I conducted exploratory analysis to identify if there were any significant stressor-response patterns observed by benthic taxa. I initiated the process by looking at scatterplots of various combinations of taxa and stressors to see if any were detected by the naked eye. Next I ran correlation analyses to determine what taxa-stressor response patterns were significant. There was a subset of amphipods, polychaetes, oligochaetes, bivalves, gastropods, and chironomids that exhibited significant response patterns to a variety of sediment contaminants and physical variables that I wanted to analyze further.

In Chapter 3, I used Generalized Additive Models (GAMs) to further examine the significant patterns that were found in Chapter 2. GAMs are a semi-parametric extension of Generalized Linear Models (GLM) that makes assumptions that link functions are additive, model components are smooth, and the Y predictor is non-linear, allowing the underlying data to do the work of highlighting emerging patterns. Ultimately, three taxa- *Ampelisca abdita*, *Ampelisca verrilli*, and *Spiochaetopterus costarum* were identified as the most parsimonious models with significant stress response curves to dissolved oxygen, Pb, Hg, and Mn.

In Chapter 4, I attempted to test the aforementioned models with data from Delaware Bay, which is the next closest large estuary near Chesapeake Bay. Both Chesapeake Bay and Delaware Bay have many of the same stressors in their respective watersheds that it made sense to test data from Delaware. Only one of the three models worked with the Delaware Bay data, indicating that some fine-tuning of the models is needed in order to make this a tool that is widely applicable beyond the confines of the Chesapeake Bay region.

The implications of the findings of this work are far-reaching. Many of the assessment tool currently used do not take individual stressor responses into account. Here, I have shown that certain taxa are indicators of the presence and effects of certain pollutants. This would be useful to use along with IBIs or other tools to add information on the possible sources of disturbance or stress that the IBIs pick up at the community and higher levels. This technique could be operationalized in such a way that someone without full knowledge of benthic ecological principles could run data through a model like I used with GAM and be able to get results that could possibly be an early warning that something is amiss in the ecosystem.

There are some refinements to this approach that could be made to enhance the functionality. For sake of simplicity, I used the default setting for the GAM models, and for the next iteration of this work, I would like to manipulate basis, distribution and smoothing functions in subsequent model runs to see if better fitting and more models result. I would also like to use this model to actually revise and test an established IBI to incorporate individual stressor response information. Lastly, one big lesson I learned is

that just because you have a lot of data to use, that is not an indication that it is all useable. While I had 20-plus years of data at my disposal, I found that large amounts were not compatible for my purposes. Much of the incompatibility had to do with discrepancies in how the data were collected and or stored. There were many instances where I had to throw out whole sections of data because one value or parameter was missing, making the entire record invalid for my needs.

Notwithstanding the fact that the models did not perform as expected in Delaware Bay, I was able to show that there are significant response patterns of particular taxa to specific stressors in estuarine systems. With some adjustments I am confident that I will be able to refine the models presented and make them applicable to other estuaries, as well as become a useful ecosystem management tool.

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