ABSTRACT

Title of dissertation:	**POLYCHAETES, HYPOXIA, AND NITROGEN CYCLING IN THE MESOHALINE CHESAPEAKE BAY**
	Jennifer Anne Bosch, Doctor of Philosophy, 2014
Dissertation directed by:	**Professor W. Michael Kemp** **Marine Estuarine and Environmental Science**

Benthic macrofauna can play an important role in facilitating some of the microbial mediated processes of nitrogen cycling in estuarine sediments. Declines in benthic macrofauna, like polychaete worms, have been attributed to long-term increases in bottom water hypoxia in Chesapeake Bay. Utilizing a large monitoring dataset including benthic macrofaunal abundance, biomass, and concurrent measures of environmental parameters, I examined how environmental conditions regulate the densities of opportunistic polychaetes in a mesohaline estuarine system. This analysis points to a benthic community dominated by euryhaline, opportunistic polychaete worms (*M. viridis, S. benedicti, H. filiformis, A. succinea*) which have well adapted but varying responses to hypoxia and other stressful conditions. Results of two laboratory experiments with the opportunistic polychaete *Alitta (Neanthes) succinea* were used to quantify the short-term influence of density and size of surface-feeding polychaetes on sediment-water

fluxes of inorganic nitrogen under varying oxygen conditions. Polychaete enhancements of O_2 and nitrogen fluxes were strongly correlated with total animal biomass. Solute fluxes were stimulated by presence of both larger and smaller worms, but per capita effects were greater for the deep-burrowing larger polychaetes. Utilizing a unique large-scale monitoring dataset collected in the Chesapeake Bay, I employed Classification and Regression Tree (CART) and multiple linear regression (MLR) analyses to assess the relationship between benthic biomass and NH_4^+ efflux within different regions of the estuary by season. In addition to labile organic matter, oligohaline and mesohaline tributary temperature and salinity control the rate of nitrogen cycling and benthic macrofaunal biomass. In deeper regions of mesohaline tributaries and the mainstem Bay, dissolved oxygen was found to be the dominating parameter regulating sediment nitrogen pathways as well as the structure of the benthic macrofaunal community. With increased macrofaunal biomass, spring regressions indicated an enhancement of NH_4^+ efflux. In contrast, fall regressions indicated the enhancement of fixed nitrogen removal from sediments. Summer data lacked a significant relationship, but high NH_4^+ effluxes under hypoxic/anoxic conditions suggested dissolved oxygen is the primary driver of summer nitrogen cycling. This study, using field and laboratory data, concludes that a complex balance between seasonal and regional dissolved oxygen, temperature and salinity conditions shape not only the benthic community but also the relationship between macrofaunal biomass and sediment nitrogen flux in this eutrophic estuarine system.

**Polychaetes, Hypoxia, and Nitrogen Cycling in the Mesohaline
Chesapeake Bay**

By

Jennifer Anne Bosch

Dissertation submitted to the Faculty of the Graduate School of the
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DEDICATION

To the three women who made me who I am and
continue to inspire me every day:

Ellen Elizabeth Vrabel

Joan Susanne Bosch

Anna Dydak

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CHAPTER 1:

Introduction and Overview

INTRODUCTION

Aquatic systems are some of the most productive ecosystems in the world. This production is primarily driven by the supply of nutrients through runoff from the land that fuels primary productivity and biomass of phytoplankton. Zooplankton and benthic invertebrate fauna consume that primary production and pass it on to fish and other carnivores to enhance production of the entire ecosystem.

Eutrophication, an increase in the rate of supply of organic matter to an ecosystem (Nixon 1995), is common in coastal systems where human activities on land increase the amount of nutrients, especially nitrogen and phosphorous, running off the watershed into estuaries and bays. A wide range of ecological problems result from eutrophication including the degradation of overall water quality and associated changes in productivity, biomass and species composition of benthic flora and fauna (Breitburg et al. 2003, Diaz & Rosenberg 2008). While an increase in nutrients can initially increase production of the whole system, over time, eutrophic coastal ecosystems and their food webs loose the ability to assimilate and process the resulting accumulation of organic matter in the system (Nixon 1995, Caddy 2000, Kemp et al. 2005). In this case, sediment and water column microbial activity tends to increase, causing higher demand on dissolved oxygen (DO) in bottom waters. If waters are vertically stratified and/or slowly flushed, like with Chesapeake Bay, the consumption of DO near the bottom can exceed the replenishment from the overlying water resulting in oxygen-stressed

conditions and hypoxia; where DO < 2.0 mg l^{-1} (Kemp et al. 2009). These low DO concentrations in bottom water threaten all bottom-dwelling animals, particularly those that cannot move away from the affected area, like sessile benthic macrofauna. The hypoxic conditions also alter biogeochemical processes occurring in sediments.

The complex biological, chemical and physical processes in marine sediments play an important, often seasonally varying, role in the functioning of a healthy productive estuary (Bianchi 2007). Under hypoxic conditions, however, sediment biogeochemical processes can be altered, shifting nitrogen cycling pathways, and other ecologically important elements. Organic material from productive surface waters is deposited on the bottom and decomposed via bacterial respiration. Subsequent biogeochemical processes can either recycle organic material to ammonium, which diffuses to the overlying water column where it sustains primary production, or remove fixed nitrogen by transforming ammonium to gaseous forms that are biologically unavailable (Kemp et al. 1990). Benthic macrofauna can play an important role in physically facilitating some of these microbial mediated processes in sediment nitrogen cycling (Welsh 2003).

Like many benthic macrofauna, polychaete worms influence rates and pathways of organic matter remineralization and the vertical distribution of sediments through burrow irrigation (bioirrigation) as well as feeding and burrow construction (bioturbation) (Rhoads 1974, Diaz 2001, Francois et al. 2001, Welsh 2003). Mechanical breakdown of particulate matter through feeding activities increases the surface area of organic particles enhancing the microbial

decomposition. Animal tubes and burrows also increase the surface area for the exchange of substrates and nutrients between sediments and the overlying water (Francois et al. 2001). Depending on the type of feeding mode (subsurface-deposit, surface-deposit or suspension-feeder) of the animals, they can have a profound effect on the nutrient and substrate exchanges with the overlying water (Schaffner et al. 2001). The role of macrofauna, like polychaetes, in the organic matter remineralization and burial processes in estuarine sediments is not completely understood and studies of benthic faunal roles in processing organic matter represent an active area of research, especially for eutrophic estuarine systems like Chesapeake Bay.

The effects of bioturbation and bioirrigation activities of polychaetes on various aspects of the nitrogen cycle have not been thoroughly explored, particularly under varying environmental conditions. As in many estuaries, summertime hypoxia is a common occurrence in Chesapeake Bay once temperatures have risen and the large input of spring bloom organic material reaches the bottom. Many polychaetes can withstand short-term hypoxic events; however, increased duration and spatial extent of hypoxia associated with eutrophication can play a role in shaping the community structure and seasonal succession of benthic macrofauna (Schaffner et al. 2001). The timing of hypoxic events relative to population recruitment is critical for survival of faunal species (Holland & Diaz 1983, Holland et al. 1987). Recent observations suggest that the timing of initial summertime hypoxia in Chesapeake Bay has been occurring earlier in the season during the last several decades (Murphy et al. 2011). With

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the loss or reduction in abundance of and/or change in species composition of benthic bioturbators under hypoxic conditions, the pathways and rates of organic matter diagenesis and nitrogen cycling may be altered. This shift may have a notable impact on the way the whole ecosystem functions. As summertime hypoxia sets up in Chesapeake Bay, the effects of macrofaunal bioturbation and bioirrigation on organic matter recycling may be altered and the sediment metabolism may become reduced. More specifically, nitrification (ammonium oxidation to nitrate) and denitrification (nitrate reduction to N_2) could be severely limited and the nitrogen cycle may shift toward enhanced ammonium recycling. This build up of ammonium in bottom waters would allow for increased vertical transport to surface waters where it would stimulate algal production. This represents a positive feedback for sustaining primary production and in turn reinforcing the eutrophication process and perpetuating hypoxia on the bottom (Kemp et al. 2005).

This dissertation research explored the influence of low oxygen concentrations and polychaete species abundance on the cycling of nitrogen in Chesapeake Bay mesohaline sediments. The approach began by developing relationships among environmental parameters and benthic community composition through an assessment of historical data collected in mesohaline Chesapeake Bay. I then quantified some of those relationships with controlled laboratory experiments evaluating the effects of different DO concentrations on nitrogen cycling and fluxes in the presence of varying densities of polychaetes. Additionally, I explored the effects of individual size and total biomass of

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polychaetes on solute fluxes and pathways of nitrogen cycling in sediments.

Finally, I extended the observed mechanistic relationships into the general context

of macrofaunal impacts on organic matter diagenesis by refining the statistical

relationships between total macrofaunal biomass and other environmental

parameters of an estuarine system based on long-term monitoring data of

biogeochemical sediment fluxes and concurrent macrofaunal species abundance,

biomass and species composition.

SYNOPSIS OF DISSERTATION CHAPTERS

Chapter 2

Utilizing a large monitoring dataset including benthic macrofaunal

abundance, biomass, and concurrent measures of environmental parameters (e.g.,

depth, sediment type, salinity, temperature, and DO) I examined how

environmental conditions regulate the densities of opportunistic polychaetes in a

mesohaline estuarine system. The examination of polychaete abundance and

biomass in the mesohaline Chesapeake Bay region pointed to a benthic

community dominated by euryhaline, opportunistic polychaete worms (*M. viridis,*

S. benedicti, H. filiformis, and *A. succinea*). Macrofaunal samples were divided

into three groups by depth ranges that often have similar sediment types, but also

have similar overlying water properties, including dissolved oxygen

concentrations. Linkages between variations in environmental factors and

abundance of animals were initially evaluated using Spearman rank correlation

and linear regression analyses. Classification and Regression Tree (CART)

analysis revealed salinity is a controlling parameter of polychaete abundance in shallow zones (< 5 m), particularly during spring recruitment, and dissolved oxygen controls abundance at depths > 10 m. Mid-depth abundances are controlled by the interaction between both parameters. Winter air temperature and in situ bottom water temperature play strong roles in the timing of spring recruitment. This study supports previous work indicating a shift in the dominant polychaete community of the mesohaline Chesapeake Bay to one made up of four species, all of which are known to be well adapted to hypoxia and other stressful conditions. My analysis further shows that the ability of these polychaetes to respond to hypoxia varies among species. Those species that can withstand temporary hypoxia (*A. succinea)* still thrive, however, dissolved oxygen appears to be the "master variable" controlling long-term trends and spatial distribution of these dominant members of the polychaete community.

Chapter 3

Once an understanding of these community characteristics and shifts was established, I examined how these shifts may have affected changes in benthic nitrogen cycling. Specifically, the biogenic activity of polychaetes can stimulate microbial ammonification, nitrification, and/or denitrification in estuarine sediments as well as increase the fluxes of inorganic nitrogen (NH_4^+, NO_2^-, NO_3^-, N_2) across the sediment-water interface. As shown in Chapter 2, chronic eutrophication and expanding seasonal hypoxia ($O_2 < 63$ μM or 2 mg l^{-1}) in estuaries like Chesapeake Bay have altered benthic faunal communities in favor

of opportunistic species. It has been suggested that the efficient decomposition of organic material is enhanced by the presence of polychaetes that can quickly populate organic-rich sediments following hypoxia events. Improved understanding of relationships among oxygen (O_2), polychaete density, and nitrogen cycling can help refine biogeochemical models of coastal ecosystems. Results of two laboratory experiments with the opportunistic polychaete *Alitta (Neanthes) succinea* were used to quantify the short-term influence of density and size of surface-feeding polychaetes on denitrification and sediment-water fluxes of inorganic nitrogen under varying oxygen conditions. Results showed that polychaete enhancements of O_2 and nitrogen fluxes were strongly correlated with total animal biomass. Fluxes of O_2, NH_4^+ and N_2 were stimulated by presence of animals for both larger and smaller worms, but per capita effects were greater for the deep-burrowing larger polychaetes. With the onset of hypoxic conditions, all animal density treatments had reductions in O_2, NH_4^+ and N_2 fluxes, with the high-density treatment showing the greatest change. Denitrification efficiency [$DE^* = N_2$ flux / ($N_2 + NH_4^+$ fluxes)] was 33% higher for experiments with large worms than for smaller worm treatments, suggesting the former were more effective in removing fixed nitrogen.

Chapter 4

It is widely understood that benthic macrofauna affect sediment biogeochemistry, but the relationship between macrofaunal biomass and ammonium (NH_4^+) efflux from estuarine sediments is challenging to generalize. I

examined a unique large-scale monitoring dataset collected in the Chesapeake

Bay to assess the relationship between benthic biomass and NH_4^+ efflux within

different regions of the estuary by season. Biomass data was separated into

different classes of benthic macrofauna (polychaetes, bivalves, and amphipods) to

isolate the different faunal type impact on nitrogen recycling across the different

regions of the Bay. Factors controlling NH_4^+ efflux were tested using three

different methods (Classification and Regression Tree (CART), multiple linear

regression (MLR) and Analysis of Variance (ANOVA). Community structure

influence on NH_4^+ efflux was evaluated by regressing total species richness

(number of unique species) with NH_4^+ efflux using least squares regression.

CART and MLR analyses identified dissolved oxygen, temperature and salinity as

the primary drivers of NH_4^+ efflux in all regions of the estuarine system.

Oligohaline and mesohaline tributary temperature and salinity control the rate of

nitrogen cycling as well as benthic macrofaunal biomass. In deeper regions of

mesohaline tributaries and the mainstem Bay dissolved oxygen was found to be to

dominating parameter regulating nitrogen pathways in sediments as well as the

structure of the benthic macrofaunal community. Spring regressions of total

macrofaunal biomass NH_4^+ efflux suggested an enhancement of efflux with

increased biomass. In contrast, fall regressions suggested the enhancement of

fixed nitrogen removal from sediments with increased benthic biomass. No

significant relationship was observed in summer data, but high NH_4^+ effluxes

under hypoxic/anoxic conditions suggested dissolved oxygen is the primary driver

of nitrogen cycling during that time of year. Individual species excretion rates

estimated using previously published allometric models (Peters 1983) estimated

polychaetes, bivalves and amphipods excretion contribute ~ 12, 20 and 16%

(respectively) of the total measured NH_4^+ efflux. Deviations from Redfield

organic matter aerobic respiration were evaluated against macrofaunal biomass

and helped to support the interpretations of NH_4^+ efflux relationships with benthic

biomass. I concluded a complex balance between seasonal and regional

environmental conditions coupled with the benthic community species richness

and dominant feeding guilds controls the relationship between macrofaunal

biomass and sediment nitrogen flux in this eutrophic estuarine system.

Chapter 5

Summary and Synthesis

CHAPTER 2:

Analysis of opportunistic polychaete abundance and

size in mesohaline estuarine environments with seasonal hypoxia

ABSTRACT

An increase in hypoxia (dissolved oxygen (DO) < 63 μM or < 2 mg L^{-1}) is an environmental stressor associated with eutrophication that can cause a shift in benthic community structure towards opportunistic macrofauna. Chesapeake Bay is a eutrophic estuary where seasonal hypoxia has been increasing since the early 1950's. Utilizing a large monitoring dataset including benthic macrofaunal abundance, biomass, and concurrent measures of environmental parameters (e.g., depth, sediment type, salinity, temperature, and DO) this study examined how environmental conditions regulate the densities of opportunistic polychaetes in a mesohaline estuarine system. The examination of polychaete abundance and biomass in the mesohaline Chesapeake Bay region points to a benthic community dominated by euryhaline, opportunistic polychaete worms (*M. viridis, S. benedicti, H. filiformis,* and *A. succinea*). Macrofauna samples were divided into three groups by depth ranges that often had similar sediment types, but also had similar overlying water properties, including dissolved oxygen concentrations. Linkages between variations in environmental factors and abundance of animals were initially evaluated using Spearman rank correlation and linear regression analyses indicating depth, sediment type, temperature salinity and dissolved oxygen have varying levels of importance with each species studied. Classification and Regression Tree (CART) analysis revealed salinity is a controlling parameter of polychaete abundance in shallow zones (< 5 m), particularly during spring recruitment, and dissolved oxygen controls abundance

at deep depths (> 10 m) of the estuary. Mid-depth abundances are controlled by

the interaction between both parameters. Winter air temperature and in situ

bottom water temperature play strong roles in the timing of spring recruitment.

This study supports previous work indicating a shift in the dominant polychaete

community of the mesohaline Chesapeake Bay to one made up of four species, all

of which are known to be well adapted to hypoxia and other stressful conditions.

Our analysis further shows that the ability of these polychaetes to respond to

hypoxia varies among species. Those species that can withstand temporary

hypoxia (*A. succinea)* still thrive, however, dissolved oxygen appears to be the

"master variable" controlling long-term trends and spatial distribution of these

dominant members of the polychaete community.

INTRODUCTION

Polychaete worms are among the most abundant and pervasive macrofaunal

populations in estuarine sediments, and they have been shown to have profound

effects on many benthic processes (Kristensen & Blackburn 1987, Christensen et

al. 2000, Aller et al. 2001a, Quintana et al. 2007). For example, polychaetes serve

an important trophic link between primary producers and primary carnivores

(Rhoads 1982), and through their bioturbation activities, these animals also tend

to accelerate and modulate diagenetic processing of sediment organic material

(Welsh 2003).

It is broadly thought that these animals have opportunistic behavior since many of them have a short lifespan (< one year), and their reproduction strategies are mostly "*r*-selected" (Vermeij 1978) with respect to the logistic growth equation.

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right)$$

Where *r* is the maximum growth rate of the population (*N*), and *K* is the carrying capacity of its local environmental setting. Polychaete exceptions, often termed "equilibrium species" (Wilson 1967, McCall 1977), are worms that tend to live longer, have a "*K*-selected" life strategy, and are characteristic of less stressed ecosystems. Typically most adult estuarine polychaetes (regardless of their life strategy) have a wide range of environmental conditions they can tolerate temporarily allowing for a continuum of species in an estuarine system (Boesch 1977, Gray & Elliott 2009). But when water quality in an estuary becomes degraded due to anthropogenic eutrophication leading to seasonal low dissolved oxygen, equilibrium species can no longer thrive, and more opportunistic species replace them. Larval or juvenile stages of many polychaetes, even for opportunistic species, can be sensitive to many environmental variables, including salinity and temperature. Therefore, in addition to eutrophication, the timing of seasonal temperature changes or freshwater runoff can affect reproduction and recruitment of polychaetes (Gray & Elliott 2009).

An increase in hypoxia (dissolved oxygen < 63 μM or < 2 mg L^{-1}) is an environmental stressor associated with eutrophic environments that can shift

benthic community structures towards opportunistic macrofauna. As hypoxia intensifies, many polychaetes migrate to the surface and in some cases emerge from the sediments in search for higher oxygen concentrations (Rosenberg 1977). Eventually, species sensitive to low oxygen conditions die or become vulnerable to predation (Gray & Elliott 2009). Opportunistic polychaetes, frequently found thriving in hypoxic environments, have the ability to survive in low oxygen conditions through physiological adaptations (Diaz & Rosenberg 1995, Gray & Elliott 2009).

A major shift in benthic community structure appears to have occurred in the mid-salinity Chesapeake Bay, where seasonal hypoxia has been documented since the early 1950's (Officer et al. 1984, Weisberg et al. 1997). Beginning in the 1950's, the volume of hypoxic water in summer increased, thereby posing a significant threat to the ecological health of the estuary (Hagy et al. 2004). Research done in the late 1970's and early 80's revealed a change in the benthic macrofaunal community towards opportunistic species (Holland & Diaz 1983, Holland 1985, Holland et al. 1985). By 1984 the Chesapeake Bay Program (CBP) initiated a long-term monitoring of Bay water quality and biology. Long-term time series datasets of benthic macrofauna and water quality in the Chesapeake Bay represent a resource for inferring how large-scale changes in estuarine environmental conditions (e.g. climate change, expansion of hypoxia) have influenced the polychaete community. Although sampling protocols have been modified in this monitoring program during the past four decades, these data provide a window to glean potential insight into benthic community temporal

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trends and shifts.   Possible drivers may also be found by examining these data in

conjunction with other environmental datasets. Information on the patterns of

long-term in situ variation are important for elucidating the complex relationship

between macrofauna (specifically polychaetes) and sediment biogeochemistry

under changing environmental conditions (Diaz & Schaffner 1990). Surprisingly

few previous studies have examined the existing monitoring data with these

possible relationships in mind (Holland et al. 1987). An assessment of specific

polychaete communities spanning all depths of the Maryland mesohaline Bay and

the physical and ecological environmental parameters that shape those

communities has yet to be specifically evaluated during the last 30 years.

The benthic ecosystem of an estuary is a continuum of the complex

interplay between bottom sediments, the overlying water and the species that live

there. When assessing macrofaunal communities in these environments it is

useful to delineate different habitats or niches that define assemblages of species.

Many studies use sediment type as the primary environmental factor defining

benthic communities because it has been shown to play a defining roll in the

structure of benthic communities (Rhoads 1982, Zajac 2001, Gray & Elliott 2009)

This approach works well in some study areas where sediment type and depth are

strongly correlated. In the mesohaline regions of Chesapeake Bay and its

tributaries, where this study is focused, sediment grain size tends to decline with

depth; however, the exact nature of this decline varies among regions. The

exceptions arise in the mid-depth ranges where sediments can be classified along

a gradient of sandy mud. Some opportunistic polychaetes (e.g. *Alitta (Neanthes)*

succinea) can be successful in a variety of sediment types and overlying water conditions (Gray & Elliott 2009). Others can change their feeding modes to adapted to sediment type or quantity of organic material available (e.g. *Streblospio benedicti*) (Levin 1986). Depending on developmental stage of these organisms, sensitivities to overlying water conditions may change (Gray & Elliott 2009). Influence of overlying water parameters can vary with season where as sediment type is often more static.

The first aim of this study is to document the seasonal pattern of abundance and individual size of the four dominant polychaetes (*Marenzelleria viridis, Streblospio benedicti, Heteromastus filiformis,* and *Alitta (Neanthes) succinea*) in the mesohaline waters of Chesapeake Bay and its tributaries along three different depth intervals. Seasonal succession of the benthic macrofauna of the Chesapeake Bay can have a strong influence on sediment metabolic processes (Kemp & Boynton 1981) including nitrogen removal via coupled nitrification-denitrification processes (Kemp et al. 1990). These seasonal dynamics have yet to be quantitatively described at different depths, where sediment type and overlying water hydrodynamics vary. The second objective of this study is to assess what overlying water parameters regulate the densities of opportunistic polychaetes in a mesohaline estuarine system. Finally, we explore how changes on overlying water parameters influence the long-term trends in abundance of these opportunistic polychaetes. This information on seasonal patterns and long-term trends may help elucidate the complex relationship between polychaetes and their changing environmental conditions.

METHODS AND APPROACH

Site Descriptions

Macrobenthic community data were assembled from five mesohaline regions of Chesapeake Bay and its tributaries where seasonal bottom-water hypoxia occurs (Fig. 2.1, Table 2.1). Those regions include the western side of mid-Chesapeake Bay (Region 1), two sections of the mesohaline Potomac River estuary (Regions 2, 3), and the lower reaches of the Patuxent (Region 4) and Choptank (Region 5) River estuaries. The first region is near Calvert Cliffs on the western shore of the mid (mesohaline) Bay where the narrow shallow shoals (<10 m) are vulnerable to lateral seiching and upwelling of deep hypoxic bottom water in summer (Malone et al. 1986, Chuang & Boicourt 1989). Two sections of the mesohaline Potomac River estuary are represented: Region 2 is near Morgantown, MD that often experiences summertime hypoxia, and Region 3 in the lower section of the river estuary where seasonal hypoxia and anoxia (< 0.02 mg $O_2 L^{-1}$) occur in the deep channel (Boesch et al. 2001, Hagy et al. 2004). Region 4 is the mesohaline lower Patuxent River estuary, where summer hypoxia is common (Boynton et al. 2008). Finally, Region 5 is the lower Choptank River estuary where short-term hypoxic events have been linked to wind and tidal forced lateral oscillations of the mainstem Bay pycnocline transporting Bay hypoxic bottom water into the Choptank (Sanford 1990, Fisher et al. 2006).

Data Assembly and Management

Benthic macrofauna and environmental data used in this analysis were collected as part of the Power Plant Sighting Program of the State of Maryland (1971-1984) and the Chesapeake Bay Long-term Benthic Monitoring Program (1984-2008) (Mountford et al. 1977, Llanso 2008). These data were merged into one data set by Versar, Inc. (Columbia, MD) and uploaded into the Chesapeake Bay Environmental Observatory (CBEO) data server (Ball et al. 2008). The Chesapeake Bay Program (CBP) spatial and temporal sampling frequencies changed over the 38-year times-series (Llanso et al. 2010) thereby constraining the analyses presented in this study.

Maryland's long-term benthic monitoring program included both probability-based and fixed-point sampling sites. The fixed-point monitoring sampling scheme was designed to identify temporal trends in the benthic community. The probability-based sampling scheme, implemented in 1994, was a stratified random design implemented to survey the areal extent of benthic community health. With each sample, abundance of each macrofaunal species and in situ environmental data (temperature, salinity, dissolved oxygen and the percent silt-clay of the sediment) were measured. Beginning with the CBP, total biomass of the most dominant species was measured or estimated (Llanso et al. 2010). In late 1970's until the mid- 1990's, macrobenthos and sediment sampling occurred on a monthly or in some cases bi-monthly basis during the spring, summer, and fall months. Sediments were sieved for invertebrates using a 0.5 mm screen and preserved in the field using a 10% formaldehyde solution (Llanso

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et al. 2010) . After 1994, sampling was restricted to twice a year (May and September). Sampling starting in 2008 was reduced to only once a year in late August or September.

Seasonal characterization of polychaetes and environmental Factors

The present study focuses on the four most abundant polychaete species in the Chesapeake Bay region (Table 2.2) collected over the 11-year period between 1984 and 1993 where a minimum of 5 months was sampled (Holland et al. 1987). Benthic polycheate data tend to be highly variable (Gray & Elliott 2009). To minimize the influence of outliers and increase the contribution of low-abundance samples, geometric means of species abundance and individual size were calculated by first transforming the data (log(x+1)), calculating the mean and then converting the data back to unlogged numbers for interpretation. Arithmetic seasonal means of concurrently measured in situ bottom temperature, salinity and dissolved oxygen were also calculated.

In this community analysis study, depth is a more consistent environmental parameter across study regions than sediment so it has been used as the parameter to frame habitats. Samples were divided into three groups by depth ranges that often have similar sediment types but also have similar overlying water properties, namely dissolved oxygen concentrations. Areas less than 5 m deep are typically shallow, well mixed environments that do not experience hypoxic conditions. In contrast, areas greater than or equal to 10 m deep in all five regions regularly experience summertime hypoxia ($< 63 \ \mu M \ O_2$)

or anoxia. The area in between these two extremes (5-10 m) have the potential to experience short-term hypoxia throughout the summer as wind and tidal conditions transport hypoxic water from the adjacent deep channel shoreward (Malone et al. 1986).

Spatial differences among regions and depth ranges for each species were examined using a 2-way ANOVA. Linkages between environmental factors and polychaete abundance and size were evaluated using a Spearman rank correlation coefficients and linear regression analyses. Geometric mean time-series trends over a 13-year period (1981-1993) were identified in the shallow zone of the Potomac-Morgantown and Calvert Cliffs regions were the greatest and most consistent samples were collected. Those trends were also analyzed using linear regression analysis and Spearman rank correlation. Change point analysis for linear regressions of polychaete abundance versus environmental variables was assessed using the "segmented" package in R version 3.0.2 (Muggeo 2008).

Classification and Regression Tree (CART) Analysis

Although linkages between environmental factors in ecology can often be complex a non-linear, classification and regression trees (CART) analysis (De'ath & Fabricius 2000) is a statistical tool that can help model and explore these complex relationships. We chose a CART analysis using regression trees to examine in more detail what environmental factors exert the greatest influence on patterns of polychaete abundance during different seasonal time periods. CART

analysis was performed using built in functions of the Matlab Statistics Toolbox version r2012_a.

For this analysis, abundance data were first averaged over 2-3 months based on season and published recruitment times for each species (Spring, Early summer and Late Summer). In this case, "Spring" represents data averaged over March, April and May. Because hypoxia typically sets up in these regions by mid-June and continues through July (Kemp et al. 2005, Murphy et al. 2011) we define "Early Summer" abundance as the average of June and July. "Late Summer" is defined as the mean of August and September, when macrofauna are recovering from summer hypoxic events (Boicourt 1992) and in some cases have a fall recruitment period (Diaz & Rosenberg 1995). Regression trees were constructed for each time period for each of the four dominant polychaetes (*Marenzelleria viridis, Streblospio benedicti, Heteromastus filiformis,* and *Alitta (Neanthes) succinea*). Factors used to build each tree included month, region, water column depth, sediment type (% silt-clay), temperature, salinity, and dissolved oxygen (DO). To minimize over fitting, the optimal tree size for each tree was established using a 10-fold cross-validation (De'ath & Fabricius 2000). With many parameters, the optimal tree can have a large number of terminal nodes resulting in a complex tree. Previous researchers (Steen et al. 2008, Poff et al. 2010, Cleveland et al. 2011) have either opted to report a smaller CART tree with higher error or to eliminate less important variables to create simpler trees. Since optimal trees for each species calculated here had more than 10 terminal nodes, only example portions of each CART are shown in the results to illustrate

notable relationships. Full regression trees are shown in Appendix I. At each node in the tree, mean squared error (MSE) is estimated as node error weighted by the node probability. The changes in MSE due to splits on every factor in a tree were summed and then divided by the number of tree nodes to compute a relative individual factor importance when building the tree. As the tree is grown, variance is reduced, therefore, factors with the greatest importance will have the highest MSE averaged over the number of nodes in the tree.

RESULTS

Seasonal Dynamics of Abundance and Environmental Parameters

Of the 62 polychaete genera (and other higher order taxa) recorded in the dataset, the top four species accounted for 77-93% of the individuals in the population collected within each region. The seasonal geometric means of abundance and total biomass of the most abundant species across the Chesapeake Bay region are summarized in Table 2.2 and Fig. 2.3. The most dominant species in all five mid-bay regions is *Marenzelleria viridis*. A close second in dominance is the tiny (< 2cm) spionid *Streblospio benedicti* with greatest annual abundances region-wide in the Patuxent, Lower Potomac, and Choptank Rivers. *Heteromastus filiformis* appear to be evenly distributed across all five regions representing 71-92% of all samples with the most dominant presence in the Lower Potomac and Choptank Rivers. *Alitta (Neanthes) succinea* is also a ubiquitous member of the benthic community found in 54-68% of all samples collected. While they are often found in lower densities, this species is the largest

polychaete of the top four animals with an average individual size range of ~ 3-5 mg AFDW. Other polychaetes found in samples (up to 58 species in some regions) represented ~7-23% of collected polychaetes over the sampling period (1981-1994).   Most of these rare polychaetes can be characterized as equilibrium species (Llanso et al. 2010).

Seasonal Dynamics of Abundance

Spring is the time of year when most polychaetes exhibit a peak in recruitment. This reproductive time can begin as early as the beginning of March for *M. viridis* or as late as mid-June for *S. benedicti* (Fig. 2.2). *M. viridis* annual mean abundance peak occurred in March in the mid-depth and deep regions of the Potomac River, and the shallow Choptank River. The Patuxent River and Calvert Cliffs had *M. viridis* peaks across all depths in April (Fig. 2.2a). The latest seasonal peak (May) was in the shallow Lower Potomac River. *M. viridis* were found in greatest abundances region-wide at depths less than 10 meters (Fig. 2.2a) in sandy sediments (<17 % silt/clay) (Table 2.1). The abundance and individual biomass of *M. viridis* varied in the shallow waters off Calvert Cliffs (Fig. 2.3a), with highest abundance and smallest individuals in spring. The mean size of individuals increased over summer and peaked in fall. In contrast, abundances decreased to seasonal minima over the same period.

S. benedicti peaked in the later spring and early summer (May and June). Their mean annual abundances were greatest at deeper depths where there is a higher percentage (>80%) of silt-clay in the sediment (Table 2.1, Fig. 2.2b, Fig.

2.3f). For example, high abundances are found at depths greater than 5 m in each region, but they are also found in high numbers in the Patuxent River shallow zone where sediments are less muddy (~39 % silt-clay) (Table 2.1, Fig. 2.2b). Moving into the summer months, abundances of *S. benedicti* decreased region-wide, particularly at depth. Unlike *M. viridis, S. benedicti* individual size does not have a clear relationship with abundance (Fig. 2.3d-f).

The timing of seasonal abundance peaks in *H. filiformis* and *A. succinea*, depend upon region, with the former being higher at shallower sites. *H. filiformis* abundance was lowest on Calvert Cliffs where shallow sandy sites have a lower silt and clay content (< 8 % silt-clay) and greatest salinity range (0-24) (Table 2.1 and Fig. 2.2c). In the Potomac-Morgantown region, abundances of *H. filiformis* steadily increased throughout the later spring (May or June) (Fig. 2.3g), while on Calvert Cliffs, they peaked in July (Fig. 2.2c). In contrast, in the Choptank River, maximum *H. filiformis* abundance was in March (Fig. 2.2c). Annual patterns of abundance and size of *H. filiformis* were incongruent, like *S. benedicti* (Fig. 2.3g-i). While *A. succinea* abundances were not significantly correlated to depth or sediment type (Table 2.3) they were often highest in sandier deep stations of the Choptank (Fig. 2.3l) and at mid-depth (5-10 m) where the sediment composition varies between regions (Table 2.1, Fig. 2.2d). *A. succinea* abundance often increased in July region-wide with a late summer/early fall die off. In Choptank and Potomac Rivers, however, highest seasonal abundances occurred at mid-depth sites during the winter months (December – February) (Fig. 2.2d, 3k). Increases in abundance in the deepest stations of the Calvert Cliffs, Patuxent river

regions were also observed in February (Fig. 2.2d). Monthly mean individual size of *A. succinea* exhibited an inverse relationship with mean individual size, being largest in the fall and spring when abundance was low and smallest in summer when abundance was greatest (Fig. 2.3j,l).

Relationships Between Abundance and Environmental Parameters

Relationships between abundances and in situ environmental parameters were generally explored initially using Spearman Rank Correlation (Table 2.3). Two polychaetes, *S. benedicti* and *A. succinea,* were significantly negatively correlated with temperature (p <0.05). Although *M. viridis* had a negative relationship to temperature, that relationship was not significant. When examining all data across regions and years, *H. filiformis* abundance was not significantly related to temperature. All polychaetes, however, were negatively correlated to salinity; *M. viridis* and *H. filiformis* had the strongest significant relationship (Table 2.3, Fig. 2.4a,b). Significant positive correlations to dissolved oxygen were also observed for *M. viridis*, *H. filiformis,* and *A. succinea* (Fig. 2.5 a,c, and d)*.* All polychaetes, including *S. benedicti,* were positively correlated to DO at the deepest stations under hypoxic conditions. *A. succinea* exhibited the highest sensitivity to DO across all depths (Table 2.3, Fig. 2.5d).

To understand these relationships between abundance and in situ environmental parameters further, we developed CART analyses for each species during the spring, as well as early and late summer seasons. While the upper branches of each regression tree split abundances based on parameters already

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discussed (depth, % silt-clay), the lower branches identified key times of year (months), study regions, and possible thresholds of separation for environmental parameters (salinity, temperature, and DO). Representative portions of trees for each polychaete, where branches and splits are of particular interest, are presented (Fig. 2.6). The relative importance of each parameter is depicted in the CART model for each species and season (Table 2.4).   All CART models were statistically significant (p< 0.005) with > 500 degrees of freedom.

For *M. viridis,* the highest abundances occurred in spring, with steadily declining abundances throughout summer (Fig. 2.3a). CART analysis showed depth, salinity and sediment type to account for 69% of the model's variability and abundance prediction in spring (Table 2.4).   The early (not shown) and late summer (Fig. 2.6a) regression trees revealed depth as the strongest predictor of abundances, with highest numbers found in shallow regions less than 3.6 m deep. The next branch of the tree indicated the Potomac-Morgantown region has the highest predicted abundance (> 1000 indiv. m^{-2}). This order of magnitude greater abundance over other study regions (Fig. 2.2a) is the central contributor to the ~17% "region" factor importance in the prediction of late summer *M. viridis* abundance (Table 2.4, Fig. 2.6a). Even into the fall, *M. viridis,* and other polychaetes sustained relatively high abundance in this region (Fig. 2.2). In the late summer months, individual *M. viridis* are larger than in the spring, indicating that adults dominate the population (Fig. 2.3a). It appears that larger polychaetes were relatively tolerant to hypoxia in the Potomac-Morgantown region where

peak late summer abundances (> 2000 indiv. m^{-2}) were found under oxygen stressed conditions (DO < 4.5 mg L^{-1}) (Fig. 2.6a).

In general, *S. benedicti* showed preference for muddy sediments at deeper depths (Fig. 2.2b, Fig. 2.3d-f). Sediment type was the most important predictor of abundance in the spring (42.5%), but, less important in early and late summer (~3.0 %). Together depth and region account for 64% in the spring but each dropped below 19% relative importance in summer. The annual mean abundances of *S. benedicti* suggested a late spring recruitment period in all five regions (Fig. 2.2b). The CART analysis indicated June as the month of greatest abundances, with these peaks being greatest at deeper depths when salinity exceeded 7.0 (Fig. 2.6b). Throughout the late summer salinity remained the strongest predictor of abundance with region, month, DO and depth, all between 11-16 % relative importance (Table 2.4). The late summer *S. benedicti* regression tree indicated a modest fall recruitment period in September particularly in shallow depths of the Lower Potomac and Choptank rivers where abundances reached a mean of 1400 indiv. m^{-2} (not shown). This regression tree also indicated *S. benedicti* were tolerant of hypoxic conditions in July (not shown).

Highest abundances of *H. filiformis* occurred in shallow, sandy sediments (Fig. 2.2c) and the spring regression tree revealed that depth, region and sediment type account for 96% of the variability (Table 2.4, Fig. 2.6c). Abundances were highest at depths < 3.7m and sandy sediments with slightly higher silt-clay content (> 9.0 % silt-clay). Among regions, Calvert Cliffs had the lowest mean abundances of *H. filiformis* and split off the regression tree early (Fig. 2.6c,

terminal node (TN) 8 & TN 9). The highest abundances were predicted in the

Potomac – Morgantown and Choptank River regions with a mean of > 2100

indiv. m^{-2} (Fig. 2.6c, TN 17). These trends continued throughout the summer,

with abundances increasing in some regions in late August and early September

and fall recruitment (not shown).

The polychaete, *A. succinea* was a ubiquitous polychaete within our study

area. However, CART analysis revealed that variability in *A. succinea* abundance

was not strongly explained by our environmental parameters (Table 2.4, Fig.

2.6d). Month accounts for 23.72 % of the variability in early summer abundance

(Table 2.4) for *A. succinea* with abundance peaks in July (Fig. 2.2). Salinity was

another important summertime parameter contributing 25-27 % to the CART

prediction. Unlike the other polychaetes in this study, dissolved oxygen (DO)

accounted for a large percentage of the variance (32-64 %, Fig. 2.6d, Table 2.4) in

summer, with a strong (Fig. 2.6d) split between anoxic (< 0.85 mg O$_2$L^{-1}) and

hypoxic to normoxic (> 0.85 mg O$_2$L^{-1}) conditions. With DO concentrations $>$

0.85 mgL^{-1} relatively high abundances were found in mid to shallow depths (< 13

m) where the salinity was < 5 (Fig. 2.6d, TN 8 & TN 10).

Trends in Abundance over 13 years

Trends in spring abundance of each polychaete were examined for the

shallow depth zone of two regions (Calvert Cliffs and Potomac-Morgantown) for

which there was a 13-year time series (1981-1993). Significant trends were found

for *H. filiformis* and *A. succinea*. *H. filiformis* had a general increasing trend on

both regions, but the trend was only significant (p<0.05) in the Potomac River

(Fig. 2.7a). Abundances of *H. filiformis* in the Calvert Cliffs region were,

however, significantly correlated to spring Susquehanna River flow (Fig. 2.4d). In

contrast, *A. succinea* showed a significant decline in abundance in both regions

and abundance in the Calvert Cliffs region was significantly correlated with

Susquehanna River flow (Fig. 2.7b). Regressions with *A. succinea* and *H.*

filiformis and Potomac river flow did not yield significant results, nor did Calvert

Cliffs abundance and bottom temperature. Despite significant trends in bottom

temperature, salinity, and winter air temperature (Fig 8), the only significant

Spearman correlation to environmental parameters was a negative correlation of

A. succinea and bottom temperature (ρ = -0.692, p-value = 0.01). Time series

data for *M. viridis* and *S. benedicti* in these two regions revealed no significant

trends for abundance at shallow depths (< 5m) (Table 2.5). There was, however,

a significant negative correlation of *M. viridis* to April river flow; either the

Susquehanna River for Calvert Cliffs or the Potomac River for Potomac-

Morgantown region (Fig. 2.4c). Consistent with a positive correlation of *S.*

benedicti to salinity (ρ = 0.769, p-value = 0.003), CART analysis shows *S.*

benedicti has a strong seasonal preference to more saline environments during the

summer months (Fig. 2.6b, Table 2.4).

Correlations with region-wide parameters through a multiple linear

regression analysis also yielded some success (Table 2.5). A multiple linear

regression (MLR) analysis in the Calvert Cliffs region indicates a significant

relationship between abundance *H. filiformis* and NAO, river flow, and two

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interaction terms. The first term is between DO and bottom temperature; the second term is between mean winter air temperature and bottom temperature. The Potomac-Morgantown MLR relationship for *H. filiformis* also had NAO and bottom temperature, but included salinity, as important parameters. The Calvert Cliffs MLR relationship for *A. succinea* included DO, river flow and an interaction term between salinity and bottom temperature. In the Potomac *A. succinea* is still strongly influenced by DO and river flow, but NAO and winter air temperature play a stronger role. MLR relationships in both regions for *M. viridis* and *S. benedicti* offer more information on the controls of these two species in the spring. Calvert Cliffs *M. viridis* abundance is controlled by river flow, salinity and winter air temperature and in the Potomac there is a strong relationship to NAO and winter air temperature.   Salinity is a strong driver of *S. benedicti* abundance in both regions, but interactions with DO and both winter air and bottom water temperature are important in the Calvert Cliffs region.

DISCUSSION

The Mesohaline Polychaete Community

The first study of Calvert Cliffs and Potomac-Morgantown data collected between 1971 and 1984 (Holland et al. 1987) described a community with a balance between longer-lived, equilibrium benthic macrofauna species and shorter-lived opportunistic species. This community was, however, showing signs of a possible shift in community "structure". Many of those longer-lived species (e.g., *Paraprionospio pinnata, Glycinide solitaria, Eteone heteropoda*)

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became relatively rare during the subsequent decade of data examined in this study and were essentially all but absent in recent benthic collections in many of the mesohaline regions of the Bay (Llanso et al. 2010). Over the past decade, polychaete abundance and biomass in the mesohaline Chesapeake Bay region have been dominated by euryhaline, opportunistic polychaete worms with much fewer large, hypoxia-tolerant longer-lived species (*M. viridis, S. benedicti, H. filiformis, and A. succinea)*. This change in community structure over the 23 year time-series of data suggest a benthic community response to documented environmental changes associated with eutrophication and hypoxia in the region since the 1950's and 60's (Kemp et al. 2005). Previous work has related declining abundance and structural shifts in the polychaete community to increased hypoxia in a mesohaline Chesapeake Bay tributary study that was focused on deeper stations where the hypoxia (and anoxia) occurred regularly (Llanso 1992). In contrast, the present study considers hypoxia and other environmental effects on animal abundance at a range of depths (2-20m) representing a continuum of the complex interplay between sediment types and overlying water properties. Sediment bottom types in this study range from sand to mud. This bottom type diversity could be the driving factor in benthic community composition (Gray & Elliott 2009), but previous work by Holland et al (1987) and CART analysis in this study has indicated dissolved oxygen and salinity also play a major role.

Changes in DO Redefine Middle and Deep Benthic Community Structure

Depth, sediment type and DO generally co-vary in an estuarine system. While the shallow, sandy habitats of the mesohaline Chesapeake Bay and its tributaries are typically unaffected by hypoxia, the deep habitats of all five study regions are characterized by muddy sediments (> 84% silt-clay) and summertime hypoxia or anoxia (Kemp et al. 2005). It is not uncommon for near total faunal depletions in these deep areas where summertime low oxygen conditions are particularly severe (Holland et al. 1977). In our analysis, even the opportunistic species, known for their hypoxia tolerance, show a significant positive relationship in the deepest zones of all study regions (Fig. 2.5). The physical characteristics of the mid-depth zone on the other hand are variable between each region. Sediment type at the depths of 5-10 meters have been termed "transition sediments" (Holland et al. 1985) ranging from sandy (9.13 % silt-clay), along the mid-bay Calvert Cliffs, to very muddy (90.56% silt-clay), in the Morgantown-Potomac River region. Hypoxia can occur in these areas on an event-scale basis (1-4 days) when physical forcing draws hypoxic water near shore from deeper areas (Chuang & Boicourt 1989). This variability in DO can create a dynamic habitat characteristic of opportunists.

The polychaete *A. succinea* illustrates best how the varying relationship between sediment-type and overlying water properties, like dissolved oxygen, in the middle and deep depth zones can influence polychaete abundance. In the 1970's and early 1980's *A. succinea* dominated the deeper depths of both the mid-Chesapeake Bay and Potomac River; suggesting they prefer muddy sediments

(Mountford et al. 1977, Holland et al. 1987). Results of this study suggest a shift

to sandier mid-depth zones in the 1990's as the areas where *A. succinea* thrives

(Fig. 2.3k). In the mid-1980's and 90's they are well represented in the shallow

and mid-depths of the Calvert Cliffs, Patuxent River, and, Potomac-Morgantown

region, but not in the deep zones of any area (Fig. 2.2d). It has been well

documented that hypoxia and in some cases anoxia in the deep zones of all study

regions has increased over the last several decades and is sustained in the summer

months (Kemp et al. 2005, Fisher et al. 2006). Across regions, CART analysis

indicates DO is an important factor driving abundance of *A. succinea* throughout

the summer (Fig. 2.6b, and Table 2.4). While these polychaetes are known to

tolerate short-term hypoxia events (1-3 days), sustained hypoxia and anoxia are

fatal to these animals (Kristensen 1981, Yokoyama 1995, Nizzoli et al. 2007,

Swan et al. 2007). *A. succinea* also have a significant positive correlation with

dissolved oxygen across all 5 regions in this study (Fig. 2.5d). In the Choptank

River, where hypoxia events are temporary and relatively moderate (Chuang &

Boicourt 1989), *A. succinea* are ubiquitous with abundances > 100 indiv. m^{-2} at

all depths and sediment types (Fig. 2.2d). True to its opportunistic nature, *A.*

succinea are known to re-colonize defaunated areas in the late summer and early

fall (Holland et al. 1977) once summertime hypoxia subsides.

The much smaller, hypoxia tolerant (Llanso 1991), opportunistic

Streblospio benedicti has replaced these larger worms as the most abundant deep

water benthic polychaete in regions experiencing the most severe seasonal

hypoxia (Calvert Cliffs, the Lower Potomac and Patuxent Rivers) (Fig. 2.2b).

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This tiny spionid was considered a rare species on a list of macrofaunal invertebrates in published 1970's work conducted in the 9-meter habitat of Calvert Cliffs (Holland et al. 1977, Mountford et al. 1977), but now dominates that region over the once abundant *A. succinea* by an order of magnitude. In this study, *S. benedicti* is the dominant species in the deep habitats of all regions, except Potomac-Morgantown where a fresher salinity range may favor *M. viridis* (Tables 1, Fig. 2.2a). *S. benedicti* is seen as a euryhaline species with central distribution in mesohaline zones of estuaries with affinity towards muddy sediments (Dauer et al. 1981, Sardá et al. 1995). CART analysis in this study predicts the highest abundances in deeper depths with salinity > 7.0 (Fig. 2.6b). Very opportunistic, this polychaete is a facultative suspension or surface deposit feeder that can broadcast spawn or brood their young (Levin 1984, Levin et al. 1987, Levin & Bridges 1994). With a high tolerance to hypoxia, *S. benedicti* is a strong competitor in the oxygen limited regions of a mesohaline environment (Llanso 1991). CART analysis here supports all of the published habitat preferences and tolerance to hypoxia. Unlike the other polychaetes in this study, there is not overall significant relationship with *S. benedicti* and DO; however, this polychaete does have a significant relationship to hypoxic DO concentrations (Fig. 2.5b) suggesting this worm is not completely immune to hypoxia. The greater slope of this relationship does suggest a higher tolerance to hypoxia than the other three polychaetes in this study. Abundance and biomass of this polychaete are well correlated with peaks in the late spring (June) as the weather

warms and a decrease throughout the summer with a modest rally in the fall (Fig. 2.3b)(Sardá et al. 1995).

While *S. benedicti* may dominate polychaete abundance at these depths, *A. succinea* still dominates the biomass. *S. benedicti* has a maximum abundance at this depth zone that ranges from 370 indiv. m^{-2} in the Calvert Cliffs region to over 2700 indiv. m^{-2} in the Choptank and lower Potomac (Fig. 2.2b). Its total biomass across seasons in this area is 0.23 – 72.67g AFWD m^{-2}. *A. succinea* has a maximum abundance at this depth zone that ranges from 53 – 227 indiv. m^{-2} but its total biomass across seasons is 5.7 – 465.0 g AFDW m^{-2}. Biomass is often a gauge used to measure the benthic macrofaunal contribution to nutrient cycling through bioirrigation or bioturbation (Welsh 2003, Kristensen et al. 2012). The shift in species dominance from one opportunistic polychaete to another in the deep-water habitats of these mesohaline regions is a reflection of changes in the over all stability of the entire ecosystem. Understanding the changes in the occurrence and abundance of these two species in muddy habitats experiencing regular summertime hypoxia may help fine tune ecosystem models that monitor the Bay's health.

Salinity Shapes Shallow Polychaete Community

The physical environment of the shallow mesohaline regions in this study is characterized first by sandy sediments with a silt-clay content of 7-40%. Hypoxia is uncommon in these shallow regions leaving other water parameters, like salinity, to have a stronger influence on the benthic community. The organic-

sandy sediments of shallow, mesohaline estuaries are places where *H. filiformis* and *M. viridis* are known to thrive (Dauer et al. 1981, Holland et al. 1985, Sardá et al. 1995, Zettler et al. 1995, Quintana et al. 2007) and are dominant in the shallow zones of all five-study regions. With plenty of fresh detritus at the surface and recalcitrant organic material buried at depth in these areas, the surface deposit feeding *M. viridis* and the head-down deposit feeding *H. filiformis* don't have the need to compete for food. Field samples and microcosum experiments containing both worms suggested they could co-exist (Dauer et al. 1981, Karlson et al. 2002, Quintana et al. 2007). Analysis in this study suggests salinity plays a controlling role in seasonal abundance variability of both species (Fig. 2.4, Table 2.3).

The findings of several authors who have explored the relationship between *M. viridis* and salinity are supported in the results presented here. Adult populations of *M. viridis* are tolerant of salinities as low as 0.03 but can be particularly sensitive to salinity changes during their reproductive period of February-March (Dauer et al. 1980, Dauer et al. 1981). Early laboratory work found that eggs cannot be fertilized and larvae cannot complete development under salinities fresher than 5 (George 1966). Consequently, early freshwater runoff from winters with heavy snowfall or early rainy springs can negatively impact the recruitment of *M. viridis*. The relatively long pelagic larval stage of *M. viridis* (~ 4 weeks) (Bochert & Bick 1995) can also add to the negative impact of high river flow through the passive advective transport of larvae and juveniles down-estuary. A large spring (March 13-14, 1993) "super storm" occurred over

the region bringing heavy snow, rain and winds similar to a category 1 hurricane (National Weather Service 1994). It is possible the outlying data points in Fig. 4c-d, from a May 1993 benthic sample, may be the result of higher abundances of *M. viridis* being advected down stream under unusually high river discharge. Spring data collected in both the Potomac-Morgantown and Calvert Cliffs regions indicate a strong inverse relationship to April Potomac and Susquehanna River flow (Fig. 2.4c). The spring *M. viridis* CART analysis (not shown) as well as a multiple linear regression analysis (Table 2.5) indicated salinity as the primary overlying water parameter predicting abundance throughout shallow mesohaline Bay waters. Previous work in the Baltic found the highest abundances in salinities ranging from 5-7 in muddy-sand (Zettler et al. 1995). Regressions of shallow (<5 m) water mean abundance across all study regions also indicate a similar salinity range (5-10) for peak abundance in the Bay (Fig. 2.4b). Peaks occur in March in the Potomac and Choptank but April in the Patuxent and Calvert Cliffs regions because of different patterns in spring salinity ranges (Fig. 2.2c). The Patuxent and Calvert Cliffs areas remain saltier (> 15) until April where as the Potomac and Choptank rivers become fresher (< 15) earlier in the spring (~ March, data not shown).

Generally known as a euryhaline polychaete, little work appears to be published on the specific salinity tolerances of *H. filiformis* but some authors have indicated a negative correlation to salinity (Ourives et al. 2011, Silva et al. 2012). Data presented here supports that relationship with a significant correlation of abundance to salinity (Fig. 2.4b). After a strong correlation to depth, sediment

type and region, CART analysis points to salinity having the greatest influence over abundances in the spring (Fig. 2.6c). Like *M. viridis*, *H. filiformis*, has a pelagic larval stage that lasts about one month (Cadee 1979). The strong negative relationship of abundance to river flow also suggests *H. filiformis* larval recruitment success may be dependent on the extent of advective transport of larvae during their pelagic development (Table 2.5).

Explaining Decadal Trends

Large fluctuations of interannual abundance of benthic macrofauna in estuarine systems are common, but attributing those change trends in an isolated suite of environmental parameters can be challenging. Factors controlling abundances in these systems have time scales that range from decades to seasons to tidal cycles. Spatially, distributions vary regionally and locally based on patterns of overlying water parameters (temperature, salinity, dissolved oxygen), food availability, competition for space, and predation pressure (Gray & Elliott 2009). The state of the polychaete community may be a reflection of past, not necessarily current, conditions. This is particularly relevant when looking at correlations between salinity or dissolved oxygen, where short-term events like storms or hypoxic events prior to the sampling date may have large impacts on the make-up of the community (Diaz & Rosenberg 1995). Second, the opportunistic nature of polychaete worms, particularly the species now dominant in our study region, lends them to be tolerant to a wide range of parameters (Gray & Elliott 2009). Depending on the time scale of observations, swings in temperature, salinity or

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dissolved oxygen may have little to no effect on these resilient species. On the other hand, at certain times of year, changes in temperature or salinity can impact recruitment (Neuhoff 1979, Dauer et al. 1981).

Finally, temporal changes in key demersal predator populations (e.g., fish and crabs) due to environmental perturbations like hypoxia can also shape the benthic community (Breitburg 1992). Under hypoxic conditions, benthic macrofauna are often more vulnerable to predation because they migrate to the sediment surface in search for higher DO concentrations (Diaz & Rosenberg 1995). At the same time, benthic-feeding fish like spot (*Leiostomus xanthurus*) and Atlantic croaker (*Micropogonias undulates*) can feed in low oxygen environments for short time periods. The expanding hypoxic conditions in Chesapeake Bay may have created a "habitat squeeze," where demersal fish were forced into shallower oxygenated waters, thus intensifying predation pressure in adjacent shoal areas (Kemp & Boynton 1981). The decline in the abundance of *A. succinea* (Fig. 2.7) was concurrent with an increase in croaker and spot abundance during that time (1984-1990, http://www.st.nmfs.noaa.gov/), suggesting possibly top-down control of benthic macrofauna abundance (Joseph 1972, Hare & Able 2007).

Despite the challenges with the timing of competing forces, by applying the results of this study on the seasonality and habitat preferences of this systems dominant estuarine polychaetes, we can explain some of the significant trends in data collected during the more intensively sampled 13-year period of the monitoring program. The shallow zones of the Calvert Cliffs and Potomac-

Morgantown regions are the most consistently sampled areas where trends can be detected. The most striking trends observed in these data are with the increase in *H. filiformis* along with the decline in *A. succinea* (Fig. 2.7). A significant trend in decreasing salinity across both regions may have aided in the success of *H. filiformis* (Fig. 2.8b). Although the time series trend was not significant, the MLR analysis suggests the decrease in salinity has aided spring abundances of *M. viridis* as well (Table 2.5). The exception to this trend was in 1992, where a severe drought (Spring Average Palmer Drought Severity Index of -8.09) impacted the recruitment of both polychaetes only in the Calvert Cliffs region (Fig. 2.7a). The 1992 Potomac-Morgantown abundances seem to be unaffected by the drought. In contrast to Calvert Cliffs, the salinity in the Potomac River that year was not statistically different from the average and polychaetes were able to still recruit to that area.

The decrease in salinity was also coupled with warming of temperatures over the 13-year study period when the North Atlantic Oscillation (NAO) index shifted from negative to positive (Fig. 2.8). Under a positive NAO regime, the northeast region of the U.S. tends to experience warmer, wetter winters that may have contributed to the success of early recruiting species like *M. viridis* and *H. filiformis*. In the Calvert Cliffs and Potomac-Morgantown regions *A. succinea*, however, had a significant negative relationship to temperature as well as NAO (Table 2.5). The warmer winter air temperatures, associated with the positive NAO (Fig. 2.8c) may have affected spring recruitment seeing as they spawn during the winter months. The subsequent result is a decline in the spring

abundances in both regions (Fig.7b). Abundances of *S. benedicti* did not show

any significant trend in the shallow zones of either region. Our analysis of *S.*

benedicti seasonality and abundance shows their peak recruitment period is in the

late spring – early summer and they tend to prefer more saline, deep, muddy

environments than the shallow zones of Calvert Cliffs and Potomac-Morgantown

regions. Decadal trends in these populations may be more difficult to detect in

these areas than if samples were available for deeper zones of these same regions

during the 13-year study period. With that said, the strong positive MLR

relationship with *S. benedicti* and salinity (Table 2.5) supports the CART (Fig.

2.6b) indicating this polychaete is more successful saltier environments that the

shallow regions of this estuarine system.

This study shows that the polychaete benthic community that now

dominates the mesohaline Bay is made up of four species known to be extremely

adaptable to stressful conditions like hypoxia. The magnitude of these polychaetes

response to hypoxia is species specific. While some opportunistic polychaetes are

known to be extremely adaptable to stressful conditions like hypoxia, most of

them have limits to the degree they can withstand those conditions (Diaz &

Rosenberg 1995). Examples highlighted in this study are the hypoxia tolerances

of all four animals at deepest depths, particularly *S. benedicti* (Fig. 2.5). In

shallow depths of a mesohaline region, hypoxia may not always impact the

benthos regularly, however, evidence of seiching events where hypoxic water

from deeper areas can upwell on to the shallow flanks of an estuary or river, can

have an impact on the abundances of polychaetes. Those species with a greater

tolerance to longer or more frequent hypoxic events appear to be thriving more in these mid-depths of the mesohaline estuary. These animals are important to the Bay's ecosystem much in the same way their hypoxia sensitive counterparts are.

The two biggest roles polychaetes play in the estuarine ecosystems are as prey for secondary consumers and as facilitators of biogeochemical cycling of nutrients in sediments (Rhoads et al. 1978, Welsh 2003). All polychaetes help an ecosystem achieve a nutrient balance though their enhancement of nutrient recycling through their bioturbation and irrigation activities. The overlying water parameters regulate the densities of these opportunistic polychaetes in this mesohaline estuarine system varies with depth and season. The mid-depth zone is where the majority of the top polychaetes thrive between the 13 years studied (1981-1993). This study shows fluctuations in temperature and salinity can impact the spring recruitment of polychaetes that inhabit shallow and mid-depth zones of each mesohaline region of the Bay. At depths greater than 10 meters, analyses presented here suggest dissolved oxygen is the dominant parameter shaping the benthic community structure, particularly in the summer. While temperature and salinity ranges may shape their distribution at different depths of the estuary, the tolerance to low DO allows the dominant polychaete worms in the mesohaline Chesapeake Bay to continue their ecosystem function.

Table 2.1: Five study regions of Chesapeake Bay and its tributaries with environmental parameter ranges and number of years sampled in the database.

Region	Years Sampled	Depth Range(m)	% Silt-Clay	Temperature (°C)	Salinity	Dissolved Oxygen (mg L⁻¹)
1. Calvert Cliffs- Mesohaline Western Shore	1971-2008	< 5	7.43	0 - 31.0	0 - 24.0	0.100 - 18.2
	1971-1995	5-10	9.13	0 - 29.5	3.50 - 23.5	0.700 - 16.2
	1971-1995	> = 10	87.8	0 - 28.5	3.50 - 24.9	0.100 - 16.2
2. Morgantown Potomac River	1980-2008	< 5	14.2	0 - 29.9	0.070 - 17.1	0 - 18.8
	1981-2008	5-10	90.5	0.100 - 28.6	0 - 13.7	0.500 - 14.7
	1980-2008	> = 10	91.3	0 - 28.0	0.600 - 21.2	0 - 17.9
3. Lower Potomac River	1981-2008	< 5	6.89	0 - 28.2	0.900 - 19.0	1.70 - 18.0
	1989-2008	5-10	82.8	6.48 - 27.1	5.40 - 17.6	0.100 - 11.7
	1981-2008	> = 10	84.5	0.500 - 26.8	7.20 - 19.9	0.200 - 11.5
4. Patuxent River	1981-2008	< 5	38.9	0.200 - 28.9	0.900 - 17.3	0 - 16.5
	1984-2008	5-10	73.3	5.48 - 28.0	6.40 - 18.3	1.00 - 12.2
	1986-2008	> = 10	84.0	0.640 - 27.7	6.80 - 20.4	0 -13.1
5. Choptank River	1986-2008	< 5	16.2	3.40 - 28.4	3.20 - 17.7	3.10 - 13.2
	1984-2008	5-10	80.2	0.420 - 28.4	4.40 - 18.5	0 - 13.9
	1985-2006	> = 10	85.7	2.90 - 27.6	7.30 - 18.0	1.90 - 12.0

Table 2.2: Dominant polychaetes of the mesohaline Chesapeake Bay and tributaries with species characteristics based on published research

Family: Species	Adult Size Range (length; weight)	Lifespan	Feeding Mode	Burrow type	Reproductive mode	References
Spionidae						
Marenzelleria viridis	20-140 mm 0.319 - 1.606 mg AFDW	3 years	Surface deposit feeder	Network of L-shaped ventilated galleries	Iteroperous - Larval development over late winter/early spring months - extended pelagic stage	George 1966, Bochert & Bick 1995, Dauer 1997, Zettler et al. 1995, Zettler 1997
Streblospio benecidti	10 - 15 mm 0.018-0.082 mg AFDW	36-75 weeks Fall cohort females - 5-6 months	Facultative suspension or surface deposit feeder	Small tubes in top 2-3 cm of muddy sediment	Semelparous - Planktotrophic or lecithotrophic larval development	Levin 1980, 1981, 1984, 1986, Levin & Huggett 1990, Levin & Bridges 1994
Capitelidae						
Heteromastus filiformis	20-150 mm 0.389-1.962 mg AFDW	6-9 months but can live upto 2 years under stable conditions	Head-down deposit feeder	Permenant verticle single burrows 5-30 cm deep	Semelparous - Spring and fall recruitment	Shaffer 1983, Abele et al. 1998, Gillet & Gorman 2002, Quintana et al. 2007
Nerididae						
Alitta (Neanthes) succinea	20-190 mm 1.0 - 8.869 mg AFDW	1 year	Surface deposit feeder	U-shaped burrow network	Semelparous - Early spring recruitment	Fauchald 1979, Neuhoff 1979, Swan et al. 2007

Table 2.3: Spearman correlation coefficients between all geometric mean

abundances of polychaetes in the 1981 – 1993 time series of the Potomac –

Morgantown and Calvert Cliffs regions and in situ environmental parameters

Factor	M. viridis	S. benedicti	H. filiformis	A. succinea
Depth	**-0.5442**	**0.2037**	**-0.6895**	0.0303
% Silt/Clay	**-0.4228**	**0.3568**	**-0.5500**	0.1233
Temperature	-0.1211	**-0.1619**	0.0004	**-0.1513**
Salinity	**-0.6507**	-0.0730	**-0.5085**	**-0.1568**
Dissolved Oxygen	**0.2956**	0.0009	**0.2632**	**0.2117**

Table 2.4: CART-based factor relative importance to abundance (1984-1993) for each seasonal regression tree for each polychaete species. All trees were significant (p< 0.005).

Species	Factor	Spring Relative Importance	r²	Early Summer Relative Importance	r²	Late Summer Relative Importance	r²
M.viridis	Depth	25.78%		69.41%		63.26%	
	% Silt/Clay	20.35%	0.60	7.31%	0.68	0.53%	0.67
	Temperature	9.08%		2.88%		1.21%	
	Salinity	22.89%		9.81%		9.59%	
	DO	0.00%		0.00%		0.61%	
	Region	17.13%		9.38%		24.81%	
	Month	4.78%		1.21%		0.00%	
S. benedicti							
	Depth	21.00%		16.31%		12.48%	
	% Silt/Clay	42.52%	0.60	3.35%	0.29	3.31%	0.25
	Temperature	2.22%		16.47%		0.00%	
	Salinity	8.09%		28.85%		39.98%	
	DO	1.15%		7.41%		11.78%	
	Region	25.02%		3.00%		16.53%	
	Month	0.00%		24.61%		15.92%	
H. filiformis							
	Depth	48.99%		63.32%		75.95%	
	% Silt/Clay	13.69%	0.64	7.14%	0.61	0.58%	0.71
	Temperature	0.57%		0.00%		2.45%	
	Salinity	3.16%		1.70%		0.64%	
	DO	0.34%		0.06%		0.73%	
	Region	33.24%		27.78%		18.40%	
	Month	0.00%		0.00%		1.27%	
A. succinea							
	Depth	6.90%		23.55%		23.40%	
	% Silt/Clay	70.61%	0.31	19.21%	0.20	5.78%	0.29
	Temperature	1.03%		0.00%		5.46%	
	Salinity	5.89%		25.79%		27.00%	
	DO	7.27%		63.59%		32.09%	
	Region	8.29%		26.58%		6.27%	
	Month	0.00%		23.72%		0.00%	

Table 2.5: Multiple linear regression models of spring abundance for species in the Calvert Cliffs and the

Potomac-Morgantown Regions. N=13

	Calvert Cliffs				Potomac- Morgantown			
Factor	*M. viridis*	*S. benedicti*	*H. filimormis*	*A. succinea*	*M. viridis*	*S. benedicti*	*H. filiformis*	*A. succinea*
(Intercept)	34.53	3.51	-25.08	21.85	5.45	0.53	4.16	-51.14
BottomTemperature	-	0.52	3.20	-2.00	-	-	0.13	0.69
Salinity	-2.24	0.49	-	-1.31	-	0.47	0.13	5.39
DO	-	1.22	3.66	-0.49	-	-	-	3.71
NAO	-	-	-0.39	-	1.48	-	0.22	1.86
April River Flow	0.00	-	0.00	0.00	-	-	-	0.00
Winter Air Temperature	7.11	5.39	1.63	-	-0.64	-	-	-0.81
Winter Air Temp. * Salinity	-0.58	-	-	0.16	-	-	-	-
Winter Air Temp. * Bottom Temp.	-	-0.46	-0.12	-	-	-	-	-
Winter Air Temp. * NAO	-	-	-	-	0.40	-	-	-
DO * Bottom Temp.	-	-0.21	-0.36	-	-	-	-	-
DO * Salinity	-	-	-	-	-	-	-	-0.49
Fit Statistics								
Degrees of Freedom	8	6	5	7	9	11	9	5
r^2	0.84	0.89	0.95	0.83	0.76	0.52	0.52	0.96
p-Value	0.003	0.01	0.006	0.01	0.004	0.005	0.07	0.003

Formula Summaries

Calvert Cliffs

  M.viridis ~ 1 + River Flow + Salinity * Winter Air Temp.
  S. benedicti ~ 1 + Salinity + Bottom Temp. * DO + Bottom Temp. * Winter Air Temp.
  H. filiformis ~ 1 + NAO + River Flow+ DO * Bottom Temp. + Bottom Temp. * Winter Air Temp.
  A. succinea ~ 1 + DO + River Flow + Salinity * Bottom Temp.

Potomac- Morgantown

  M.viridis ~ 1 + NAO * Winter Air Temp.
  S. benedicti ~ 1 + Salinity
  H. filiformis ~ 1 + Salinity + NAO + Bottom Temp.
  A. succinea ~ 1 + NAO + Winter Air Temp. + River Flow + Bottom Temp. + Salinity * DO

Figure Captions

Fig. 2.1:   The mesohaline Chesapeake Bay showing the location of 5 study regions.

Fig. 2.2:   For each species bar plots show the maximum mean annual abundance at three different depths (black – shallow < 5m, gray – mid-depth 5-10 m, and white – deep > 10 m) in each region. Numbers above each bar indicate the month of maximum peak abundance for that region and depth.

Fig. 2.3:   Examples of monthly mean abundance (blues bars) and biomass (red squares and line) (1981-1993) for each polychaete studied at each depth range. The 12-grid matrix has species across the top and depth range along the side. Calvert Cliffs data was chosen (a – c) to represent patterns of *M. viridis*. The lower Potomac river estuary data (d-f) is representative of *S. benedicti*, Potomac-Morgantown data (g-h) shows *H. filiformis* seasonal patterns, and the Choptank river estuary data (j-l) illustrates *A. succinea* seasonality.

Fig. 2.4:   Linear regressions between shallow (< 5m) log abundance of a.) *M. viridis* and b.) *H. filiformis* and in situ salinity were both significant (p< 0.001). Regression equations and lines presented are for the Potomac river data (dark circles) that demonstrate a stronger relationship within region (r = 0.8, p<0.001). Open circles are data from the other four regions of the study. Linear regressions between shallow log abundance of c.) *M. viridis* and d.) *H. filiformis* and mean Susquehanna (or Potomac) April river flow are plotted. Regressions were significant for both species in the Calvert Cliffs region (p < 0.01).

Fig. 2.5:   Linear regression between log summertime abundances and in situ dissolved oxygen. Regressions (dot-dashed lines) are significant (p<0.01) across shallow (green diamonds), middle (light blue circles) and deep (dark blue squares) depth for a.) *M. viridis* $y = 0.73x - 0.61$, $r^2 = 0.54$, b.) *S. benedicti* no significant relationship, c) *H. filiformis* $y = 1.4x - 0.19$, $r^2 = 0.67$ and d) *A. succinea* $y = 0.34x + 1.5$, $r^2 = 0.38$. Linear regressions were significant under hypoxic conditions of deep stations for all polychaetes a.) *M. viridis* $y = 10.0x - 8.3$, $r^2 = 0.32$, $p < 0.05$ b) *S. benedicti* $y = 2.1x + 0.59$, $r^2 = 0.44$, $p < 0.001$ c) *H. filiformis* $y = 1.4x - 0.19$, $r^2 = 0.36$, $p < 0.02$ d) *A. succinea* $y = 1.3x + 0.03$, $r^2 = 0.57$, $p < 0.001$.

Fig. 2.6:   Example sections of CART analysis trees for abundance of a) Late

Summer *M. viridis*, b) Early Summer *S. benedicti*, c) Spring

H. filiformis, and d) Early Summer *A. succinea*. Within each node

box is the splitting parameter (x) and the number of samples (n)

that were split. Along each connecting line is the splitting

threshold for the node parameter. Terminal Node (TN) circles

contain the mean abundance (# indiv. m^{-2}) and the number of

samples (n) contained in that node.

Fig. 2.7:   Shallow (< 5 m) spring geometric mean abundance time series

(1981 – 1993) for a) *H. filiformis*, and b) *A. succinea* in the Calvert

Cliffs (solid line with solid circles) and Potomac-Morgantown

(dashed line with open circles). Significant (p<0.01) regression

lines are drawn for a) *H. filiformis* in the Potomac-Morgantown

region (y = 0.041x -80.0, r^2 = 0.54), and for b) *A. succinea* in both

the Potomac-Morgantown region (y = -0.088x+177, r^2 = 0.66) and

Calvert Cliffs (y = -0.081x + 162, r^2 = 0.55)

Fig. 2.8: Time series (1981 – 1993) annual Shallow (< 5 m) in situ mean spring a) bottom temperature and b) bottom salinity for Calvert Cliffs (solid line and squares) and Potomac – Morgantown (dashed line and open circles). Also plotted are the annual mean Hurrell PC-Based North Atlantic Oscillation Index (solid line and squares) and Patuxent River Naval Air Station mean winter air temperature (dashed line and open circles). Time series regressions of

a) bottom temperature for Calvert Cliffs ($y = 0.41x – 810$, $r^2 = 0.51$) and Potomac-Morgantown ($y = 0.48x -940$, $r^2 = 0.44$) are significant, $p < 0.01$. Time series regressions of b) bottom salinity for Calvert Cliffs ($y = -0.3x + 600$, $r^2 = 0.35$) was significant, $p < 0.05$. Time series regression of c) winter air temperature ($y = 0.39x – 780$, $r^2 = 0.54$) is also significant, $p < 0.001$.

Figure 2.1

1. Calvert Cliffs
2. Potomac- Morgantown
3. Lower Potomac River
4. Patuxent River
5. Choptank River

Chesapeake Bay
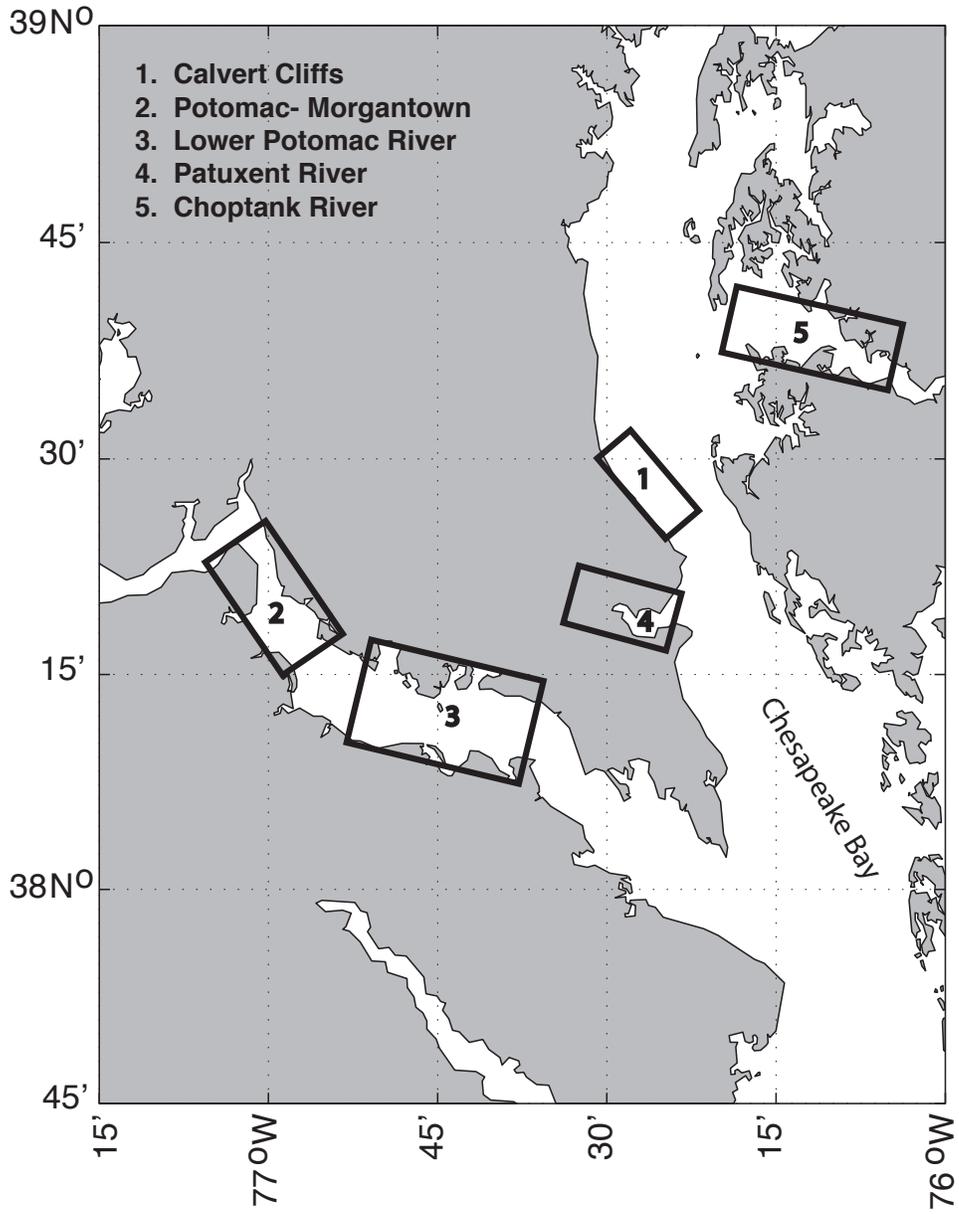
Figure 2.2

Figure 2.3

Figure 2.4

Shallow Abundance vs Salinity

M. viridis
y = -0.25x + 4.9, r² = 0.84

H. filiformis
y = -0.08x + 3.5, r² = 0.50

Shallow Abundance vs Mean April Flow

M. viridis
y = -0.0007x + 4.0, r² = 0.61

H. filiformis
y = -0.0003x + 2.85, r² = 0.4

Figure 2.5

Summary Abundance and Oxygen

a) *M. viridis*

Legend:
- < 5 m
- Btwn 5–10 m
- >=10 m

b) *S. benedicti*

c) *H. filiformis*

d) *A. succinea*

Abundance (log(indiv.+1) m⁻²)

Dissolved Oxygen (mg l⁻¹)
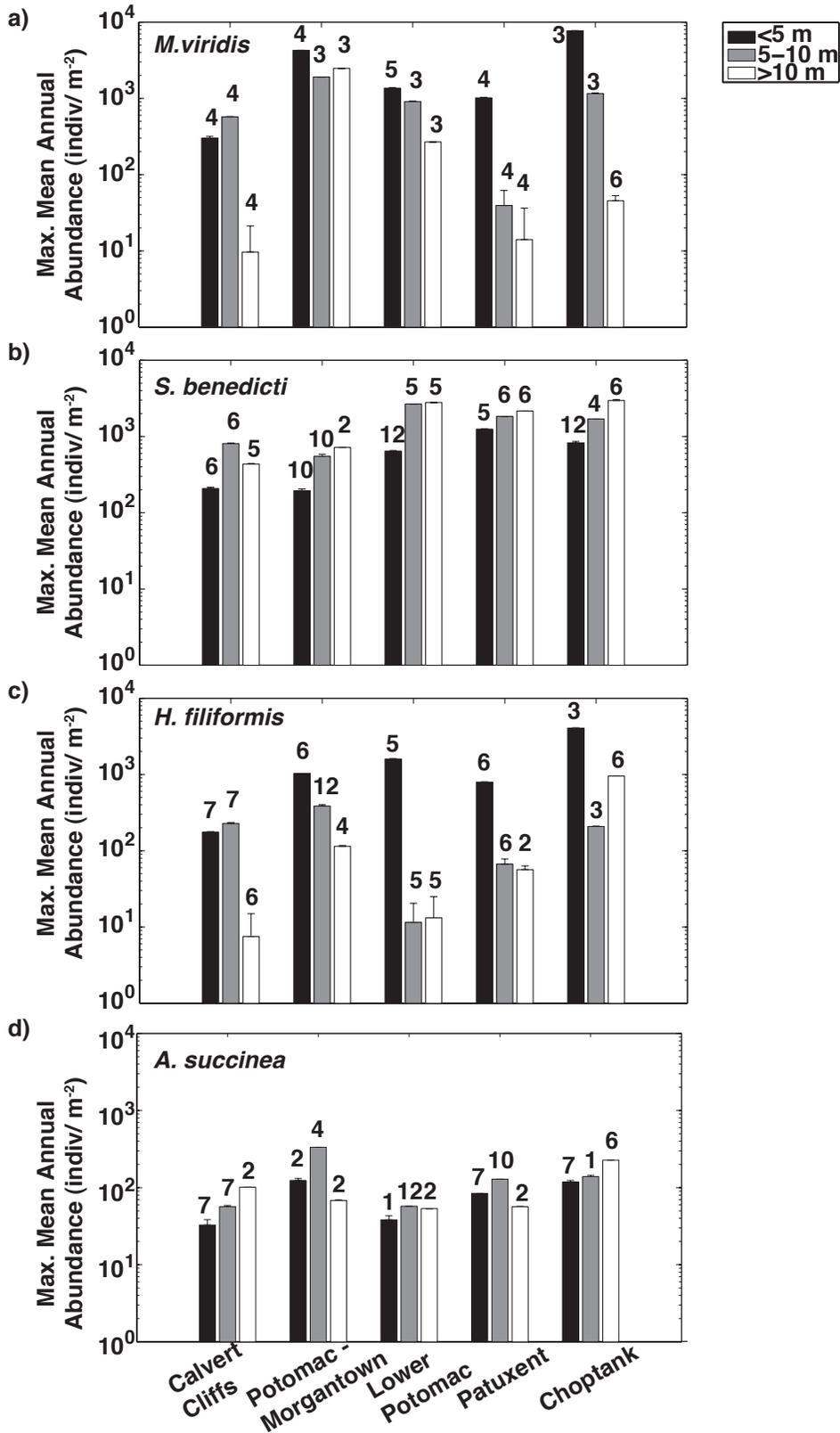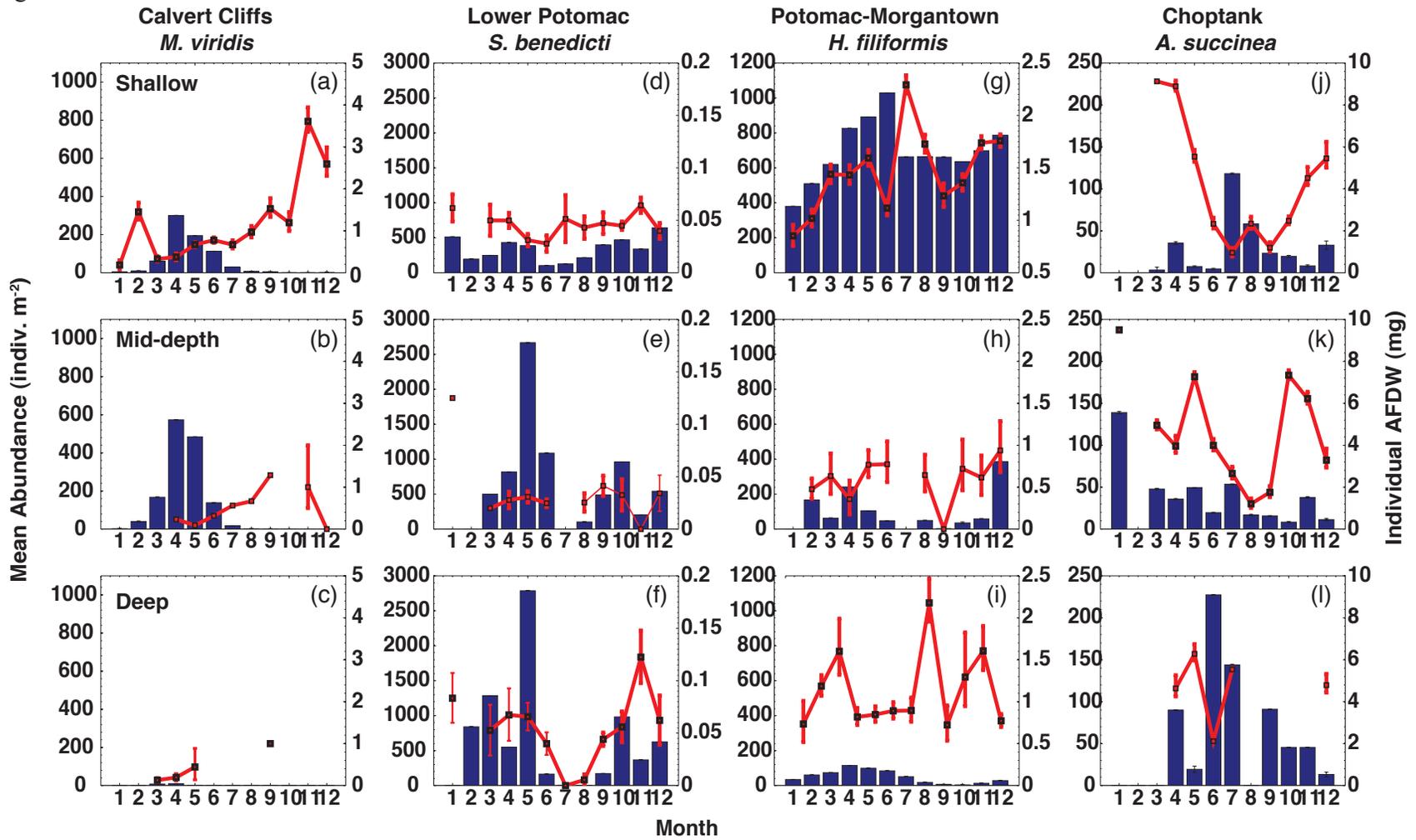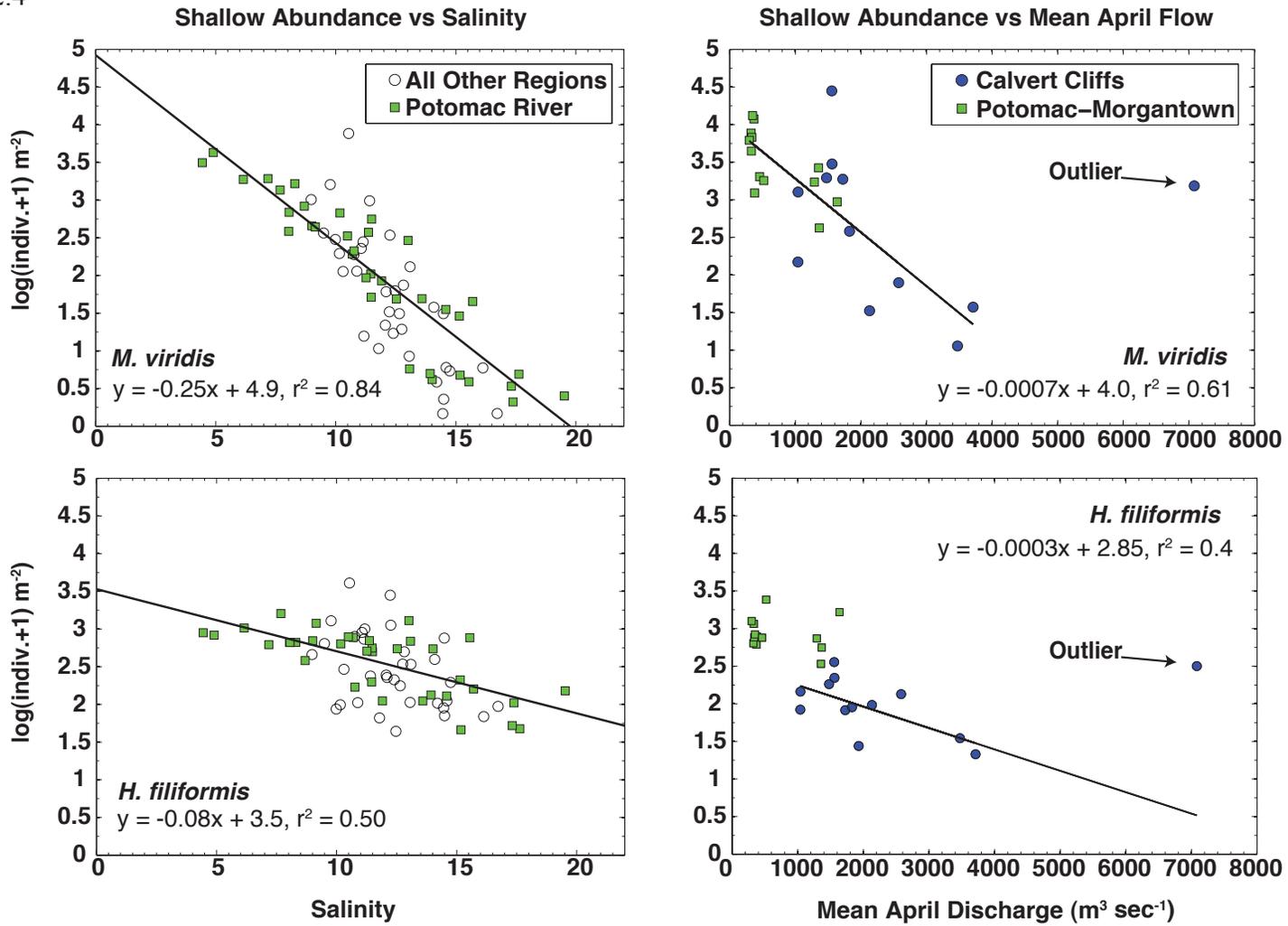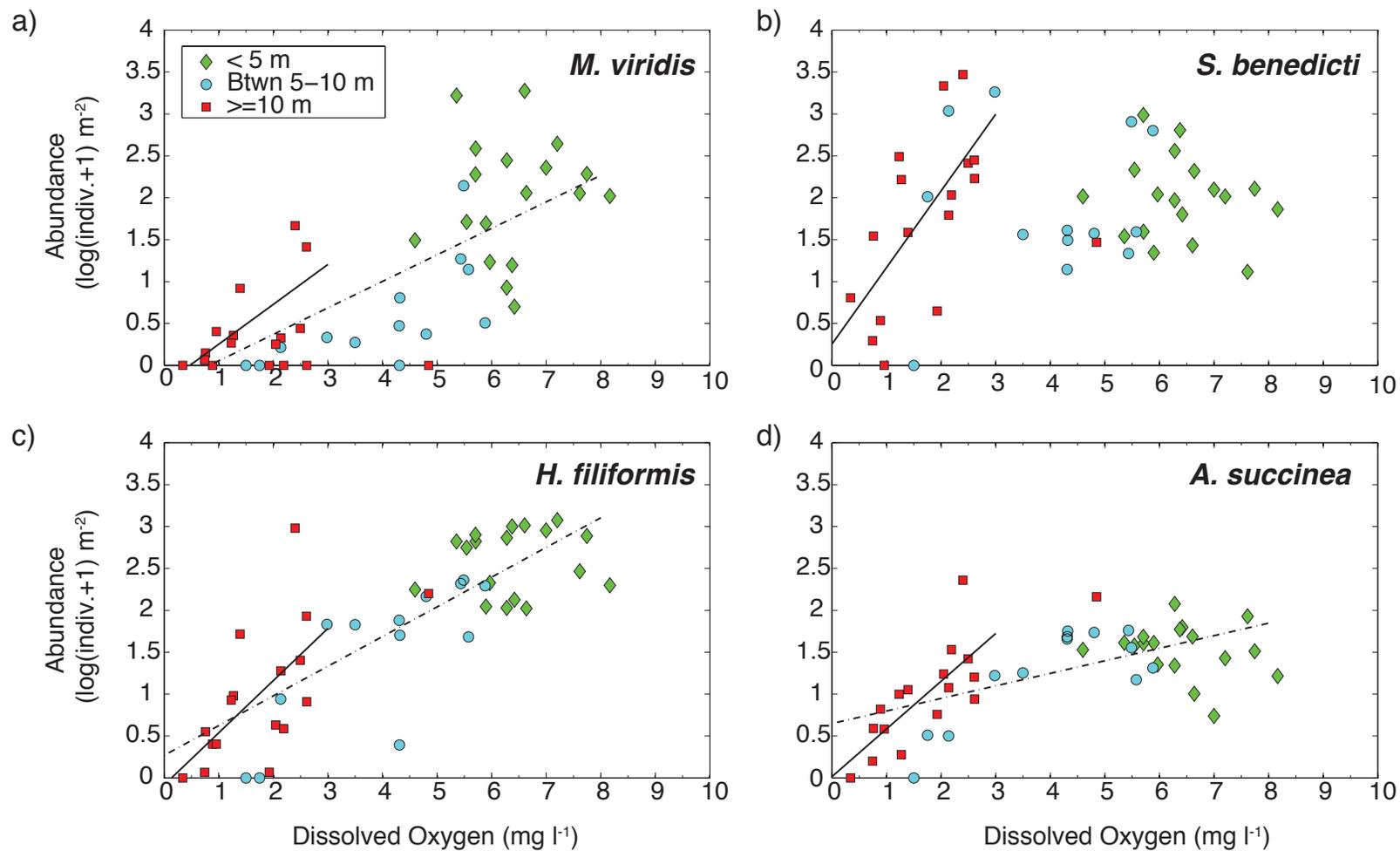
Figure 2.6

a) *M. viridis* Late Summer
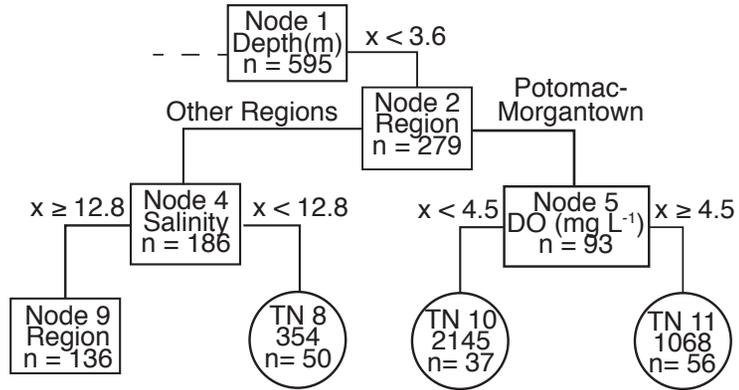
b) *S. benedicti* Early Summer
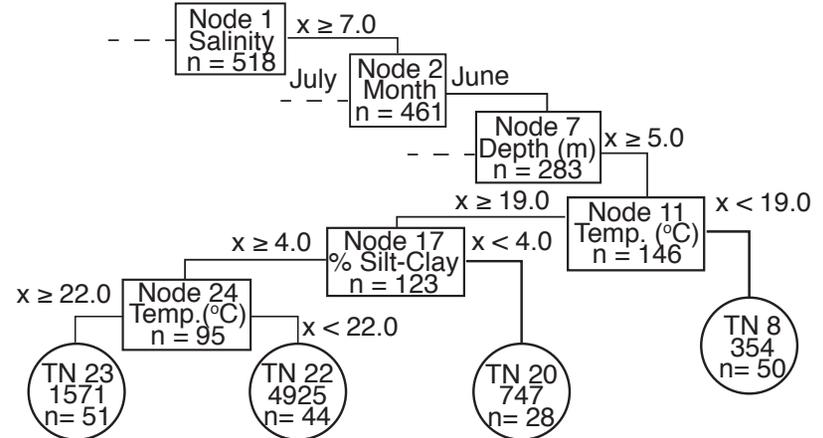
c) *H. filiformis* Spring
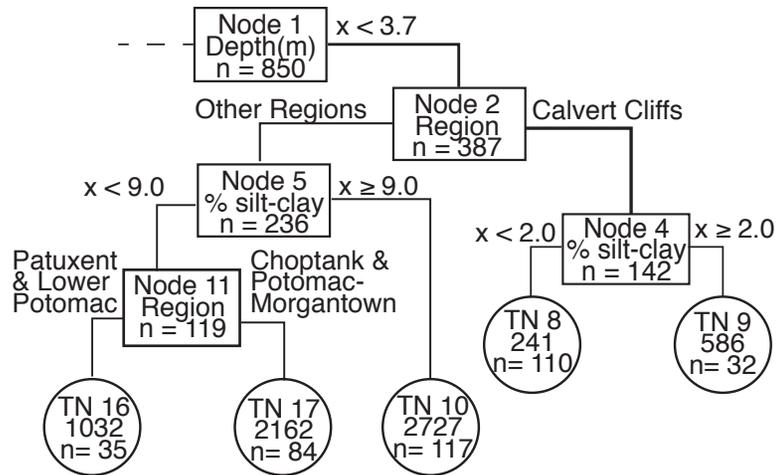
d) *A. succinea* Early Summer
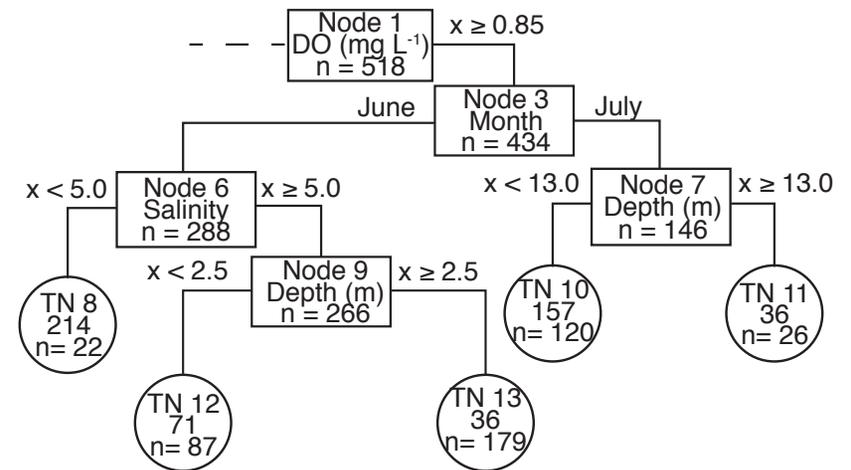
Figure 2.7

Shallow Spring Abundance
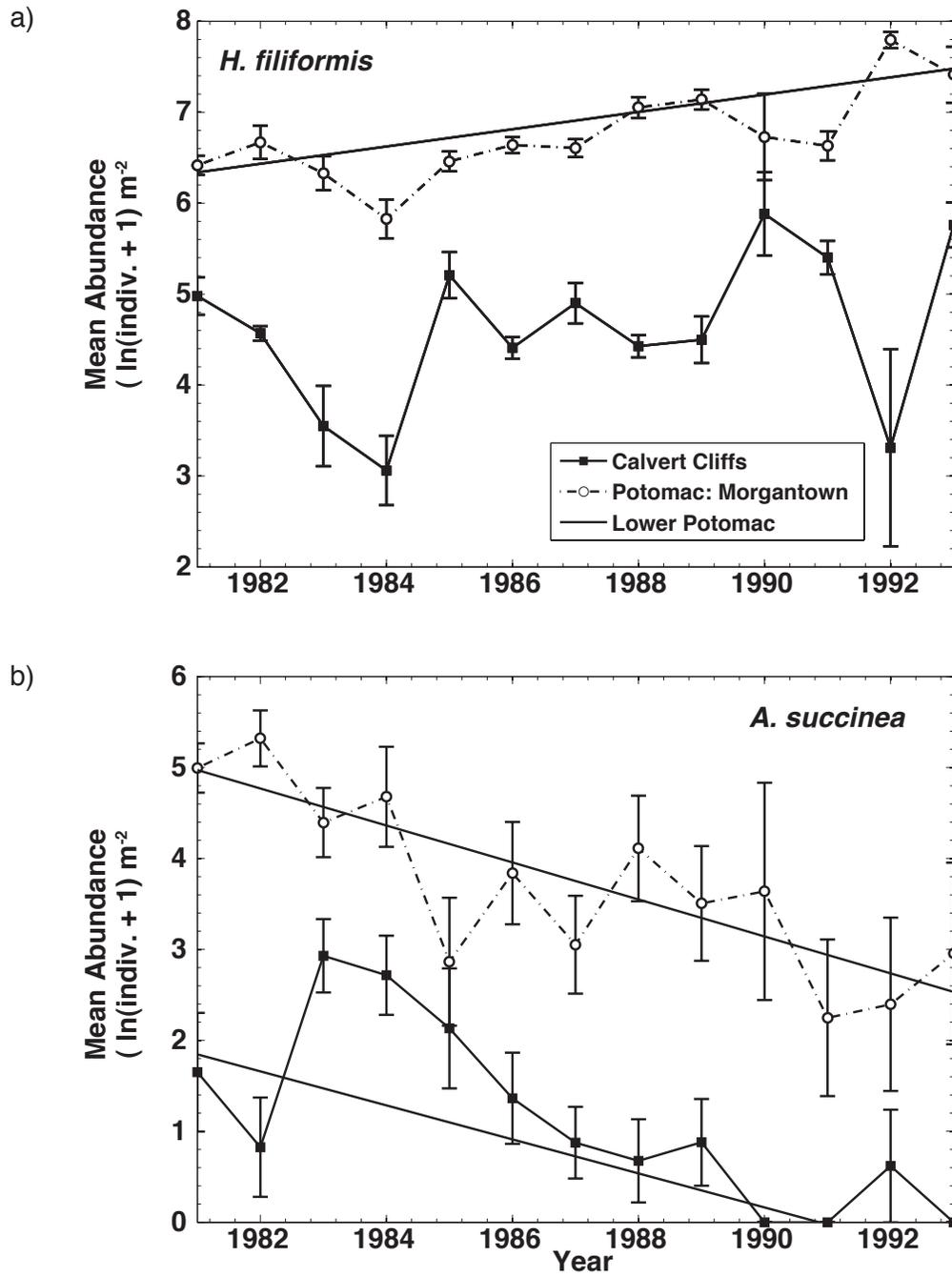
Figure 2.8
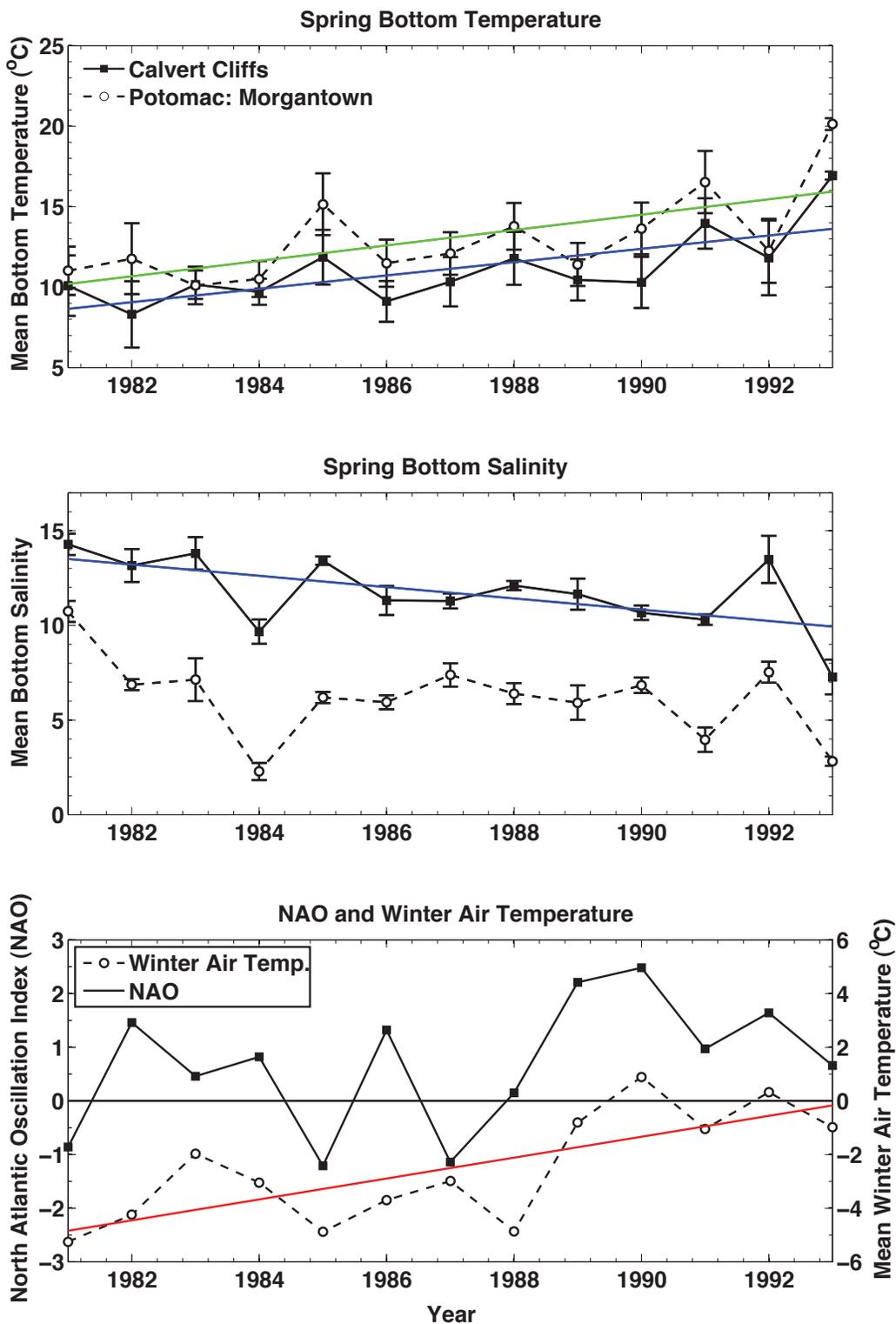
CHAPTER 3:

Short-term Effects of Nereid Polychaete Size and Density on Sediment Inorganic

Nitrogen Cycling under Varying Oxygen Conditions

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ABSTRACT

The biogenic activity of polychaetes can stimulate microbial ammonification, nitrification, and/or denitrification in estuarine sediments as well as increase the fluxes of inorganic nitrogen (NH_4^+, NO_2^-, NO_3^-, N_2) across the sediment-water interface. Chronic eutrophication and expanding seasonal hypoxia ($O_2 < 63\ \mu M$) in estuaries like Chesapeake Bay have altered benthic faunal communities in favor of opportunistic species. It has been suggested that the efficient decomposition of organic material is enhanced by the presence of polychaetes that can quickly populate organic-rich sediments following hypoxia events. Improved understanding of relationships among oxygen (O_2), polychaete density, and nitrogen cycling can help refine biogeochemical models of coastal ecosystems. Results of two laboratory experiments with the opportunistic polychaete *Alitta (Neanthes) succinea* are used to quantify the short-term influence of density and size of surface-feeding polychaetes on denitrification and sediment-water fluxes of inorganic nitrogen under varying oxygen conditions. This study shows that polychaete enhancements of O_2 and nitrogen fluxes were strongly correlated with total animal biomass. Fluxes of O_2, NH_4^+ and N_2 were stimulated by presence of animals for both larger and smaller worms, but per capita effects were greater for the deep-burrowing larger polychaetes. With the onset of hypoxic conditions, all density treatments had reductions in O_2, NH_4^+ and N_2 fluxes, with the high-density treatment showing the greatest change. Denitrification efficiency [$DE^* = N_2$ flux / ($N_2 + NH_4^+$ fluxes)] was 33% higher

for experiments with large worms than for smaller worm treatments, suggesting the former were more effective in removing fixed nitrogen.

INTRODUCTION

The complex biological, chemical, and physical processes in coastal sediments play an important, often seasonally varying, role in the functioning of healthy productive estuaries (Bianchi 2007). Early diagenesis of organic material in sediments, which involves a series of biochemical processes mediated by microbes, is influenced by the bioturbation activity of polychaete worms and other benthic macrofauna. This bioturbation can be particularly effective in the cycling of nitrogen (Welsh 2003). While the topic of benthic faunal bioturbation has been widely studied, many questions remain unresolved about how different animals directly and indirectly affect sediment biogeochemistry and sediment matrices (Meysman et al. 2006, Kristensen et al. 2012).

In many estuaries, these benthic communities are under stress from expanding and intensifying hypoxia (Diaz & Rosenberg 2008). In Chesapeake Bay, summertime hypoxia ($O_2 < 63$ μM) is a common occurrence in response to large inputs of algal bloom organic material that sinks to the bottom (Officer et al. 1984). Over the past four decades, increased seasonal hypoxia in estuaries such as Chesapeake Bay has altered the structure of the entire ecosystem (Kemp et al. 2005), shifting benthic macrofaunal communities to favor opportunistic species.

In many cases, these species are polychaete worms that are resistant to short-term oxygen (O_2) limitation (Diaz & Rosenberg 1995). Depending on feeding type (subsurface deposit-, surface deposit-, or suspension-feeder), polychaetes of different size and abundance have varying effects on organic matter diagenesis and associated nutrient and solute exchanges with the overlying water (Schaffner et al. 2001, Kristensen & Kostka 2005).

In areas experiencing short-term hypoxic events, the maintenance of opportunistic polychaete populations can mitigate some of the biogeochemical impacts of reduced oxygen, allowing an ecosystem to maintain healthy nutrient remineralization cycles (Gray et al. 2002, Welsh 2003). Observations show that some nereid polychaetes are able to switch from aerobic to anaerobic respiration during periods of O_2 limitation (Jorgensen & Kristensen 1980, Kristensen 1983a). Recolonization of sediments by these animals, after a defaunating hypoxic event, tends to alter sediment biogeochemistry in ways that differ from those associated with mature benthic systems (Nizzoli et al. 2007). Few studies have explored the transitional biogeochemical effects of sediment colonization by opportunistic polychaetes following large disturbances associated with hypoxia or other drastically altered conditions (Bartoli et al. 2000, Nizzoli et al. 2007).

While polychaete worms are among the most pervasive benthic macrofauna in estuarine systems, their role in sediment biogeochemical processes has been characterized for surprisingly few species (Kristensen & Kostka 2005). Much of that work has involved two nereid worms, *Nereis (Neanthes) virens* and *Nereis (Hediste) diversicolor*, (Diaz & Rosenberg 1995, Kristensen 2000,

Kristensen et al. 2012). Influences of these polychaetes on sediment biogeochemical processes tend to be variable due to differences in feeding and bioirrigation behaviors (Kristensen 1983b, a, Papaspyrou et al. 2006). *Alitta (Neanthes) succinea* is a versatile species common to North American estuarine systems, with facultative deposit- and suspension-feeding habits (Jorgensen & Kristensen 1980, Miron & Kristensen 1993). Indeed, *A. succinea* is an opportunistic worm that can both withstand temporary hypoxia and repopulate defaunated areas following major perturbations (Kristensen 1983a). Biogeochemical effects of *A. succinea's* bioturbation activities have, however, not been well described (Fauchald & Jumars 1979, Holland et al. 1987, Llanso et al. 2002).

This study expands on previous research by examining short-term effects of *A. succinea* abundance, biomass, and size on sediment-water fluxes of O_2 and nitrogen solutes under aerobic, transitional, and hypoxic conditions. Rates of O_2, NH_4^+, NO_3^- (plus NO_2^-), and N_2 fluxes were measured for triplicate experimental systems, and vertical profiles of porewater NH_4^+ and bromine-tracer concentrations were also measured to quantify responses to treatments. This study was designed to test the following two working hypotheses. (1) Polychaete enhancement of net inorganic nitrogen fluxes and sediment O_2 consumption are functions of total macrofaunal biomass, and these sediment-water fluxes will change as overlying water O_2 is decreased from aerobic to hypoxic levels. (2) Under aerobic conditions the polychaete enhancements of these sediment-water fluxes are greater for systems with larger worms.

METHODS

Sediment Sampling and Preparation

The Choptank River estuary is a tributary system of Chesapeake Bay whose watershed covers 1,756 km^2 (Fisher et al. 2006). All sediment samples were collected from a near shore cove located in the mesohaline region of the Choptank estuary (salinity 8-10) in 1-2 m of water with soft, muddy sediment (Porter et al. 2006).

For each incubation experiment, the sediment was defaunated using one of two different methods (see below) followed by a minimum of 2 weeks submerged in a chemostat-like equilibration system. This system consisted of a greenhouse tank equipped with an inflow of unfiltered Choptank estuary water. A standpipe in the outflow allowed for the tank water height to be maintained covering the defaunated cores. The tank was loosely covered with a Styrofoam board to maintain shade and prevent algal growth, and O$_2$ was maintained near saturation using aquarium bubblers.

In the first experiment, sediments were collected by hand coring with acrylic cylinders (6.5 cm diameter, 30.5 cm length). Sample cores were then defaunated by capping the cores with rubber stoppers and placing them in the dark for 12 hours to induce anoxia. After that time, the top 10 cm of sediments were removed from each core along with the macrofauna that had migrated toward the surface in response to low O$_2$ treatment (Porter et al. 2006). In the second experiment, sediments were sieved using a 500-μm sieve to remove infauna and larger material; the sediment slurry was then allowed to settle in 6

buckets (20 L). After 12 hours of settling, the sediments were equilibrated using the same flow-through system described above. These sediments were allowed to equilibrate for ~30 days before being sub-cored with the acrylic cylinders.

Experimental Design: Effects of Polychaete Abundance and Oxygen (A-O)

The A-O experiment measured effects of changing O_2 levels on sediment-water flux of nitrogen and O_2 under different abundances of polychaetes (2-6 cm resting length). Individual *A. succinea* polychaetes were collected from a Choptank oyster reef samples maintained in an oyster hatchery. All collected worms were immediately sorted into two size (resting length) categories [*Small* (1.0 - 4.9 cm) and *Large* (5.0 - 14.0 cm)] and placed in specimen dishes containing aerated estuarine water. Worms were added to sediment cores in triplicate for two polychaete abundance categories (Table 3.1) of *Low* abundance (5/core as 1 *Large* and 4 *Small*) and *High* abundance (11/core as 3 *Large* and 8 *Small* worms), where experimental *Low* and *High* abundance levels were equivalent to ~1500 and 3300 worms m^{-2}. These experimental abundances compare well with historical summer densities of polychaetes in the Choptank estuary, which range from ~500 to 4000 worms m^{-2} (Llanso et al. 2010) and to the worm densities used in previous published experiments (e.g. Bartoli et al 2000 and Swan et al 2007). Cores were then allowed to equilibrate overnight in cylindrical tanks filled with filtered (0.2 μm) estuarine water under continuous aeration and water circulation in a dark, temperature-controlled (25 $^{\circ}$C) chamber at ambient salinity (11.4).   After 12 hours of equilibration, all experimental cores,

including sediment-free water blank cores, were sealed without bubbles, with an o-ring fitted top with sampling valves. Cores were arranged around a central magnetic stirring motor that turned magnetic stirrers in each core at rates below the threshold of sediment resuspension.

The sediment incubations were carried out over 28 hours, to allow continuous O_2 depletion to hypoxic levels. Visual observations of worm behavior in each core were recorded and water samples were taken from each chamber in 2-hour intervals for the first 12 hours. Three final samples were collected at hours 20, 24, and 28. At each sampling point ~ 30 ml of water was collected by gravity flow through valves in the core tops. The replacement water that refilled each core at sampling was collected from Choptank estuary when cores were moved to the incubation chamber. Water samples were filtered using a 25 mm diameter, 0.45 μm cellulose acetate syringe filter (Nalgene #191-2045) into vials and frozen for analyses of ammonium (NH_4^+), and nitrate-plus-nitrite (heretofore referred to as nitrate or NO_x). Dissolved gas samples were collected in ~7 ml ground glass stoppered test tubes that were filled with a dip tube; samples were preserved with 10 μl of 50% saturated mercuric chloride ($HgCl_2$) solution. Dissolved gas samples were held under water at ambient bottom water temperatures until analysis.

The incubation was divided into three phases based on overlying water O_2 concentrations: (1) aerobic ($O_2 > 94$ μM), (2) transition (64 - 93 μM O_2), and (3) hypoxic ($O_2 < 63$ μM). The duration and onset of the transition phase varied between treatments depending on the number of polychaetes added to each core.

Overlying water in the *High* and *Low* abundance cores was hypoxic in approximately 8 and 18 hours, respectively. Solute fluxes were calculated for aerobic, transition, and hypoxic phases. Following experiments, all recovered worms per core were dried and weighed (Table 3.1).

Experimental Design: Effects of polychaete Abundance and Size (A-S)

Experimental *A-S* activities measured short-term (3- to 6-hour) effects of different sized (*Small* and *Large*) polychaetes on the sediment-water fluxes of O_2 and nitrogen at different worm densities. As described for the *A-O* experiment, *A. succinea* individuals were immediately sorted by size and placed in specimen dishes and then transferred to sediment cores in triplicate at densities of 4 and 8 worms per core (Table 3.1). Due to equipment availability *Large-* and *Small-*worm treatments were run in separate incubations (parts 1 and 2, respectively). During the incubations, visual observations of worm behavior were recorded and overlying water was sampled every 1.5 hours. Overlying water O_2 conditions remained aerobic for the duration of the incubations for all treatments except the *High*-abundance *Large*-worm treatments, which approached hypoxia after ~3 hours. For these latter cores, however, only flux rates during the aerobic portion of the incubation were used in our analysis.

To estimate how polychaete size influenced the relative contribution of direct worm excretion to total NH_4^+ efflux, we compared calculated rates for cores with *Large* and *Small* worms. Specifically, we contrasted rates (Table 3.2) for duplicate cores with one *Large* worm (mean biomass = 166 g wet m^{-2}) with

triplicate cores containing 8 *Small* worms (mean total biomass = 194 g wet m^{-2}).

These cores were incubated concurrently and experienced the same temperatures

and pre-incubation sediment treatments. We used an allometric relationship for

nereid worms (Table 3.2) to calculate excretion, $V = 0.27W^{0.62}$, where W is

individual wet weight (Kristensen 1984).

Sediment-Water Gas and Solute Flux Analysis

Ratios of gas concentrations (O_2:N_2 and N_2:Ar) were measured using a

membrane inlet mass spectrometer (MIMS) calibrated with an air saturated

standard (Kana et al. 1994). Nitrate was analyzed via segmented flow analysis

after cadmium (Cd) reduction and ammonium was manually analyzed with a

phenylhypochlorite colorimetric technique (Parsons 1984). Sediment flux rates of

solutes were calculated based on the change in solute concentration during the

incubations. Blank core incubations had only minor changes in solute

concentration over the incubation period. Corrections were made to account for

water column effects when significant linear changes in the blank were observed

for an analyte. We did not correct for replacement water additions because mean

differences with and without corrections were small (< 2 %). Statistical analyses

of these data were performed using SAS version 9.2 statistical software. One-way

or two-way mixed model ANOVAs with repeated measures and a Tukey-Kramer

multiple comparison tests were used to test for significant differences ($p < 0.05$)

in responses to treatments for both experiments.

Bromide Tracer Diffusion Model

Upon completing incubations in the *A-S* experiment, ~8 mM sodium bromide (NaBr) was added to the overlying water of experimental cores, which were continuously aerated and stirred at constant temperature. After 24 h, cores were sliced into vertical sections (1 cm), and sediments were placed into 50 ml centrifuge tubes. Porewater was centrifuged at 2000 G, filtered (0.2 μm), and frozen for subsequent Br and NH_4^+ analysis. Porewater Br concentrations were measured using a Dionex ICS 2000 ion chromatograph, and NH_4^+ was diluted (20:1) and measured as above.

A simple model was used to compute the effective diffusion of the bromide ion tracer in pore waters for each treatment. Changes in vertical distribution of Br concentration (*C*) were computed iteratively across the layers over 24-hours using Fick's First Law of diffusion: $J_s = -\varphi D_s (dC/dX)$, where J_s is the flux (mol cm^{-2} s^{-1}), φ is porosity, D_s is the molecular diffusion coefficient (cm^{-2} s^{-1}), and dC/dX the concentration gradient (mol Br cm^{-4}). Temperature and initial Br concentrations were set according to experimental conditions, and values for D_s in sediments were estimated (Yuan-Hui & Gregory 1974), with tortuosity (θ) calculated as $\theta^2 = 1 - \ln(\varphi^2)$, where ln is the natural log (Boudreau 1996). These model values were compared to measured Br values in experimental core sediments.

RESULTS

Sediment Fluxes of Oxygen and Nitrogen Species: Experiment A-O

Under the aerobic phase of the experiment, the addition of *A. succinea* significantly increased sediment O_2 demand (SOD), with the most significant increase in the *High*-density treatment (ANOVA, $p < 0.05$) (Fig. 3.1a). Aerobic NH_4^+ efflux in the *High*-density treatment was significantly greater ($p < 0.003$) than in the other treatments (Fig. 3.1b); however, fluxes for *Control* and *Low*-density treatments were not significantly different from each other ($p > 0.96$). Although effluxes of NO_x ($NO_3^- + NO_2^-$) were observed in the control and *Low*-density treatments, NO_x uptake occurred in *High*-density treatments (Fig. 3.1c). Denitrification rates under aerobic conditions, as measured with N_2 efflux (μmol N_2-N m^{-2} h^{-1}), were increased by 3-fold higher ($p < 0.05$) for *High*-density worm abundance compared controls and *Low*-density treatments (Fig. 3.1d).

As overlying water O_2 concentrations declined from aerobic to transitional to hypoxic conditions, solute fluxes generally decreased. For example, O_2 flux (SOD) in the *High*-density treatment declined by 40% ($p < 0.05$) during the "transition" phase and was reduced to $< 10\%$ of aerobic rates with hypoxia onset (Fig. 3.1a) and rates in the *Control* and *Low*-density treatments were only slightly lower through the transition but were reduced to $< 20\%$ of aerobic rates ($p < 0.05$). Although NH_4^+ fluxes were low for *Control* and *Low*-density treatments, they increase slightly as O_2 levels declined from aerobic to hypoxic conditions, and the increase in rates was significant for control systems (Fig. 3.1b). In contrast, NH_4^+ efflux rates for *High*-density treatments decreased

significantly by ~ 50% and 60% from aerobic to transitional to hypoxic

conditions. NO_x fluxes for *Controls* and *Low*-density treatments declined from

effluxes to influxes as O_2 conditions deteriorated from aerobic to hypoxic, while

small influxes remained unchanged across the O_2 gradient for *High*-density

treatments (Fig. 3.1c). Denitrification rates decreased significantly for control,

Low-density and *High*-density treatments as oxygen declined significantly (p <

0.05) from aerobic to hypoxic conditions (Fig. 3.1d). A decline in N_2 fluxes

followed the gradual shift from aerobic to transition to hypoxic conditions for

Control and *High*-density treatments, whereas the decline in flux rates was more

abrupt for the Low-density treatment when O_2 reached hypoxia.

To understand treatment effects on the balance between NH_4^+ efflux and

denitrification (N_2 efflux), a modified index of "denitrification efficiency" (*DE**)

was calculated for each density treatment at each experiment. Here we define

DE* as $100[(N_2$ efflux) / (N_2 efflux + NH_4^+ efflux)]. Because rates of direct

versus coupled denitrification or rates dissimilatory nitrate reduction to NH_4^+

(DNRA) were not measured, and because mean values for NO_x fluxes were

generally consistent (ranging between 30 and 55 μmol m^{-2} h^{-1}), we omitted NO_x

fluxes from our definition of *DE**. We also did not calculate *DE** values for

control systems that had negative effluxes of N_2 and/or NH_4^+. A comparison of

DE* for worm density treatments suggests that *DE** values were higher for *Low*-

density polychaete treatments (Table 3.3). In addition, there was a marked

decline in *DE** for *Low*-density treatments, as O_2 levels decreased from aerobic to

transition to hypoxic conditions. In contrast, the *High*-density treatments

maintained a modest *DE** level of ~50% across all O_2 levels.

Sediment Fluxes of Oxygen and Nitrogen Species: Experiment A-S

As in experiment *A-O*, the addition of *A. succinea* worms to sediment

cores in this *A-S* experiment resulted in significantly increased (p < 0.05)

sediment O_2 and NO_x influxes, as well as NH_4^+ and N_2 effluxes (Fig. 3.2, Table

3.3). Influx of O_2 in *Low-* and *High*-density treatments increased significantly by

> 2.5- and 4-fold, respectively, compared to the *Control* treatment. NH_4^+ flux

increased with polychaete density for Small worms and Large worms; however,

differences between *Low-* and *High*-density treatments were significant (p < 0.02)

only for *Large* worms (Fig. 3.2B). Mean rates of NO_x influx were not different

from *Controls* for *Small*-worm treatments, but rates for Large-worm treatments

were significantly different from *Controls* for both Low- and *High*-density worm

additions (Fig. 3.2C). Denitrification rates were significantly increased (p < 0.05)

with worm addition at both densities and worm sizes (Fig. 3.2D); however, N_2

fluxes were not different between *Low-* and *High*-density for either size worms.

For the treatment with *One-Large*-worm, O_2 and nitrogen fluxes were

significantly less (p < 0.05) than rates for the *High*-density *Large*-worm treatment

but were not different from *Controls* or *Low*-density treatments (Table 3.3).

Mean polychaete biomass levels for the *One-Large*-worm treatment were 194 g

wet m^{-2}, which are significantly different from mean biomasses in *Control* and

Large-worm treatments, but are not different from values for the *High*-density

Small-worm treatment (166 g wet m^{-2}). Mean NH_4^+ effluxes for *One-Large* worm (343 μmol m^{-2} h^{-1}) and *High*-density *Small*-worm (337 μmol m^{-2} h^{-1}) treatments were also not statistically different from each other. Thus, although mean values for polychaete biomass and NH_4^+ effluxes were 14% lower and 2% higher, respectively, *One-Large*-worm treatment values were not significantly different from those for the *High*-density *Small*-worm treatment (Table 3.2).

Logistic constraints caused the timing of *Small*- and *Large*-worm phases of the *A-S* experiment to be separated by 20 days; however, comparison of sediment-water fluxes in the respective *Control* treatment cores represents a measure of how different experimental conditions were between the two phases of this experiment. Mean fluxes of O_2 and N_2 were both directed into sediments for both *Small*- and *Large*-worm *Controls*, and while rates for the *Large*-worm *Controls* were 50% lower for O_2 and 20% higher for N_2 compared to *Small*-worm *Controls*, these differences were not significant. NH_4^+ and NO_x fluxes were also not significantly different for *Large*- and *Small*-worm *Controls*.

Major sediment N cycling processes measured or inferred by mass-balance calculations (Table 3.3, Fig. 3.6) compare mean N fluxes and transformation processes for *High*-density *Large*- (A) and *Small*-worm (B) treatments for mean *Control* (C) systems. For all processes, *Control* rates were very low, compared to those for *Large*- and *Small*-worm treatments, with measured denitrification rates increasing by ~ 10-fold from *Control* to *Small*-worm treatments and by ~ 4-fold from *Small* to *Large*-worm treatments. On the other hand, calculated rates of net nitrification increased by ~ 5-fold from *Control*

to *Small*-worm and again by ~ 5-fold from *Small*- to *Large*-worm treatments (Fig. 3.6).

Vertical Profiles of Pore Water Solute Concentration

The mean bromide porewater profiles for each *A-S* experiment treatment exhibited clear patterns for the *Control,* model, *Small*- and *Large*-polychaete treatments (Fig. 3). These profiles reflect differences in "effective depth" of solutes influenced by polychaetes of different size. The *Control* treatments closely followed the molecular diffusion model profile. Compared to *Controls*, *Small*-worm bromide profiles showed enhanced diffusion to a depth of ~ 4.5 cm. *Large*-worm bromide profiles, however, indicate enhanced diffusion to the bottom of the core (12 cm). In fact, at the end of the experiment, large worms were observed moving at the bottom edges of the core.

Porewater NH_4^+ profiles of *Control* treatments in *Small*- and *Large*-worm incubations showed increasing concentrations with depth (Fig. 3.4). For the *Small*-worm treatments, vertical profiles of NH_4^+ did not appear to be substantially different between Controls and treatments; however, the absence of replicate cores precludes testing for statistical significance among all treatments. In contrast, the vertical profiles of NH_4^+ in *Large*-worm treatments were sampled in triplicate, and these mean profiles were markedly different for *Controls* compared to worm treatments. Below 4.5 cm *Control* cores had significantly higher ($p = 0.008$) NH_4^+ concentrations than did *High*- or *Low*-density *Large*-

worm cores, while below 4.5 cm depth, the *High*-density cores had significantly higher (p = 0.03) concentrations compared to *Controls*.

DISCUSSION

Polychaete Effects on Sediment N-Cycling

Sediment-water fluxes of O_2, NH_4^+, and N_2 were consistently increased with the addition of the polychaete *A. succinea* in this study, and these responses can be attributed to several biological and chemical mechanisms. These polychaete worms alter nitrogen fluxes through many processes including: (1) direct NH_4^+ excretion, (2) stimulated microbial decomposition of organic material, (3) enhanced nitrification and coupling to denitrification by ventilating O_2 into deep anoxic sediments, and (4) liberation of porewater NH_4^+ from deep sediments to overlying water. It appears that the short-term responses to worm addition measured in this study may involve all but the second of these mechanisms (Bartoli et al (2000). Regardless of the mechanisms, our experiments reveal strong linear relationships of polychaete abundance and biomass to NH_4^+, O_2 and N_2 fluxes across the sediment-water interface (Figs. 3.1, 2, 5). These effects of polychaete activity are most likely attributable: to increases in surface area separating oxidized and reduced sediments; to increased pore-water circulation; and to solute transport through deep animal burrows (Aller 1994). NO_x fluxes across the sediment-water interface were relatively small but highly variable, presumably because these fluxes result from the net sum of diverse redox processes (Henriksen et al. 1983, Nizzoli et al. 2007) including

nitrification, assimilation, denitrification, anammox and DNRA. In most instances, however, the magnitude and direction of NO_x fluxes were directly related to overlying water column NO_x levels (Kristensen 1984).

The enhancement of nitrification and denitrification with the addition of *A. succinea* was illustrated in both the *A-O* and *A-S* experiments. N_2 effluxes were significantly stimulated with worm additions in all experiments, but particularly with addition of deep-burrowing large polychaetes (Figs. 3.1-3). Similar measured N_2 effluxes have been shown to be directly related to denitrification rates (e.g., Kana et al. 1994, An et al. 2001). Although the present study did not directly measure nitrification and its coupling to denitrification, mass-balance calculations suggest that the vast majority (~90%) of denitrification in these experiments was driven by coupling with sediment nitrification (Fig. 3.6). While it is unlikely that nitrifier abundance grew substantially during these short experiments (Pelegri & Blackburn 1995, Bartoli et al. 2000), it has been shown that dormant nitrifiers in marine sediments are rapidly revived and metabolizing when moved to new habitats with ideal growth conditions (Henriksen & Kemp 1988). Experimental polychaetes may also have inadvertently inoculated their burrows with active nitrifying microbes from surface sediment. In addition, it is unlikely that the relatively small N_2 influxes measured in dark incubations of *A-S* Control treatments were associated with nitrogen fixation (Gardner et al. 2006) and previous studies have suggested such N_2 influxes to be artifacts of O_2 in gas-stripping bubbles or to interference with mass-spectrometry (Kana & Weiss 2004, Eyre & Ferguson 2006).

Effects of Polychaete Biomass versus Size

When considering relationships between benthic macrofauna sediment-water fluxes of ecologically relevant solutes, animal effects are often measured in terms of biomass. Macrofaunal biomass can be controlled experimentally by changing either density of animals or their average size. In this study we manipulate both metrics (density and size) to investigate impacts of polychaetes on O_2 and nitrogen solute fluxes. Previous laboratory studies of nereid enhancement of solute fluxes reported results similar to ours, with relatively small densities and/or size of individuals in each treatment (Bartoli et al. 2000, Christensen et al. 2000, Swan et al. 2007). One of these studies (Bartoli et al. 2000) used *A. succinea* worms of a size similar to that used in our *Small*-worms treatment, and these authors found a remarkably similar linear relationship between animal biomass and denitrification (Fig. 3.5C). Our results indicated the relationship also holds at higher worm biomass, suggesting a broader relationship between N_2 flux and animal biomass (Fig. 3.5). The present study further revealed that sediment-water fluxes of O_2 and NH_4^+ were also significantly correlated to total *A. succinea* biomass (Fig. 3.5a, b), indicating that worm biomass is a general predictor of many sediment diagenetic processes. This result is consistent with previous reports for other nereid species (Henriksen 1980, Pelegri & Blackburn 1995, Kristensen 2000, Nizzoli et al. 2007).

Macrofaunal body size is known to influence both physiological and behavioral processes (Ahrens & Lopez 2001). By constructing deeper burrows, larger infauna ultimately increase advective and diffusive transport of key solutes

(Kristensen & Hansen 1999) and enhance rates and coupling of important redox reactions, including nitrification and denitrification. Deeper macrofaunal burrows effectively increase the area across which electron acceptors (e.g., O_2 and NO_3) and electron donors (e.g., NH_4^+ and S^{2-}) can readily diffuse between aerobic and anaerobic zones (Aller et al. 2001b, Francois et al. 2001). This increased scope of bioirrigation by larger polychaetes is illustrated in our *A-S* experiment where porewater profiles of Br and NH_4^+ suggest that larger *A. succinea* transported of these solutes from core sediment depths >12 cm, whereas impact of smaller worms appears to have been confined to the upper 4 cm of sediments (Figs. 3, 4).

The present study also explored the potential importance of macrofaunal excretion rates and how these differed between our larger and smaller experimental animals (Table 3.2). Allometric relationships reflect the widely observed phenomenon that biomass-specific NH_4^+ excretion tends to be higher for smaller animals (e.g., Kristensen 1984), and calculated rates were 0.9 and 0.4 μmol g^{-1} h^{-1}, respectively, for small and large worms (Table 3.2). Not surprisingly, larger individual worms had higher biomass and metabolic rates than did smaller ones, and thus per-capita excretion rates were > 4-fold higher for larger compared to smaller worms (Table 3.2). The proportion of total NH_4^+ effluxes comprised by excretion rates among our experimental treatments varied widely from ~ 20% to 70% (Table 3.2), which is considerably higher than the 10-40% range reported in previous work (Kristensen 1985, Boynton et al. 1997, Welsh 2003).

We further compared NH_4^+ effluxes for small polychaete (*High*-density *Small*-worm) and large polychaete (*One-Large*-worm) treatments with similar worm biomass values of 166 and 194 g WW m^{-2}, respectively (Table 3.2). As expected from Fig. 3.5B, rates of total NH_4^+ effluxes for these two treatments were virtually identical. Calculated rates (per m^2) for NH_4^+ excretion for these two treatments were, however, 2.4-fold higher for the *One-Large* worm treatment compared to the *Small*-worm treatment (Table 3.2). In addition, comparing our steady-state mass-balances for nitrogen fluxes in *High*-density treatments (Fig. 3.6) we see that, while NH_4^+ effluxes were 2.4-fold higher for large worms than for small worms, respective rates of net nitrification and denitrification were even higher with large worms (5- and 4-fold). Although ~ 90% of the total denitrification rates for both *Large*- and *Small*-worm treatments were supported by in situ sediment nitrification, *DE** values were 30% higher for cores with large compared to small polychaetes, with respective values of 58% and 44% (Fig. 3.6). We speculate that higher rates of bio-irrigation by larger worms may have led to relatively higher advective fluxes of NH_4^+ liberated from deep porewaters (e.g., Fig. 3.4). Thus, although the total biomass values and NH_4^+ efflux rates were comparable for experimental cores with one large worm and eight small worms, it appears that the relative importance of excretion, nitrification and bio-irrigation varies with worm size.

Effects of Oxygen Limitation

Effects of short-term, transient hypoxia in estuaries on benthic biogeochemical processes can be complex and difficult to predict (Weissberger et al. 2009). Depending on the timing and duration, hypoxic events may have a large impact on chemical equilibriums, microbial activity, and macrofaunal physiology; however, effects may not be severe enough to kill-off macrobenthic populations (Meyer-Reil & Koster 2000). As O_2 is depleted from the overlying water to below 94 μM O_2, worms migrated closer to the surface, maintain their irrigation rates, but reduce burrow construction and eating (Long & Seitz 2009). Once the overlying dissolved O_2 reaches 63 μM O_2 or lower, polychaetes stop constructing burrows, decrease their burrow ventilation and migrate closer to the sediment surface (Diaz & Rosenberg 1995). Experiment *A-O* data documents how these altered behaviors caused declines sediment-water fluxes (Fig. 3.1).

As O_2 declined in experiment *A-O*, each treatment underwent similar relative shifts in solute flux but at different rates. It is assumed that experimental cores did not reach steady-state in terms of solute flux over the brief duration of these experiments. The *Control* and *Low*-density treatments had relatively modest increases in NH_4^+ flux as dissolved O_2 decreased in each treatment core (Fig. 3.1B). NO_x flux for *Low*-density treatments also followed a pattern driven by concentration gradients, with an initial small efflux due to net nitrification under aerobic conditions, followed by a gradual decrease during transition, shifting to negative fluxes (driving denitrification) under hypoxia.

The *High*-density treatment, on the other hand, had a much greater relative NH_4^+ efflux compared to the measured NO_x influx, suggesting strong bioirrigation effects. The significant decrease in NH_4^+ efflux during transition from aerobic to hypoxic conditions within the *High*-density treatment cores implies marked reductions in worm metabolism, excretion and bioirrigation (Forbes & Lopez 1990). The notable difference in denitrification rates with respect to *High*- and *Low*-density treatments may also be attributed to a change in *Large*-worm behavior. Under hypoxia, nereid worms are far more likely to be found on the sediment surface compared to normal O_2 conditions (Diaz 2001). Visual observations during experiment *A-O* revealed that as worms moved closer to the surface, their burrow depths shoaled and irrigation frequency declined, thus decreasing their depth of influence on biogeochemical processes. During the transition period, when animals experience initial O_2^-limitation stress (Diaz & Rosenberg 1995, Gray & Elliott 2009), denitrification rates in experiment *A-O* remained elevated and significantly higher than in *Control* treatments (Fig. 3.1D). However, the inability of *A. succinea* to continue stimulating denitrification under transitional and hypoxic conditions represents a pivotal impact on sediment nitrogen cycling. It appears that the precipitous decline of O_2, NH_4^+, NO_x and N_2 fluxes across all treatments under hypoxic conditions resulted, in part, from a general down-shifting in polychaete metabolism as conditions degraded from aerobic to hypoxic (Gray & Elliott 2009).

Opportunistic Polychaetes and Ephemeral Hypoxia

Many studies have explored effects of infaunal bioturbation on organic matter diagenesis, and most of these studies structured experiments to allow infauna to equilibrate with their new surroundings prior to measurements (Henriksen et al. 1983, Kristensen 1984, Kristensen & Blackburn 1987, Christensen et al. 2000). This approach tends to mimic longer-term macrofaunal effects under more stable conditions. Although many coastal systems commonly experience short-term hypoxic events, surprisingly little research has addressed the biogeochemical effects of macrofauna under these transient conditions (Bartoli et al. 2000, Nizzoli et al. 2007, Bartoli et al. 2009).

As for many estuaries, the increase of hypoxia in Chesapeake Bay has been attributed as a prime driver in an overall shift in benthic community structure, leading to reduced species diversity and biomass (Holland & Diaz 1983, Dauer et al. 1992, Kemp et al. 2005). This Bay community is now dominated by hypoxia-tolerant opportunistic species, like *A. succinea*, with seasonal cycles characterized by peak abundances in the spring and small resurgences in the late summer and fall, (Llanso et al. 2010). Upon repopulating sediments in the spring, these small opportunistic worms can achieve high density due to limited competition for food resources (Gray et al. 2002). Their tolerance for short-term hypoxic events, however, makes these polychaetes important players in spring and early summer remineralization of organic material in this system.

As shown in previous work (Kristensen & Blackburn 1987, Pelegri & Blackburn 1995, Bartoli et al. 2000, Marinelli & Williams 2003, Welsh 2003, Braeckman et al. 2010), our study demonstrates that polychaete enhancement of net sediment-water fluxes is linked to total polychaete biomass. This study also supports the hypothesis that effects of macrofauna on these fluxes decline as overlying water O_2 levels decrease to hypoxic levels. Our experiments further explain effects of worm size on sediment biogeochemical processes. Compared to small polychaetes, larger animals burrow deeper in sediments, irrigate greater volumes of water and solutes through these burrows, but excrete less per biomass. These attributes of larger worms tend to enhance rates of sediment nitrification and denitrification, effectively helping to remove fixed-nitrogen from nutrient-rich environments. However, smaller opportunistic worms with less pronounced stimulation of denitrification often dominate eutrophic systems that regularly experience low-O_2 conditions. Thus, the hypoxia-induced shift from larger to smaller polychaetes, and the associated decline in nitrification and denitrification rates, represents a "positive feedback" process whereby high nutrient levels stimulate low O_2, which selects for smaller polychaetes that stimulate efficient nitrogen recycling, which in turn reinforces the eutrophication process (Kemp et al. 2005, Conley et al. 2007).

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Table 1: Experiment Core Setup

Treatment (No. worms added core^{-1})	Worm Density (indiv m^{-2})	Mean length (cm)	Mean Dry Mass (g m^{-2})	Incubation Temperature (°C)	Incubation Salinity
Experiment A-O (24-hours)					
Control (0)	-	-	-		
Low biomass (5)	1515	2.7	1.96	25.0	11.4
High biomass (11)	3333	3.0	11.63		
Experiment A-S (6-hours)					
Control (0)	-	-	-		
Large worm only (1)	303	10.5	22.1 -L	15.0 -S, 19.8 -L	9.9 -S, 9.0 -L
Low biomass (4)	1212	3.2	15.32 -S, 158.32 -L		
High biomass (8)	2424	8.5	26.70 -S, 171.70 -L		

Table 3.1- Worm-addition study design details for both *A-S* and *A-O* experiments.

Mean total dry weights per core measured post experiment (Small-worms = S,

Large-worms = L). Experiment *A-O* polychaetes experienced anoxia after 6-15

hours of the 24-hour incubation. Therefore, biomass may be reduced due to

change in feeding behavior under low oxygen conditions. (Forbes & Lopez 1990).

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Table 2: Calculated rates of NH_4^+ excretion for Small and Large-worm High- and Low-density Treatments

Mean Parameter	Small worms		Large worms		
	Low	High	Low	High	One
Worm Biomass (g WW m^{-2}) [a]	95	194	986	2344	166
Total NH_4^+ Flux (μmol NH_4^+ m^{-2} h^{-1}) [b]	263	337	665	738	343
NH_4^+ Excretion Rate					
(μmol NH_4^+ worm^{-1} h^{-1}) [c]	0.07	0.07	0.31	0.34	0.24
(μmol NH_4^{+1} core^{-1} h^{-1}) [d]	0.29	0.58	1.23	1.72	0.24
(μmol NH_4^+ m^{-2} h^{-1}) [e]	86	175	374	520	73
(μmol NH_4^+ g^{-1} h^{-1}) [f]	0.91	0.90	0.38	0.35	0.43
% of Total Flux [g]	33%	52%	56%	71%	21%

Table 3.2 – Polychaete excretion contribution to ammonium flux at different size and biomasses. a.) mean biomass of triplicate cores. b.) mean total ammonium flux Excretion rates (V) calculated based on $V = 0.27W^{0.62}$ where W = biomass (g wet weight) Kristensen (1984). c.) Excretion rate based on the mean weight of the worms recovered from core after incubation. All worms add to incubations were recovered except in the *Large*-worm *high*-density treatment where the average number of worms recovered was 5 instead of 8. d.) Excretion rate per worm multiplied by the number of worms recovered from each core. e.) Excretion rate per m^2 for each treatment. f.) Excretion rate per gram wet weight of worm. g.) Excretion rate base as a percentage of the total ammonium flux rate for each treatment.

Table 3: Calculated Experiment Mean Solute Fluxes and Denitrication Efficiency*

Experiment	Treatment	Worm Density	NH_4 Flux	NO_x Flux	N_2-N Flux	O_2 Flux	Denitrification Efficiency*
A-O	Oxic	Control	-42	61	62	564	-
		Low (5 core^{-1})	9	31	72	924	89%
		High (11 core^{-1})	225	-47	243	2399	52%
	Transition	Control	-9	-2	34	467	-
		Low (5 core^{-1})	19	-30	75	845	80%
		High (11 core^{-1})	127	-51	142	1402	53%
	Hypoxic	Control	8	-34	7	52	45%
		Low (5 core^{-1})	25	-48	27	174	51%
		High (11 core^{-1})	46	-54	43	199	48%
A-S	Small Worms	Control	142	-16	-	521	-
		Low (4 core^{-1})	188	-27	229	1363	55%
		High (8 core^{-1})	302	-29	234	2196	44%
	Large Worms	Control	-41	12	-	171	-
		One worm	343	-13	269	704	44%
		Low (4 core^{-1})	665	-56	695	2316	51%
		High (8 core^{-1})	738	-97	1030	7323	58%

Table 3.3 – Calculated solute fluxes, where all values represent mean of each treatment and are expressed in μmol m^{-2} h^{-1}. Denitrification efficiency (*DE**) was calculated as % N_2-N of Total NH_4^+ plus N_2-N effluxes. Negative N_2-N and NH_4^+ effluxes for "Control" systems were not included in mass-balance and *DE** calculations.

Figure Captions

Fig. 3.1:   Sediment-water solute fluxes measured over 24-h core incubations

in *A-O Experiment* (mean ± SE, n=3) for: a) O_2, b) NH_4^+,

d) NO_x, and d) N_2-N. Three primary treatments include *Control*

(no worms), *Low*-density (5 worms), and *High*-density

(11 worms). At each density results of three O_2 treatments are also

shown: *Aerobic* (> 94 µM O_2) as open bars, *Transition* (64 – 93

µM O_2) as gray bars, and *Hypoxic* (< 63 µM O_2) as black bars.

Fig. 3.2:   Sediment-water solute fluxes measured over 6-hour core

incubation in *A-S Experiment* (mean ± SE, n=3) for: a) O_2,

c) NH_4^+, c) NO_x, and d) N_2-N. Treatments include a sediment

Control (no worms), *Low*-density (4 worms), and *High*-density (8

worms). At each density, results of two categories of worm size

are also shown: *Small*-worm treatments (1 - 4.9 cm) as gray bars,

and *Large*-worms (5-14 cm) as black bars.

Fig. 3.3:   Bromide distribution (mean ± SE, n=3) for *A-S* Experiment *Large*-

worm (circles), *Small*-worm (triangles), and *Control* (squares)

treatments. Dotted line represents a diffusion model based on

Fick's First Law adjusted for the background bromide

concentration.

Fig. 3.4:   Porewater NH_4^+ concentration (mean ± 1 SE, n=3) for *A-S Experiment*: a) *Large-worm* treatments and b) *Small*-worm treatments. In both panels *High*-abundance (diamonds), *Low*-abundance (squares), and *Control* (circles) treatments are presented. Standard errors were computed for all depths, but for clarity, bars are plotted here only for *Large*-worm *Control* and *Low*-abundance treatments.

Fig. 3.5:   Relationship between *A. succinea* biomass and sediment-water fluxes of a) O_2, b) NH_4^+, and c) N_2-N. Data points represent the biomasses and rates determined for the individual experimental units (cores with sediments and overlying water). Positive fluxes are out of the sediment and negative fluxes are in to the sediment. Triangles are data from *Experiment A-O*. Open symbols are for fluxes under *Aerobic* conditions, gray symbols are under *Transitional* conditions, and black symbols are *Hypoxic* conditions. The remainder symbols are data from the aerobic *A-S Experiment*, with squares representing fluxes for *Small*-worm treatments and diamonds for *Large*-worm treatments. Solid lines are for linear regressions (equations given) on date from this study, and dotted line (Panel c) is for a previous published equation (Bartoli et al. 2000).

Fig. 3.6:   Summary diagram with mean values of measured sediment-water

fluxes, calculated polychaete NH_4^+ excretion fluxes (Table 3.2)

and mean calculated N transformation processes for net

ammonification, net nitrification and denitrification. Here we

assume steady-state mass-balance for arrows going to and from

boxes (representing three porewater N pools), and we also assume

that rates of dissimilatory nitrate reduction to ammonia (DNRA)

and anammox are negligible. Diagram compares mean fluxes and

processes for a) *High*-density *Large*-worm treatments, b) *High*-

density *Small*-worm treatments, and c) *Control* treatments for *A-S*

Experiment.

Figure 3.1
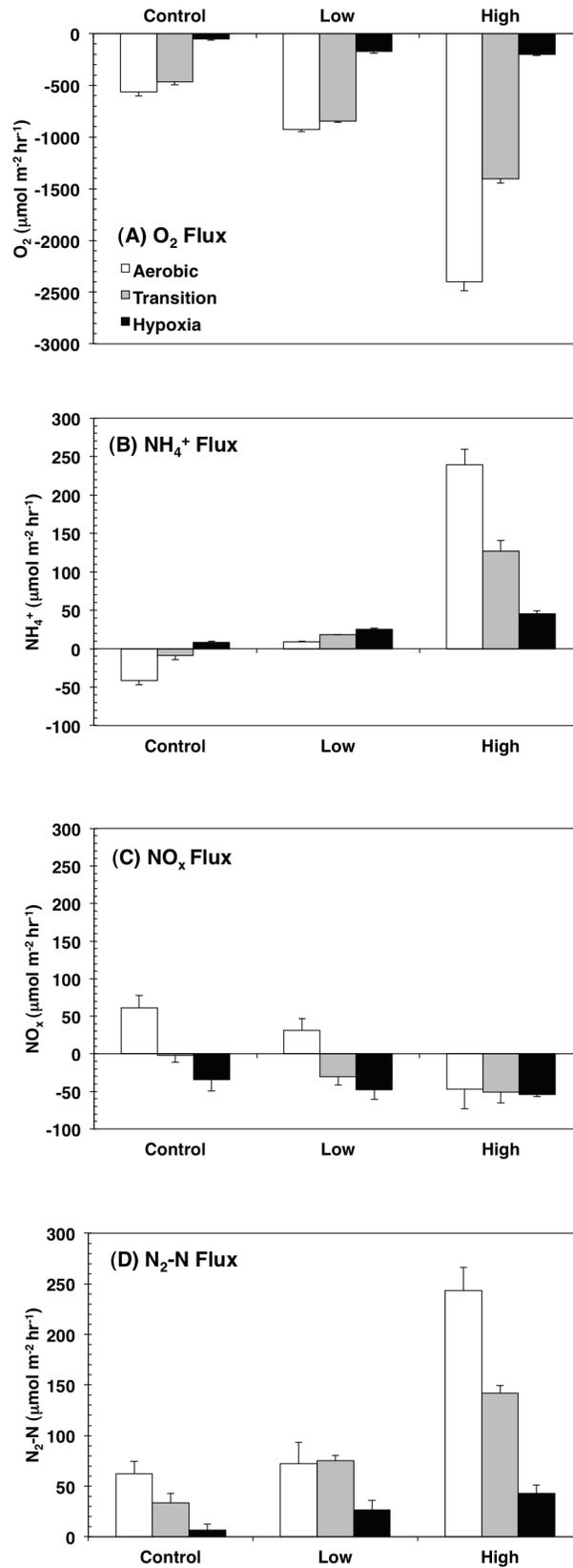
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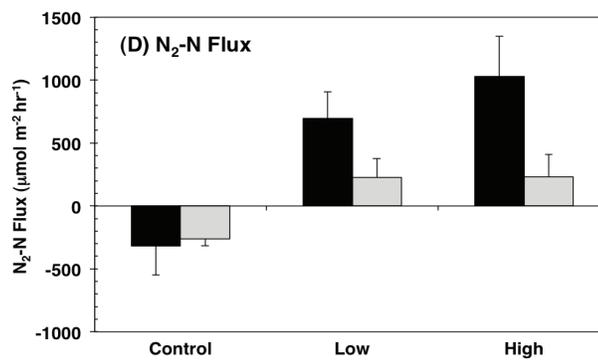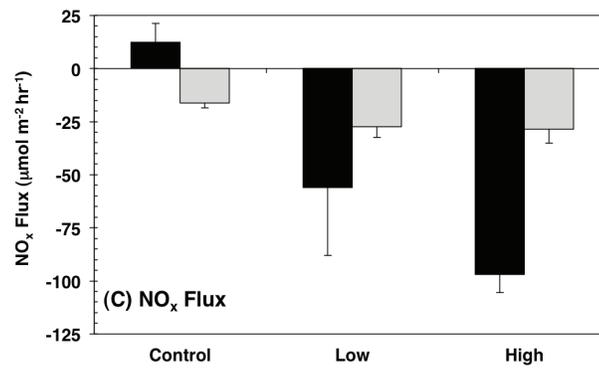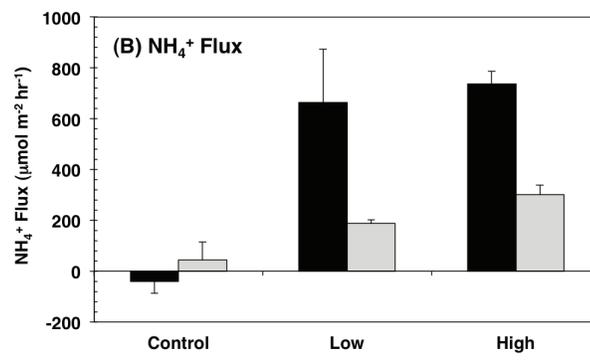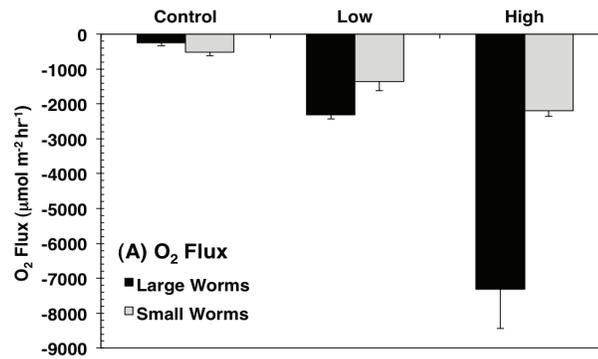
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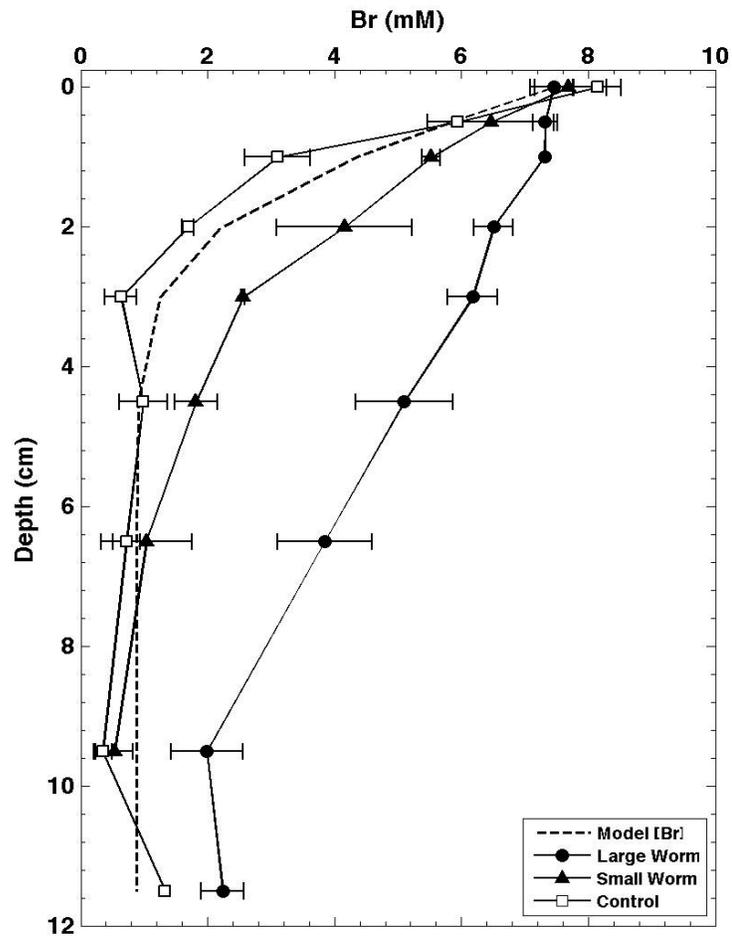
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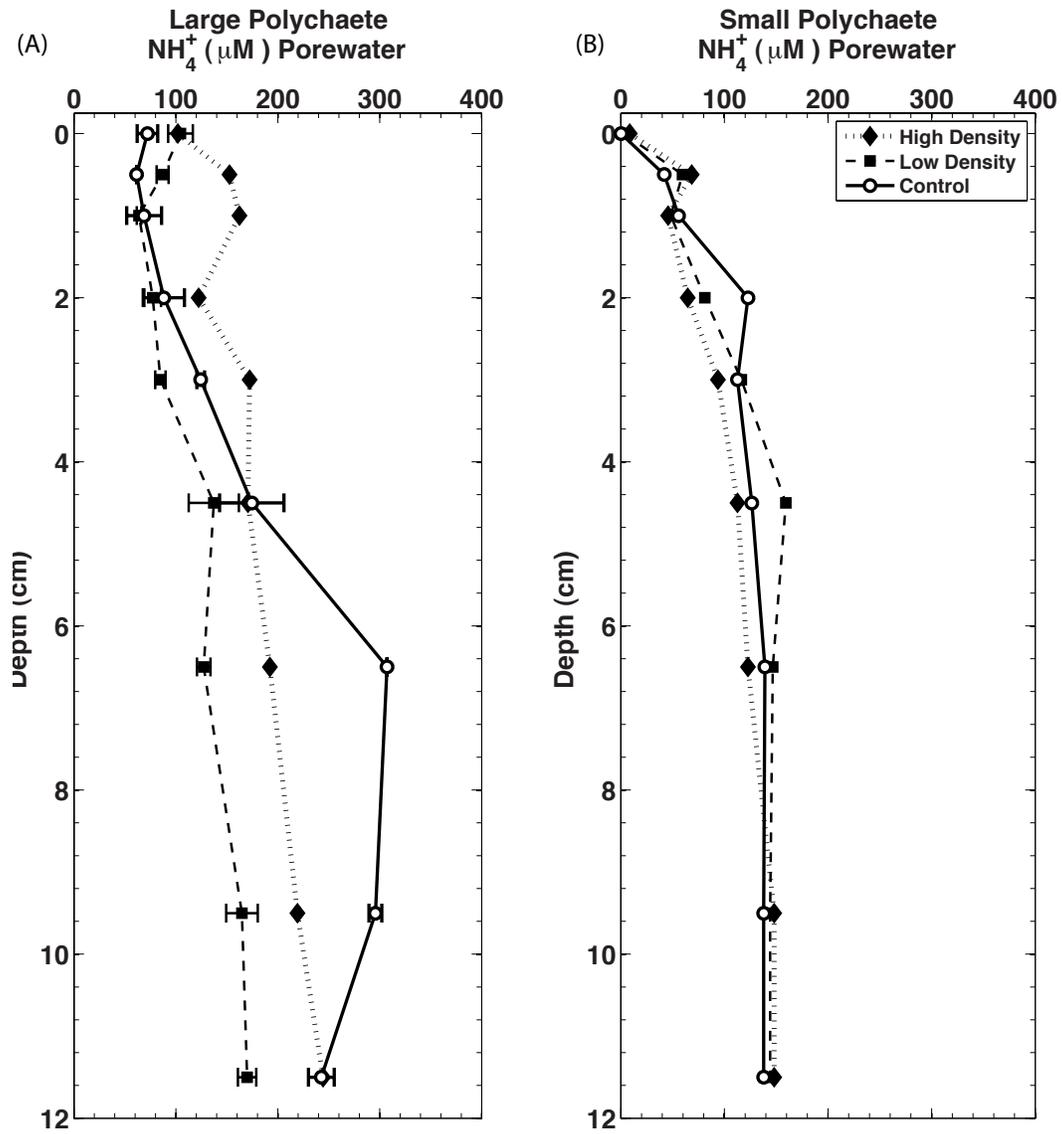
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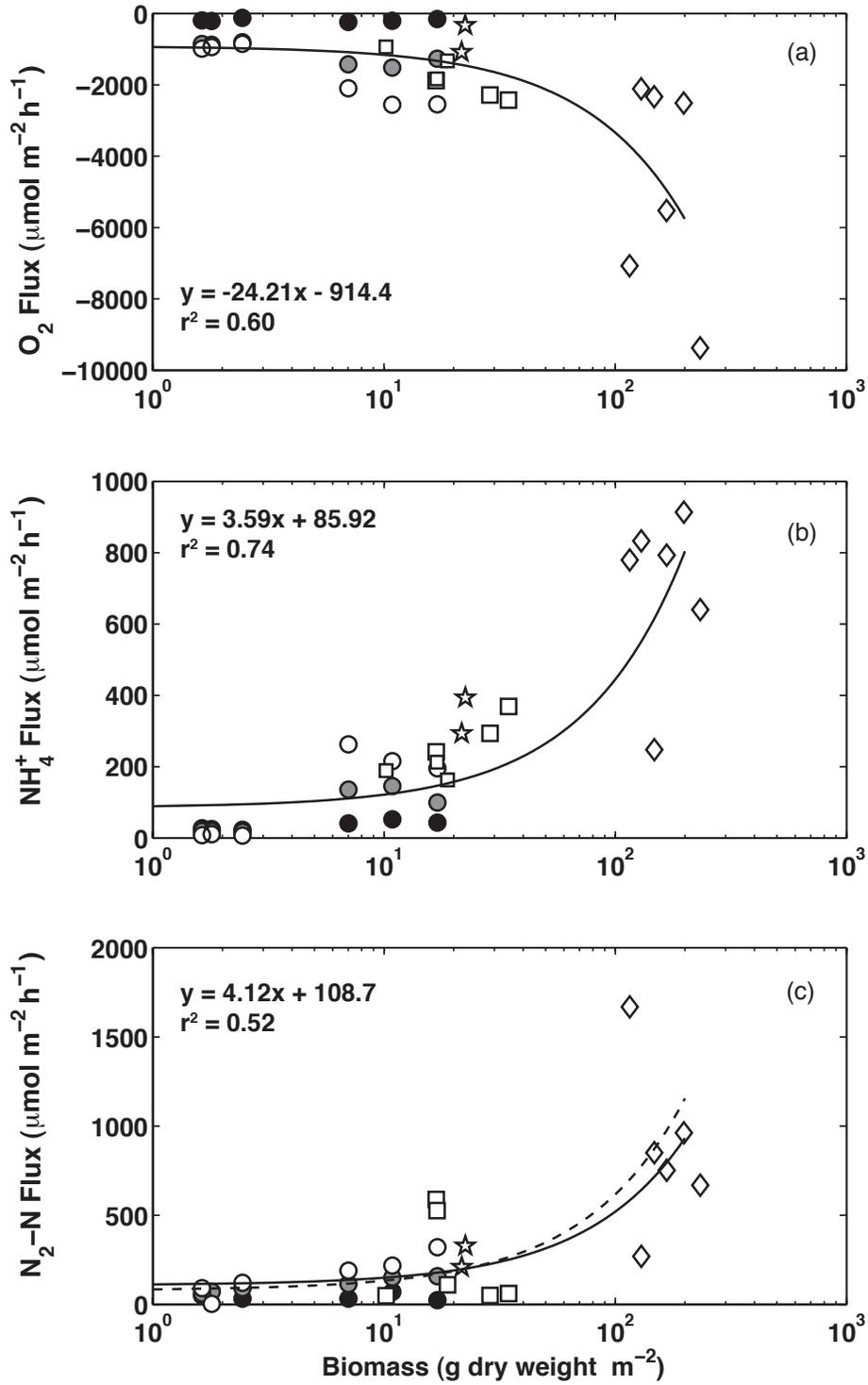
Figure 3.6

a) High-density Large-Worm Addition

	NH$_4^+$		NO$_x$		N$_2$-N
	738		97		1030

Sediment Surface

Net Ammonification 1226

PON → NH$_4^+$

445 Polychaete Excretion

Net Nitrification 933 → NO$_x$

Denitrification 1030 → N$_2$-N
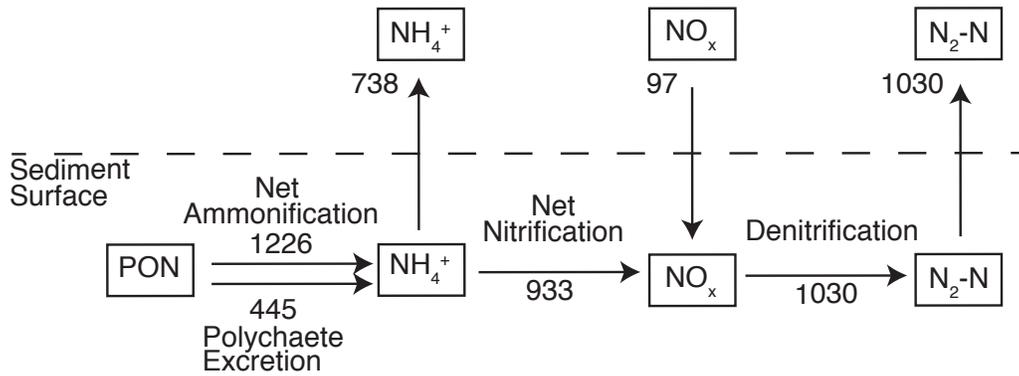
b) High-density Small Worm Addition

	NH$_4^+$		NO$_x$		N$_2$-N
	302		29		234

Sediment Surface

Net Ammonification 381

PON → NH$_4^+$

126 Polychaete Excretion

Net Nitrification 205 → NO$_x$

Denitrification 234 → N$_2$-N

c) Control Average

	NH$_4^+$		NO$_x$		N$_2$-N
	20		19		21

Sediment Surface

Net Ammonification 20

PON → NH$_4^+$

0 Polychaete Excretion

Net Nitrification 40 → NO$_x$

Denitrification 21 → N$_2$-N
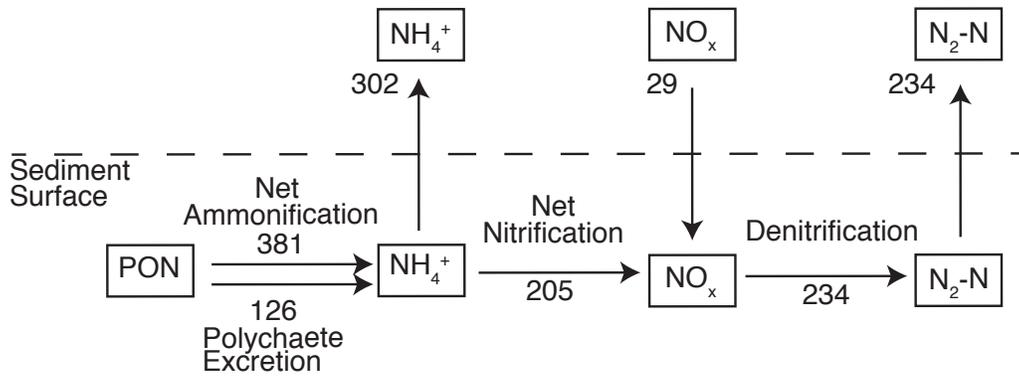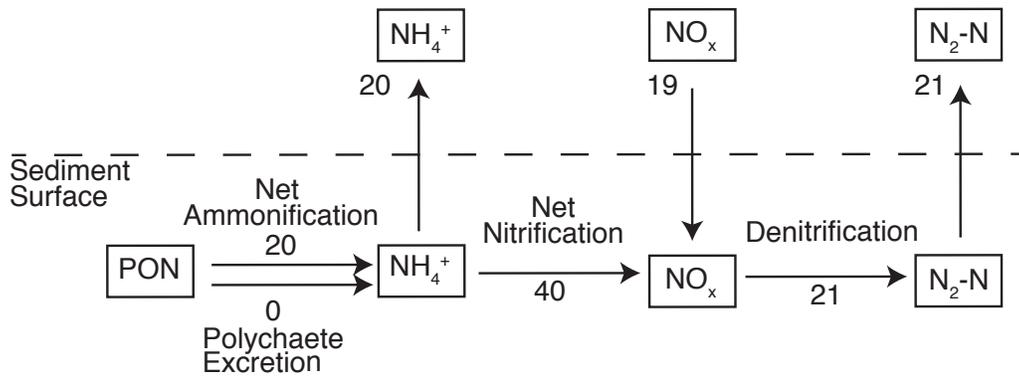
CHAPTER 4:

Macrofaunal Biomass Effects On Nitrogen Recycling

In A Eutrophic Estuarine System

ABSTRACT

It is widely understood that benthic macrofauna affect sediment biogeochemistry, but the relationship between macrofaunal biomass and ammonium (NH_4^+) efflux from estuarine sediments is challenging to generalize. This study examines a unique large-scale monitoring dataset collected in the Chesapeake Bay to assess the relationship between benthic biomass and NH_4^+ efflux within different regions of the estuary by season. Biomass data was separated into different classes of benthic macrofauna (polychaetes, bivalves, and amphipods) to isolate impact on nitrogen recycling across the different regions of the Bay. Factors controlling NH_4^+ efflux were tested using three different methods (Classification and Regression Tree (CART), multiple linear regression (MLR) and ANOVA). Community structure influence on NH_4^+ efflux was evaluated by regressing total species richness (number of unique species) with NH_4^+ efflux using least squares regression. CART and MLR analyses identified dissolved oxygen, temperature and salinity as the primary drivers of NH_4^+ efflux in all regions of the estuarine system. Oligohaline and mesohaline tributary temperature and salinity influence the rate of nitrogen cycling as well as benthic macrofaunal biomass. In deeper regions of mesohaline tributaries and the mainstem Bay dissolved oxygen was found to be to dominating parameter regulating nitrogen pathways in sediments as well as the structure of the benthic macrofaunal community. Spring regressions of total macrofaunal biomass NH_4^+ efflux suggested an enhancement of efflux with increased biomass. In contrast,

100

fall regressions suggested the enhancement of fixed nitrogen removal from sediments with increased benthic biomass. No significant relationship was observed in summer data but high NH_4^+ effluxes under hypoxic/anoxic conditions suggested dissolved oxygen is the primary driver of nitrogen cycling during that time of year. Individual species excretion rates estimated using previously published allometric models (Peters 1983) estimated polychaetes, bivalves and amphipods excretion contribute ~ 12, 20 and 16% (respectively) of the total measured NH_4^+ efflux. Deviations from Redfield organic matter aerobic respiration were evaluated against macrofaunal biomass and helped to support the interpretations of NH_4^+ efflux relationships with benthic biomass. This study concludes that a complex balance exists between seasonal and regional environmental conditions, coupled with the benthic community's species richness and dominant feeding guilds, controls the relationship between macrofaunal biomass and sediment nitrogen flux in this eutrophic estuarine system.

INTRODUCTION

Springtime inputs of organic carbon to estuarine sediments are primarily in the form of phytoplankton production, but also include allochthonous suspended material. These "food sources" tend to initiate the annual cycle with an increase in estuarine production that often peaks in the mid-summer and declines through the fall (Kemp & Boynton 1981, Malone et al. 1986, Herman et al. 1999). This organic input feeds heterotrophic processes in sediments, where it is hydrolyzed and remineralized into inorganic nutrients and other by-products consumed by microbes and invertebrate infauna. Several factors can affect the rate and quantity of sediment decomposition, including the magnitude of organic matter deposition to the sediments (Nixon 1981, Graf et al. 1982). Environmental conditions, chiefly temperature, oxygen, and salinity, additionally impact the rate and pathways of organic matter remineralization (Kemp & Boynton 1984, Aller 1994). Benthic macrofaunal animals also facilitate microbial processes through their bioturbation and irrigation activity (Rhoads 1974, Aller 1994, Aller & Aller 1998). All of these factors tend to vary substantially over seasons and across different regions within the estuarine system.

Macrofauna are typically modeled in terms of biomass contribution to sediment biogeochemistry (Cerco 2000, Meyers et al. 2000, Di Toro 2001, Welsh 2003, Timmermann et al. 2012, Sturdivant et al. 2013). The relationship between macrofaunal biomass and organic matter breakdown to nutrients, in this case, NH_4^+, is not simple. Macrofaunal effects on diagenesis and nutrient recycling

vary with season and regions of an estuary. Benthic macrofauna can impact

sediment biogeochemistry through several mechanisms including: (1) increased

rate of particle mixing and burial through feeding and burrow construction

(bioturbation), (2) increased rates of solute transfer to the overlying water through

burrow irrigation (bioirrigation), (3) enhanced penetration of oxygen into the

sediment porewaters and (4) increased sediment-water fluxes of oxygen and

nutrients associated with direct animal respiration and excretion (Rhoads 1974,

Diaz 2001, Francois et al. 2001, Welsh 2003). All of these mechanisms vary with

animal species, size, abundance and behavior, making it challenging to generalize

the effects of estuarine macrofaunal biomass on nutrient cycling. Laboratory

incubation experiments, including those discussed in Chapter 3, have shown that

more biomass of macrofauna often results in an increase in sediment-water fluxes

of key solutes including NH_4^+, NO_3^- phosphate, oxygen, carbon dioxide and

nitrogen gas (Kristensen et al. 1991, Bartoli et al. 2000, Nizzoli et al. 2007). As

shown in Chapter 3, the specific individual size of animals also affects their own

metabolic contribution of NH_4^+ flux via excretion.

Additionally, not all macrofauna interact with the sediment in the same

way. Differences related to functional feeding groups (e.g. surface-deposit, deep-

deposit or suspension-feeders) can be important; however, some species can

complicate that approach by facultatively feeding in more than one way (Welsh

2003). For example, the bivalve *Macoma balthica* and many spionid polychaetes

(e.g. *Paraprionospio pinnata* and *Streblospio benedicti*) can consume both

suspended and deposited particles (Dauer et al. 1981, Hummel 1985). Deeper

burrowing and deep-deposit-feeding animals (e.g. *Heteromastus filiformis*) can also enhance the remineralization of buried organic material where as smaller surface deposit feeding animals (e.g. *Leptocherius plumulosus*) enhance recently deposited material (Rosenberg et al. 2000, Quintana et al. 2007).

Spatial and temporal variations in estuarine environmental parameters and benthic macrofauna can complicate quantifying general patterns as trends. Environmental parameters (temperature, salinity, dissolved oxygen, sediment organic matter concentration) can influence nutrient cycling, and independently they can also modulate the influence of macrofauna on these processes. These parameters shape benthic communities in terms of both total biomass and species composition (Boesch 1977, Gray & Elliott 2009). For example, dissolved oxygen is a particularly important parameter to ammonification and nitrification in estuaries (Henriksen & Kemp 1988). Summertime hypoxia can limit the rate of nitrification and inhibit coupled nitrification-denitrification in sediments, often resulting in a midsummer efflux of NH_4^+ (Kemp et al. 1990). Under hypoxic conditions many macrofauna stop bioturbating or bioirrigating, thus reducing their impacts on sediment-water solute exchange (Diaz & Rosenberg 1995). Persistent hypoxia is often fatal to most macrofauna (Vaquer-Sunyer & Duarte 2008). Declines in benthic abundance, in estuaries like the Chesapeake Bay, have been attributed to recurrent seasonal hypoxia (Holland et al. 1977, Holland et al. 1987).

In this study we statistically analyze large-scale monitoring data collected in the Chesapeake Bay estuarine system to explore the complex interactions between benthic macrofaunal biomass and sediment-water fluxes of NH_4^+, NO_3^- +

NO_2^- (referred to as NO_x in further narrative), and O_2 to assess how the relationships are modified by environmental parameters. To determine the primary drivers of nitrogen recycling in the Bay we use a Classification and Regression Tree (CART) analysis and multiple linear regressions (MLR). We explore if the relationship between benthic biomass and NH_4^+ flux within different regions of the estuary can be characterized by season. And finally, we separate biomass data into different classes of benthic macrofauna (polychaetes, bivalves, and amphipods) to assess if the impact of on nitrogen recycling can be isolated across the different regions of the Bay. The analysis presented here shows environmental parameters, including macrofaunal biomass, can influence nitrogen recycling. However, not all will be operative at all times or in locations of an estuarine system. A close examination of these data shows the relationship between macrofaunal biomass and nitrogen flux changes seasonally and with the classes of macrofauna dominating the benthic community.

METHODS

Study Regions

The dataset used in this study was collected, as part of a U. S. Army Corps of Engineers project in the 1980s, to understand the impact sediment biogeochemical processes have on water quality in the Chesapeake Bay. The flux data is part of a larger Sediment Oxygen and Nutrient Exchanges (SONE) program and a component of the Maryland Chesapeake Bay Water Quality Monitoring Program from 1984 until 2001 (Boynton 2011). The data spanning

from 1985 – 1988 is unique because it combines the measurement of biogeochemical fluxes over a broad area of the estuary with concurrent documentation of the benthic community. The motivation to collect benthic macrofaunal species composition data was to further refine the Bay's water quality model predictions. While not the original intent upon collection, the uniqueness of these data provides an opportunity to enhance the understanding of the complex relationship between macrofaunal biomass and NH_4^+ recycling.

This chapter uses data on sediment-water fluxes of O_2, NH_4^+ and NO_x and benthic macrofauna abundance and biomass collected seasonally at multiple stations over a four-year period (Boynton et al. 1997). Intact sediment samples were collected in triplicate from eight stations in the Chesapeake Bay and tributary rivers. Four stations were located in the mainstem of the Bay (Still Pond, buoy R-78, buoy R-64, Point No Point), and four stations in the Patuxent River (Buena Vista, Saint Leonards Creek) and Potomac River estuaries (Maryland Point, Ragged Point) (Fig. 4.1). Most of these stations were sampled four times each year, once in the spring and fall and twice during the summer months (Table 4.1). A total 251 cores were selected for examination from the total number of cores collected (768 cores) at the eight stations of the four-year period (1985 – 1988). All sampling stations were characterized by fine-grained sediment.

Benthic Macrofauna Sample Processing

Benthic macrofuanal samples were sorted and identified by Cove

Corporation and counted to the level of species or species groups. Various studies

have shown that macrofauna loose ~10% weight when preserved in 10% formalin

(Donald & Paterson 1977). Preserved wet weights were converted to live wet

weights multiplying by a factor of 1.1. Bivalve weights from Cove Corporation

included the weight of the shells. The mean ratio of preserved bivalve tissue

weight to preserved whole bivalve (shell + tissue) weight was 0.47 (Boynton et al.

1997). This factor was used to convert whole bivalve weights to bivalve tissue

weights before converting the preserved weights to live wet weights. A

conversion of 0.2 was then applied to the data to convert live wet weights to dry

weights (Wetzel et al. 2005). All biomass data (x) were log-transformed using

log (x+1) prior to statistical analysis. Biomass is reported in Table 4.2 as g dry

weight m^{-2}; however, the data were plotted in the log form.

Calculation of Macrofaunal Excretion Rates

Dry weights of total polychaete, bivalve and amphipod biomasses for each

sample core were fitted to three respective allometric models found in the

literature (Table 4.3) of the forms:

$$V = aW^b \ \ \text{or} \ \ logV = log \ a \ + b*logW + c*logT \qquad \text{(Peters 1983)}$$

where V = NH_4^+ excretion rate in units of µmol N individual dry weight^{-1} h^{-1}. W is the weight (mg of dry weight) for the organism and T is the in situ temperature in ^{o}C. b and c are an exponential constants (slopes) for animal weight and in situ temperature respectively. Coefficient "a" is a constant of proportionality (intercept). Excretion rates were first calculated per individual and then multiplied buy the total abundance of that species in each core.

Data Analysis

Factors controlling NH_4^+ flux were tested using three different methods (CART, Linear regression, and ANOVA). First, a non-linear, classification and regression tree (CART) analysis (De'ath & Fabricius 2000) was used to examine what environmental factors, including macrofaunal biomass, exert the greatest influence on patterns of sediment-water NH_4^+ fluxes. CART analysis was performed using built in functions of the Matlab Statistics Toolbox version r2012_a. Changes in the mean squared error (MSE) due to splits on every predictor in the regression tree were summed and then divided by the number of tree nodes to calculate a quantitative estimate of predictor importance. At each node, MSE is estimated as the node error weighted by the node probability. Variable importance associated with each split is computed as the difference between MSE for the "parent node" and the total MSE for the two "children".

The results of the CART analysis suggested that "season" might be an over-arching parameter controlling relationships between estuarine parameters, nitrogen recycling and remineralization. We separated the monthly data by spring

(April and May), summer (June and August) and fall (October and November) to further investigate seasonal relationships. Environmental parameters (temperature, salinity, dissolved oxygen, total surface sediment chlorophyll) were then used in multiple linear regression analyses with and without macrofaunal biomass to understand the strength of macrofaunal effects on nitrogen recycling within spring, summer and fall seasons. Relationships between macrofaunal biomass and NH_4^+ flux were grouped into logged bins and analyzed using 1 and 2-way ANOVAs with a Tukey-Kramer multiple comparison test. Community structure influence on NH_4^+ flux was evaluated by the total species richness (number of unique species). These data were regressed with NH_4^+ flux using least squares regression.

Evaluating Fluxes Against Redfield Stoichiometry

We assumed that the main source of labile organic matter to sediments was associated with phytoplankton, and therefore that organic decomposition would yield approximately 1 atom of N for 6.6 atoms of O_2 consumed or CO_2 produced (Redfield et al. 1963). Measured NH_4^+ effluxes were evaluated against predicted NH_4^+ efflux based on the Redfield stoichiometric ratio of (O/N) 6.6:1 assuming that O_2 uptake is proportional to total benthic system respiration (Nixon 1981). If predicted NH_4^+ efflux exceeds measured rates, we hypothesize that the difference is attributable to nitrification, denitrification and/or nitrogen burial. On the other hand, if predicted NH_4^+ efflux is less than measured rates, we hypothesize that O_2 consumption under estimates respiration rates indicate more

NH_4^+ is fluxed to the anaerobic domain by anaerobic metabolic processes. We subtracted the predicted total nitrogen from the sum of measured NH_4^+ and NO_x flux to estimate the amount of nitrogen unaccounted in our flux measurements (*Denitrification index*).

Regression Dilution Correction (Type II errors)

Random measurement error is a pervasive problem when measuring biological systems. It has been shown that random measurement error can bias a regression slope coefficient downwards towards the null if the parameter containing the error is used as the independent variable. This phenomenon has been termed regression dilution bias (Liu 1988, Hutcheon et al. 2010). In this study we have chosen a components-of-variance method to calculate a regression correction factor (Rosner et al. 1992).

$$\lambda = 1 + \frac{\sigma_{replicate}^2}{\sigma_{total}^2}$$

Where $\sigma_{replicate}^2$, the variance of the replicate (triplicate) cores on each sample date at each station and is σ_{total}^2 is the variance of the total measured NH_4^+ fluxes. The calculated slope of the least squares regression line (m) was then multiplied by the correction factor ($y = \lambda m x + b$). Next, by applying the corrected slope and the means of both the independent and dependent variables to the original least squares fit equation, we solved for a corrected y-intercept ($b_{corrected}$) where $b_{corrected} = y_{mean} - \lambda m x_{mean}$.

RESULTS

Study Region Species Composition

Estuary-wide five classes of macrofauna were found (Table 4.2), although only three of them are the focus in this study (polychaetes, bivalves and amphipods). The seasonal biomass of these three groups was evaluated for eight stations within three estuarine regions (Fig. 4.2). Oligohaline stations include Still Pond, the most northern mainstem Bay site and Maryland Point in the tidal fresh Potomac. Three mesohaline tributary stations include Buena Vista and St. Leonard's Creek in the Patuxent River estuary, and Ragged Point in the Potomac River estuary. Finally, the mesohaline mainstem Bay stations are Point No Point, Buoy R-64, and Buoy R-78.

Polychaete abundance is highest in the spring at oligohaline stations and these communities are dominated by *Marenzelleria viridis* (Table 4.4). The spring abundance value is also significantly ($p = 0.002$) greater than spring polychaete abundances in either mesohaline regions (Table 4.4). Across seasons, *M. viridis* abundance decreased in both the oligohaline and mesohaline tributaries (Table 4.4). In oligohaline stations, summer and fall abundances were significantly different ($p \ll 0.001$) from the spring but not significantly different from each other. While *M. viridis* was present in all regions, spring and summer mesohaline tributary polychaete abundances are dominated by *H. filiformis* and small spionids like *S. benedicti* and *P. pinnata* (Table 4.4). These abundances were significantly different ($p = 0.01$) from each other, but fall polychaete abundances in these two mesohaline regions were not significantly different from

the spring or summer. Mainstem Bay stations saw an increase in polychaete

abundance in the summer; however, that increase was not significantly different

from the spring or fall abundances. Mainstem polychaete abundance was chiefly

dominated by *S. benedicti* and *P. pinnata*. Biomass of polychaetes was greatest in

spring oligohaline stations (Fig. 4.2). As the seasons progress, the biomass of

polychaetes decreased and abundance also declined (Fig. 4.2, Table 4.2). The

biomasses of spionid polychaetes that dominate both of the mesohaline regions

were smaller than the polychaetes occupying the oligohaline region (Table 4.2),

but the abundances remained relatively consistent throughout the year (Table 4.4).

Bivalve abundances were highly variable across all three regions,

particularly in the spring. The mainstem stations, however, had variable and high

abundances of relatively large *Mulinia lateralis* that dominated spring bivalve

abundance and biomass at these stations (Fig. 4.2, Table 4.3, 4). Across seasons,

spring bivalve abundance in mesohaline tributaries was significantly higher than

in the summer and fall. These stations were dominated by *M. lateralis* in the

spring with a biomass range of $0.53 - 1.09$ (g m^{-2}, dry weight).   Summer

abundances in the oligohaline and mesohaline tributaries were not significantly

different. In the summer, the mainstem can have high, but variable, abundances

of small *Macoma sp.* and *M. lateralis* (Table 4.4). While abundances were lower,

biomasses for both of these clams peaked in summer in mesohaline tributaries

$(10.38 - 28.28$ g m^{-2}, dry weight). In the fall across all three regions, abundances

were low. but variability remained high. Oligohaline fall bivalve abundances

were significantly different from both mesohaline regions. While their biomasses

were not large (Table 4.3), amphipods had greatest abundances in the mesohaline

tributaries (especially Buena Vista). Amphipod abundances also peaked in the

summer (> 2400 indiv. m^{-2}) at the oligohaline stations. These abundances were

similar within and across both regions (Table 4.4).

Classification and Regression Tree Analysis

To initially understand the relationships between macrofuanal biomass and

in situ environmental parameters, we developed a CART analysis to identify

controls and thresholds for NH_4^+ flux. Representative portions of the regression

tree highlight branches and splits where biomass plays a role (Fig. 4.3). The

relative importance of each parameter is also depicted as an inset table in this

figure. This regression tree has an overall $r^2 = 0.83$ with 233 degrees of freedom

and relationships were significant to 95% confidence. The most important

parameter in determining NH_4^+ flux was dissolved oxygen (46%) followed by

temperature (18%), depth (14%) and salinity (13%). Total surface sediment

chlorophyll, a proxy for fresh organic matter, was most important in oxygen-rich,

cooler and fresher environments (Node 12). Polychaete and amphipod biomass

contributed modestly (1-2%) to the NH_4^+ flux prediction and bivalve biomass was

not important. Polychaete biomass had a negative effect on NH_4^+ flux with higher

sediment chlorophyll (Node 21). Conversely, under more shallow (< 10 m), salty

(>10.4 psu), normoxic (>= 5 mg L^{-1}) conditions, higher polychaete biomass

resulted in greater NH_4^+ flux (Node 47). Amphipods exhibited a negative impact

on NH_4^+ flux under aerobic, mesohaline (Node 41) and oligohaline (Node 4) conditions.

Seasonal Sediment Solute Fluxes

Patterns of seasonal variability in nitrogen flux can vary among different estuarine regions. To assess those seasonal pattern differences in measured NH_4^+ and NO_x flux were plotted along with sediment oxygen demand (SOD) for each region (Fig. 4). In the spring and fall NH_4^+ flux had a positive correlation with SOD. Oligohaline and mainstem SOD was relatively constant throughout the year, and the greatest peak in SOD occurred in May mesohaline tributaries. In the summer, the positive relationship between SOD and NH_4^+ flux becomes variable in regions that are known to experience summertime hypoxia or anoxia. Oligohaline peak NH_4^+ fluxes occurred in the late summer and early fall. NH_4^+ flux in both mesohaline regions remains high from June through October. NO_x influx was greatest in the spring, particularly in May across all regions. Small rates of NO_x efflux minimally occurred throughout the rest of the year in mesohaline stations. At oligohaline stations NO_x fluxes into the sediment predominated in August and October.

The relationship between SOD and NH_4^+ flux revealed many points out of stoichiometric balance to the Redfield ratio of (O/N) 6.6:1 (Fig. 4.5). Points found above the Redfield ratio have more NH_4^+ released than what is expected from the respiration (as measured by SOD) of fresh organic material. Points below the line released less NH_4^+ from sediments than expected, suggesting

nitrogen has been oxidized to NO_x and/or denitrified. These latter points are referred to here as "*Denitrification index*". This assumption was tested by regressing the calculated *Denitrification index* with N_2-N flux measurements of intact sediment cores collected from oligohaline and mesohaline mainstem stations (Still Pond and Buoy R-64) (J. Cornwell, unpublished data). The significant positive regression indicated our assumption was reasonable (Fig. 4.6). Oligohaline stations have points balanced on either side of the Redfield line (Fig, 4.5a) with most oligohaline points below Redfield in the fall (Fig. 4.5b). Mesohaline tributaries had greater SOD than oligohaline or mainstem regions, and most of the data points fell either on or below the Redfield line (Fig. 4.5a,b). Spring mainstem stations had very low NH_4^+ efflux with respect to SOD. In contrast, mainstem points had the highest fluxes above the Redfield ratio during the summer months (Fig. 4.5a,b).

Macrofuanal Biomass and Nitrogen Flux

Total macrofaunal biomass was regressed against NH_4^+ flux and the flux of *Denitrification index* for each season. Regression computations were made using both a standard linear regression method and one that corrects for error in the biomass measurements (Type II). Only the standard regression lines were plotted on all regressions of biomass to nitrogen fluxes for simplicity but both equations are noted in the figure captions. While the r^2 values changed slightly because Type II regression accounts for error in the independent variable, the overall significance of the regression did not change. In the spring the regression

between biomass and NH_4^+ flux was significantly positive ($p \ll 0.001$) (Fig. 4.7a). The *Denitrification index* flux was also significantly positive. The three data points for the May 25, 1986 sample at the Ragged Point in the Potomac River estuary were deemed to be statistical outliers because they were above the ninety-fifth percentile of the full data set and were thus omitted from the regression analysis (Fig. 4.7). Total sediment chlorophyll was particularly high in all three sample cores and historical weather records from Andrews Air Force Base (www.wunderground.com) revealed a weather system that stalled over the region that week (May 19 – 25, 1986) dropped more than an inch of rain over the area leading to higher stream discharge and greater turbidity.

Mainstem macrofaunal biomasses were relatively small in the spring with NH_4^+ efflux typically less than 100 μm N $m^{-2} h^{-1}$. These lower biomasses were typically dominated by at least 50% polychaete worms (not shown). Oligohaline biomass levels ranged between 0.43 and 1.1 g log dry weight m^{-2}, and had NH_4^+ fluxes less than 250 μm N $m^{-2} h^{-1}$. Mesohaline macrofauna had the most variable biomass ranging from 0.22 to 2.1 g log dry weight m^{-2}. The higher biomass values were dominated by at least 50% bivalves (not shown) and had the greatest NH_4^+ flux. In the summer, there was no significant relationship between macrofaunal biomass and either NH_4^+ or *Denitrification index* (Fig. 4.7). There were, however, elevated NH_4^+ effluxes as well as negative *Denitrification index* under summer anoxia in the mesohaline mainstem. Under hypoxic conditions there was little denitrification at higher biomasses. Under biomasses less than 1.1 g log dry weight m^{-2}, NH_4^+ flux was slightly elevated in some samples but there

were also a greater number of negative *Denitrification index* points. With the

exception of 2 observations, all hypoxic and anoxic data points with biomass

between 0.43 and 1.1 g log dry weight m^{-2} were either zero or negative. CART

analysis revealed that fall N fluxes (Fig. 4.3, Node 3) were strongly influenced by

temperature. NH_4^+ fluxes from fall samples collected under temperatures greater

than 16.0 ^{o}C had a significant negative relationship ($p \ll 0.001$) to biomass (Fig.

4.9a). In contrast, the *Denitrification index* had a significant positive relationship

to biomass (Fig. 4.9b). Regressions of NH_4^+ and *Denitrification index* were

computed against amphipod biomass for all sites where amphipod abundances

were greater than 3500 indiv. m^{-2} (Fig. 4.10). While most instances occurred in

the summer, a few fall and spring samples also contributed to the significant

relationships. Collectively NH_4^+ fluxes from all seasons had a significant

negative relationship to amphipod biomass, and *Denitrification index* had a

significant positive relationship to biomass.

NH_4^+ Flux and Macrofaunal Community Composition

The two most dominant groups of macrofauna in the Bay regions analyzed

here were polychaetes and bivalves. To evaluate the relative influence of these

two groups on NH_4^+ flux, the ratio of polychaetes to bivalves was computed for

biomass data. These data were then log-binned for an ANOVA analysis

evaluating the relative influence of each group on NH_4^+ flux (Fig. 4.11a). As the

ratio of polychaetes to bivalves increased, there was a steady decline in NH_4^+ flux

until the ratio became greater than 1000. The decline was the most significantly

different between the smallest bin (bivalve dominated community) and the third

bin where polychaetes dominated the community by 10-fold. A ratio of

polychaetes to the bivalve *Macoma sp.* was also computed and analyzed with

respect to NH_4^+ flux (Fig. 4.11b). As the ratio of polychaetes to *Macoma sp.*

increased there was a decline in NH_4^+ flux. That decline was significantly

different between a smallest bin (*Macoma sp.* dominated community) and the

second bin that was most balanced between polychaetes and *Macoma sp.*

Because most spionid polychaetes can also be facultative suspension and deposit

feeders (Dauer et al. 1981), the benthic community structure was measured using

species richness (the number of species per sample). Species richness and NH_4^+

flux had a significant negative relationship (Fig. 4.12) despite an outlying point

with one species (*S. benedicti*) and high NH_4^+ flux (380 μm m^{-2} h^{-1}) that was

attributed to summertime mainstem anoxia at station R-64.

Seasonal multiple linear regressions (MLR) of environmental parameters

and NH_4^+ flux with and without macrofaunal biomass revealed the shifting

importance of macrofauna to seasonal N-fluxes (Table 4.5). Dissolved oxygen

and salinity were the most important parameters to all MLR relationships.

Temperature was the third most important in the summer and fall but not

important in the spring. Depth was also important in all MLR relationships.

With the exception of the summer regression without biomass, total sediment

chlorophyll was the fourth or fifth most important regression parameter. In all

three seasons macrofauna biomass was an important independent parameter that

increases the r^2 value of the regression. Co-varying parameters involving biomass

and salinity, temperature or depth also enhance the MLR fit in the summer and fall. Co-varying parameters in the spring and summer for regressions without biomass include dissolved oxygen, sediment chlorophyll and depth.

Macrofaunal excretion rates were also estimated for polychaetes, bivalves and amphipods using the equations in Table 4.3. A histogram distribution of the percent NH_4^+ flux attributed to each species class shows that macrofauna excretion accounted for ~ 20% of the total measured NH_4^+ flux (Fig. 4.13). Bivalves contributed the most excretion to the total NH_4^+ flux (20%) (Fig.12b). Amphipods, when present, contributed a mean of 16% (Fig. 4.13c) and polychaetes 12% (Fig. 4.13a). The median contributions for all species groups, however, were very small (4-6%).

DISCUSSION

Regional and Seasonal Variation and Controls of Biogeochemistry and Macrofauna

Benthic macrofaunal abundance and biomass levels generally peak in spring (Fig. 4.2, Table 4.4) and tend to diminish throughout the year, presumably due to diminished food, low-oxygen and/or increased predation (Kemp & Boynton 1981, Holland et al. 1987). Oligohaline polychaetes have the greatest seasonal shift in abundance from spring to fall, and this pattern is consistent with the life cycle of the region's dominant polychaete, *M. viridis* (Table 4.4, see also Chapter 3). In the mesohaline tributaries, biomass is dominated by the bivalve

Macoma sp. Biomass of these species remains relatively constant even when the abundance numbers decline indicating the growth of the surviving spring cohort. This pattern is consistent also with polychaete biomass in both mesohaline regions. As a semelparous organism, *H. filiformis* can have two recruitment periods but reproduction doesn't occur until the second year of life (Shaffer 1983). This life cycle may help maintain the relatively stable polychaete biomass in the mesohaline tributary community. In the mesohaline mainstem, as well as some stations in the mesohaline tributary region (Ragged Point and St. Leonad's Creek), where summer hypoxia (Kemp et al. 2005) occurs, polychaete biomass, is also maintained by small opportunistic polychaetes (e.g. *P. pinanta and S. benedicti*) that can tolerate hypoxic events (Llanso 1991, Llanso 1992). Summer biomasses of bivalves in the mainstem region are very variable and are often attributed to an isolated larger *Macoma sp*. Amphipods (predominantly *Leptocheirus plumulosus*) are a small but a consistent presence in the oligohaline and mesohaline tributaries throughout the year. Their peak abundances occur in June (Table 4.2) with highest abundances are found mainly at the Buena Vista and Still Pond sites, where hypoxia is rare (Fig. 4.10). While amphipods occasionally appear in the spring at mainstem sites, their numbers are very low in these more saline environments.

It is known that macrofauna, through their bioturbation and bioirrigating activities, facilitate key biogeochemical reactions that remineralize organic matter (Welsh 2003). A number of studies have demonstrated that sediment diagenetic rates (including NH_4^+ production and denitrification) tend to increase with

increased abundance or biomass of animals (Bartoli et al. 2000, Swan et al. 2007).

Other research has shown that animal effects on nitrogen cycling and sediment-

water fluxes also vary for different animal species and different life cycles and

feeding habits (Pelegri & Blackburn 1995, Waldbusser et al. 2004). Both the

CART analysis and the seasonal MLRs indicate macrofaunal biomass is the 5th or

6th most important parameter (Fig. 4.3, Table 4.5), perhaps because factors that

control nitrogen recycling also control benthic macrofaunal communities. In

environments where temperature, salinity, and dissolved oxygen are less variable,

the role of macrofauna may be more discernable.

The three most important factors (temperature, salinity and DO) identified

and quantified by Classification and Regression Tree (CART) analysis and

multiple linear regressions (MLR) have long been understood to be strong

controlling forces behind seasonal and regional variation in estuarine nutrient

recycling processes (Nixon 1981). Statistical models relating nitrogen recycling

to these primary factors vary in space and time. MLRs showed DO and salinity,

two seasonally and regionally varying parameters, play a strong role at all times

of the year in all parts of the estuary (Table 4.5). DO is essential to the aerobic

breakdown of organic material and the production of NH_4^+. An initial CART split

of and aerobic DO value (6.05 mg L^{-1}) (Fig. 4.3, Node 1) suggests an underlying

set of conditions where the interaction of other factors control the magnitude of

NH_4^+ efflux from sediments. Along the estuarine salinity gradient, the other

factors in the CART analysis (temperature, depth, and total sediment chlorophyll)

also change spatially and temporally. Ultimately relationships between DO,

NH_4^+, and NO_x fluxes along the estuarine salinity gradient reflect the interactions

between freshwater nutrient loading and nitrogen regeneration in sediments. In

the spring and summer, organic matter deposition and water column production

drive these sediment-water fluxes (Kemp & Boynton 1984, Hagy et al. 2005). In

this study we use total sediment chlorophyll as a proxy for organic matter

deposition. Labile phytoplankton-derived organic material can be decomposed

faster than it can build up in the sediment. The deposition rate of chlorophyll to

the sediment, rather than the sediment stock of chlorophyll, is a better determinant

of nutrient fluxes like NH_4^+ (Hagy et al. 2005), which might explain why

sediment chlorophyll was ranked so low in importance in the CART analysis and

MLRs. Temperature also plays a major role in defining the seasonal cycles of

enzyme regulated diagenetic processes with seasonal peaks of NH_4^+ efflux

occurring in the summer (Boynton et al. 1989). Seasonal patterns of NH_4^+ flux

within the three study regions of the Bay allude to how these three parameters,

along with factors that control differences in organic matter deposition, like depth,

can control the magnitude of NH_4^+ flux (Fig. 4.4).

Estimation of NH_4^+ Pathways

In the absence of N_2 flux measurements, an analysis of NH_4^+ fluxes to

SOD was evaluated against elemental ratios assuming aerobic decomposition of

phytoplankton (Fig. 4.5). In estuaries such as Chesapeake Bay, the majority of

organic material deposited to the sediments in the spring and summer is of

planktonic origin (Kemp et al 1999, (Graf et al. 1982, Kemp & Boynton 1984).

To infer which diagenetic transformation processes are active with regard to sediment-water nitrogen fluxes we examined departures from the Redfield O:N ratio (6.625 : 1) for NH_4^+ effluxes and SOD assuming aerobic respiration of phytoplankton organic matter (Redfield et al. 1963). Departures from the Redfield ratio in this study varied with season and region (Fig. 4.5). If the ratio of SOD : NH_4^+ efflux is higher than Redfield proportions we assume excess nitrogen or sulfide burial (Cornwell & Sampou 1995), whereas disproportionately lower ratios suggest loss of fixed nitrogen (primarily as N_2) via denitrification (Nixon 1981) or dissimilatory nitrate reduction to ammonium (DNRA).

Seasonal relationships between Macrofauna and Biogeochemical Fluxes

In the spring warming temperatures and the increased nutrient loading from freshwater runoff initiates the annual cycle in increased phytoplankton production. It is also the peak recruitment period for most benthic macrofauna (Gray & Elliott 2009). A significant positive relationship was observed between macrofaunal biomass with NH_4^+ during this time (Fig. 4.7a). A few higher points in the mesohaline tributaries and oligohaline stations contribute to a weakly significant, positive relationship between biomass and *Denitrification index* (Fig. 4.7b). The rapid pace of springtime production and organic matter deposition along an estuarine salinity gradient may control both significant positive relationships. With unlimited oxygen availability high microbial remineralization rates could potentially mask the impact of macrofauna on biogeochemical processes and impair the interpretation of computed *Denitrification index* values.

Also, under aerobic conditions where macrofaunal biomasses are highest, like in the oligohaline and mesohaline tributaries (Fig. 4.2,3), macrofaunal excretion may comprise a substantial fraction of total NH_4^+ efflux in sediment.

Previous work in the Chesapeake Bay has shown that most (70%) of the total nitrogen delivered to the estuary in river flow is in the form of NO_x (Hagy et al. 2004). The relatively high rates of NO_x uptake by sediments in the oligohaline stations in this study along with the reduced NH_4^+ flux in the spring suggests potentially high rates of denitrification (Kana et al. 2006). The mean May oligohaline SOD of 1042 μmol O_2 $um^{-2} h^{-1}$ the Redfield predicted NH_4^+ regeneration is 158 μmol N $mm^{-2} h^{-1}$ and the measured NH4+ flux was only 106 μmol NH_4^+ $um^{-2} h^{-1}$ (Fig. 4.4a). Previous work has demonstrated strong correlations between temperature, microbial activity and sediment – water exchange rates (Nixon 1981, Cowan & Boynton 1996) presumably due to increased microbial and macrofaunal activity. With the biomass and abundance of polychaetes in the oligohaline region at its peak during the spring months (Fig. 4.2, Table 4.2), it is possible the bioturbation activity of worms shunts NH_4^+ production away from recycling pathways (NH_4^+ efflux) towards denitrification (Henriksen et al. 1983, Jenkins & Kemp 1984, Kemp et al. 1990)(Fig. 4.4a). This hypothesis is also supported in part by the oligohaline NH_4^+ data points falling below the Redfield proportion when regressed with macrofauna biomass (Fig 6b); however, the contribution of NH_4^+ excretion may be clouding this macrofauna enhanced nitrogen pathway in the spring.

The greatest springtime NH_4^+ flux occurs in the mesohaline tributaries where oxygen is high and there is a high amount of production and organic matter deposition. These tributaries also have the highest annual abundance and biomass macrofauna in the spring (Tables 4.2). Response to the seasonally high organic matter deposition is also suggested in the mesohaline tributaries by elevated NH_4^+ efflux in late spring and early summer (May and June) (Fig. 4.4b). Rates of nutrient regeneration in the sediments increase as temperature increases especially in the transition zone between fresh and salt water (Kemp & Boynton 1984). The mesohaline tributary data analyzed here includes the turbidity maximum section of the Patuxent River (station Buena Vista). This region has been shown to have higher than average particle deposition rates for the Patuxent river estuary due to the increased flocculation of particles as fresh and salt water mix (Kemp & Boynton 1984). The Redfield predicted N value for the mean May mesohaline tributary SOD (2912 $\mu mol\ O_2\ \mu m^{-2} h^{-1}$) is 441 $\mu mol\ N\ \mu m^{-2} h^{-1}$ but the measured NH_4^+ flux was only 173 $\mu mol\ N\ \mu m^{-2} h^{-1}$, suggesting a high denitrification rate. More recent field measurements at this station confirm that denitrification rates are also highest at Buena Vista, the turbidity maximum of the Potomac River estuary (Cornwell, personal communication). The diversity in total biomass in these tributaries suggests that production supports a variety of benthic species. The largest biomasses are attributed to bivalves, specifically *Macoma sp.* at Buena Vista and *M. lateralis* at Ragged Point (Table 4.2).

In the mainstem of the Bay, the breakdown of spring phytoplankton blooms can be temperature limited. NH_4^+ fluxes in April and May are the lowest across all stations sampled. Organic material not remineralized in the water column and subsequently deposited on the surface of sediment can become buried because temperatures are below 15^oC, a threshold for microbial activity (Kemp & Boynton 1984, Cowan & Boynton 1996). Variable abundances and biomasses of small polychaetes and clams dominate most of the mainstem sites (Table 4.2,4) in the spring where NH_4^+ fluxes are typically low (100 μmol NH_4^+ $\mu m^{-2} h^{-1}$). These low fluxes could be attributed to a cool mean bottom temperature ($\sim 10.5^oC$) at these deeper sites in the spring, along with a lower rate of organic matter deposition (Kemp & Boynton 1984). The majority of the *Denitrification index* points are positive indicating they are in excess of what would be expected from the break down of fresh phytoplankton organic material. Because oxygen is not limiting, these positive *Denitrification index* values would not be attributed to sulfide burial but they could possibly be attributed to the breakdown of older organic material through the transport of oxygen to deeper sediments though bioturbation by macrofauna (Kristensen 2000).

Occasionally in the spring, but mostly in the summer, salinity can control the degree of stratification and ultimately bottom water DO concentrations that in turn can control the pathways of nitrogen cycling in sediments. Hypoxic conditions are also known to be particularly deleterious to macrofaunal populations (Diaz & Rosenberg 1995). Summertime fluxes of nitrogen appear to be driven by dissolved oxygen (hypoxia) leaving a scattered relationship between

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flux and biomass for both NH_4^+ and *Denitrification index* (Fig. 4.7) for most

regions. The most notable pattern with regard to summer nitrogen flux and

biomass is when there are little to no macrofaunal present. June and August NH_4^+

effluxes in the mainstem stations are the highest across all regions and well above

the Redfield predicted NH_4^+ flux (~150-175 μmol N mm^{-2} h^{-1}) presumably due to

hypoxia restricting nitrification causing NH_4^+ to build-up in sediments (Fig. 4.5).

The concurrent negative *Denitrification index*, with macrofauna present, suggests

that under these conditions buried NH_4^+ may also be released from sediments due

to the dominance of anaerobic respiratory processes (Kemp et al. 1990).

There is one central limitation to this method of estimating the fate of

inorganic nitrogen in sediments that must be noted. Not all organic material in

sediments is broken down by aerobic respiration. Anaerobic respiration processes

such as sulfate reduction may remineralize organic matter but is not be accounted

for in this Redfield O_2: NH_4^+ ratio because of end-product burial and SOD flux is

zero. Therefore, this method may under estimate the amount of organic matter

diagenesis occurring in estuarine sediments especially under summer hypoxic

conditions (Roden & Tuttle 1993, Cornwell & Sampou 1995, Marvin-DiPasquale

et al. 2003). Never the less, this method provides a first-order understanding of

where or when denitrification is reducing NH_4^+ recycling by shunting fixed

nitrogen salts to biologically unavailable gaseous forms (e.g. N_2 and N_2O).

Due to the intense mixing, Buena Vista doesn't normally experience low

levels of dissolved oxygen but the two tributary stations in this study (St.

Leonard's Creek and Ragged Point) can experience hypoxic events in the

summer. These temporary hypoxic events, along with the high rates of remineralization in the turbidity maximum zone of Buena Vista, may maintain the observed high NH_4^+ fluxes in this region through the summer and into the fall (Fig. 4.4b). Additionally, peak abundances of amphipods in the summer (Table 4.4) can enhance nitrification (Pelegri & Blackburn 1994) in both the oligohaline and mesohaline tributaries. Also, NO_x uptake by oligohaline sediments continues because NO_x concentrations remain high in this region (Cowan & Boynton 1996, Hagy et al. 2004). As in the spring, high microbial remineralization rates in these areas along with a roughly 12-20% enhancement of NH_4^+ flux from macrofaunal excretion (Fig 12) makes the interpretation of divergences from Redfield proportions challenging.

Along with salinity and an interaction term between DO and depth, both suggesting regional differences, the fall MLR with macrofaunal biomass indicated interaction terms between biomass and temperature as well as biomass and sediment chlorophyll (Table 4.5). CART analysis also suggested temperature might control processes differently at a threshold of 16^oC (Fig. 4.3). While this split was ultimately a mixture of samples collected in all regions during all seasons, both sides of the temperature split included animal biomass as a subsequent predicting parameter (Fig. 4.3). We found this split inline with previous work noting a threshold of 15^oC in the spring was linked with increased sediment fluxes (Cowan & Boynton 1996). Laboratory experiments presented in Chapter 3 also indicated a shift in control core flux rates with a temperature increase from $15 - 19.8$ ^{o}C in the two phases of the A-S Experiment.

Based on the results of both statistical models, we chose to investigate the 16°C grouping of fall data along this split and found a distinct shift in flux rates between the two temperature groupings (Fig. 4.9). Much of the data was collected when bottom water temperatures were greater than 16°C in October. Data collected with temperatures less than 16°C were in November. Curiously, the biomass data collected in warmer waters were dominated by polychaetes, whereas bivalves dominated data collected in colder temperatures. In the fall, as microbial activity is beginning to shut down for the year, temperature can regulate the rates of microbial processes like ammonification (Kemp & Boynton 1981). It is not surprising that total sediment chlorophyll does not become important except when biomass is included as a parameter. It has been suggested that modest fall blooms may be a controlling factor on the magnitude of macrofuanal biomass in terms of food availability (Gray & Elliott 2009).

Biomass data collected in warmer temperatures shows a significant, inverse relationship to NH_4^+ efflux (Fig. 4.9a) in the spring. This inverse trend to the spring pattern suggests with increasing biomass, polychaetes decrease NH_4^+ flux in the fall. The contribution of polychaete NH_4^+ excretion, while estimated to be relatively low (~15%), should theoretically increase with increasing biomass (Fig.12a). Despite this increase in NH_4^+ excretion, we still observe a decrease in NH_4^+ flux. The relationship between macrofaunal biomass and *Denitrification index* was significantly positive for these warmer data points indicating a greater deviation from Redfield with increasing biomass. Under temperatures less than 16°C there was no relationship for either the NH_4^+ flux or *Denitrification index*.

This result supports the MLR and CART analyses that bivalve biomass plays very little role in NH_4^+ flux in the fall. These results suggest that benthic community composition plays an important role in controlling the magnitude and the source of measured NH_4^+ flux.

Effects of Macrofaunal Community Structure

A regression of mean NH_4^+ flux and species richness shows a decrease in NH_4^+ flux with an increase in diversity (Fig 4.11). Increased diversity brings together animals that interact with the sediment in varying space (burrow depth) and time (ventilation frequency) dimensions maximizing remineralization pathways. The CART analysis specifically isolated polychaetes and amphipods as having the most influence over NH_4^+ flux and found no relationship with bivalve biomass (Fig. 4.3). These results are in line with previous work that found the polychaete *Nereis diversicolor* and the amphipod *Corophium volatator* doubled the sediment-water solute flux while the bivalve *Cerastoderma edule* had only minor effects on biogeochemical processes (Mermillod-Blondin et al. 2005). Amphipods and polychaetes construct burrows in sediments and consume sediment deposits assisting in the breakdown of organic material. Many polychaetes have intervals of burrow irrigation in between rest periods (Kristensen 1983b). These intervals of irrigation alter the diffusional gradients within their burrows, often involving oxygen, impacting chemical processes in the sediment at depth (Mayer et al. 1995, Kristensen et al. 2012) further assisting in organic matter breakdown. Enhancement of nitrification potential (the ability of a

sediment volume to oxidize ammonium to nitrate) was positively associated with the percentage of time a benthic species spends irrigating (Mayer et al. 1995). Bioirrigation flushes nutrients like ammonia and nitrate out of the sediment and brings dissolved oxygen to sediment depth potentially increasing nitrification. Researchers have reported bioturbation can have a 1.5 to 4-fold increase in sediment-water oxygen and ammonium fluxes in sediments initially colonized by *Nereis spp.* (Nizzoli et al. 2007). The result is the alteration of overall reactivity of labile organic material and the succession of an active microbial community that efficiently breaks down organic material and rematerializes nutrients (Aller et al. 2001b). Unlike polychaetes and amphipods, suspension feeders don't typically consume sediment. Their contribution to NH_4^+ recycling is mostly through their excretion in the form of urea, feces or pseudo-feces derived from the consumption of overlying water organic material. Bivalves must continuously irrigate their burrows in order to feed (Welsh 2003). This may limit the dynamics of the diffusional gradients within their burrows (Forster & Graf 1992, Mayer et al. 1995). It is also possible that the seasonal and/or spatial biomass variability of bivalves with respect to other macrofauna, may also limit their contribution to the overall prediction of NH_4^+ flux.

Burrow geometry and spacing of individual macrofauna and the overall population abundance can affect the relative impact of a macrofaunal community on sediment biogeochemistry (Aller 2000, Aller et al. 2001b). Distribution and diversity of either deposit or suspension feeding macrofauna is shaped by food availability (Kemp & Boynton 1981, Gray & Elliott 2009). Commonly, benthic

communities exhibit regions of high diversity and abundance of deposit feeders accompanied with a low diversity and biomass of suspension feeders (Rhoads & Young 1970). This pattern was particularly noted in the oligohaline regions of is study. Data analysis showed that an increasing dominance of polychaetes over bivalves decreases the flux of NH_4^+ (Fig. 4.11a). This pattern supports the suggesting that the increase biomass of deposit feeders, like polychaetes, results in less NH_4^+ flux by facilitating nitrogen removal from the sediment through pathways like coupled nitrification-denitrification.

While ecosystem functions may not only be affected by increased species richness, they may also depend on the relative contribution of the dominant species in the community (Emmerson et al. 2001, Solan et al. 2004). The dominance of facultative suspension and deposit-feeding macrofauna, like the bivalve *Macoma sp*. (Hummel 1985) and many spionid polychaetes, suggest the impact of macrofauna on NH_4^+ efflux in this estuarine system may be linked to species-specific traits in addition to species richness. An evaluation of the balance between the dominant deposit feeding species, polychaetes and *Macoma sp.*, in this estuarine system suggest that polychaetes are more effective at nitrogen removal than other deposit feeders (Fig. 4.11b). Estimates of excretion indicate bivalves contribute more excretion to the total NH_4^+ flux than polychaetes or amphipods (Fig. 4.13). *Macoma sp.*, like amphipods, consume surface sediment that can enhance the rapid break down of newly deposited organic material and facilitate microbial processes at the sediment–water interface (Mermillod-Blondin & Rosenberg 2006). Polychaetes, particularly larger

individuals, bury deeper into the sediment, enhancing the decomposition of buried, older organic material and deeper sediment microbial processes (Chapter 3) (Kristensen 2000). The oligohaline region is dominated both in abundance and biomass by polychaetes in the spring. On the other hand, mesohaline tributaries of the Bay have high springtime bivalve abundance and biomass (Fig. 4.2, Table 4.4). The difference in species composition between these two regions may contribute to the differences in the relative NH_4^+ flux.

Many of the environmental parameters that control NH_4^+ efflux also control the occurrence and abundance of various macrofaunal species. By minimizing variability in the three primary drivers of temperature, salinity and DO by partitioning data by season and salinity regions of the Bay we further characterized the relationship between benthic biomass and NH_4^+ efflux. In the spring there is generally an increase in NH_4^+ efflux with and increase in benthic macrofaunal biomass. Regional ranges in temperature and salinity largely control the species composition and the magnitude of efflux. In the summer, DO (mainly hypoxia) controls the dominant pathway of nitrogen cycling by limiting nitrification and favoring the efflux of NH_4^+ while also shaping the benthic community composition, particularly in deeper regions. Fall changes in temperature and sediment chlorophyll concentrations cause a different relationship between biomass and NH_4^+ flux not seen in the spring or summer. As the annual cycle of estuarine production declines, but temperatures are still relatively warm, the relationship between macrofaunal biomass and NH_4^+ efflux shifts to negative, suggesting increases in biomass may aid in the elimination of

nitrogen from sediments through pathways like coupled nitrification-denitrification. An increase in species numbers also shows a decrease in NH_4^+ efflux. Further investigation revealed polychaetes as dominant deposit feeding macrofauna may be the primary driver in that species richness relationship. A complex balance between seasonal and regional environmental conditions coupled with benthic community species richness and dominant feeding guilds controls the relationship between macrofaunal biomass and sediment nitrogen flux.

Table 4.1: A listing of station names, locations, station depths, salinity, sediment characteristics, and years sampled

Region	Station	Location Latitude	Longitude	Depth (m)	Salinity Code	Sediment Type (% Slit/Clay)	CBP Benthic Data Years Sampled
Chesapeake Mainstem	Still Pond	39º 20.81'	76º 10.72'	10.4	Oligohaline	82.6	1989 - 1994
	Buoy R-78	38º 57.81'	76º 23.62'	15.8	Mesohaline	88.2	1989 - 1994
	Buoy R-64	38º 33.59'	76º 25.63'	16.8	Mesohaline	84.4	1989 - 1994
	Point No Point	38º 07.99'	76º 15.13'	14.2	Mesohaline	76.8	1985 - 1993
Patuxent River	Buena Vista	38º 31.12'	76º 39.82'	5.8	Oligo-Mesohaline	86.7	1984 - 1994
	St. Leonard Creek	38º 22.88'	76º 30.06'	7	Mesohaline	79.6	1988 - 1995
Potomac River	Maryland Point	38º 21.32'	77º 11.64'	10.3	Oligohaline	91.9	1981 - 1994
	Ragged Point	38º 09.86'	76º 35.52'	16.5	Mesohaline	82.7	1981 - 1994

Table 4.2: Seasonal mean biomass (g m^{-2}, dry weight) of different macrofaunal groups found in three salinity zones of Chesapeake Bay

Order/Family/Species	Mainstem Chesapeake Bay			Mesohaline Tributaries			Oligohaline		
	Spring	Summer	Fall	Spring	Summer	Fall	Spring	Summer	Fall
Annelida : Oligochaeta	0.00	0.02	0.04	0.09	0.02	0.07	0.03	0.02	0.01
Annelida : Polychaeta	0.24	0.53	0.32	0.40	0.39	0.50	1.95	1.05	0.39
Capitellidae									
Heteromastus filiformis	0.02	0.03	-	0.33	0.17	0.05	0.04	0.05	0.08
Nereididae									
Alitta succinea	0.58	0.84	0.55	2.09	1.77	1.44	-	0.02	0.06
Spionidae									
Marenzelleria viridis	-	-	-	0.23	0.22	0.24	3.20	1.85	0.94
Paraprionospio pinnata	0.13	0.32	0.42	0.13	0.11	1.04	0.02	-	-
Streblospio benedicti	0.05	0.05	0.02	0.04	0.02	0.02	0.01	0.01	0.02
Other	0.24	0.98	0.20	0.12	0.33	0.24	0.03	0.03	0.01
Arthropoda : Amphipoda	0.12	-	-	0.79	0.73	0.69	0.47	0.65	0.19
Mollusca : Bivalvia									
Mactridae									
Mulinia lateralis	0.56	6.54	0.01	1.09	1.17	0.10	0.00	0.10	0.02
Rangia cuneata	-	-	-	-	0.01	0.00	48.14	27.95	30.53
Other	0.01	-	0.01	0.48	3.47	0.00	-	-	-
Tellinidae									
Macoma sp.	0.02	3.19	-	10.38	16.32	28.28	1.00	1.94	0.73
Mollusca : Gastropoda	0.10	0.05	0.09	0.20	0.08	0.11	-	0.03	-
Miscellaneous Taxa	0.04	0.03	0.08	2.44	1.07	1.52	0.32	0.37	0.24

Table 4.3: Nitrogen excretion rates (V) of macrofauna reported in the literature and used in estimating macrofaunal group ammonium release. The abbreviations DW and WW represent dry weight and wet weight of flesh only (shell not included). (Kristensen 1984, Shumway & Newell 1984, Sereda & Hudson 2011)

Rate	Species (Group)	Temperature	Excretion Rate Units	Reference
$V=0.27W^{0.62}$	Neries (Polychaete)	16	μmolN h^{-1}, g WW	Kristensen 1984
$V=0.0038W^{0.564}$	Mulinia lateralis (Bivalve)	20	mgN d^{-1}, mg DW (flesh)	Shumway and Newell 1984
$\log V = -0.254 + 0.96\log(W) + 0.012\log(T)$	Various benthic macroinvertebrates (Amphipod)	in situ	μgN h^{-1}, mg DW (flesh)	Sereda and Hudson 2011

Table 4.4: Seasonal mean Abundance (indiv. m^{-2}) of different macrofaunal groups found in three salinity zones of Chesapeake Bay

Order/Family/Species	Mainstem Chesapeake Bay			Mesohaline Tributaries			Oligohaline		
	Spring	Summer	Fall	Spring	Summer	Fall	Spring	Summer	Fall
Annelida : Oligochaeta	145	347	2387	4058	1001	4629	1107	469	868
Annelida : Polychaeta									
Capitellidae									
Heteromastus filiformis	72	145	-	694	311	249	96	108	96
Nereididae									
Alitta succinea	118	101	138	164	209	145	-	72	169
Spionidae									
Marenzelleria viridis	-	-	-	196	135	90	2919	736	317
Paraprionospio pinnata	193	2393	847	203	236	526	72		
Streblospio benedicti	755	635	325	514	544	481	96	72	195
Other	404	434	386	371	219	289	108	130	201
Arthropoda : Amphipoda	108	-	-	2329	2306	2541	851	2408	1109
Mollusca : Bivalvia **Mactridae**									
Mulinia lateralis	4581	2737	96	5387	579	217	72	72	127
Rangia cuneata	-	-	-	-	72	72	181	2413	279
Other	271	-	72	262	83	96	-	-	-
Tellinidae									
Macoma sp.	174	4268	-	994	643	408	118	165	141
Mollusca : Gastropoda	461	108	747	755	338	682	-	195	-
Miscellaneous Taxa	313	96	90	542	653	406	366	427	450

Table 4.5: Seasonal multiple linear regression coefficients, number of observations, degrees of freedom, and r^2 for the regression of environmental parameters and ammonium flux with and without macrofaunal biomass. All regressions are significant with p-Values << 0.0001.

Parameter	With Biomass			Without Biomass		
	Spring	Summer	Fall	Spring	Summer	Fall
(Intercept)	-1372.60	-1609.70	-2439.80	-1249.50	263.21	-2425.30
DO	170.58	79.76	183.11	161.49	25.20	183.45
Salinity	-3.87	-13.28	-6.07	-6.04	-35.83	-7.59
Temperature	-	50.62	60.34	-	-4.38	-3.19
Depth	40.39	52.81	96.90	35.45	41.75	182.67
Sediment CHL	6.72	1.33	0.37	6.73	-6.02	-
DO * Salinity	-	-	-	-	4.34	-
Biomass	24.84	319.05	185.80	-	-	-
DO * Temperature	-	-	-	-	-	6.74
DO * Depth	-4.46	-8.31	-9.50	-3.89	-9.28	-18.44
DO * Sediment CHL	-0.82	-	-	-	-	-
Biomass * Salinity	-	7.70	-	-	-	-
Biomass * Temperature	-	-13.03	-9.38	-	-	-
Biomass * Depth	-	-7.71	-6.94	-	-	-
Sediment CHL * Biomass	-	-	-0.41	-	-	-
Sediment CHL * DO	-	-	-	-0.80	-	-
Sediment CHL * Salinity	-	-	-	-	0.15	-
Sediment CHL * Temperature	-	-	-	-	0.21	-
Fit Statistics						
# of observations	50	109	72	50	109	72
degrees of freedom	42	98	61	43	99	65
RMSE	50.7	121	71.2	56.4	128	76.8
R-squared	0.78	0.51	0.74	0.72	0.45	0.68
F-statistic vs constant Model	21.5	10.2	17.6	18.7	8.93	23.1
p-Value	5.51E-12	1.43E-11	1.74E-14	1.46E-10	9.40E-10	2.10E-14

Formula Summaries

With biomass
    Spring
        NH_4^+ Flux ~ 1 + Salinity + Biomass + DO*Depth + DO*Sediment CHL
    Summer
        NH_4^+ Flux ~ 1 + Sediment CHL + DO*Depth + Salinity*Biomass + Temp.*Biomass + Depth*Biomass
    Fall
        NH_4^+ Flux ~ 1 + Salinity + DO*Depth + Temp.*Biomass + Depth*Biomass + Sediment CHL*Biomass
Without Biomass
    Spring
        NH_4^+ Flux ~ 1 + Salinity + DO*Depth + DO*Sediment CHL
    Summer
        NH_4^+ Flux ~ 1 + DO*Salinity + DO*Depth + Salinity*Sediment CHL + Temp.*Sediment CHL
    Fall
        NH_4^+ Flux ~ 1 + Salinity + DO*Temp. + DO*Depth

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Figure captions

Fig. 4.1: Map of Chesapeake Bay showing water depth and major tributary systems. Sampling stations are labeled and marked in red. Inset map indicates location of estuary and watershed.

Fig. 4.2: Mean monthly biomass of polychaetes (black bars), bivalves (gray bars) and amphipods (white bars) in oligohaline, mesohaline tributary and mainstem Bay stations. Error bars represent standard error. Letters denote significantly different groups across regions. Numbers denote significantly different groups across seasons within a region.

Fig. 4.3: Example sections of a CART analysis tree predicting ammonium flux based on measured environmental parameters at each station. Within each node box is the splitting parameter (x) and the number of samples (n) that were split. Along each connecting line is the splitting threshold for the node parameter. Terminal Node (TN) circles contain the mean NH_4^+ flux ($\mu m\ m^{-2}\ h^{-1}$) and the number of samples (n) contained in that node. Also included is a table of CART-based factor relative importance to ammonium flux prediction. This regression tree has an $r^2 = 0.83$ with 233 degrees of freedom and is significant to 95% confidence.

Fig. 4.4:   Monthly sediment oxygen demand (SOD – blue line) and flux of

ammonium (dark green bars) and nitrate + nitrite (NO_x - yellow

bars) is shown for each study region. Negative values are fluxes

into the sediment and positive values are fluxes out of the

sediment. SOD values are all plotted here as positive even though

they are measured oxygen flux into the sediment. Error bars

represent standard error. Both y-axes are scaled in line with the

Redfield ratio of (O_2/N) 6.6 :1 for ease of interpretation.

Fig. 4.5:   SOD regressed with ammonium for each region and season. For

each a) region yellow squares are the oligohaline stations, green

inverted triangles are the mesohaline tributary stations, and the

purple circles are the mesohaline mainstem stations. For each b)

season green triangles are spring, light blue circles are summer and

dark blue squares are fall samples. The black line drawn on both

plots represents Redfield 6.6 O_2 to N.

Fig. 4.6:   Measured N_2-N flux regressed with calculated *Denitrification*

index at oligohaline station Still Pond (green circles) and

mesohaline mainstem station Buoy R-64 (blue triangles).   The

least squares regression line is plotted and is significant

($p < 0.001$). These flux data are presented courtesy of J. Cornwell.

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Fig. 4.7:   Spring macrofaunal biomass regressed against spring a) NH_4^+ and

b) *Denitrification index* flux. In both figures, yellow squares are

the oligohaline stations, green inverted triangles are the mesohaline

tributary stations, and the purple circles are the mesohaline

mainstem stations. Clear inverted triangles are outlying tributary

samples (Ragged Point, June 1986) that have been removed from

the regressions. The least squares regression lines are plotted for

both plots. The Type II regression equations are a) $y = 100x -$

67.3, $r^2 = 0.32$ and b) $y = 136x + 149$, $r^2 = 0.15$. All regression

lines are significant with p-values < 0.05.

Fig. 4.8:   Summer macrofaunal biomass regressed against summer a) NH_4^+

and b) *Denitrification index*. In both figures, red stars are

hypoxic, dark blue circles are normoxic, and black triangles are

anoxic dissolved oxygen conditions.

Fig. 4.9:   Fall macrofaunal biomass regressed against fall a) NH_4^+ and

b) *Denitrification index* flux. In both figures, red circles have

bottom water temperatures greater than or equal to 16.0 ^oC and

light blue squares have bottom water temperatures less than

16.0^oC.   The least squares regression lines are plotted for both

plots. The Type II regression equations are a) y = -393x – 400,

$r^2 = 0.30$ and b) y = 392x - 175, $r^2 = 0.46$. All regression lines are

significant with p-values < 0.005.

Fig. 4.10:   Amphipod biomasses greater them 3500 indiv. m^{-2} regressed

against a) NH_4^+ and b) *Denitrification index* flux. Green triangles

are spring, light blue circles are summer and dark blue squares are

fall samples. The single clear blue circle is a summer tributary

outlier (Buena Vista, June 1986) that has been removed from the

regressions. The least squares regression lines are plotted for both

plots. The Type II regression equations are a) y = -1191x – 756.8,

$r^2 = 0.40$ and b) y = 1163x – 742.1, $r^2 = 0.30$. All regression lines

are significant with p-values < 0.005.

Fig. 4.11:   Ammonium flux of log-binned macrofaunal group composition

ratios. NH_4^+ flux for computed a) polycheate to bivalve biomass

ratios and b) polychaete to *Macoma sp.* biomass ratios are log-

binned into 5 bins. Letters indicate statistical significance and

error bars are standard error. The mean standard error for all mean

ammonium fluxes is 3.1 μmol N m^{-2} h^{-1}.

Fig. 4.12:   Regression of species richness and mean ammonium flux. The

least squares regression is significant with a p-value < 0.001.

Fig. 4.13   Distribution of percent of the total measured NH_4^+ flux attributed

to macrofaunal excretion for a) polychaetes, b) bivalves, and

c) amphipods binned into 5 log-bins. Mean and median values

reported represent the geometric mean and medians after estimates

greater than 100% were set to 90%.

Figure 4.1
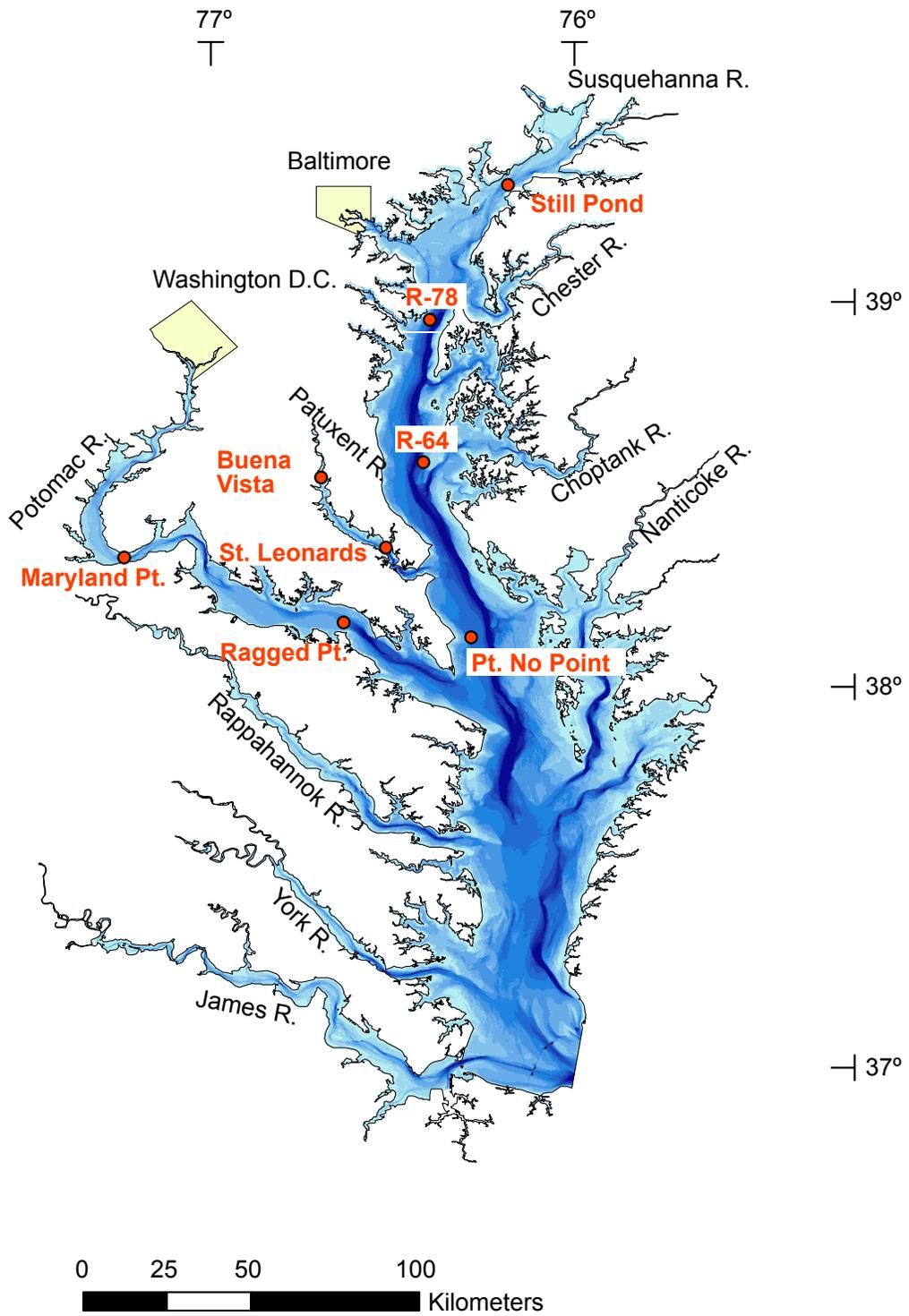
Figure 4.2
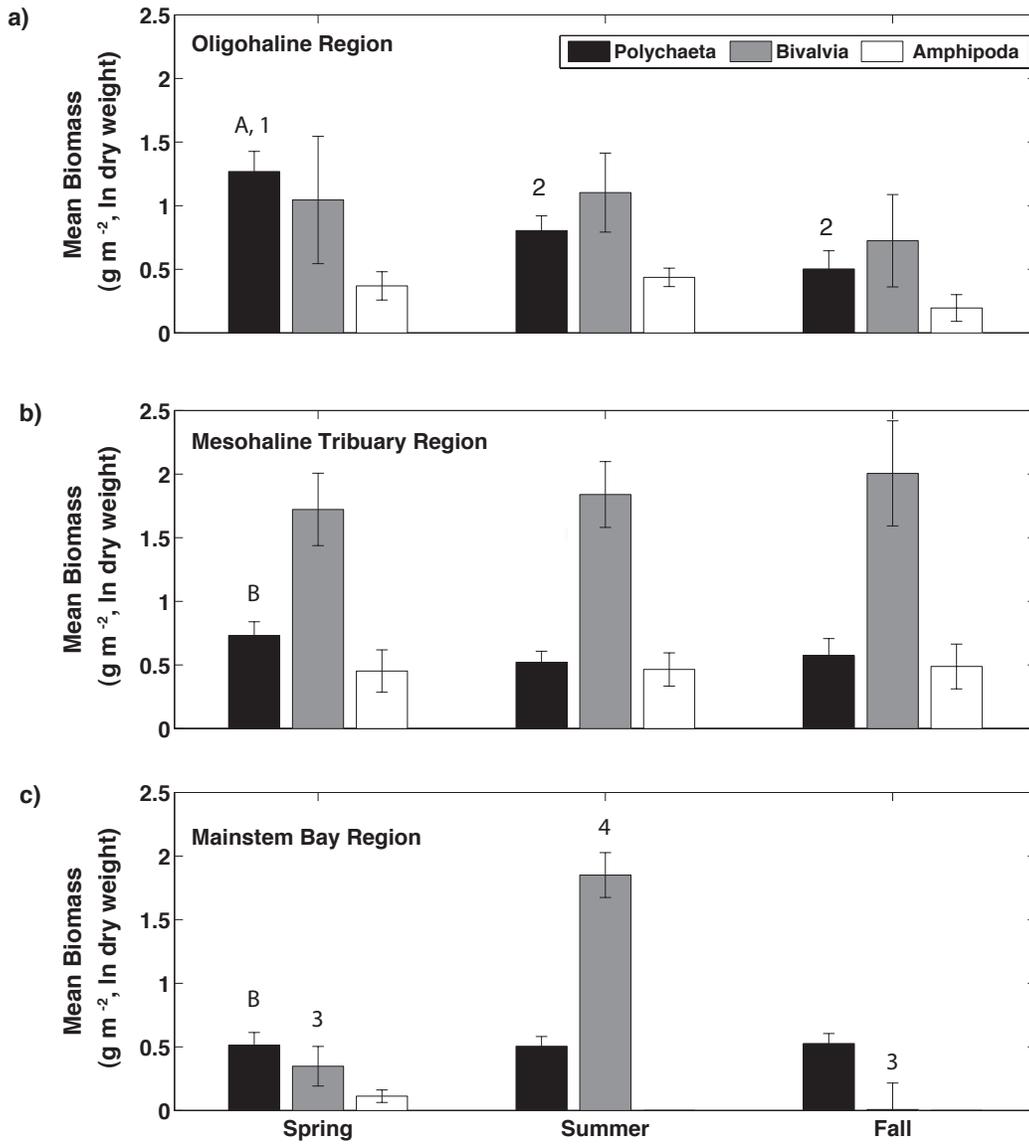
Figure 4.3
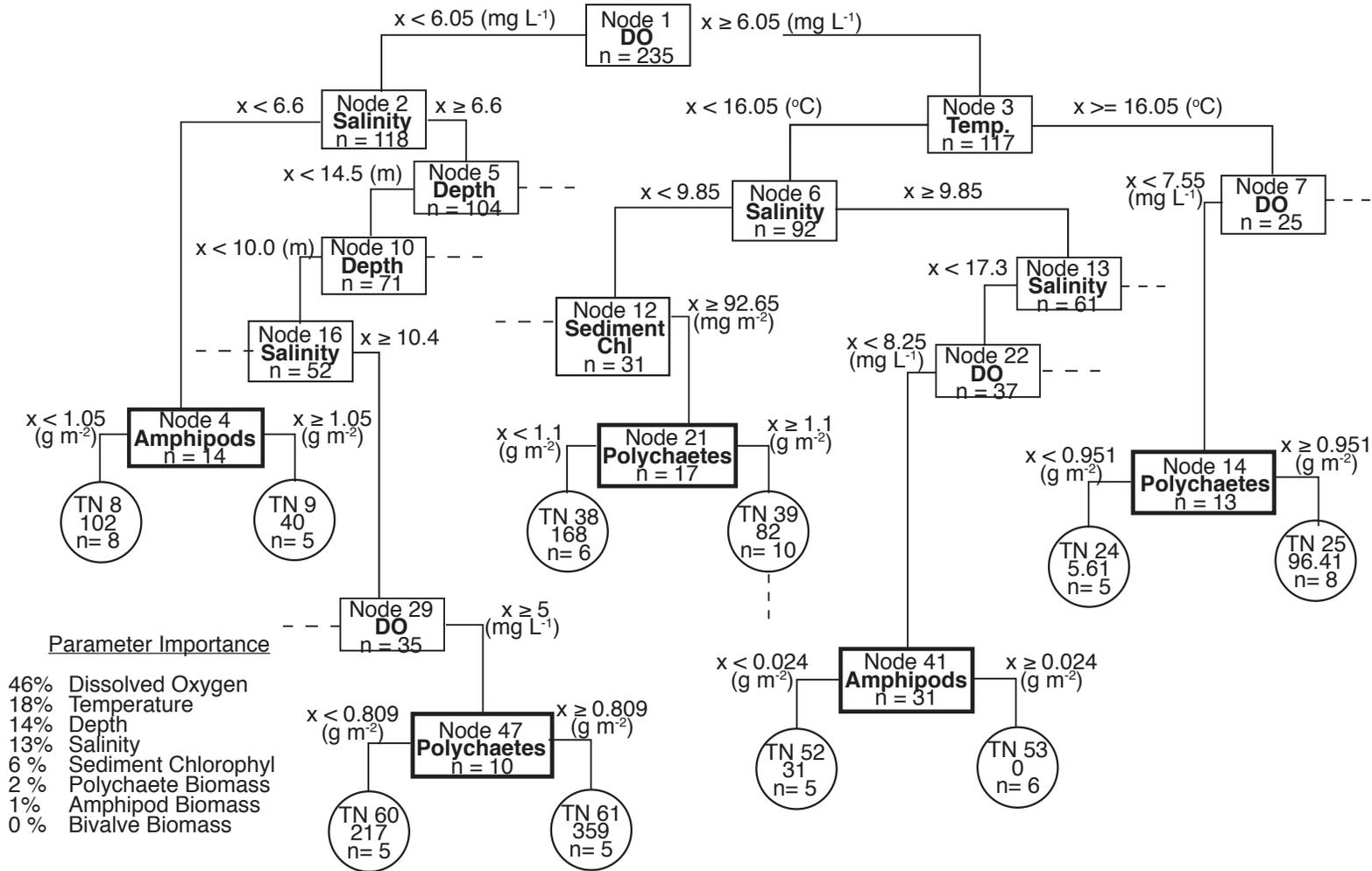
Figure 4.4

Figure 4.5

Figure 4.6

N$_2$–N Flux vs Denitrification Index

Figure 4.7

Spring NH$_4^+$ Flux and Denitrification Index vs Biomass

a)

b)

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Figure 4.8

Summer NH$_4^+$ Flux and Denitrification Index vs Biomass

a)

NH$_4^+$ Flux (μmol N m^{-2} h^{-1})

- ● Normoxic
- ★ Hypoxic
- ▲ Anoxic

b)

Denitrification Index (μmol N m^{-2} h^{-1})

Biomass (g m^{-2}, log dry weight)

Figure 4.9

Fall NH$_4^+$ Flux and Denitrification Index vs Biomass

a)

NH$_4^+$ Flux (μmol N m^{-2}h^{-1})

Legend:
- $\geq$ 16.05^{O}C
- $<$ 16.05^{O}C

$y = -280x + 349, r^2 = 0.36$

b)

Denitrification Index
(μmol N m^{-2}h^{-1})

$y = 280x - 125, r^2 = 0.55$

Biomass (g m^{-2}, log dry weight)

Figure 4.10

NH$_4^+$ Flux and Denitrification Index vs Amphipod Biomass

a)

NH$_4^+$ Flux (μmol N m^{-2} h^{-1})

Fall
Spring
Summer

$y = -791x + 560$, $r^2 = 0.50$

b)

Denitrification Index
(μmol N m^{-2} h^{-1})

$y = 772x - 550$, $r^2 = 0.40$

Biomass (g m^{-2}, log dry weight)

Figure 4.11

NH$_4^+$ Flux and Macrofaunal Group Composition

Figure 4.12

Richness vs Mean NH$_4^+$ Flux

$y = -14x + 263$, $r^2 = 0.66$

y-axis: **NH$_4^+$ Flux (μmol N m^{-2} h^{-1})**

x-axis: **Species Richness**

Figure 4.13

Distribution of % Excretion of Total NH$_4^+$ Flux

Polychaete

Mean 12%
Median 4%

Bivalve

Mean 20%
Median 6%

Amphipod

Mean 16%
Median 4%

Number of Occurences

0–20   21–40   41–60   61–80   >81

Calculated NH$_4^+$ Excretion / Total Measured NH$_4^+$ Flux, %

CHAPTER 5:

Summary and Synthesis

Spatial and temporal expansion of hypoxia in Chesapeake Bay has been accompanied by changes in the benthic macrofaunal community (Holland et al. 1987, Diaz & Rosenberg 1995, Kemp et al. 2005, Long & Seitz 2009). In aquatic systems macrofaunal bioturbation and bioirrigation play important roles in organic matter processing and nutrient cycling at the sediment-water interface (Rhoads et al. 1978). Eutrophication of estuarine systems like Chesapeake Bay can alter benthic community structure and function through the effects of organic enrichment and hypoxia. The motivation behind this dissertation was to explore the feedback loop between changes in the benthic macrofaunal, specifically polychaete, community relative to eutrophication and climate change, and sediment nitrogen cycling in their environment.

In Chapter 2, using long-term monitoring data collected in the Chesapeake Bay, I explored changes in the mesohaline polychaete community and characterized the regional habitat preferences of today's dominant species. Over the past two decades, small opportunistic polychaete worms (*M. viridis, S. benedicti, H. filiformis, A. succinea)* have dominated the polychaete abundance and biomass in the mesohaline Bay, with the historically important larger longer-lived, but hypoxia-sensitive, species (e.g. *Glycinde solitaria, Mediomastus ambiseta, Loimia medusa*) being virtually extirpated (Llanso 1992, Diaz & Rosenberg 1995). This change in community structure over the several decades appears to be related to eutrophication and hypoxia in the region since the 1950's and 1960's (Kemp et al. 2005). Despite the ability of these now dominant opportunistic macrofauna to adapt to a wide range of environmental parameters,

CART analysis revealed key environmental factors that regulate the densities of these opportunistic polychaetes. For the 13-year period (1981-1993) during which mcarofauna abundance was monitored seasonally and across depths, these analyses showed that fluctuations in temperature and salinity influence spring recruitment of polychaetes that inhabit shallow and mid-depth zones in the mesohaline Bay. At depths greater than 10 m, my analyses strongly suggested that dissolved oxygen is the dominant parameter shaping the benthic community structure, particularly in the summer. While temperature and salinity ranges may influence macrofaunal distribution at shallower depths, low DO is the key environmental variable regulating polychaete abundance in deeper waters of the mesohaline Chesapeake Bay.

The two key roles polychaetes play in the estuarine ecosystems are as prey for secondary consumers and as facilitators of biogeochemical cycling of nutrients in sediments (Rhoads et al. 1978, Welsh 2003). As members of the food web, polychaetes are an important food source for demersal fish and mobile invertebrates, like crabs and snails. Polychaetes also stimulate microbial remineralization of organic matter in estuarine sediments and increase the recycling and release of inorganic nitrogen solutes (e.g., ammonium and nitrate) to the water column through bioturbation activities. Other experiments have demonstrated how macrofaunal bioirrigation enhances the coupling of nitrification and denitrification, which effectively reduces nitrogen pollution by removing excess fixed nitrogen from coastal waters (Kristensen 1984, Aller 1994, Kristensen 2000, Welsh 2003). Much of this work has been done with larger

animals that dominated the macrofaunal community 30-40 years ago. Although the role of polychaetes in sediment biogeochemical processes has been characterized for surprisingly few species (Diaz & Rosenberg 1995, Kristensen 2000, Kristensen et al. 2012), macrofauna influence on sediment biogeochemical processes have been attributed to differences in feeding and bioirrigation behaviors (Kristensen 1983b, a, Papaspyrou et al. 2006).

Further investigating the role of polychaetes in sediment biogeochemical processing of nitrogen in Chapter 3 laboratory experiments, I used an opportunistic nereid polychaete *Alitta (Neanthes) succinea*, that is the largest of the four dominant species now found in the Bay and many other estuarine systems (Jorgensen & Kristensen 1980, Miron & Kristensen 1993). Shown to repopulate defaunated areas following major perturbations, *A. succinea* is an opportunistic worm that withstand temporary hypoxia (Kristensen 1983a). Until now, biogeochemical effects of this species' bioturbation activity have, however, not been well described (Fauchald & Jumars 1979, Holland et al. 1987, Llanso et al. 2002). The results of my laboratory experiments with this polychaete have improved our understanding of the relationships among oxygen (O_2), polychaete density, and nitrogen cycling and can help refine biogeochemical models of coastal ecosystems. This study showed that polychaete enhancements of O_2 and nitrogen fluxes were strongly correlated with total animal biomass. As suggested by my analysis of historical *A. succinea* populations (Chapter 2), these animals are relatively tolerant of short-term hypoxic events, making them important players in spring and summer remineralization of organic material. These

laboratory experiments also support the hypothesis that macrofaunal effects on solute fluxes decline as overlying water O_2 levels decrease to hypoxic levels. Fluxes of O_2, NH_4^+ and N_2 were stimulated by presence of animals for both larger and smaller worms, but per capita effects were greater for the deep-burrowing larger polychaetes. This is an important result with in the context of the shift in Chesapeake Bay macrofaunal dominance towards smaller species.

The results of Chapter 3 experiments further explain the effects of worm size on sediment biogeochemical processes. Compared to small polychaetes, larger animals burrow deeper in sediments, irrigate greater volumes of water and solutes through their burrows, but excrete less NH_4^+ per unit biomass. These larger worm attributes tend to enhance rates of sediment nitrification and denitrification, effectively helping to remove fixed-nitrogen from nutrient-rich environments. Smaller opportunistic worms that often dominate eutrophic systems that regularly experience low-O_2 conditions, on the other hand, have limited impact on denitrification. Thus, the hypoxia-induced shift from larger to smaller polychaetes, and the associated decline in nitrification and denitrification rates, represents a "positive feedback" process. High nutrient levels stimulate low O_2, which selects for smaller polychaetes that stimulate efficient nitrogen recycling, which in turn reinforces the eutrophication process (Kemp et al. 2005, Conley et al. 2007).

Chapter 4 brings together elements of the historical analysis in Chapter 2 as well as the laboratory experimental results of Chapter 3 by examining statistically the relationship between macrofaunal biomass and solute fluxes from

intact sediment cores. A previous analysis of a 14-year (1971-1984) time-series

of the mesohaline Bay macrofaunal community (Holland et al. 1987) suggested

that salinity and DO were major factors in regional distributional patterns of

macrofauna and that food availability limited annual recruitment. The observed

increase in salinity and decrease in DO over the study period was accompanied by

an increase in opportunistic species, like polychaetes, suggesting that the observed

changes may have played a role in the decline of equilibrium species (Holland et

al. 1987). Additionally, Chapter 4 analyses revealed that regional ranges in

temperature and salinity largely control the benthic species composition and the

magnitude of NH_4^+ efflux. In the spring, there is generally an increase in NH_4^+

efflux with an increase in benthic macrofaunal biomass. However, rates of NH_4^+

efflux in some salinity regions appear to be temperature controlled. In the

summer, low DO controlled the dominant pathway of nitrogen cycling by limiting

nitrification and favoring the efflux of NH_4^+. In turn, DO also shaped the benthic

community composition, particularly in deeper regions that experience hypoxia.

As the annual cycle of estuarine production declined in the fall, but temperatures

remained relatively warm, a negative relationship between biomass and NH_4^+ flux

suggested increases in biomass may aid in the shunting of nitrogen from recycling

pathways to favor production of gaseous forms through coupled nitrification-

denitrification. A fall increase in species abundance in some areas, particularly of

deposit feeding polychaetes, also corresponded to a decline in NH_4^+ efflux.

Chapter 4 also brought to light while polychaetes are important members of the benthic community; an increase in species diversity had a positive effect on the removal of fixed nitrogen from the system (Fig 4.11). Increased diversity brought together animals that interact with the sediment in varying space (burrow depth) and time (ventilation frequency) dimensions maximizing remineralization pathways. CART analysis suggested polychaetes and amphipods had the strongest relationship with NH_4^+ flux. In higher abundances, particularly in fresher parts of the estuary, amphipods can have a strong impact on coupled nitrification-denitrification (Fig. 4.10). Bivalves contribute greater ammonium excretion per biomass allometrically and, through their filter feeding, they also process organic material from the overlying water, but not the sediment. These activities make their relationship with sediment NH_4^+ flux relatively weak. Analysis of the benthic community species composition ultimately suggested that not only species diversity but also the dominant presence of animals within the deposit-feeding guild have the greatest impact on sediment nitrogen cycling processes.

Long-term benthic datasets, like the two used in this dissertation, are rare and valuable. They are sometimes characterized by inconsistencies in sampling frequency and methods, but they can provide a unique understanding of changes in the benthic ecosystem. By examining these data in conjunction with other environmental datasets, insight can be gleaned into benthic community shifts that are correlated with other environmental parameters and ecological changes in the Bay. Information on the patterns of long-term in situ variation is essential in

quantifying and confirming the complex relationship between macrofauna (specifically polychaetes) and sediment biogeochemistry under changing environmental conditions. The research conducted in this dissertation has shown that a complex balance between seasonal and regional environmental conditions, coupled with the benthic community's the species richness and feeding guilds, control the relationship between macrofaunal biomass and sediment nitrogen flux. Despite changes in benthic community composition due to eutrophication and increased hypoxia in Chesapeake Bay, work presented here suggests the benthic polychaete community continues to serve as important facilitators of sediment biogeochemical processes

Despite the continued positive influence of macrofauna on sediment biogeochemistry, research suggests that the Chesapeake Bay ecosystem is still out of ecological balance. Natural resource managers strive to get the system back in healthy nutrient balance through restoration efforts like reducing the runoff of nutrients into the Bay, restoring habitats by planting seagrasses, and seeding regions of the Bay with oysters. The results of this dissertation suggest polychaete worms also play an important role in balancing the fate of nutrient inputs to the estuary; however, animal size and density are key factors that dictate their significance. While the volume of summer hypoxic water in the Bay may be of interest to the modeling community, the analysis of regional habitats of polychaetes in Chesapeake Bay (Chapter 2) suggests the spatial footprint of the hypoxic water impacts the benthic ecosystem ecology. A management goal of gradually approaching a threshold of a limited hypoxic water footprint may help

the Bay achieve a more healthy nutrient balance by providing a habitat for sustained benthic populations. Studies around benthic ecological succession show the initial dominance of opportunistic species in an environment. Over time, as the ecosystem becomes more stable, the benthic community composition evolves to sustain greater populations of longer-lived, larger species (Gray & Elliott 2009). These deeper burrowing animals can further facilitate the diagenesis of organic material and balance of nutrients in sediments. The analysis of historical data suggests there is a seed population of larger, longer-loved species residing in the Bay. To facilitate the process of succession, the Bay may benefit from a seeding of longer-lived deposit feeders, namely polychaetes, in areas showing signs of improvement. Shallow waters and tributaries are often easier to restore than deeper habitats. Hypoxia tends to be less common in these areas making restoration efforts involving deposit-feeders potentially more successful.

Future benthic ecology research in Chesapeake Bay should aim to characterize habitats the mid-depth (5-10 m) region of the Bay where periodic exposure to hypoxic water degrades ecosystem health. Predation effects on macrofauna should also be investigated, particularly looking for impacts due to a hypoxia induced habitat squeeze at those mid-depths. The design of the CBP benthic monitoring program between 1981 and 1993 is an example of an optimal sampling frequency for seasonal review of the benthic community. Recognizing the limited financial and personnel resources of the Chesapeake Bay Program, an ideal benthic monitoring program would sample targeted depth and salinity zones over at least 7 months every five years. In between those more intensive

sampling years, the monitoring would continue with a stratified, random sampling

protocol over representative depth and salinity regions of the Bay in the spring

and fall. In addition, there should be an intensive examination of changes in the

Bay's ecosystem to detect impacts of ongoing restoration and load reduction

efforts along with interannual fluctuations and long-term changes in climate

conditions. This monitoring design would allow the program to conserve costs

but still track changes in the distribution of species and provide increased data for

modeling food webs and biogeochemical process of the Chesapeake Bay.

Appendix I: Chapter 2 CART Analysis Full Regression Trees

Figure 2.6 of Chapter 2 showed portions of example seasonal CART analysis for abundance of *M. viridis, S. benedicti, H. filiformis, and A. succinea*. This appendix includes a series of statistics tables for each CART and the CART analysis full regression trees for Figure 2.6. AI.1), Late Summer *M. viridis* AI.2) Early Summer *S. benedicti*, AI.3) Spring *H. filiformis*, and AI.4) Early Summer *A. succinea*. Within each node box is the splitting parameter (x) and the number of samples (n) that were split. Along each connecting line is the splitting threshold for the node parameter. Parameters include temperature (temp.) (^{o}C), salinity (psu), % silt-clay, dissolved oxygen (DO) (mg L^{-1}) , and Region. Region numbers correspond to indexes in Fig 2.1. Terminal Node values contain the mean abundance (natural log # indiv. m^{-2}) and the number of samples (n) contained in that node.

Table A1.1: CART Regression Tree Statistics

a) **_M. viridis_- Spring**

Linear regression model: y ~ 1 + x1

Estimated Coefficients:		Std Error	t-Stat	p-value
(Intercept)	2.13	0.10	20.72	1.69E-77
x1	0.60	0.02	35.40	2.98E-169

Number of observations:	850			
Degrees of Freedom:	848			
RMSE:	1.5			
r^2:	0.6			
F-statistic vs. constant model:	1250	p-value =	2.98E-169	

b) **_S. benedicti_- Early Summer**

Linear regression model: y ~ 1 + x1

Estimated Coefficients:		Std Error	t-Stat	p-value
(Intercept)	3.37	0.11	29.39	2.92E-112
x1	0.31	0.02	15.24	1.50E-43

Number of observations:	518			
Degrees of Freedom:	516			
RMSE:	1.22			
r^2:	0.31			
F-statistic vs. constant model:	232	p-value =	1.50E-43	

c) **_H. filiformis_- Spring**

Linear regression model: y ~ 1 + x1

Estimated Coefficients:		Std Error	t-Stat	p-value
(Intercept)	1.63	0.08	19.40	1.20E-69
x1	0.64	0.02	38.86	1.68E-190

Number of observations:	850			
Degrees of Freedom:	848			
RMSE:	1.12			
r^2:	0.64			
F-statistic vs. constant model:	1510	p-value =	1.68E-190	

d) **_A. succinea_- Early Summer**

Linear regression model: y ~ 1 + x1

Estimated Coefficients:		Std Error	t-Stat	p-value
(Intercept)	2.12	0.08	27.93	3.01E-105
x1	0.30	0.02	14.93	3.56E-42

Number of observations:	518			
Degrees of Freedom:	516			
RMSE:	0.998			
r^2:	0.3			
F-statistic vs. constant model:	223	p-value =	3.56E-42	

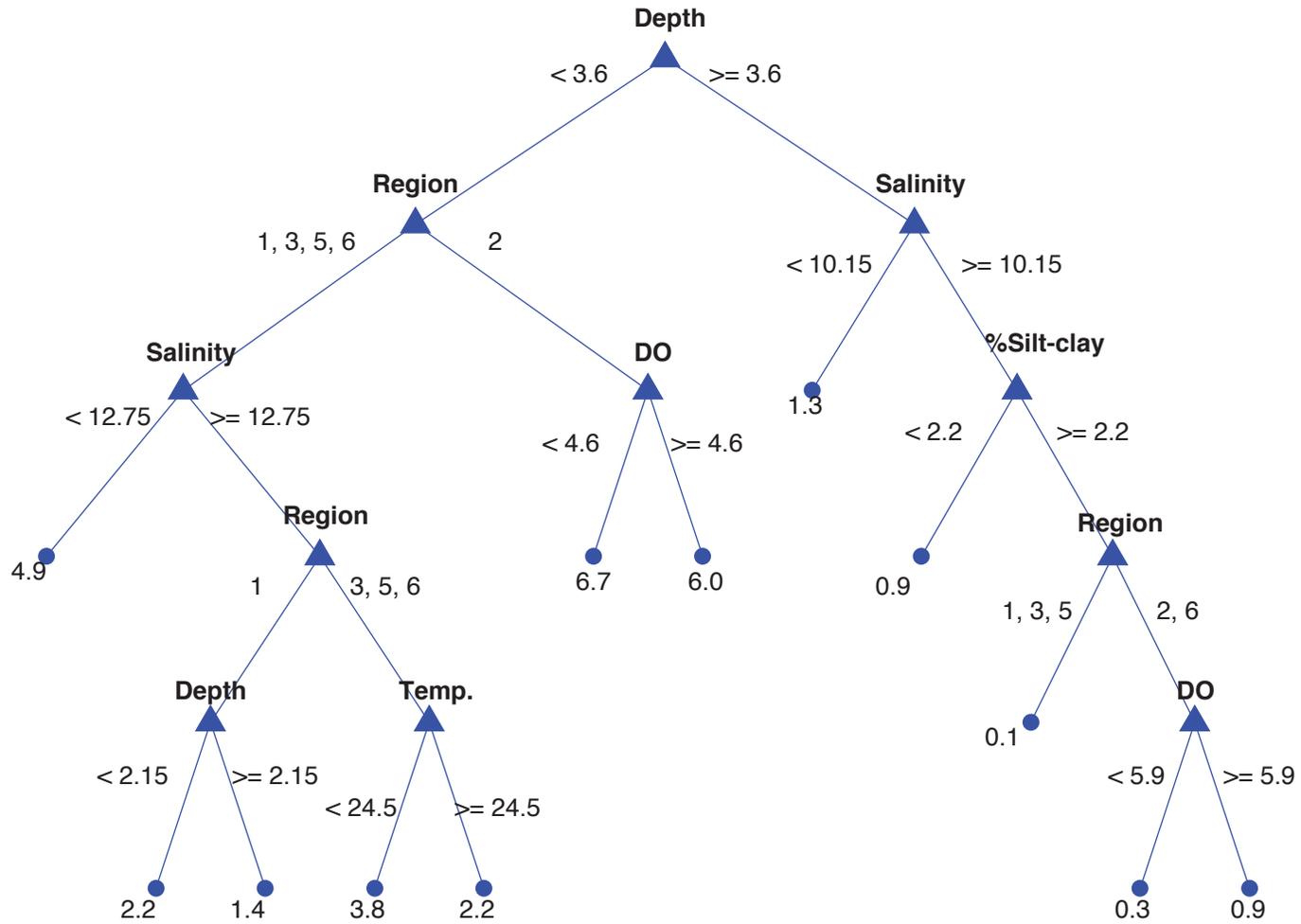Figure AI.1: Late Summer CART - *Marenzelleria viridis*

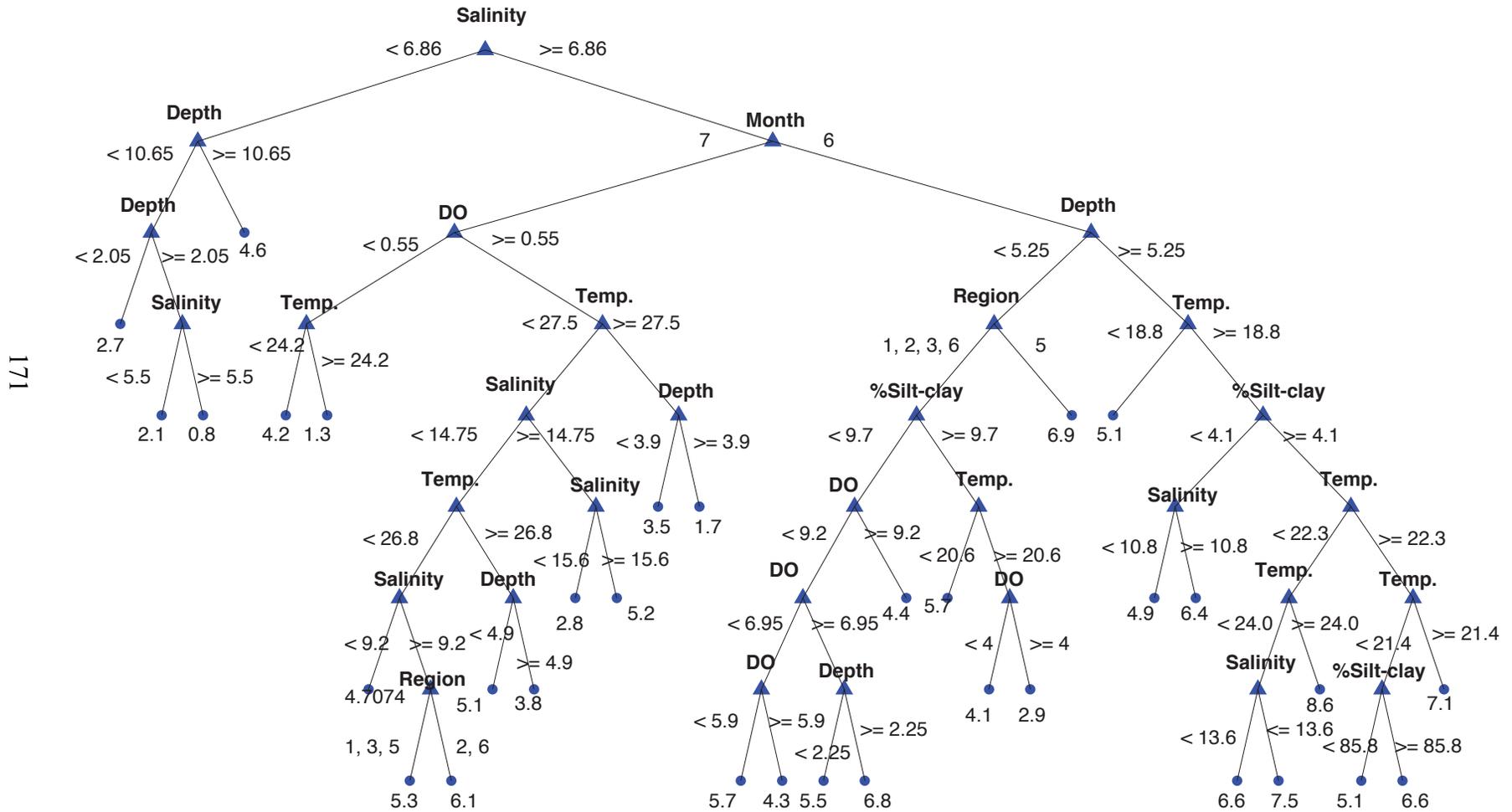Figure AI.2: Early Summer CART - *Streblospio benedicti*

Figure AI.3:   Spring CART - *Heteromastus Filiformis*
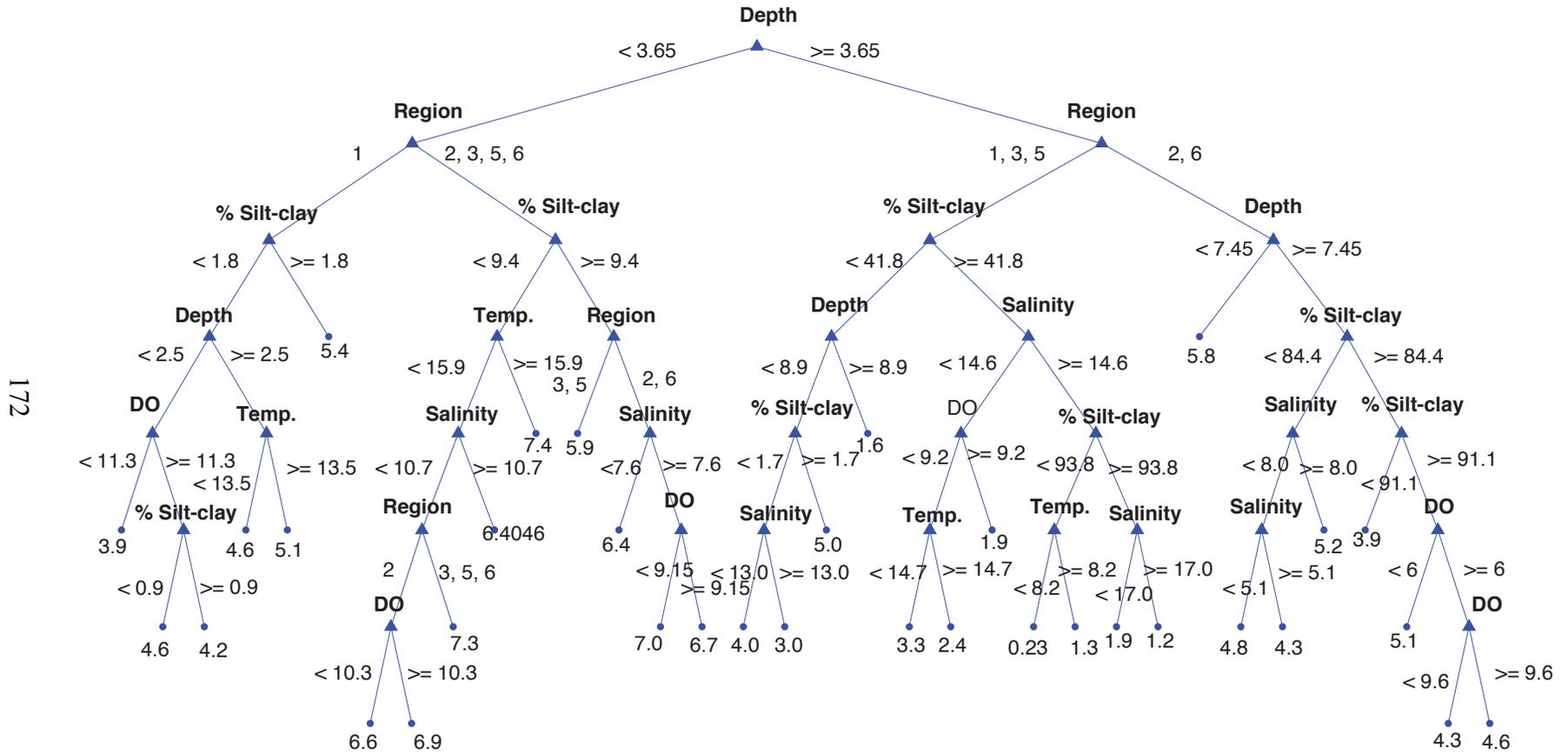
Figure AI.4: Early Summer CART - *Alitta succinea*
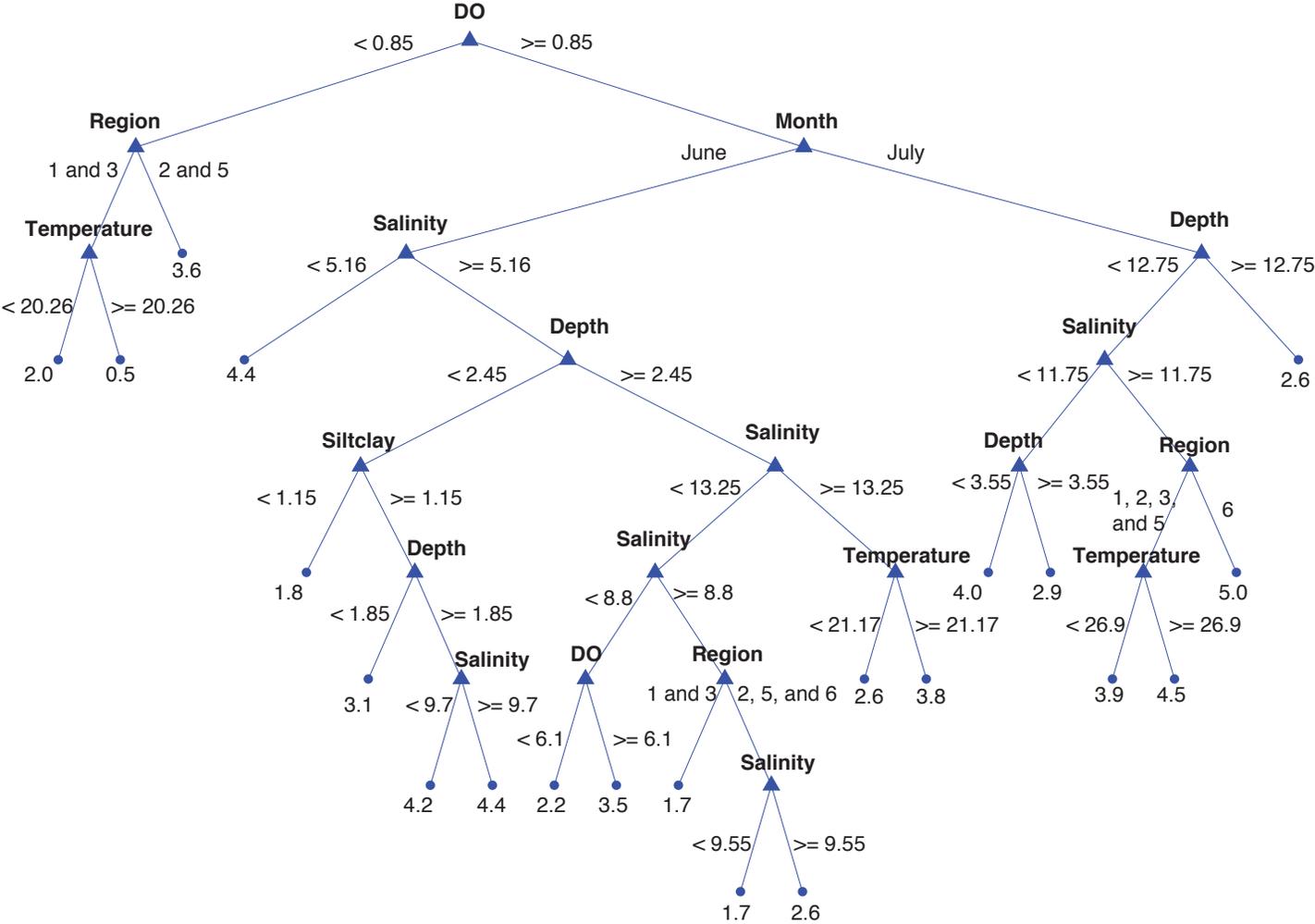
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