ABSTRACT

Title of Dissertation:	STABLE NITROGEN ISOTOPES ($\delta^{15}N$) IN THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) AS AN INDICATOR OF NITROGEN SOURCE
	Benjamin Meir Fertig, Doctor of Philosophy, 2010
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This dissertation demonstrates that stable nitrogen isotope signatures ($\delta^{15}N$) in oysters (*Crassostrea virginica*) can identify anthropogenic nitrogen sources (a cause of degraded water quality) at multiple spatial scales in Chesapeake Bay and Maryland's Coastal Bays. Fieldwork, monitoring and land use data, spatial analyses, and modeling techniques were employed. Due to minimal tissue $\delta^{15}N$ variations between individuals as replicates (standard error < 0.5 ‰), a sample size of five individuals optimally balanced error with effort. Transplantation verified convergence of oyster $\delta^{15}N$ after changes in nitrogen source while modeling quantified temporal integration (four months for muscle, two to three months for gill and mantle) and measurements over two years demonstrated seasonal $\delta^{15}N$ increases in seston (summer) and oysters (winter). At the small scale (10s of km^2), oyster tissues in Monie Bay's creeks (varying by watershed land use) were dominated by

anthropogenic nitrogen transported to Monie Bay from Wicomico River whose watershed inputs were predominantly manures (6.8×10^4 to 2.4×10^6 kg N yr^{-1}), not sewage (2.0×10^5 kg N yr^{-1}) or septic (1.1×10^5 kg N yr^{-1}). This has large implications for Delmarva Peninsula: home to 4,630 poultry feeding houses (generating 3.9×10^6 to 1.3×10^8 kg N yr^{-1}) and 1.2×10^6 people (combined sewage and septic generating 3.7×10^6 kg N yr^{-1}), thus a poultry:human nitrogen generation ratio of 1:1 to 91:1. At the medium spatial scale (100s of km^2), water quality in Maryland's Coastal Bays was susceptible to runoff. Macroalgae $\delta^{15}N$ (*Gracilaria* sp.) responded rapidly (4 days) over 100s of km^2, while oyster $\delta^{15}N$ responded slowly (2 months) over 10s of km^2. Broadly, in Chesapeake Bay (large scale, 10,000s of km^2), oyster $\delta^{15}N$ was correlated to land use, stream and tributary water quality, and it reflected tributary wastewater plumes. The overall oyster $\delta^{15}N$ gradient (16.0‰ in Eastern Bay, 8.3‰ in Lynnhaven River) decreased with flushing time, with increased salinity, and with increased shell height. Denitrification remains potentially confounding as it elevates nitrate $\delta^{15}N$ signals, potentially before oyster assimilation (via plankton). Nevertheless, oyster $\delta^{15}N$ is a powerful tool for indicating nitrogen sources across spatial and temporal scales.

STABLE NITROGEN ISOTOPES ($\delta^{15}N$) IN THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) AS AN INDICATOR OF NITROGEN SOURCE

By

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

To my wife, Elana: deeply grounded, steadfast, and encouraging, always urging me to push harder, more than I ever knew possible.

And:

To He Who Shall Be Named: in anticipation of years of laughter and wisdom to carry on,

L'Dor va Dor.

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CHAPTER 1: INTRODUCTION

This dissertation examines distributions of stable nitrogen isotope values ($\delta^{15}N$) in eastern oyster (*Crassostrea virginica*) tissues to investigate the potential of this species to serve as a bioindicator of nitrogen sources. Nitrogen has been identified as a limiting nutrient and a key factor in eutrophication in many estuarine ecosystems (e.g. Vitousek et al. 1997, Kennish 2002, Bricker et al. 2008,) including Chesapeake Bay (e.g. Horton 2003, Kemp et al. 2005, Fisher et al. 2006) and Maryland's Coastal Bays (Wazniak et al. 2007, Dennison et al. 2009).

The current conceptual model of eutrophication (Cloern 2001) includes linkages to degraded water quality and hypoxia (Paerl et al. 1998, Smith et al. 1999), (in some cases harmful) algal blooms (Paerl 1997, Anderson et al. 2002, Glibert et al. 2007), changes in seagrass populations (Kiddon et al. 2003, Orth and Moore 1987), and declines in diversity/ abundance of fisheries (Boynton 1993, Wazniak and Glibert 2004, Tyler 2007). Numerous anthropogenic nitrogen sources are delivered to estuaries, including wastewater effluents, septic systems, animal manures, runoff from agricultural and residential fertilizers (both synthesized and manures), atmospheric deposition. (Valiela 1995, Schlesinger 1997, Vitousek et al. 1997).

Once mixed in estuaries, nitrogen can be difficult to link back to specific sources, which is often overlooked in monitoring programs. Yet tracking and mapping nitrogen sources through the utilization of $\delta^{15}N$ bioindicators could target

nutrient reduction efforts and potentially help reach established nitrogen management and restoration goals (Boesch et al. 2001).

Nitrogen measurements, sources, transport and cycling

Nitrogen has a complicated biogeochemical cycle (Figure 1.1) that includes numerous transport and transformation mechanisms between global and local sources and sinks (Valiela 1995, Schlesinger 1997). The major reservoir of nitrogen is the atmosphere, in the form of N_2, which is stable and generally biologically unreactive due to a triple bond. N_2 is made more biologically available to terrestrial and aquatic ecosystems naturally by fixation, i.e. transformation to other compounds by a few species including nitrogen-fixing cyanobacteria, legumes that host rhizobium bacteria in root nodules, and certain trees (Schlesinger 1997). Additionally, the Haber-Bosch process is an industrial method for nitrogen fixation and has been employed to yield munitions and synthetic fertilizers, among other products (Smil 2000). Anthropogenic nitrogen fixation, including soybean cultivation, now exceeds natural nitrogen fixation processes on a global scale (Galloway et al. 2004).

Associated human activities reliant on industrial nitrogen fixation (e.g agriculture) are increasingly dominating the global nitrogen cycle, resulting in accumulation of reactive nitrogen in most environmental reservoirs (Galloway et al. 2004). Aside from atmospheric N_2 gas, nitrogen bonds with hydrogen and oxygen through various processes including nitrification (oxidation to nitrate),

ammonification (reduction to ammonium), and volatilization (phase transformation to gas), and is most available biologically as ammonium (NH_4^+) and nitrate (NO_3^-).

Nitrogen applied agriculturally can remain in soils, be incorporated by primary producers (crops), or oxidized into highly soluble nitrate and exported to aquatic ecosystems via overland flow, groundwater, or surface water, eventually entering surface water, estuaries, and coastal waters. There, it can be taken up by aquatic primary producers and subsequently assimilated by consumers (which lose nitrogen via excretion and defecation), both of which can be consumed or decay and either enter the dissolved organic nitrogen pool or be regenerated as NH_4^+. Denitrification and annamox (an abbreviation for ANaerobic AMMonium OXidation) complete the cycle, returning inorganic nitrogen to the atmosphere as unreactive N_2.

Denitrification is a multi-step reduction of nitrate (NO_3^-) or nitrite (NO_2^-) to nitric (NO) and nitrous (N_2O) oxides and further reduction to unreactive nitrogen gas (N_2) thereby returning nitrogen to the atmosphere. Annamox was recently discovered (Mulder et al. 1995) to directly reduce ammonium (NH_4^+) and nitrite (NO_2^-) to nitrogen gas (N_2) and water (H_2O). These reactions are mediated by microbial heterotrophs under anaerobic conditions (Arrigo 2005) with a sufficient carbon supply, as the bacteria use these compounds as terminal electron acceptors in the absence of oxygen. Globally, denitrification is most prevalent in terrestrial ecosystems, in suboxic oceanic water columns, and in continental shelf sediments but only minimally in estuaries (Wada et al. 1975, Seitzinger et al. 2006). Possibly, coupling of denitrification and annamox may be the dominant process for nitrogen

removal from the world's marine oxygen minimum zones (Gruber and Sarmiento 1997).

Ecologically, denitrification has remained an elusive aspect of the nitrogen cycle for three main reasons: 1) Very high precision is required as the small signal of N_2 production is difficult to measure accurately with a N_2 dominated atmospheric background (Davidson and Seitzinger 2006, Groffman et al. 2006) and older techniques (acetylene inhibition) underestimated rates compared to newer techniques (membrane inlet mass spectrometry; Seitzinger et al. 1993, Kana et al. 1994, Cornwell et al. 1999). 2) Environmental drivers have not fully been identified (Cornwell et al. 1999), though water residence time is recognized as one such factor due to rate-limiting nitrate diffusion across the water-sediment interface (Dettman 2001, Sebilo et al. 2003, Seitzinger et al. 2006, Klocker et al. 2009). 3) Denitrification rates are highly variable both spatially (e.g. at the km scale; Scala and Kerkhof 2000) and temporally (low in spring and summer; Kemp et al. 1990, Chen et al. 2009).

Isotope cycling and biogeochemistry

In addition to its biogeochemical cycle, nitrogen has several isotopes (nitrogen atoms with seven protons but various numbers of neutrons yielding atomic masses of 10 to 25). Of the ten nitrogen isotopes produced synthetically, ^{13}N (6 neutrons) has a half-life of less than 10 minutes and is used in medicine to label ammonia for positron emission tomography (PET, e.g. Muzik et al. 1993) while the others have half-lives on the order of seconds or less. In contrast, both ^{14}N (7 neutrons) and ^{15}N (8 neutrons)

are stable and are the current focus. For definition, $\delta^{15}N$ (units of per mille, ‰) is the ratio of atomic ratios ($R = {}^{15}N/{}^{14}N$) in a sample compared to a standard, which is amplified partly to help visualize very precise and subtle numerical differences (Equation 1). Atmospheric N_2 is the standard reference for comparison, with ^{15}N abundance of 0.3663% and is defined as 0 ‰ with instrumental error approximately ± 0.2 ‰ (e.g. Fry 2006).

$$\delta^{15}N = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 10^3 \qquad \text{Equation 1.1}$$

Two main processes can alter $\delta^{15}N$ values throughout various aspects of the nitrogen cycle: *mixing* (which yields $\delta^{15}N$ values intermediate with respect to its original constituents) and *fractionation* (which yields products with values both enriched and depleted in $\delta^{15}N$ relative to the starting value).

While mixing effects on $\delta^{15}N$ values are relatively intuitive, Isotopic fractionation occurs when nitrogen bonds are broken or formed during chemical reactions, and most isotope effects discriminate against the heavier isotope due to a faster kinetic reaction rate for the 'lighter' ^{14}N as compared to the 'heavier' ^{15}N; the latter requires slightly larger activation energy (Fry 2006). Since a particular pool of nitrogen undergoing a reaction contains ^{14}N and ^{15}N, the ratio of their kinetic reaction rate constants yields a fractionation factor for that reaction; in other words, a difference in $\delta^{15}N$ value due to that reaction. For example, from the fractionation factor which occurs during digestion and waste elimination, there is a mean enrichment or 'trophic shift' of ~ +3.4 ‰ at each trophic step (Minagawa and Wada

1984, Adams and Sterner 2000) due to preferential loss of the lighter ^{14}N during deamination. Variations in trophic shifts are associated with the processes of nitrogen assimilation and excretion (Vanderklift and Ponsard 2003) or sample type and method (McCutchan et al. 2003). Values of $\delta^{15}N$ at the food chain base are used to establish organisms' trophic positions and food webs (Pasquaud et al. 2007).

Fractionation effects can be utilized to track human or animal wastes (Figure 1.2) since these nitrogen sources become enriched in $\delta^{15}N$ relative to food to measured values ranging 5 to 8 ‰, (Sweeny and Kaplan 1980, Tucker et al. 1999, Leavitt et al. 2006, Kendall et al. 2007) during a combination of ammonia volatilization and denitrification in waters contaminated with waste products (McClelland and Valiela 1998, Altabet 2006, Kendall et al. 2007). Volatilization is kinetically faster for ^{14}N and denitrifying microbes preferentially reduce ^{14}N (Wellman et al. 1968, Mariotti et al. 1982). Thus, both volatilization and denitrification processes discriminate against the heavier ^{15}N and enrich the remaining nitrogen with ^{15}N compared to the gaseous NH_3 (ammonia) or N_2 product (Miyake and Wada 1971, Cline and Kaplan 1975, Kendall 1998). Many wastewater treatment plants implement Biological Nutrient Removal, promoting microbially mediated denitrification to remove nitrogen at rates higher than those found in natural ecosystems and leaving effluent enriched in $\delta^{15}N$. In contrast, nitrogen fixation (both biological and industrial) does not result in measurable fractionation (Hoering and Ford 1959), thus fertilizers synthesized from atmospheric N_2 (0 ‰) have corresponding $\delta^{15}N$ values, generally ranging from -4‰ to +4‰ (Hübner 1986,

Macko and Ostrom 1994, Vitoria et al. 2004). In general, $\delta^{15}N$ values from these nitrogen sources can serve as comparative signatures that vary predictably through food chains (i.e. adjusted for fractionation due to assimilation and trophic shifts) and can be measured in selected bioindicator species. Since dissolved inorganic nitrogen can be transformed, fractionate, and fluctuate quickly (e.g. Horrigan et al. 1990), its $\delta^{15}N$ values reflect the very short-term and may be considered a 'snapshot' in time.

Bioindicator $\delta^{15}N$ can determine nitrogen sources

The expanding literature on bioindicator $\delta^{15}N$ suggests that organisms in a variety of taxonomic groups can identify anthropogenic nitrogen sources. Examples include: macrophytes (e.g. McClelland et al. 1997, Fourqurean et al. 1997, Costanzo et al. 2001, Cole et al. 2004, Cohen and Fong 2006, Benson et al. 2008), finfish (Lake et al. 2001, Schlacher et al. 2005), mollusks (Fila et al. 2001, McKinney et al. 2001), or a combination (Schlacher et al. 2001, Gartner et al. 2002, Fry et al. 2003).

Analyzing $\delta^{15}N$ in different functional groups can provide insight into nitrogen cycling and how human and animal wastes are incorporated into different subsets of the overall community within the ecosystem. Dissolved inorganic nitrogen concentrations and $\delta^{15}N$ values, along with particulate nitrogen $\delta^{15}N$ values, have shown the dominance of spring phytoplankton and fall microbially-mediated transformations in Chesapeake Bay (Horrigan et al. 1990, Montoya et al. 1990). Species from different functional groups have been able to provide insights into nitrogen source over different time scales (Gartner et al. 2002, Lorain et al. 2002),

and particulate nitrogen has been shown to change over roughly a week, with variability greater in spring than in fall (Montoya et al. 1990) suggesting greater influence on nitrogen cycling by phytoplankton and zooplankton in spring than fall. Macroalgae uptake nitrogen directly from the water column, generally preferring NH_4^+, and rapidly undergo nitrogen turnover. This integrates nitrogen sources over a period of days (Costanzo et al. 2001, Montoya et al. 1990), while shellfish integrate over months (Moore 2003) due in part to feeding on phytoplankton and rates of new tissue growth and tissue repair. Discrepancies between primary producer and primary consumer $\delta^{15}N$ values might be magnified by a time lag due to different rates or modes of assimilation (direct uptake from water column vs. indirect via consuming a mixture of microorganisms, phytoplankton, detritus and inorganic particles) to reflect ambient $\delta^{15}N$ (Langdon and Newell 1990, Cohen and Fong 2005). Primary producer $\delta^{15}N$ values might provide direct incorporation of dissolved inorganic nitrogen, but consumer $\delta^{15}N$ values may provide longer temporal integration.

Bioindicator applications in consumers can benefit from measuring $\delta^{15}N$ in multiple tissues within a species because nitrogen turnover rates vary by tissue, offering monitoring resolution at various time intervals within a single species (Figure 1.3). Australian Sydney rock oysters (*Saccostrea commercialis*) tissues have a nitrogen half-life of one week (mantle), two weeks (gills), and more than three months (adductor muscle; Moore 2003). Similarly, bioindicator studies could benefit from deployment of multiple species within a functional group because temporal integration varies among species (a pattern observed in mammals, birds, and

mollusks; DeNiro and Epstein 1981, Tieszen et al. 1983, Hobson and Clark 1992, Garton et al. 2005), thereby also offering monitoring resolution at desired intervals while controlling assimilation mechanism and avoiding potential confounding effects due to $\delta^{15}N$ trophic shifts. In either scenario, staggered deployment of bioindicators could provide a more complete inference of changes in nitrogen source to a biological community, e.g. the benthos, over weeks, months, or even a year (Fila et al. 2001, McKinney 2002, Moore 2003, Dattagupta 2004).

Interpreting $\delta^{15}N$ values in bioindicators requires caution because terrestrial nitrogen sources may have complicated pathways with the potential for modification of $\delta^{15}N$ values before reaching aquatic biological indicators (Kendall 1998, Altabet 2006, Fry 2006). Nitrogen from animal manures, for example, can be further fractionated during volatilization (Cline and Kaplan 1975, Kendall 1998, McClelland and Valiela 1998, Altabet 2006, Fry 2006), and dissolved nitrate can undergo denitrification in terrestrial ecosystems before transport to aquatic ecosystems (Mariotti et al. 1982, Shearer and Kohl 1988, Seitzinger et al. 2006). Further, $\delta^{15}N$ signatures may be modified by fractionation associated with the bioindicator species itself due to metabolic factors such as nutritional stress (Hobson et al. 1993, Fuller et al. 2005), weight change, life stage (Grant and Kopple 2009), gestation (Fuller et al. 2005), and starvation (Hobson et al. 1993, Haubert et al. 2005, Boag et al. 2006). Careful attention to species selection, bioindicator growth and %N is necessary to minimize these potential sources of error and confounding effects.

Oysters, macroalgae as bioindicators of nitrogen source

Ideally, candidate bioindicator species intended for deployment meet several pragmatic criteria. These species should be 1) able to be collected in sufficient quantities; 2) hardy and able to be transplanted across a variety of environmental regimes (e.g. salinity); 3) resistant to expected concentrations of anthropogenic nitrogen and yield minimal mortalities; 4) sedentary to facilitate deployment and collection; and 5) easily prepared for sample analysis to facilitate timely examination of numerous samples.

In addition to serving as representative primary producer and consumer respectively, the red macroalgae *Gracilaria* sp. and the native eastern oyster *Crassostrea virginica* meet all of these ideal criteria. Therefore, these two species were deemed suitable for analysis and potentially successful bioindicator species. *Gracilaria* sp. is found abundantly in natural communities along Maryland's Coastal Bays (Jones et al. 2004) and can thus be collected for deployment at desired locations. In contrast, *C. virginica* must be cultured for deployment in Chesapeake Bay and Maryland's Coastal Bays because historically abundant populations in these areas were decimated by over-fishing, pollution and disease (Kennedy and Breisch 1983). Cultured oysters also result in a cohort characterized by similar ages and genetic diversity, controlling for these potentially confounding variables. Cultured oyster specimens are available from the University of Maryland Center for Environmental Science Horn Point Laboratory Aquaculture and Restoration Ecology Laboratory and from the Chesapeake Bay Foundation's Oyster Gardening Program.

Assessing water quality changes in Chesapeake Bay

Chesapeake Bay and Maryland's Coastal Bays are ideal locations to examine nitrogen sources and linkages with water quality at multiple spatial scales. Chesapeake Bay is the largest (~300 km long, 11,600 km^2 surface area) and arguably most studied estuary in the United States with a mixed-use watershed (164,200 km^2). Maryland Coastal Bays (~70 km long, 600 km^2 surface area) provide a complementary study site and are also well studied (though to a lesser extent). Total nitrogen loads to Chesapeake Bay per estuarine area (14 g N m^{-2} $year^{-1}$; Boynton et al. 1995) vary between wet or dry years (Hagy et al. 2004), and inputs are derived from diffuse watershed sources (60%), watershed point sources (28%), and atmospheric deposition (12%) while the majority of nitrogen loss is due to denitrification (26% of total nitrogen inputs) and long-term burial (35%), with only minimal loss by fisheries (9%; Kemp et al. 2005). Phytoplankton absorb both NH_4^+ and NO_3^- roughly in proportion to concentration despite an overall gradient of dissolved inorganic nitrogen concentrations (Montoya et al. 1990) and $\delta^{15}N$ values (Horrigan et al. 1990) in Chesapeake Bay. These studies showed that local processes are critical in determining isotopic values, thus particulate $\delta^{15}N$ distribution lacked a consistent Bay-wide pattern (Horrigan et al. 1990, Montoya et al. 1990). Since nitrogen largely enters Chesapeake Bay as inorganic forms and is largely exported to the ocean as organic forms (Kemp et al. 1997), Chesapeake Bay does not merely transport nutrients to the ocean (Borum 1996, Nixon et al. 1996), but rather facilitates the processes of active uptake, transformation, and production.

Anthropogenic pressures on these natural processes increased dramatically over the 400 years of European settlement. Collectively, they changed watershed characteristics (Brush et al. 1980, Curtin et al. 2001), increased eutrophication (e.g. Brush 1984, Kemp et al. 2005), and depleted bottom water oxygen concentrations (hypoxia/anoxia during the last 50 years; Cooper & Brush 1991, 1993, Karlsen et al. 2000, Zimmerman & Canuel 2000, Adelson et al. 2001, Hagy et al. 2004). Further, water clarity declined due to algal blooms (Gallegos and Jordan 2002), which altered primary production (Harding 1994, Harding and Perry 1997, Kemp et al. 2004, 2005, Glibert et al. 2007) and food webs (Holland et al. 1987, Hagy 2002). Overall, habitats such as marshes (Kearney et al. 2002) and seagrass meadows (Orth and Moore 1987, Kiddon et al. 2003) have been lost, fisheries have been depleted (Kennedy and Breisch 1981, Rothschild et al. 1994, Breitberg 2002, Smith et al. 2003) or in summary, ecosystem services have been degraded (Newell 1988). Vertical patterns of $\delta^{15}N$ (increasing by ~ 4‰) in sediments cores collected from Chesapeake Bay indicate periods of oxygen depletion, intense denitrification, and nutrient recycling starting around A.D. 1750 to 1800 that are coincident with the advent of widespread land clearing, tillage, erosion and sedimentation (Bratton et al. 2003).

To a great extent, physical, chemical, and biological indicators are routinely used for monitoring the spatial and temporal extent of eutrophication, its impacts, and associated nutrient cycling and transport. Water quality monitoring is crucial to observe and document eutrophication symptoms. Yet merely monitoring and summarizing water quality data has not resulted in eutrophication reversal in

Chesapeake Bay (Kemp et al. 2005). Innovative reporting of integrated ecosystem health assessment (Williams et al. 2009, Longstaff et al. 2010) and comparisons with restoration goals (Chesapeake Bay Program 2010) can track progress but these techniques have associated challenges. Reaching consensus on defining metrics and thresholds to assess water quality has been difficult to achieve (for examples, see Suter 1993; Ferreira 2000, Ebersole et al. 2002, Kennish 2002, Kiddon et al. 2003, Borja et al. 2004, 2009, Williams et al. 2009). Furthermore, water quality data are resource intensive to generate over the long-term at appropriate time and spatial intervals, may not indicate underlying causes or nitrogen sources, and often do not reflect linkages with living resources. Bioindicators may address these limitations.

Analyzing oyster $\delta^{15}N$ at three scales in Chesapeake Bay

To quantify relationships between oyster $\delta^{15}N$ and overall water quality and to identify potential influencing or confounding factors, this dissertation examines baseline information on oyster $\delta^{15}N$ at various locations and then applies this tool at three spatial scales: 1) a small scale (~50 km^2) at Monie Bay, National Estuarine Research Reserve; 2) an intermediate scale (~160 km^2) in Maryland's Coastal Bays; and at 3) a large scale (~11,600 km^2) in Chesapeake Bay's tributaries (Figure 1.4).

Baseline oyster $\delta^{15}N$ observations were made in mesohaline tributaries of Chesapeake Bay: Monie Bay (38°13'30"N, 75° 50'00"W), South River (38°57'11"N, 76°34'21"W), Severn River (38°56'42"N, 76°28'1"W), and Choptank River (38°35'36"N, 76°07'43"W). Manipulative fieldwork, monitoring, and modeling

datasets are explored in these tributaries to verify convergence of oyster $\delta^{15}N$ upon a local ambient $\delta^{15}N$ signature after transplantation and quantify the time required to expose oyster bioindicator deployments. Additionally, seasonal patterns of $\delta^{15}N$ values and oyster tissue integration are explored. This chapter examines pragmatic questions related to understanding the utility and deployment of oyster bioindicators.

Oysters were deployed at a small spatial scale (~50 km^2 water surface area) in Monie Bay National Estuarine Research Reserve (38°13'30"N, 75°50'00"W) to 1) link living resources with water quality; 2) identify nitrogen in this shallow estuary (1.9 ± 0.1 m mean depth) derived from various land uses (including poultry production, crop agriculture, and protected wetlands); and 3) examine implications of nitrogen generation by poultry production across Delmarva Peninsula. Monie Bay's watershed serves as a 'natural laboratory' because different proportions of multiple anthropogenic nitrogen sources are available to three tributary creeks.

Comparison of macroalgae and oyster $\delta^{15}N$ bioindicators was conducted at an intermediate spatial scale (160 km^2 water area) throughout Maryland's (and some of Virginia's) Coastal Bays including Chincoteague Bay (38°15′14″ N, 75°11′57″ W in the north to 37°54′14″ N, 75°24′38″ W in the south). Unusual precipitation events in 2006 afforded an opportunity to examine the response by macroalgae and oysters to a nutrient pulse. Further, these events enabled inferences to be made regarding bioindicator temporal/spatial integration in comparison to water quality metrics.

The utility of deploying oyster bioindicators at a very large scale (11,600 km^2) subject to physical gradients such as salinity (Pritchard 1956, 1967) and tributary

flushing time (Wazniak et al. 2009) was assessed in Chesapeake Bay, from tributaries extending from Annapolis, MD to Virginia Beach, VA. This broad survey seeks to identify the maximum spatial extent at which oysters can serve as nitrogen source bioindicators. Further, this project demonstrates how existing large-scale citizen scientist and monitoring programs can be leveraged for mutual benefit.

Collectively, the importance of these projects is to establish oyster $\delta^{15}N$ as a bioindicator of nitrogen sources that integrates temporally and is capable of deployment at multiple spatial scales for mapping nitrogen sources, even if extant populations are unavailable. This dissertation seeks to understand the factors that influence oyster $\delta^{15}N$ at multiple spatial scales and increase understanding of bioindicator functionality and application relevant to eutrophication in Chesapeake Bay and coastal ecosystems worldwide.

Dissertation outline

This dissertation examines the distribution of $\delta^{15}N$ values in oyster (*Crassostrea virginica*) tissues, assesses its utility as a bioindicator species, and applies this technique at three spatial scales to verify the ability to infer nitrogen source from this data. Chapters 2 through 5 (inclusive) are in various stages of publication (Table 1.1). Specific aims and questions addressed are listed here.

- Chapter 1 has provided detailed background on the theory, application, and potential caveats relevant to employing bioindicator $\delta^{15}N$ values to identify and track anthropogenic nitrogen sources.
- Chapter 2 examines preliminary baseline information, including variability in $\delta^{15}N$ across individuals, tissues, and seasons, to identify key physiological factors controlling $\delta^{15}N$ and answer practical questions for bioindicator deployment.
    - What sample size optimally balances error with effort?
    - After shared deployment in new locations, do $\delta^{15}N$ in oysters from two locations converge to water column $\delta^{15}N$ values?
    - How long must oysters be deployed and exposed to nitrogen sources to reflect a change in source, and what is the duration of their temporal integration?
    - To what extent does oyster $\delta^{15}N$ vary seasonally?
- Chapter 3 addresses linkages of oyster $\delta^{15}N$ with water quality and land use (particularly poultry operations, crop agriculture, and wetlands) at the small scale (10s of km^2) in Monie Bay (National Estuarine Research Reserve) and Delmarva Peninsula.
    - Can oyster $\delta^{15}N$ link water quality, nitrogen source, and living resources?

- What role does denitrification play in the nitrogen isotope ratios observed in Monie Bay and its creeks?

- What nitrogen sources are available to Monie Bay and its creeks?

- Chapter 4 compares relative roles for biological indicators in different functional groups (oysters as consumers, macroalgae as primary producers) at an intermediate spatial scale (100s of km^2) in Maryland's Coastal Bays.

    - What are the relative capabilities of macroalgae and oysters to detect nitrogen from human and animal wastes?

    - What are the broad scale spatial patterns of nitrogen from wastes spanning these coastal bays (~600 km^2)?

    - What are the fine-scale spatial patterns of influence by nitrogen from human and animal wastes within regions (ranging from ~10 to 50 km^2) of Maryland's Coastal Bays?

- Chapter 5 applies oyster $\delta^{15}N$ as a bioindicator to identify relationships with flushing time, salinity, and oyster size and to map nitrogen sources at a large scale (1,000s of km^2) throughout Chesapeake Bay and its tributaries.

    - Do broad spatial gradients of salinity or water flushing time influence oyster $\delta^{15}N$ or $\delta^{13}C$, and are these affected by oyster size?

- By integrating nitrogen sources temporally and spatially, is oyster $\delta^{15}N$ related to land use, overall stream health, or overall health of Chesapeake Bay at either the tributary or entire bay scale?
- What broad spatial patterns of nitrogen sources are evident in Chesapeake Bay and its tributaries?
- What spatial evidence does oyster $\delta^{15}N$ provide for the relative contribution of direct inputs vs. diffuse anthropogenic inputs?

- Chapter 6 summarizes the overall successes and limitations of oyster $\delta^{15}N$ to identify and map anthropogenic nitrogen sources at these spatial scales, and comments on the contribution of this dissertation to ecology and its applications.

Chapter 1 Tables

Table 1.1: Dissertation outline and publication schedule

	Chapter 1	Chapter 2	Chapter 3	Chapter 4	Chapter 5	Chapter 6
Title	Introduction	Eastern oyster (*Crassostrea virginica*) $\delta^{15}N$ as a bioindicator of nitrogen sources: Observations and modeling	Oyster $\delta^{15}N$ as a bioindicator of human and animal nitrogen and degraded water quality in a sub-estuary of Chesapeake Bay	Oyster and Macroalgae Bioindicators Detect Elevated $\delta^{15}N$ in Maryland's Coastal Bays	Oyster $\delta^{15}N$ in Chesapeake Bay: large-scale bioindicator of nitrogen source and water quality in tributaries and streams	Discussion
Target Journal		Marine Pollution Bulletin	Journal of Coastal Research	Estuaries and Coasts	Estuaries and Coasts	
Authors		B. Fertig, T.J.B. Carruthers, W.C. Dennison, E.J. Fertig, M.A. Altabet	B. Fertig, T.J.B. Carruthers, W.C. Dennison	B. Fertig, T.J.B. Carruthers, W.C. Dennison, A.B. Jones, F. Pantus, B. Longstaff	B. Fertig, T.J.B. Carruthers, W.C. Dennison	
Status		In press. Online.	Minor revisions necessary before 2nd submission.	Published 2009. 32:773–786	In preparation.	
DOI		doi:10.1016/j.marpolbul.2010.03.013		DOI 10.1007/s12237-009-9148-x		

Chapter 1 Figures

Figure 1.1: Nitrogen biogeochemistry (modified from Valiela 1995)

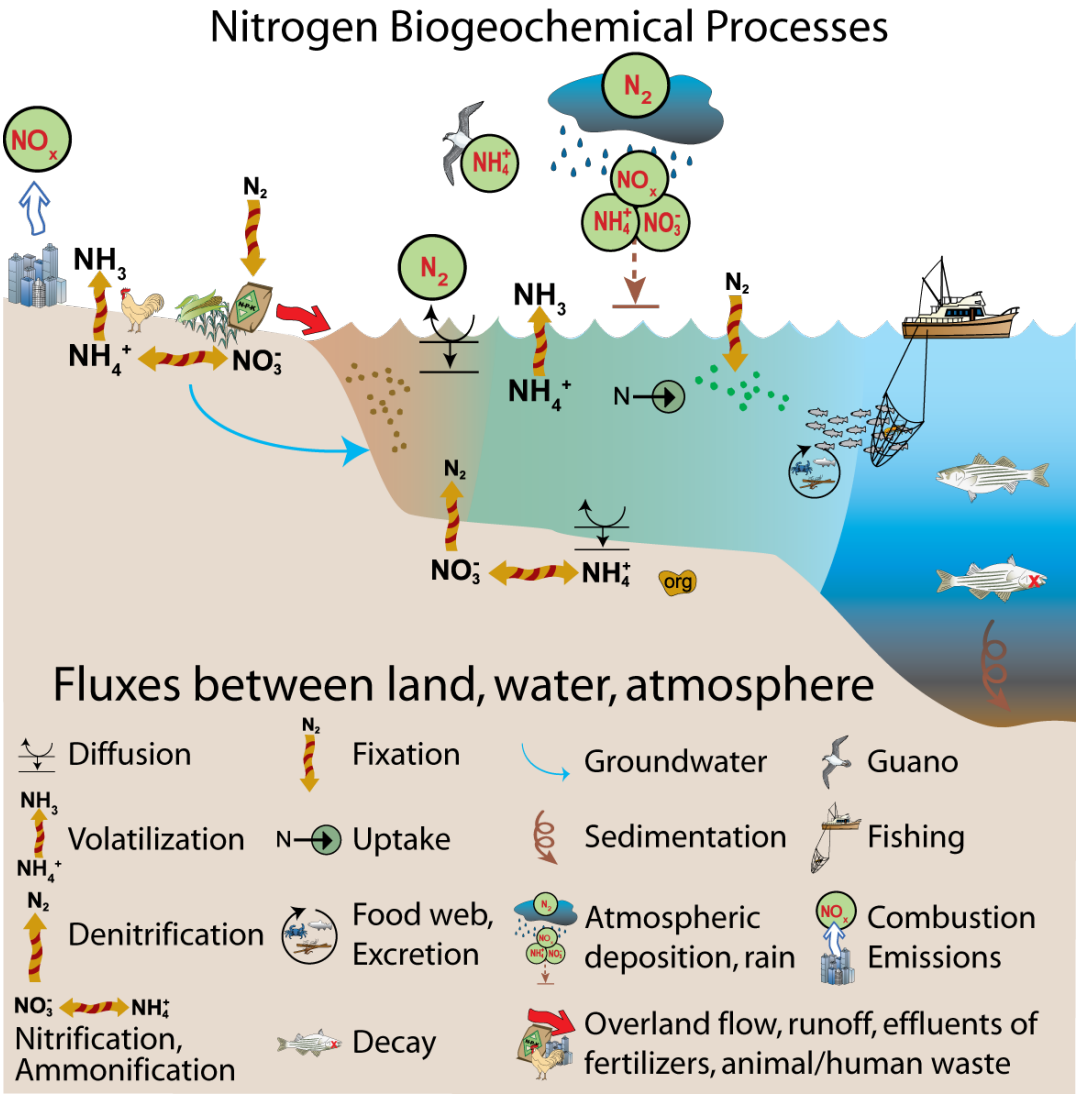


Figure 1.2: Oyster and macroalgae $\delta^{15}N$ values in relation to nitrogen source

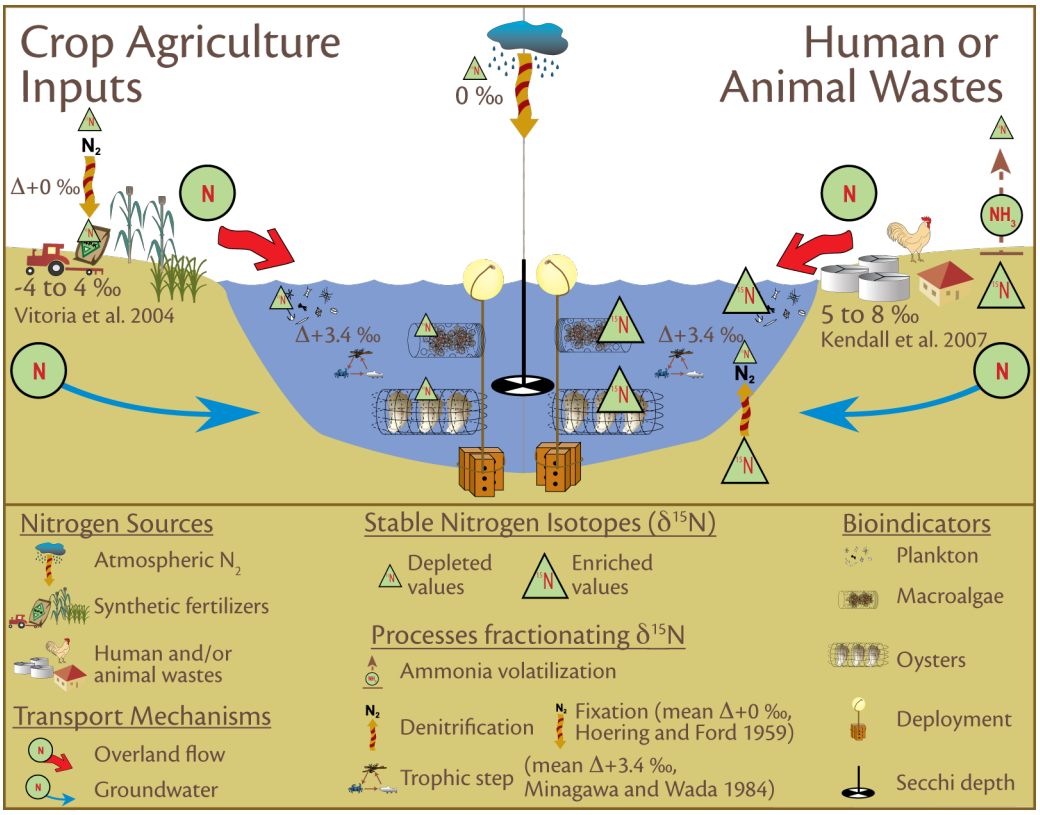


Figure 1.3: Turnover and integration time periods vary by oyster tissue

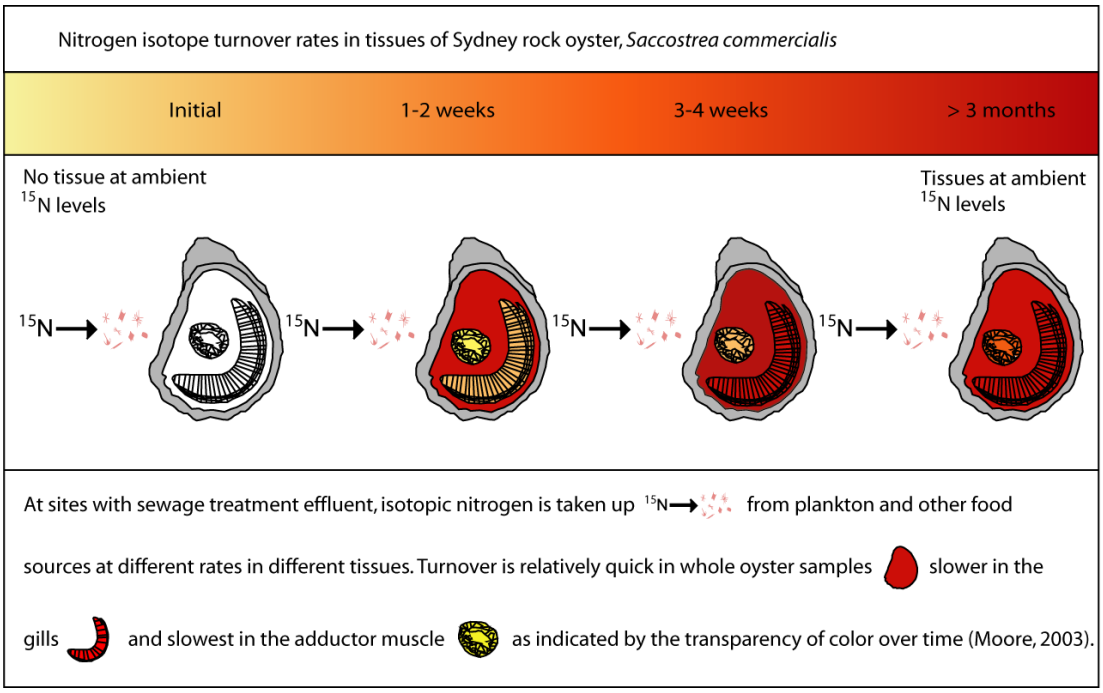
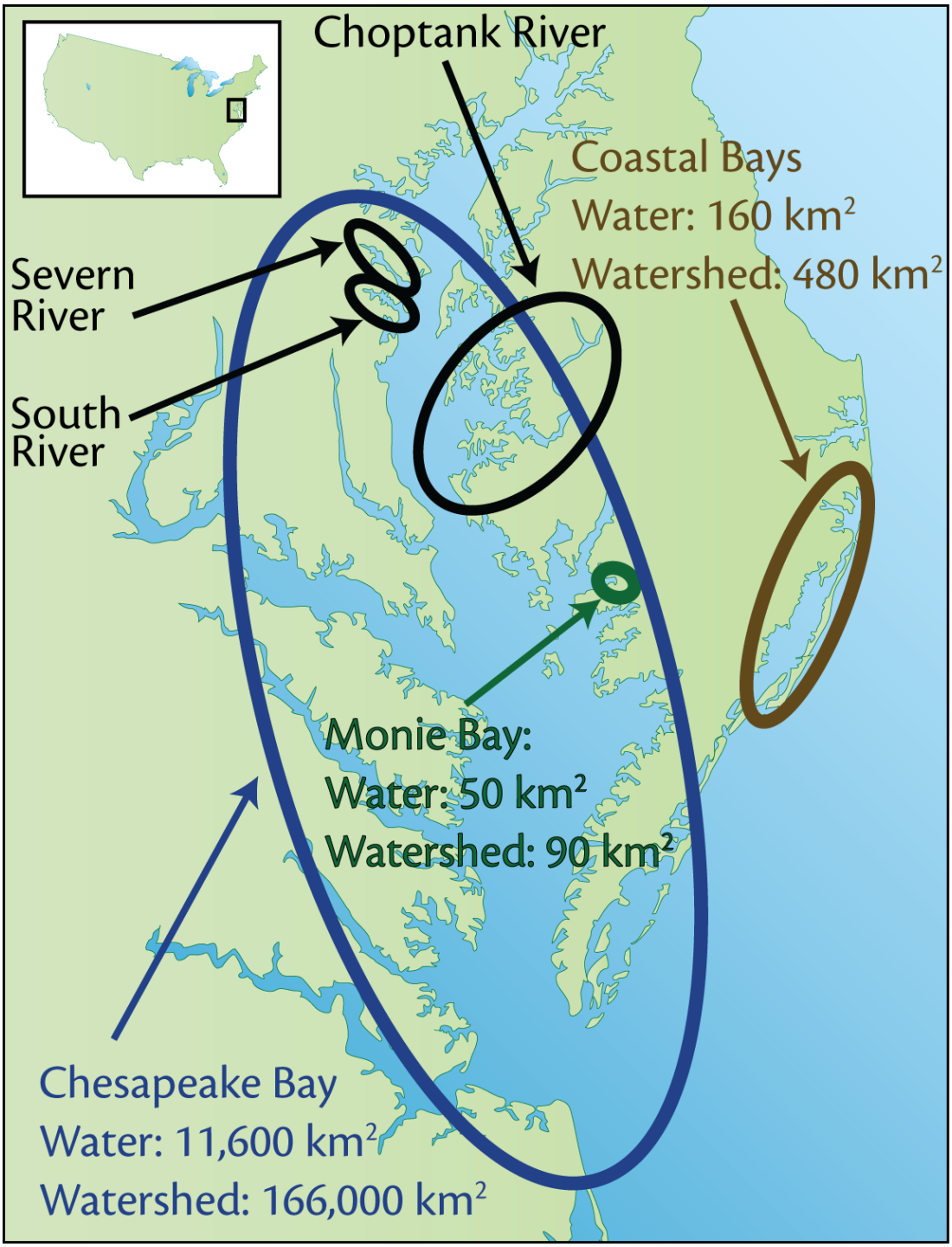


Figure 1.4: Applying oyster bioindicators at three spatial scales



CHAPTER 2: EASTERN OYSTER (*CRASSOSTREA VIRGINICA*) δ^{15}N AS A BIOINDICATOR OF NITROGEN SOURCES: OBSERVATIONS AND MODELING

Chapter Citation:

Fertig, B., Carruthers, T.J.B., Dennison, W.C., Fertig, E.J., Altabet, M.A. In Press. Eastern oyster (*Crassostrea virginica*) δ^{15}N as a bioindicator of nitrogen sources: observations and modeling. Marine Pollution Bulletin

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Abstract

Stable nitrogen isotopes ($\delta^{15}N$) in bioindicators are increasingly employed to identify nitrogen sources in many ecosystems, and biological characteristics of the eastern oyster (*Crassostrea virginica*) make it an appropriate species for this purpose. To assess nitrogen isotopic fractionation associated with assimilation and baseline variations in oyster mantle, gill, and muscle tissue $\delta^{15}N$, manipulative fieldwork in Chesapeake Bay and corresponding modeling exercises were conducted. This study 1) determined that five individuals represented an optimal sample size; 2) verified that $\delta^{15}N$ in oysters from two locations converged after shared deployment to a new location reflecting a change in nitrogen sources; 3) identified required exposure time and temporal integration (four months for muscle, two to three months for gill and mantle); and 4) demonstrated seasonal $\delta^{15}N$ increases in seston (summer) and oysters (winter). As bioindicators, oysters can be deployed for spatial interpolation of nitrogen sources, even in areas lacking extant populations.

Introduction

Measurements of stable nitrogen isotopes ($\delta^{15}N$) are increasingly used to identify nitrogen inputs from anthropogenic sources; agricultural runoff as well as human and animal wastes (Costanzo et al., 2001; Kendall, 1998; McClelland et al., 1997). Many examples suggest the suitability of $\delta^{15}N$ in many organisms for this purpose, including macrophytes (e.g. Benson et al., 2008; Cohen and Fong, 2006; Cole et al., 2004; McClelland et al., 1997), finfish (Lake et al., 2001; Schlacker et al. 2005), and mollusks (Fila et al., 2001; McKinney et al., 2001; McKinney et al., 2002), or some combination (Fry et al., 2003; Gartner et al., 2002). Enriched $\delta^{15}N$ signatures in sewage or animal waste arise from isotopic discrimination due to a combination of ammonia volatilization and denitrification at the source or by microbial processing employed by wastewater treatment facilities (Fry, 2006; McClelland and Valiela, 1998; Sweeny and Kaplan, 1980; Tucker et al., 1999). In contrast, synthetic fertilizers are 'fixed' from atmospheric N_2 (0‰) and have corresponding $\delta^{15}N$ values: generally -4 to +4 ‰ (Hübner, 1986; Macko and Ostrom, 1994; Vitoria et al., 2004).

Terrestrial nitrogen sources may have complicated pathways before reaching aquatic biological indicators, and isotopic signatures can be modified or mixed en route; therefore, interpretations must consider alternative hypotheses (Fry, 2006; Kendall, 1998). Nitrogen from animal manures, for example, can be fractionated

during volatilization which favors ^{14}N and enriches the remaining nitrogen pool with ^{15}N (Altabet, 2006; Cline and Kaplan, 1975; Fry, 2006; Kendall, 1998; McClelland and Valiela,1998), and dissolved nitrate can be microbially denitrified to gaseous N_2, elevating the remaining nitrate pool (Mariotti et al. 1982; Shearer and Kohl 1988). Once entering estuarine waters, dissolved inorganic nitrogen is assimilated by phytoplankton and subsequently consumed by oysters with an accompanying enrichment of 3 – 4 ‰ at each trophic step as fractionation occurs during digestion and waste elimination (Adams and Sterner, 2000; Minagawa and Wada, 1984). Further, $\delta^{15}N$ signatures may vary due to metabolic factors including nutritional stress (Fuller et al., 2005; Hobson et al., 1993), weight change, life stage (Grant and Kopple, 2009), gestation (Fuller et al., 2005), and starvation (Boag et al., 2006; Haubert et al., 2005; Hobson et al., 1993).

Measuring $\delta^{15}N$ in biological indicator species, particularly bivalves such as the eastern oyster (*Crassostrea virginica*), can provide different information than direct measurements made on nitrogen in groundwater (Aravena et al., 1993; Jin et al., 2004; Lefebvre et al., 2007), the water column (Cole et al., 2006), or sediments (Tucker et al., 1999), as oysters integrate spatial and temporal variability. Bivalves indicated a variety of contaminants (Bebianno et al., 2004; Carmichael et al., 2008; Kimbrough et al., 2008) including nitrogen sources (Daskin et al., 2008; Fila et al., 2001; McKinney et al., 2002; Moore and Suthers, 2005), and $\delta^{15}N$ in filter feeders has been compared to that in primary producers as bioindicators of nitrogen sources (Fertig et al., 2009; Fry et al., 2003). In Chesapeake Bay, oysters' historic prevalence

(Newell, 1988) and ecological roles (Kemp et al., 2005) make this species an ideal bioindicator, and additionally oysters are sessile, hardy, euryhaline, and tolerant to transplantation (Powell and Ashton-Alcox, 2004), enabling deployment in numerous locations for spatial assessments. Further, oysters assimilate nitrogen from suspended particulate organic matter (phytoplankton, other microbes, as well as detritus; Langdon and Newell, 1996) throughout their lives, and do not substantially change diets post-metamorphosis or with developmental stages (i.e. ontogenetic shifts). Therefore, the length of usefulness is not limited by life cycle patterns, in contrast to some species of arthropods (Haubert et al., 2005), crustacean zooplankton (Ventura and Catalan, 2008), and estuarine fish (Griffin and Valiela, 2001; Witting et al. 2004).

Knowledge gaps about stable isotopes in organisms persist, including the influence of variables such as locality, tissue type, seasonality, and temporal integration and are addressed in this work. Convergence of $\delta^{15}N$ to new conditions was observed experimentally after diet changes (Adams and Sterner, 2000) and post-deployment in the field (Daskin et al., 2008; Dattagupta et al., 2004), but not previously for *C. virginica* in a water body with a large, multi-use watershed. To account for $\delta^{15}N$ variation in *C. virginica*, a baseline was established through fieldwork and modeling exercises to investigate oyster muscle, gills, and mantle in areas of Chesapeake Bay. Specifically, the following research topics were addressed: 1) optimization of sample size; 2) verification that $\delta^{15}N$ in oysters from two locations converged to water column $\delta^{15}N$ values after shared deployment in new locations;

3) identification of required exposure time and duration of temporal integration; and 4) assessment of seasonal variations.

Methods and Materials

Study Location

Fieldwork was conducted in Monie Bay and the South, Severn, and Choptank Rivers (Figure 2.1a-d), which are tributaries in the mesohaline region of Chesapeake Bay (Chesapeake Bay Program, 2004). Measurements of standard water quality metrics (salinity, temperature, and dissolved oxygen concentration and saturation) in these regions were conducted using a standard YSI 85 handheld dissolved oxygen and conductivity instrument (YSI Inc.), and Secchi depth was also measured.

Isotope Analyses

Upon collection, oysters were kept on ice until frozen (-20 °C) at the laboratory until preparation for isotopic analysis. For preparation, oysters were thawed, shell height (mm) was measured with calipers, and individuals were dissected to obtain the adductor muscle, gills, and mantle. Tissues were rinsed to avoid contamination by carbonates from the shell and then oven dried (60 °C) until completely dry (48 hours minimum). Dried tissues were ground and homogenized by mortar and pestle (see Coleman and Fry, 1991; Knowles and Blackburn, 1993).

Tissue sub-samples (1.0 ± 0.2 mg) were packed into tin capsules (Elemental Microanalysis, pressed, standard weight, 8 × 5 mm).

Where specified, seston (60 ml) was also collected on pre-combusted glass fiber filters (25 mm Wattman GF/F) for $\delta^{15}N$. Filters were packaged in tin foil and kept on ice in the field and frozen in the laboratory until preparation for analysis. Filters were thawed, thoroughly oven dried (60 °C), rolled, then pressed into tin foil discs for isotopic analysis. Nitrogen content (μg N) and $\delta^{15}N$ of oysters and seston were analyzed by the University of California Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Calculations of molar %N in oysters were also conducted to assess nutritional condition based upon the calculated molar ratio of the sub-sample nitrogen content (μg) to sub-sample dry weight (mg). For definition, $\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 10^3$, where R was defined as the $^{15}N/^{14}N$ ratio. The standard reference was atmospheric N_2 (air), with 0.3663 atom % ^{15}N, defined as 0‰ (e.g. Fry, 2006), and instrumental error was ± 0.2 ‰.

Isotopic analysis of dissolved inorganic nitrogen was conducted on 125 ml filtered water column samples collected in May and June 2009. Methods described by McIlvin and Altabet (2005) were used to reduce NO_3^- to NO_2^- and those described by Zhang et al. (2007) were used to oxidize NH_4^+ to NO_2^-. All fractions converted to NO_2^- were then reduced to N_2O through a reaction with azide. These methods require very low nitrogen sample size (15 nmols). For the McIlvin and Altabet (2005)

method, Cd powder used for NO_3^- reduction was prepared as a slurry with deionized water and 10% HCl, which was added drop-wise and mixed with an Aerolatte™ coffee stirrer. Addition of acid continued until the cadmium lost its brown color and the overlying liquid was clear. Care was taken to avoid agglutinization. Modifications from Zhang et al. (2007) included use of 4 ml of the BrO^- working solution for a 20 ml of sample, immediate vigorous shaking for 30 seconds, 1 hour reaction time, and 0.8 ml arsenite reagent to stop the reaction. Also, 0.8 ml of 6 N HCl was added to a 5 ml aliquot to lower pH sufficiently for the colorimetric determination of concentration (sulfanilamide method, Strickland and Parsons, 1972) of NO_2^- after oxidation. For both NO_3^- reduction and NH_4^+ oxidation, , 0.87 mg NaCl was added to bring chlorinity to that of seawater for samples with salinity < 5 (Millero, 2005). Dissolved N_2O gas was then analyzed on an isotope ratio mass spectrometer. Reproducibility of $\delta^{15}N$ values was ± 0.5 ‰.

Determination of Optimal Sample Size

Oysters were collected randomly from the Choptank River (38°34'19"N, 76°3'14"W) on 14 June 2005 and from the South River (38°55'51"N, 76°32'16"W) on 17 June 2005 (Figure 2.1a-c). Variability of standard errors was assessed to optimize the trade-off between resolving power and expended effort using methods described by Bros and Cowell (1987). The standard error of the deviations for $\delta^{15}N$ data was calculated for various sample sizes (2 – 20), which were randomly selected ten times

for each tissue, and the mean, maximum, and minimum standard error of the deviations of these selections was plotted against sample size.

Verification of Convergence After Shared Deployment

A transplantation study was conducted to verify that $\delta^{15}N$ in oysters from two locations converged towards a common $\delta^{15}N$ value after shared deployment. Oyster spat on shell were transplanted (Figure 2.1a, 2.1b, 2.1d) in June - October 2006 from two Chesapeake Bay tributaries (the South River: 38°57'11" N, 76°34'21" W and the Severn River: 38°56'42" N, 76°28'1" W) to 10 stations in Monie Bay and its three creeks (38°13'30" N, 75°50'00" W). Two mesh (3.61 cm^2 holes) bags were deployed at each of the 10 stations such that both source tributaries were represented by a mesh bag at each of the ten sampling stations (Figure 2.1a, 2.1b, 2.1d). Mesh bags contained 20 oysters each, from their respective sources, and were anchored with three bricks and suspended 0.5 m off the bottom using a marked buoy to minimize sediment smothering. Five randomly selected surviving oysters (23% mortality rate from 100% recovery of bags) from each source tributary were sampled for isotopic analyses. The oysters were in apparent good health based on observations of new growth and stable nutritional condition (%N). Fouling organisms and trapped sediments were removed from mesh bags fortnightly or as needed to maintain water flow through the mesh bags.

Multiple assessments were made to quantify the convergence of oyster $\delta^{15}N$ values after deployment and to verify that the post-deployment $\delta^{15}N$ values resembled

isotopic signatures of nitrogen sources at the location of deployment. Post-deployment $\delta^{15}N$ values in oysters from the Severn River were plotted and regressed (Proc Reg, SAS Institute) against post-deployment $\delta^{15}N$ values in oysters from the South River and regression slopes were tested for significant difference from the 1:1 line and from each tissue (Proc GLM, SAS Institute). Additionally, the convergence of oyster $\delta^{15}N$ was modeled (based on inputs of measured seston $\delta^{15}N$ values) and compared to measured $\delta^{15}N$ values, as described below, to identify acclimatization patterns over seasons and a range of environmental conditions and seston $\delta^{15}N$ values. Dissolved inorganic nitrogen $\delta^{15}N$ values were compared to seston and oyster tissue $\delta^{15}N$ values to verify that each reflected ambient conditions in the water column based on the assumption that most available nitrogen is assimilated by seston and oysters. Isotopic fractionation varies widely (Wada et al. 1975; Hoch et al. 1992; Montoya and McCarthy 1995; Waser et al. 1998; Pennock et al. 2006) due to algal assimilation of nitrate (+1 to +19‰), nitrite (-9 to +4‰) and ammonium (-7 to +2‰), and can be affected by nitrogen concentrations and algal species. Expected $\delta^{15}N$ values were generated by adding 3 to 4‰ per trophic level (i.e. seston assimilation by oysters; Minagawa and Wada, 1984) to dissolved inorganic $\delta^{15}N$ values. A trophic fractionation of 3 to 4 ‰ is reasonable due to mesohaline salinities, likely dominance of flagellate algal species, and low nutrient concentrations in Monie Bay (Wada et al. 1975; Montoya and McCarthy 1995; Waser et al. 1998). Expected oyster muscle tissue $\delta^{15}N$ values were adjusted by +1.0‰ based on observations and reported muscle and other tissue $\delta^{15}N$ values (Lorrain et al. 2002, Piola et al. 2006).

Quantifying Temporal Integration and Required Exposure Time

To identify temporal patterns of isotopic signatures and assess integration by $\delta^{15}N$ values in oyster tissues, periodic measurements of seston $\delta^{15}N$ values and other water quality metrics were conducted in Monie Bay and the Choptank River. In Monie Bay (Figure 2.1a, 2.1d), seston $\delta^{15}N$ values were measured six times during the four-month deployment period, and means of each time period combination were regressed against oyster tissue $\delta^{15}N$ values. Additionally, $\delta^{15}N$ values from each tissue were assessed to identify if tissues integrated over different periods of time. In the Choptank River, (38°35'36"N, 76°07'43"W, Figure 2.1a, 2.1c), variations of salinity, temperature, dissolved oxygen, total nitrogen, total phosphorus (three replicate 20 ml nutrient samples), seston (three 60 ml replicates collected and analyzed for isotope values as described above), and oyster $\delta^{15}N$ (mantle, gills, and muscle tissues from five individuals) were observed monthly over two years. Total nutrient concentrations were analyzed by standard methods (D'Elia et al. 1977; Kerouel and Aminot 1987).

Required exposure times, seasonal oyster $\delta^{15}N$ values, and optimal timing of deployment were inferred by modeling the incorporation of seston $\delta^{15}N$ into oyster tissues. The model assumed the tissue in question (mantle, gills, or muscle) responded due to a temperature dependent tissue turnover, which reflected a mixture of the seston isotopic signal encountered (model input values) as well as a trophic shift (a constant 3.0‰). Instantaneous scaled temperatures were calculated by interpolating

temperature measurements between each time step and then normalizing the temperature range between 1 (maximum in summer) and 0 (minimum in winter). In Monie Bay and its creeks, the winter temperature minimum was assumed to be ~0°C (Apple et al. 2006).

Specifically, each tissue was modeled according to the equation:

$$\delta_O(t) = \delta_O(t - \Delta t) - \Theta\tilde{T}(t)\delta_O(t - \Delta t)\Delta t + \Theta\tilde{T}(t)\left(\delta_S(t) + \tau\right)\Delta t \qquad \text{Equation 2.1}$$

In Equation 2.1,

t = current model time (day),

Δt = time step between model times (1 day),

δ_O = $\delta^{15}N$ of the oyster tissue (‰),

δ_S = $\delta^{15}N$ of the seston (‰),

$\tilde{T}(t)$ = normalized temperature as a function of time (°C),

Θ = tissue turnover rate at maximum normalized temperature (% tissue day^{-1}),

τ = the change in $\delta^{15}N$ due to a trophic shift (‰).

The input seston $\delta^{15}N$ values, δ_S, was obtained by interpolating measured seston $\delta^{15}N$ values at each simulation time step, t. The normalized temperature values ($\tilde{T}$) were obtained from water measurements interpolated to model times and normalized to 1 at maximum observed summer temperature and 0 at minimum

observed winter temperature. When sampling occurred over only one season (i.e. in Monie Bay), the maximum summer temperature was assumed to be 30°C and the minimum water temperature to be 0°C. Alternatively, the variable $\tilde{T}$ could be obtained from a $\log_{10}$ transform of temperature as in Hall (1984) for greater accuracy in future studies.

Finally, the maximum turnover rate (Θ) was determined by minimizing the least squares model error (E) for the seasonal data set from the Choptank River:

$$E = \sqrt{\sum_{i=1}^{n} \left(\delta_O^{\text{measured}}(t_i) - \delta_O^{\text{modeled}}(t_i) \right)^2}, \qquad \text{Equation 2.2}$$

In Equation 2.2, *i* indexes each of the time steps at which oyster $\delta^{15}N$ was measured and σ_i^2 is the standard deviation of that measurement. This error measure is standard to quantify the average deviation between the model and measured $\delta^{15}N$ values of the oyster, and is used implicitly in the parameter inference by regression in Moore (2003), Witting et al. (2004), and Sweeting et al. (2005). The resulting parameters for maximum tissue turnover (Θ) were 1.9% day^{-1} in mantle with (E = 4.7 ‰), 2.6% day^{-1} in gill (E = 3.0 ‰), and 0.7% day^{-1} in muscle (E = 1.5 ‰). Literature values report an average trophic shift of 3.4‰ per trophic level (Minagawa and Wada, 1984), so scenarios of 3.0‰ to 4.0‰ were used for model verification and error assessment and 3.0‰ was selected as it yielded minimal error. Using these parameters, oyster tissues were modeled and compared to measured data using measured seston $\delta^{15}N$ values over time in the Choptank River and further validated against an independent data set

from Monie Bay. Finally, a constant value of seston $\delta^{15}N$ (8.0 ‰) within the observed range of seston $\delta^{15}N$ values in Monie Bay was used as input to identify the length of time it takes for oyster $\delta^{15}N$ to stabilize under a constant maximum summertime temperature (30°C) and under temperatures observed in the Choptank River across seasons (starting in winter), providing the optimal deployment duration.

Results

Optimal Sample Size

Because of individual variability in $\delta^{15}N$ values in oysters (58 ± 2 mm shell height), it was necessary to determine the number of individual $\delta^{15}N$ measurements to average over (the sample size) that optimally balanced error with effort. For sample sizes of 2 to 20, the mean, maximum, and minimum standard errors were compared (Figure 2.2a-c) and as sample size increased, the maximum and minimum converged upon the mean standard error, becoming increasingly balanced around the mean value, with optimum balance at sample size five. The range of standard errors for $\delta^{15}N$ at sample size five for muscle (0.08), gill (0.07), and mantle (0.14) was larger than the minimum range of standard errors for each tissue: muscle (0.03 at sample size 20), gill (0.02 at sample size 17), and mantle (0.02 at sample size 19). However, a minimum of five replicate samples were required (Figure 2.2a-c) to optimize standard error.

Convergence and Stabilization of $\delta^{15}N$ Values

Oyster transplantations were conducted in the mesohaline region of Chesapeake Bay, which was characterized by salinity ranging from 0.9 in upper portions of tributaries to 15.0 near tributary mouths and temperatures that ranged from 0.2 °C in winter to 29.1 °C in summer. Oxygen saturation fluctuated from 15.0 % to 115.6 % and dissolved oxygen concentrations were below 5.0 mg L^{-1} during 32 % of measurements in Monie Bay and 61 % of those in Choptank River. Secchi depth ranged from 0.3 m to 2.8 m, with a mean of 1.0 m.

Initially, oyster $\delta^{15}N$ was significantly and consistently higher in oysters from the Severn River (48 ± 2 mm shell height) as compared to the South River (57 ± 1 mm shell height; all tissues $p < 0.01$, Table 2.1a, Figure 2.3a) but after transplantation to Monie Bay, $\delta^{15}N$ values in oysters converged to common values at each of the ten stations (Figure 2.3a). Oyster tissue $\delta^{15}N$ from both rivers declined from initial values (12.3 ± 0.2 ‰ in South River and 13.7 ± 0.1 ‰ in Severn River, mean of all tissues; Table 2.1a) after deployment in Monie Bay (11.3 ± 0.1 ‰, grand mean of all tissues post-deployment; Table 2.1b; Figure 2.3a) as they fed on seston which also declined in $\delta^{15}N$ values during the deployment period (Figure 2.4a,b). Post- deployment, gill and mantle $\delta^{15}N$ in oysters from different initial rivers were not significantly different at each of the stations along the $\delta^{15}N$ gradient in Monie Bay although muscle $\delta^{15}N$ values were still significantly different between sites (Table 2.1b, Figure 2.3a). In contrast to $\delta^{15}N$ values, deployment in Monie Bay did not alter oyster nitrogen

content (%N), as %N did not vary among initial rivers before or after deployment in Monie Bay (Table 2.1a,b; Figure 2.3b).

Convergence rates of $\delta^{15}N$ values varied by tissue and metabolic activity. When post-deployment $\delta^{15}N$ values were plotted to compare oysters from the Severn and South Rivers and exhibited a slope of 1.0, complete convergence of $\delta^{15}N$ during deployment was achieved, while plots with slopes < 1.0 indicated incomplete convergence. Mantle (fastest tissue metabolism) had a slope of 1.0 (Figure 2.5a), gills had an intermediate slope (0.92; Figure 2.5b), while muscle (slowest tissue metabolism) had the lowest slope value (0.74; Figure 2.5c). Regressions of tissue $\delta^{15}N$ from both collection rivers were significant (all tissues $p < 0.0001$; $R^2 > 0.45$), but they did not significantly differ across all tissues ($p > 0.05$). Contrast analyses between slopes indicated that muscle significantly differed from both gill and mantle ($df = 1$, $SS < 4.2$, $F = 14.22$, $p < 0.01$) but that mantle and gill (more metabolically active tissues) did not significantly differ ($df = 1$, $SS < 0.10$, $F = 0.33$, $p < 0.57$; Proc GLM, SAS Institute).

Regardless of completion of convergence in each tissue, the post-deployment oyster $\delta^{15}N$ value resembled seston and dissolved inorganic nitrogen $\delta^{15}N$ values in Monie Bay (Figure 2.6). Measured $\delta^{15}N$ values in each oyster tissue matched expected values generated from a 50/50 mixture of ammonium and nitrate $\delta^{15}N$ values modified by two trophic shifts (Figure 2.6). Additionally, measured $\delta^{15}N$ values in oyster tissues matched both measured seston $\delta^{15}N$ values (Figure 2.4a) and

modeled oyster $\delta^{15}N$ values (Figure 2.4b). Therefore, both measurements and modeling indicate that oyster $\delta^{15}N$ values resembled ambient signatures in Monie Bay after deployment.

Required Exposure Time and Temporal Integration

Seasonal variations in seston and oyster $\delta^{15}N$ values each related differently to water quality metrics. Oysters grew (from a mean of 16 to 61 mm) over the course of the study. Both seston and all oyster tissue $\delta^{15}N$ values varied seasonally in the Choptank River (Table 2.2), and were related to total nitrogen concentrations (Table 2.3). Seston $\delta^{15}N$ values were strongly related to temperature, and were elevated during spring and summer (May – July), depleted in cooler months (November to March; Table 2.2, Figure 2.7a), exhibited a significant positive correlation with temperature and salinity and a significant negative relationship with dissolved oxygen concentrations (Table 2.3). The relationship between seston $\delta^{15}N$ values and temperature was significant in both simple linear ($p < 0.03$) and multiple regressions (seston $\delta^{15}N = 7.881 + (0.236 \text{ Salinity}) + (0.0847 \text{ Temperature}) – (0.0745 \text{ Dissolved Oxygen mg } L^{-1})$; mean squared error = 0.795, $R^2 = 0.73$, $p < 0.001$).

In contrast to seston $\delta^{15}N$, oyster $\delta^{15}N$ values tended to be depleted during warmer months (Table 2.2) and oyster muscle $\delta^{15}N$ was not significantly related to any physical metric. However, mantle and gill $\delta^{15}N$ values were negatively correlated to temperature almost as strongly as seston $\delta^{15}N$ was positively correlated (Table 2.3). Mantle tissue $\delta^{15}N$ values were most variable between samples while muscle tissue

$\delta^{15}N$ values were least (Figure 2.7b, Table 2.1a,b, Table 2.2). Despite apparent variations in oyster %N (Figure 2.7c), these were not significantly different by season (Table 2.2). Modeling exercises reflected the variability in oyster tissue $\delta^{15}N$ values over time in the Choptank River (Figure 2.8a-c). Modeled gill $\delta^{15}N$ best resembled measured values (total error of 2.99 ‰) but had moderate variability, while muscle had minimum variability but moderate total error (1.53 ‰), followed by mantle, which varied the most and had the highest total model error (4.66 ‰; Figure 2.8a-c).

Modeled response of oyster $\delta^{15}N$ over time in a hypothetical scenario with constant seston $\delta^{15}N$ determined the required exposure time to attain stable tissue $\delta^{15}N$. Tissue $\delta^{15}N$ values decreased exponentially when a constant summer temperature was assumed and rapidly during warm months and slower during cooler months when seasonal temperatures were used as inputs. Oyster tissue $\delta^{15}N$ values stabilized at 11.0 ± 0.5 ‰, which was offset from the constant seston $\delta^{15}N$ input (8 ‰) by the trophic fractionation factor (+3 ‰). Under a constant summer temperature, stabilization was achieved quickly (79 days in gills, 103 days in mantle, and 258 days in muscle) compared to seasonal temperature fluctuations: 165 days in gills, 189 days in mantle, and 522 days in muscle (Figure 2.9). Maximum turnover rates were reached during summer conditions in this model. Model results indicated longer required exposure times than observations, where oyster tissue $\delta^{15}N$ values lagged seston $\delta^{15}N$ values by an average of 33 to 57 days (Table 2.4), producing the best fit as compared with all other time period combinations during the four month deployment in Monie Bay.

Discussion

Sample size requirements

Quantifying the required replication at the convergence of minimum, mean, and maximum standard errors (Bros and Cowell, 1987) optimized sample size at five individuals (Figure 2.2a-c). Relatively low variability (Figure 2.2a-c) among individuals required few replicate samples, and is due to temporal integration, but individual variability likely persists due to isotopic heterogeneity in the food supply. Many previous studies (e.g. Costanzo et al., 2001; Elliot and Brush, 2006; Fry and Allen 2003; McClelland and Valiela 1998) identified nitrogen source via opportunistic sample collection, while others manipulatively examined $\delta^{15}N$ in the laboratory or field (Adams and Sterner, 2000; Fertig et al., 2009); however few bioindicator studies explicitly identified appropriate sample size. Small sample size requirements reduced the likelihood of incomplete sampling due to mortality (Ford et al., 2006; Newell et al., 2000), disease (Ford and Smolowitz, 2007), or predation (Kennedy et al., 2009) in addition to enabling future studies at broad spatial scales.

$\delta^{15}N$ converges to reflect deployment locations

Oyster $\delta^{15}N$ tissues converge upon a common isotopic signature influenced by ambient isotopic signatures because they are assimilating local nitrogen sources. In Monie Bay, both nitrate and ammonium are available to and assimilated by plankton, with concentrations higher in upstream areas due to anthropogenic terrestrial runoff

(Chapter 3, this dissertation). Both $\delta^{15}N$ values from seston and oyster tissues fit expected $\delta^{15}N$ values generated from a 50/50 mixture of dissolved inorganic nitrogen $\delta^{15}N$ values modified by two trophic shifts (Figure 2.6). Additionally, measured $\delta^{15}N$ values in oyster tissues matched modeled values based upon measured seston $\delta^{15}N$ values (Figure 2.4a) and measured initial oyster $\delta^{15}N$ values (Figure 2.4b). Therefore, after deployment, oyster $\delta^{15}N$ values resembled the ambient gradient of $\delta^{15}N$ signatures in Monie Bay (Figure 2.5a-c), and can be averaged to elucidate this convergence (Figure 2.3a). Convergence with local ambient conditions enables organisms initially grown in various locations to be utilized, and expands potential areas for deployment even if lacking extant or sufficient populations (Smith et al., 2005), as has been done with macroalgae (Costanzo et al., 2001; Udy and Dennison, 1997), filter feeders (Gartner et al., 2002), or a combination (Fertig et al., 2009).

While oyster mantle and gill $\delta^{15}N$ values were sensitive to changing conditions, %N as a measure of nutritional condition was not. Tissue $\delta^{15}N$ values post-deployment were lower than upon collection (Figure 2.3a) while %N did not change substantially (Figure 2.3b). Similarly, oyster $\delta^{15}N$ values from the Severn and South Rivers initially varied by collection location (Table 2.1a), but did not significantly differ after shared deployment in Monie Bay (Table 2.1b). Meanwhile, oyster %N from these two rivers did not significantly differ either before or after deployment (Table 2.1a,b, Figure 2.3b). As further corroboration, post-deployment $\delta^{15}N$ values in oysters from the South and Severn Rivers aligned along the 1:1 line (Figure 2.5a-c), from which $\delta^{15}N$ responsiveness was inferred. Therefore, $\delta^{15}N$ values

were sensitive to different nitrogen sources while %N was less responsive, likely because nutritional condition of oysters was relatively stable and because source of dissolved inorganic nitrogen was not directly related to algal production or assimilation by oysters.

Oyster tissues integrate nitrogen over different time periods

Due to tissue turnover rate variations, nitrogen flux through the trophic structure is integrated over different time periods (e.g. Fry 2006). The shortest integration period was in the most metabolically active tissues (mantle, gills), and the longest was in less active tissues (muscle). Muscle tissue integrated the longest of the three tissues, as inferred from minimal temporal variability (Figure 2.7b, 2.8c). Further, all tissues from deployments in Monie Bay significantly differed by the initial growth location (Table 2.1a), but only muscle remained different after deployment (Table 2.1b). Measurements (Figure 2.5a-c), data regressions (Table 2.4), and modeling exercises (Figure 2.9) indicated that muscle tissue converged most slowly, though modeling provided an overestimate (258 days for muscle, 103 days in mantle, and 79 days in gills for a constant summertime temperature), (33 to 57 days), possibly due to temperatures and growth rates observed in Monie Bay. The longer length of time required for the muscle to stabilize is likely why this tissue has an apparent enrichment (of approximately +1 ‰) compared to gills or mantle (Figure 2.9). Similarly, bivalve muscle tissues were less sensitive than viscera (Garton et al., 2005; Moore, 2003; Piola et al., 2006) though likely more sensitive

than the shell matrix which integrates over the life of the bivalve (Carmichael et al. 2008). Variations in time integration may result in a longer required deployment time for muscle tissue than mantle or gills, though four months should be sufficient for all tissues and still enables short-term manipulative fieldwork with *C. virginica*, in comparison to species with very slow metabolism, such as the hydrocarbon seep mussel *Bathymodiolus childressi,* which takes over a year for complete tissue turnover (Dattagupta et al., 2004). Regardless of tissue or species selected, exposure and integration duration should be considered when interpreting nitrogen sources.

Reliance upon on measured temperatures and seston $\delta^{15}N$ values enabled the oyster isotope model to estimate seasonal uptake rates and turnover times *in situ* via tissue $\delta^{15}N$ value outputs (Figure 2.7). Variable environmental conditions and seston $\delta^{15}N$ values over seasons extends tissue turnover estimation beyond laboratory conditions (e.g. Witting et al. 2004, Sweeting et al. 2005) or single season field studies (e.g. Moore 2003) because assumption of a constant input $\delta^{15}N$ values is unnecessary. When applied to a scenario with constant temperature and isotopic signature over time (Figure 2.9), the oyster isotope model reduces to similar exponential tissue turnover models found in the literature (e.g. Moore 2003, Witting et al. 2004, or Sweeting et al. 2005).

Exposure timing and integration duration influenced seasonal patterns of $\delta^{15}N$ values in oysters and their relationship with dietary seston $\delta^{15}N$ values. In the literature, few of the numerous studies using $\delta^{15}N$ values to indicate nitrogen source

account for seasonal variability (Finlay and Kendall, 2007). Summertime enrichment in seston (12.2 ± 0.5 ‰) was observed in the Choptank River (Figure 2.7a) similar to seasonal patterns in Waquoit Bay (York et al. 2007) and in Florida Bay (Anderson and Fourqurean, 2003; Fourqurean et al., 1997; Fourqurean et al., 2005; Fourqurean et al., 2007). As a food source, cyclical patterns of seston $\delta^{15}N$ influences oyster $\delta^{15}N$ values (Matthews and Mazumder, 2005), which also significantly differed by season (Table 2.2), but opposite in sign (depleted in summer, enriched in winter) to that of seston $\delta^{15}N$ (Figure 2.7a,b). As oysters integrated over time and were influenced by previous seasons, they were also more metabolically active in warmer months due in part to temperature-related biochemical reactions; (Dame 1972). Consistent with assumptions, oyster isotope model outputs exhibited faster responses in warmer months and longer retention of these values into cooler months (Figure 2.8a-c) as well as with constant temperature (Figure 2.9), implying maximal turnover during summer, the importance of temperature dependence and confirms that spring and summer are ideal times for deployment. Similar to scallops (Lorrain et al. 2002), oyster tissue $\delta^{15}N$ values converged in late summer and fall, as the gonads utilize metabolite stores during maturation (Figure 2.7b). Therefore, oysters were most sensitive when acclimated during spring for summer deployments. Furthermore, mantle was the most responsive tissue to these seasonal shifts (Figure 2.7b), reaching lowest $\delta^{15}N$ values by summertime (12.7 ± 0.3 ‰). Muscle $\delta^{15}N$ values were also depleted in summer months (13.8 ± 0.1 ‰), but less so than either gills (13.1 ± 0.2) or mantle (Figure 2.7b; Table 2.2), which suggested this tissue integrated over the

longest time period, likely due to slow turnover rates compared to other tissues (Bosley et al., 2002; Frazer et al., 1997; Sakano, 2005). Oysters were clear temporal integrators of nitrogen sources as they reflected seasonal $\delta^{15}N$ values with less variability than primary producers.

Baseline variations between tissues were possibly due to amino acid composition and associated affinities to $\delta^{15}N$ (Gaye-Siessegger, 2004), but regardless of mechanism these variations are important to consider when interpreting $\delta^{15}N$ values across multiple tissues. Mean muscle $\delta^{15}N$ values in all samples except for those in the Choptank River during winter 2005 were greater than mantle or gill $\delta^{15}N$ values by 0.4 to 1.1‰ or 0.2 to 0.7‰, respectively (Figures 2.2a, 2.6, 2.7b). Relatively higher muscle $\delta^{15}N$ values compared to other tissues have also been observed in other species including mammals, fish, birds, and bivalves (Frazer et al., 1997; Heikoop et al., 2000; Hobson and Clark, 1992; Lorrain et al., 2002; Piola et al., 2006; Tieszen et al., 1983). If differences between tissue $\delta^{15}N$ values were solely due to nitrogen source and integration times, the tissue type that was most enriched would vary more often, especially in the case of a pulse of anthropogenic nitrogen source, however this scenario was not observed.

Conclusions

Multiple biological characteristics make oysters appropriate biological indicators of nitrogen sources. Sample size can be optimized at five individuals, which can be achieved with 7 to 8 oysters (accounting for mortality) collected from

multiple locations for deployment lasting four months (or two to three months, if only analyzing mantle or gill) at new locations to identify nitrogen types (NO_3^- or NH_4^+) and to infer nitrogen sources. Manipulative field deployments can provide spatial and temporal integrations (e.g. Costanzo et al., 2001; Fertig et al., 2009). Staggering deployments would enable nitrogen source detection at desired intervals to provide an inference of changes in nitrogen source over weeks, months, or even a year (Dattagupta et al., 2004; Fila et al., 2001; McKinney et al., 2002; Moore, 2003), however due to variations in integration periods and seasonal fluctuations in primary producers, deployment in springtime would optimize integration over summer months. For studies concerned with chronic or long-term nitrogen sources, muscle tissues best provided temporal integration but required a longer exposure time than gills or mantle. Since mantle and gill tissue $\delta^{15}N$ values responded similarly, the mantle would suffice for short-term studies due to slightly quicker response times than gills. This simple and straightforward method may aid detection and monitoring of nitrogen sources over multiple spatial and temporal scales.

Chapter 2 Tables

Table 2.1. Descriptive statistics and ANOVAs before and after deployment

Descriptive statistics and ANOVA analysis of $\delta^{15}N$ values and %N in oyster tissues from the South and Severn Rivers (a) and after shared deployments in Monie Bay and its tributary creeks (b). Sample size (n), mean, standard error (SE), degrees of freedom (df), mean square error (MSE), F value (F), and p value (p) are reported.

(a)

Parameter	Tissue	South River		Severn River		ANOVA				
		n	*Mean (SE)*	*n*	*Mean (SE)*	*n*	*df*	*MSE*	*F*	*p*
$\delta^{15}N$ (‰)	Muscle	10	12.5 (0.1)	10	14.3 (0.1)	20	1,18	0.0902	174.86	**<0.0001**
	Gills	5	12.2 (0.2)	5	13.3 (0.1)	10	1,8	0.1281	23.78	**0.0012**
	Mantle	4	12.2 (0.3)	5	13.4 (0.1)	9	1,7	0.1966	16.86	**0.0045**
%N	Muscle	10	12.3 (0.2)	10	12.6 (0.2)	20	1,18	0.5547	1.12	0.3043
	Gills	5	9.7 (0.2)	5	9.8 (0.2)	10	1,8	0.2011	0.31	0.5958
	Mantle	4	10.1 (0.5)	5	9.8 (0.3)	9	1,7	0.5790	0.29	0.6046

(b)

Parameter	Tissue	Monie Bay		Little Creek		Little Monie Creek		Monie Creek		ANOVA				
		n	*Mean (SE)*	*n*	*Mean (SE)*	*n*	*Mean (SE)*	*n*	*Mean (SE)*	*n*	*df*	*MSE*	*F*	*p*
$\delta^{15}N$	Muscle	20	12.9 (0.2)	20	11.9 (0.1)	30	11.9 (0.1)	30	12.4 (0.1)	100	1,98	0.4843	15.68	**0.0001**
	Gills	20	12.0 (0.2)	20	10.7 (0.1)	30	10.5 (0.1)	30	10.7 (0.1)	100	1,98	0.6921	2.26	0.1356
	Mantle	20	11.8 (0.2)	20	10.7 (0.1)	30	10.5 (0.1)	30	10.7 (0.1)	100	1,98	0.5945	1.54	0.2172
%N	Muscle	20	13.9 (0.1)	20	13.1 (0.1)	30	13.1 (0.2)	30	13.5 (0.2)	100	1,98	0.8760	0.20	0.6539
	Gills	20	9.9 (0.1)	20	9.7 (0.1)	30	9.7 (0.1)	30	10.2 (0.1)	100	1,98	0.4593	0.12	0.7351
	Mantle	20	9.6 (0.1)	20	9.8 (0.1)	30	9.2 (0.2)	30	9.3 (0.1)	100	1,98	0.6606	0.26	0.6087

Table 2.2. Descriptive statistics and ANOVAs across seasons

Descriptive statistics and ANOVA analysis of oyster $\delta^{15}N$ values and %N in muscle, gills, and mantle tissues in the Choptank River, 2006-2007. Spring: March-May, Summer: June-August, Fall: September–November, and Winter: December–February. Seasons were analyzed by ANOVA. Sample size (n), degrees of freedom (df), mean squared error (MSE), F value (F), and p value (p) are reported.

Parmeter	Tissue	Spring		Summer		Fall		Winter		ANOVA				
		n	*Mean (SE)*	*n*	*Mean (SE)*	*n*	*Mean (SE)*	*n*	*Mean (SE)*	*n*	*df*	*MSE*	*F*	*p*
$\delta^{15}N$	Muscle	32	14.3 (0.1)	23	13.8 (0.1)	27	14.4 (0.1)	27	14.2 (0.1)	109	3,105	0.33	4.41	**0.006**
	Gills	17	14.1 (0.2)	18	13.1 (0.2)	23	14.0 (0.1)	24	14.5 (0.2)	82	3, 78	0.45	17.04	**< 0.001**
	Mantle	15	13.9 (0.2)	19	12.7 (0.3)	23	13.6 (0.2)	23	14.3 (0.1)	80	3, 76	0.83	10.37	**< 0.001**
%N	Muscle	32	11.9 (0.2)	23	12.0 (0.3)	27	12.2 (0.1)	20	11.2 (0.6)	102	3, 98	2.46	1.64	0.186
	Gills	17	9.9 (0.3)	18	9.5 (0.1)	23	9.3 (0.2)	16	9.8 (0.4)	74	3, 70	1.17	1.19	0.318
	Mantle	15	10.5 (0.6)	19	10.3 (0.5)	23	9.3 (0.2)	14	9.6 (0.3)	71	3, 67	3.07	2.09	0.110

Table 2.3. Correlations of physical, chemical, and biological measurements

Correlations between physical, chemical, and biological measurements conducted in the Choptank River (2006-2007). Correlations use the lower of the two sample sizes. Significant correlations are in bold and asterisks reflect p values (* : $p < 005$; ** : $p < 0.01$; *** : $p < 0.001$).

			Muscle $\delta^{15}N$	Gill $\delta^{15}N$	Mantle $\delta^{15}N$	Seston $\delta^{15}N$	Total Phosphorus	Total Nitrogen	Dissolved Oxygen	Dissolved Oxygen	pH	Temperature	Salinity
	Units		‰	‰	‰	‰	µM	µM	%	mg L^{-1}		°C	
	Sample size	19	15	15	17	13	13	18	18	17	19	19	
Salinity		19	0.158	-0.029	-0.278	**0.678****	-0.069	**-0.635***	0.272	0.061	-0.262	0.340	1.000
Temperature	°C	19	-0.436	**-0.627***	**-0.589***	**0.704***	**0.612***	**-0.653***	-0.140	**-0.568***	-0.415	1.000	
pH		17	0.283	0.283	0.210	-0.006	-0.174	0.288	-0.015	0.053	1.000		
Dissolved Oxygen	mg L^{-1}	18	-0.008	0.532	0.209	**-0.513***	-0.256	0.349	**0.865*****	1.000			
Dissolved Oxygen	%	18	-0.181	0.253	-0.150	-0.117	0.264	0.089	1.000				
Total Nitrogen	µM	13	**0.569***	**0.672***	**0.684***	**-0.628***	-0.106	1.000					
Total Phosphorus	µM	13	-0.216	-0.199	-0.073	0.149	1.000						
Seston $\delta^{15}N$	‰	17	-0.219	-0.385	-0.552	1.000							
Mantle $\delta^{15}N$	‰	15	**0.650****	**0.905*****	1.000								
Gill $\delta^{15}N$	‰	15	**0.725****	1.000									
Muscle $\delta^{15}N$	‰	19	1.000										

Table 2.4. Regressions of oyster $\delta^{15}N$ vs. seston $\delta^{15}N$

Regression analysis between oyster mantle, gill, and muscle $\delta^{15}N$ values as the dependent variable and mean seston $\delta^{15}N$ values during various time periods as the independent variable. Regression R^2 values are presented for each tissue.

		$\delta^{15}N$ in oysters vs. seston		
Averaging Seston Since	Days Integrated	Mantle R^2	Gills R^2	Muscle R^2
22-Jun	110	0.24	**0.51**	**0.55**
11-Jul	91	**0.52**	**0.58**	0.20
25-Jul	77	**0.52**	**0.57**	0.16
14-Aug	57	**0.65**	**0.69**	0.32
7-Sep	33	**0.83**	**0.83**	**0.56**
10-Oct	1	0.14	0.21	0.36

Chapter 2 Figures

Figure 2.1: Map of Chesapeake Bay regions studied.

Chesapeake Bay study locations (a). White circles indicate collection sites in the South and Severn Rivers (b) for deployment (grey circles) in Monie Bay (d). Black triangles indicate collection sites in the South and Choptank Rivers for the sample size determination (b and c). The black star (c) indicates where seasonal variations were observed in the Choptank River.

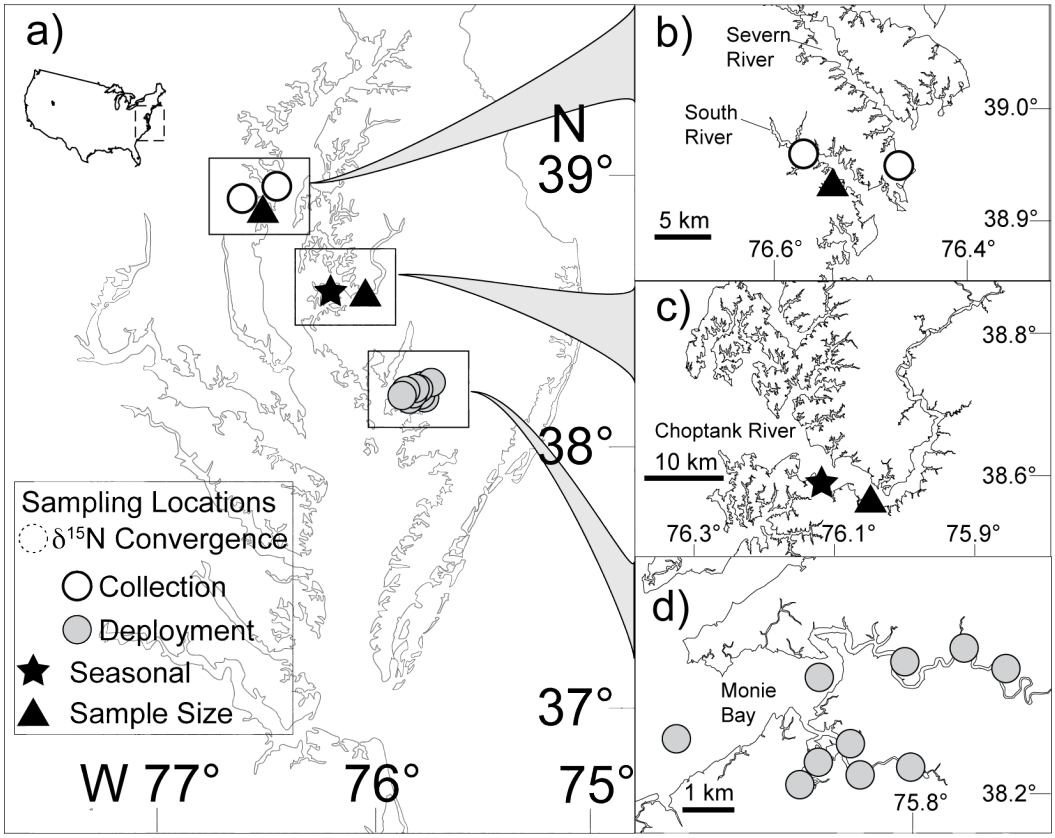


Figure 2.2: Trade-off between error and sample size

Oyster mantle (a), gills (b), and muscle (c) tissues. Panels include the maximum (squares), mean (circles), and minimum (triangles) standard errors of various sample sizes drawn randomly from 35 samples (Bros and Cowell 1987).

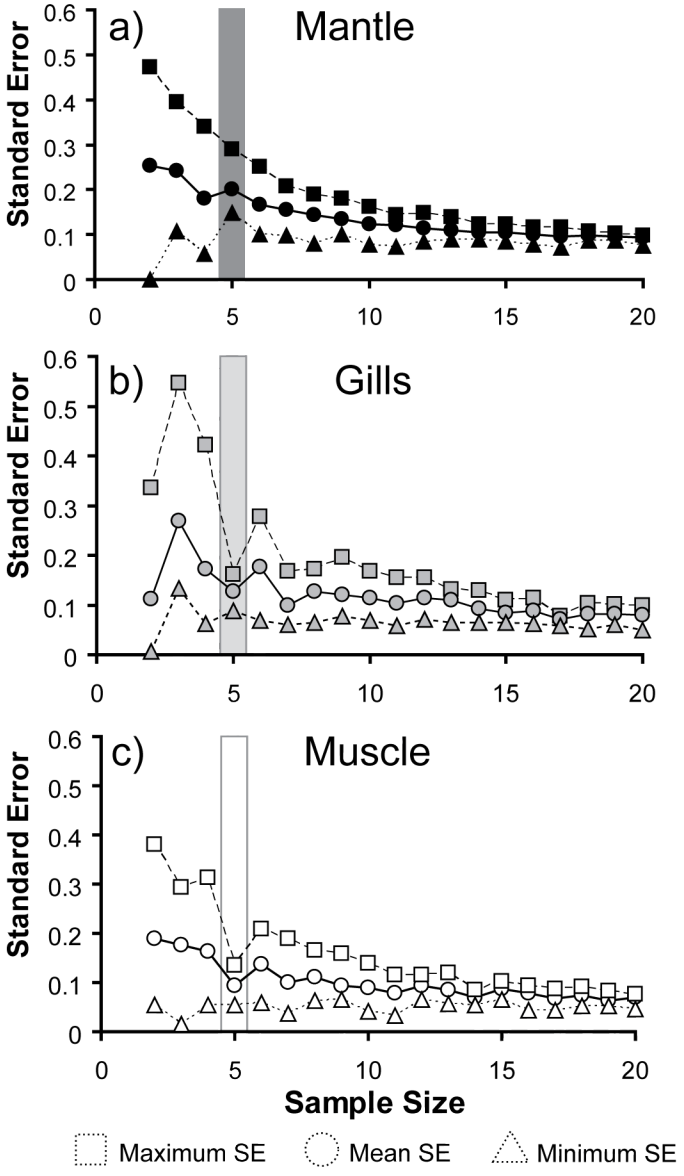


Figure 2.3: Oyster $\delta^{15}N$ upon collection and post-deployment

Mean oyster mantle (black), gills (grey), and muscle (white) $\delta^{15}N$ (a) and %N (b) upon collection in the South River (circles) and Severn River (squares) and after deployment in Monie Bay. Standard error bars are plotted, but in most cases are smaller than symbols representing means.

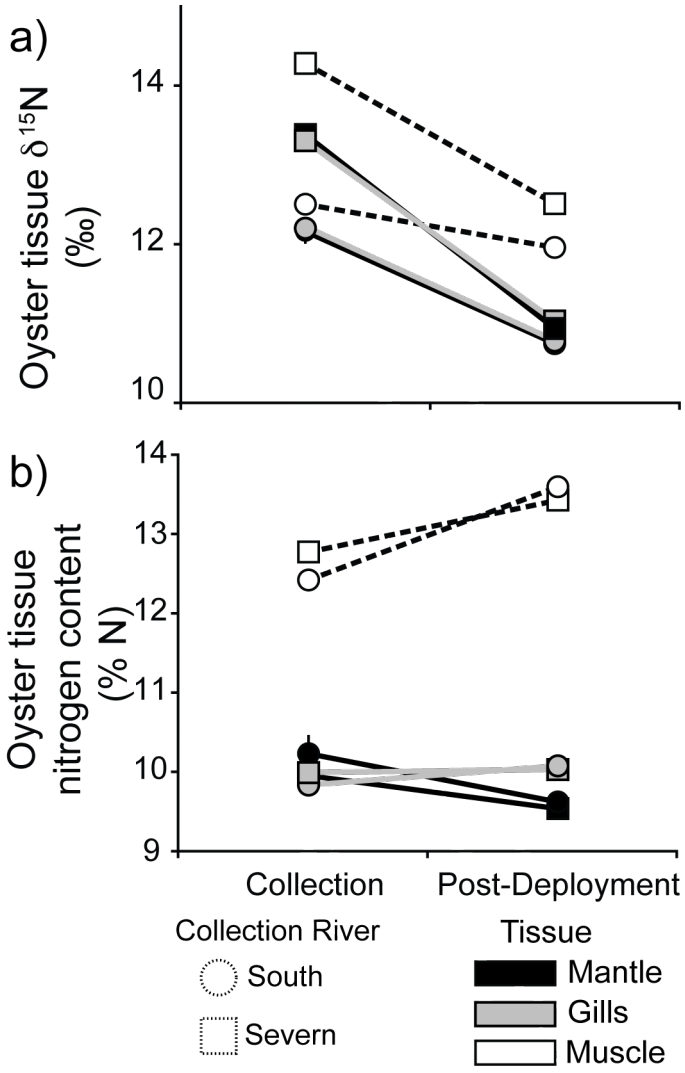


Figure 2.4: Measured and modeled seston and oyster $\delta^{15}N$ in Monie Bay

Measured mean a) seston $\delta^{15}N$ values and b) modeled and measured oyster $\delta^{15}N$ values for mantle (black), gill (grey), and muscle (white) tissues in Monie Bay and its tributary creeks. Standard error bars are presented for measurements, but are sometimes smaller than symbols representing means.

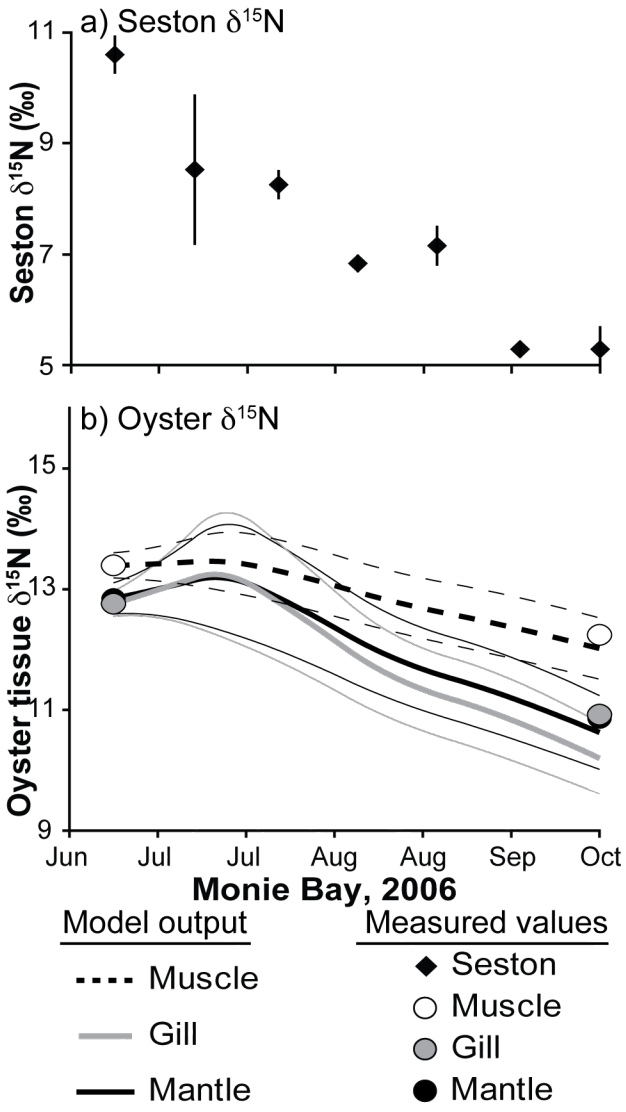


Figure 2.5: Post-deployment oyster $\delta^{15}N$ in oysters from different rivers

Comparison of oysters initially grown in the Severn or South Rivers after a shared deployment in Monie Bay. Mantle (a), gills (b), and muscle (c) $\delta^{15}N$ values at the initial collection (triangles) and post-deployment (circles) are regressed and plotted against the 1:1 line.

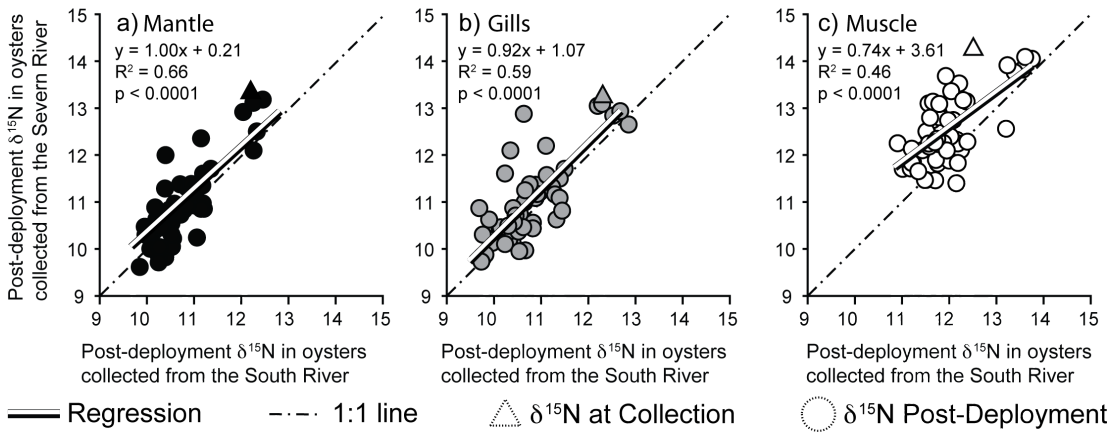


Figure 2.6: $\delta^{15}N$ values of dissolved inorganic nitrogen, seston, and oysters

Mean nitrite, nitrate, ammonium, seston, and oyster tissue (mantle, gills, and muscle) $\delta^{15}N$ values. Standard error bars are shown, though in some cases are smaller than data points.

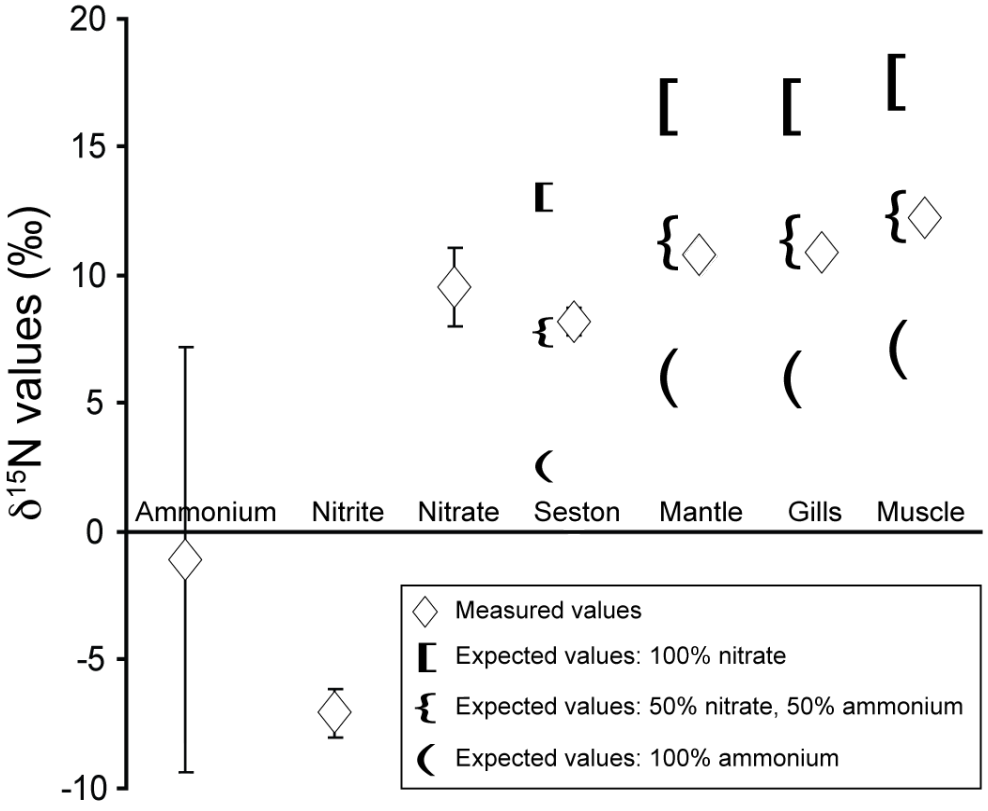


Figure 2.7: Seasonal seston and oyster $\delta^{15}N$ variations

Seasonal variations observed in the Choptank River of a) seston (black circles), b) oyster $\delta^{15}N$ values and c) nitrogen content in oyster tissues. Oyster tissues are represented by squares: mantle (black), gills (grey), and muscle (white).

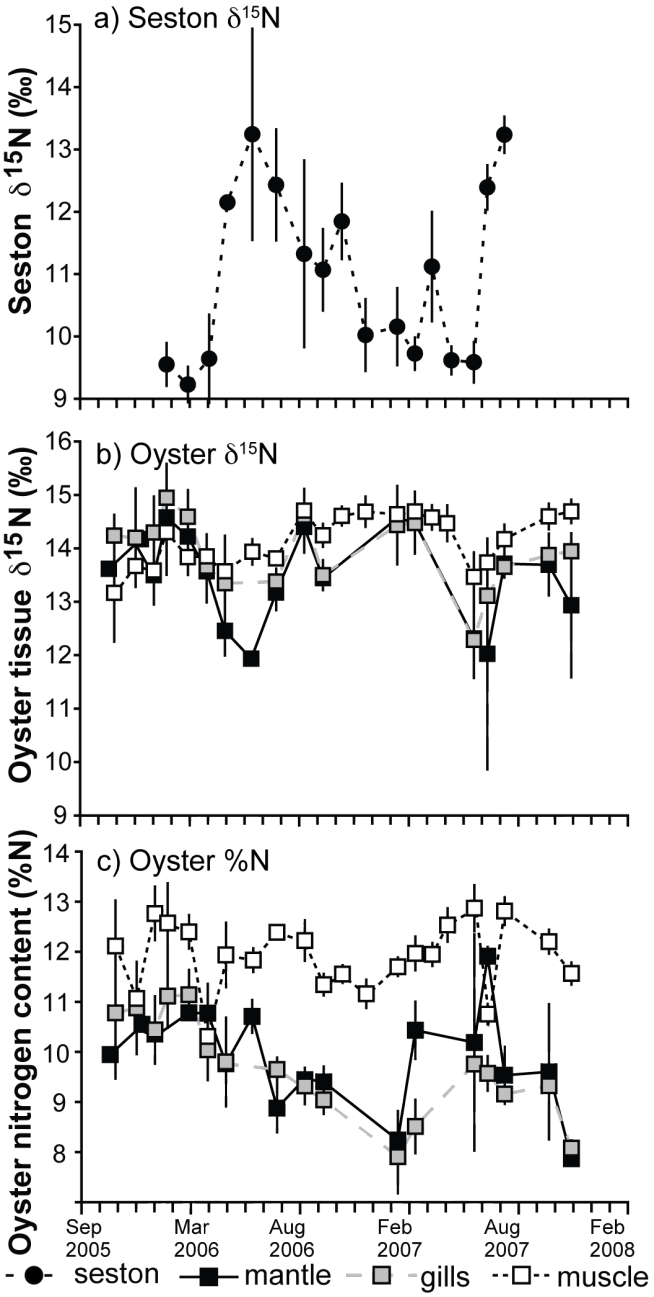


Figure 2.8: Modeled and measured oyster $\delta^{15}N$ in the Choptank River

Modeled and measured values of oyster $\delta^{15}N$ values in a) mantle, b) gills, and c) muscle during 2006-2007 in the Choptank River.

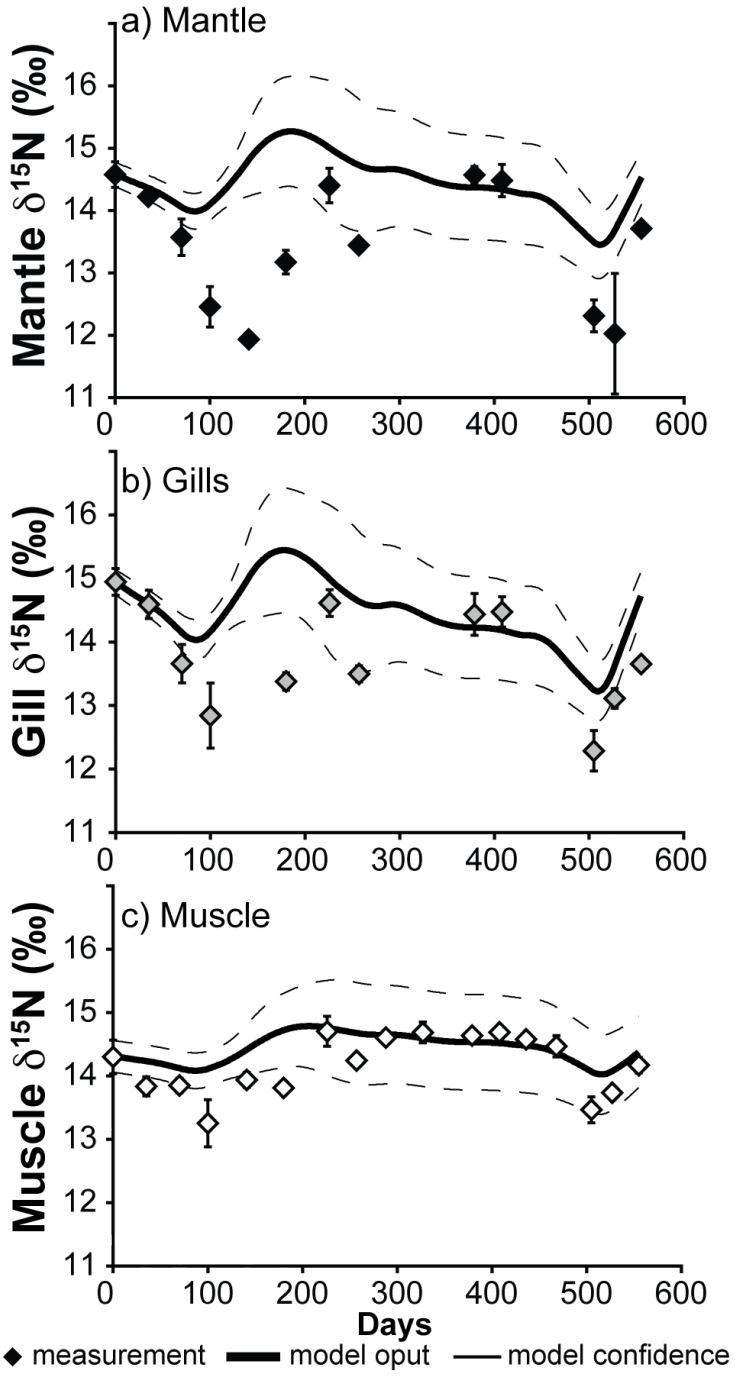
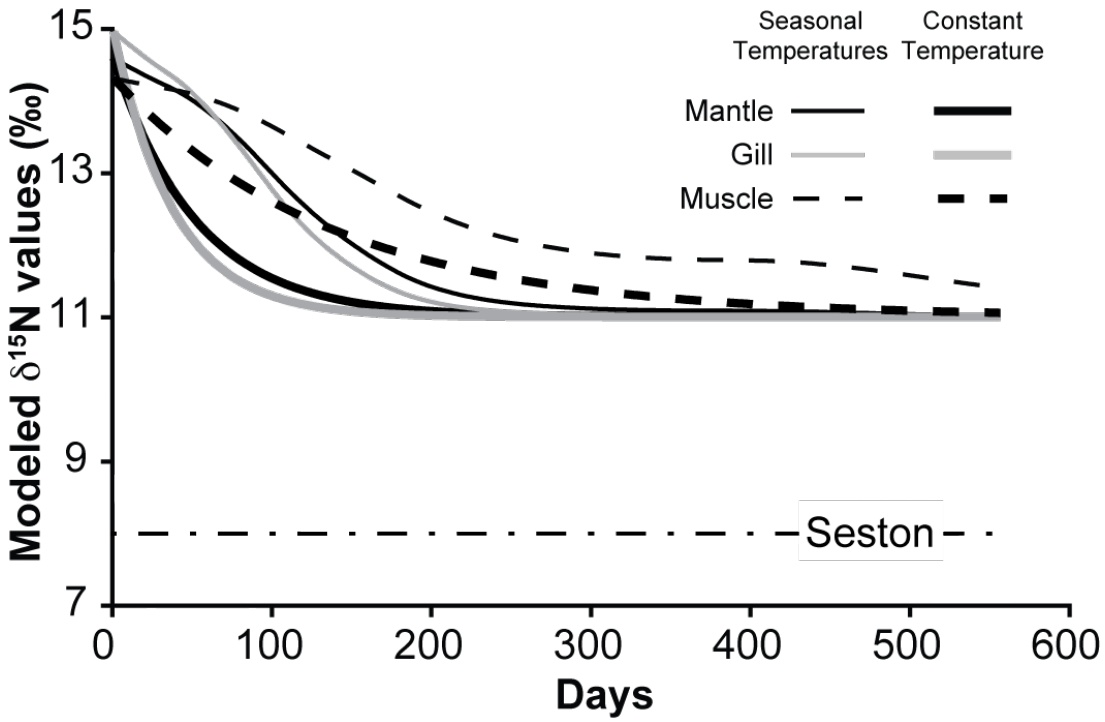


Figure 2.9: Modeled constant seston $\delta^{15}N$ scenario

Hypothetical scenario with a constant seston δ15N value and modeled oyster δ15N values for mantle (black), gill (grey), and muscle (white).



CHAPTER 3: OYSTER δ^{15}N AS A BIOINDICATOR OF HUMAN AND ANIMAL NITROGEN AND DEGRADED WATER QUALITY IN A SUB-ESTUARY OF CHESAPEAKE BAY

Abstract

Nitrogen from manures, septic systems, and wastewater treatment plants contribute to water quality degradation and are difficult to distinguish once transported to and mixed in aquatic ecosystems. Monie Bay, a small, shallow, tidal sub-estuary situated within the Chesapeake Bay (Maryland) National Estuarine Research Reserve, provided a 'natural laboratory' to test if nitrogen sources could be identified by analyzing $\delta^{15}N$ in oyster (*Crassostrea virginica*) tissues. Monie Bay receives freshwater inputs from three creeks (with flushing times of 1.2, 1.9, and 12.4 days) in its rural watershed that vary in sub-watershed size and land use: residential septic systems and poultry operations (Monie Creek), crop fertilizer (Little Monie Creek), and wetlands/forest (Little Creek). Potential nitrogen loss from denitrification (calculated from an established relationship with residence time) was estimated to be low (12.1 – 19.5 %), and elevated oyster $\delta^{15}N$ values (12.4 ± 0.1‰ in Monie Creek and 12.9 ± 0.2‰ in Monie Bay) indicated anthropogenic nitrogen sources. Spatial patterns supported the inference that animal waste entered Monie Bay from within and human waste from outside the watershed. Nitrogen was interpreted to be derived from both 19 poultry houses delivering manure from the Monie Creek watershed (~8.6×10^5 kg N yr-1 input, the approximate equivalent to ~200,000 people yr^{-1}) and human/animal wastes from Wicomico River and its watershed (including wastewater facilities servicing ~29,500 people, ~7,000 septic systems, and estimated poultry manure inputs of 3.7×10^6 kg N yr^{-1}). Therefore, living resources (oysters) linked

nitrogen sources with a Water Quality Index ($R^2 = 0.89$, $p < 0.05$) that was rated 'poor' (mean 36 ± 4 out of 100) due to nutrient concentrations. Nutrient reductions in Monie Bay will require input reductions from human/animal wastes derived from inside and outside its topographic watershed.

Introduction

Nitrogen from human and animal waste sources (e.g. wastewater treatment plants, septic systems, and manures) and abiotic sources (e.g. chemically synthesized agricultural fertilizers) continue to result in degradation of water quality in Chesapeake Bay (Kemp et al. 2005; Fisher et al. 2006) and other estuaries in the United States (Howarth et al. 2002; Bricker et al. 2008) and worldwide (Smith 2003). Sources are difficult to distinguish with conventional water quality monitoring data (e.g. total nitrogen). The burgeoning field of biological indicators (e.g. McClelland and Valiela 1998; Costanzo et al. 2001; and Leavitt et al. 2006) report that stable nitrogen isotopes ($\delta^{15}N$) in a wide range of organisms (Vanderklift and Ponsard 2003) such as macrophytes (e.g. McClelland et al 1997; Cole et al. 2004; Cohen and Fong 2006; Benson et al. 2008), finfish (Lake et al. 2001), and mollusks (Fila et al. 2001; McKinney et al. 2002; Daskin et al. 2008) can be used to distinguish between chemically synthesized agricultural fertilizers and human or animal wastes. As filter feeders, oysters integrate nitrogen derived from microorganisms, phytoplankton, detritus and inorganic particles (Langdon and Newell 1996). Furthermore, oyster muscle $\delta^{15}N$ integrates these sources over four months (see Chapter 2) while direct measurements on groundwater (Aravena et al. 1993) or the water column (Lefebvre et al. 2007) only provide an instantaneous measure.

Determination of nitrogen source by measuring $\delta^{15}N$ is possible as natural sources and synthetic fertilizers are 'fixed' from atmospheric N_2 (0‰) and have correspondingly low $\delta^{15}N$ values: generally -4 to +4 ‰ (Hübner, 1986; Macko and Ostrom, 1994; Vitoria et al., 2004) while human and animal wastes are fractionated to +5 to +8 ‰ (Leavitt et al. 2006; Fry 2006). Fractionation is due to a combination of ammonia volatilization and denitrification at the source of the signal or by microbial processing employed by wastewater treatment facilities (Fry, 2006; Kendall 1998; McClelland and Valiela, 1998; Sweeny and Kaplan, 1980; Tucker et al., 1999). Interpretation of source must be balanced against alternative hypotheses as terrestrial nitrogen sources have a complicated pathway to aquatic biological indicators and isotopic signatures can be modified or multiple sources can be mixed (Fry, 2006; Kendall, 1998). Nitrogen from manures can be further fractionated by volatilization (Altabet, 2006; Cline and Kaplan, 1975; Fry, 2006; Kendall, 1998; McClelland and Valiela, 1998) or dissolve and be denitrified, both of which elevate $\delta^{15}N$ in the remaining nitrate (Mariotti et al. 1982; Shearer and Kohl 1988). Phytoplankton assimilate dissolved inorganic nitrogen and are consumed by oysters, with an enrichment of 3 to 4 ‰ at each trophic step due to digestion and waste elimination (Adams and Sterner, 2000; Minagawa and Wada, 1984).

Measurements of oyster $\delta^{15}N$ present an opportunity to link land use, nitrogen sources, and aquatic living resources (e.g. Dennison et al. 1993; Wazniak et al. 2007) to understand water quality degradation in a rural sub-estuary of Chesapeake Bay. Nitrogen source identification can direct nutrient reduction priorities in Monie Bay, a

component of the Chesapeake Bay National Estuarine Research Reserve System (NERRS), which is designated for monitoring, education, and conservation (Kennish 2004). The current study addressed three questions: 1) Can oyster $\delta^{15}N$ link water quality, nitrogen source, and living resources? 2) What role does denitrification play in the nitrogen isotope ratios observed in Monie Bay and its creeks? 3) What are the nitrogen sources available to Monie Bay and its creeks?

Methods and Materials

Study Location and Experimental Design

The Chesapeake Bay, Maryland NERRS site includes Monie Bay (38°13'30"N, 75° 50'00" W), a sub-estuary of Chesapeake Bay, USA that served as a 'natural laboratory' (Figure 3.1) for linking terrestrial nitrogen sources with aquatic living resources. Monie Bay is a small (1 - 2 km wide, 4 km long), shallow (1.9 ± 0.1 m), tidally influenced embayment that receives freshwater inputs from three creeks, varying in watershed size and flushing time. Tidal flushing from Monie Bay, springtime flows, and intermittent precipitation act to control salinities in Little Monie Creek and Little Creek, while a stream provides freshwater to Monie Creek year-round (Jones et al. 1997). Tidal scouring, rather than fluvial input, formed these creeks (Ward et al. 1998), but freshwater nutrient delivery, associated with land use, over spatial and seasonal patterns is a key driver of their overall variability (Apple et al. 2004).

Forests and wetlands generally dominate Monie Bay's rural and remote watershed (located in Somerset County, Maryland) but comparisons between creeks were made to identify anthropogenic sources of nitrogen, which include agricultural fertilizers, residential septic systems, and industrial poultry production. Land use in the watersheds of Monie Creek (45.0 km^2) and Little Monie Creek (17.9 km^2) are similar, with over 50% forest cover, only 3% developed and the remainder roughly split between wetlands and agriculture (Figure 3.1). Comparisons of nitrogen sources can be made between septic and poultry (Monie Creek) and crop agriculture (Little Monie Creek) due to minimal residential development or poultry production in the Little Monie Creek watershed. Little Creek, with the smallest sub-watershed (9.4 km^2) is dominated by wetlands (63%) and forests (35%), and was used as a reference creek as virtually no agriculture (1%) or development (1%) was present (Figure 3.1). Most forests in the watersheds of Monie Bay's tributaries are largely managed as unfertilized tree farms (due to economic constraints; Fykes pers. com.). Poultry production is also located in the watershed, and poultry houses were counted from tiled digital ortho-imagery (resolution of 1 meter ground sample distance) collected during the agricultural growing season (USDA 2005). While no wastewater treatment plants are located in the watershed of Monie Bay, the watershed of the adjacent Wicomico River contains three (2002 nitrogen loads: Salisbury ~ 1.8×10^5 kg N y^{-1}, Fruitland ~ 9.1×10^3 kg N y^{-1}, and Delmar ~ 5.9×10^3 kg N y^{-1}). To link land use, nitrogen source, and water quality, conventional water quality monitoring and oyster $\delta^{15}N$ values were measured at ten stations situated to compare inputs from

septic and poultry feeding operations (Monie Creek), crop fertilizers (Little Monie Creek), and reference wetlands and forests (Little Creek) as well as downstream effects in Monie Bay (Figure 3.1). Station 1 was located at the mouth of Monie Bay, at its intersection with the mouth of Wicomico River.

Deploying, sampling, and analyzing oyster biological indicators

Sufficient spatial variability of oyster $\delta^{15}N$ to identify nitrogen sources available to the different creeks was ensured by deploying oysters according to the methods described earlier (see Chapter 2) for four months, from 21 June 2006 (Monie Bay and Monie Creek) or 22 June 2006 (Little Monie Creek and Little Creek) to 10 October 2006 (Figure 3.1). At each station, fouling organisms, predators, and trapped sediments were removed from mesh bags fortnightly or as needed to maintain water flow through the bags.

Analytical methods

Upon collection, oysters were kept on ice in the field and frozen (-20° C) at the laboratory until processing. Five surviving oysters were dissected to recover individual adductor muscles. Tissues were thawed, rinsed, thoroughly dried (60 °C for 48 hours minimum) and ground (with mortar and pestle). Sub-samples (1.0 ± 0.2 mg dry weight) were placed in tin capsules (Elemental Microanalysis, pressed, standard weight, 8 × 5 mm) for analysis of nitrogen content (%N) and $\delta^{15}N$ (where $\delta^{15}N = (^{15}N/^{14}N_{sample}/^{15}N/^{14}N_{standard} - 1) \times 10^3$ and the standard was atmospheric N_2, defined as 0‰; Fry 2006) by University of California Davis Stable Isotope Facility

which used a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

Water Quality

To quantify the effects of anthropogenic nitrogen on water quality, standard physical, nutrient, and biological metrics were measured. Water quality monitoring was conducted at all 10 stations according to standard protocols employed by the NERRS System-Wide Monitoring Program (SWMP), including fortnightly measurements of Secchi depth, salinity, temperature, dissolved oxygen concentration, dissolved oxygen saturation, total nitrogen, total phosphorus, and chlorophyll *a* (Mills et al. 2008). The Department of Health and Mental Hygiene Division of Environmental Chemistry analyzed chlorophyll a, while the Chesapeake Biological Laboratory Nutrient Analytical Services Laboratory measured total nutrients by standard techniques (D'Elia et al. 1977; Technicon 1977, 1986, 1987; US EPA 1979a,b; Valderrama 1981).

Estimating flushing times and nitrogen removal

Creek flushing rates affect nutrient transport and potential for denitrification and recycling. Therefore, non-advective water exchange and flushing time between Monie Bay and each of its tributaries were quantified with a simple conservative box model (Pritchard 1969; Officer 1980; Hagy 1996). The non-advective water exchange was quantified using two linear equations:

$$Q_f = Q_{out} = 1/3 * \text{precipitation} \quad \text{Equation 3.1}$$

$$V\, ds_{in}/d_t = 0 = -Q_{out}s_{in} + E(s_{out} - s_{in}) \quad \text{Equation 3.2}$$

In Equations 3.1 and 3.2, Q_f = freshwater input ($m^3\ day^{-1}$), Q_{out} = advective transport out of the creek ($m^3\ day^{-1}$), E = non-advective exchange between Monie Bay and each of the creeks ($m^3\ day^{-1}$), s_{in} = salinity inside the creek (ppt), s_{out} = salinity in Monie Bay (ppt), and V = creek volume (m^3).

Assumptions for utilizing these equations were 1) that each creek's water volume remained constant and not stratified, 2) that differences in groundwater inputs were negligible as topography and soils were similar, and that therefore, 3) Q_f was 1/3 * precipitation (the other 2/3 precipitation underwent evapotranspiration). Precipitation volumes were obtained from average daily precipitation from 1971-2000 across the area of each creek's watershed (Maryland State Climatologist, 2008) while creek mean measured salinity values (in 2006) were utilized as a constant to solve the equation for E (non-advective exchange). Flushing times were calculated by dividing creek volume by non-advective exchange.

Nitrogen removal (%) was calculated as $r = 23.4m^{0.204}$, where m is residence time in months and r is % nitrogen removal due to denitrification across lakes, river reaches, estuaries, and the continental shelf (Seitzinger et al. 2006). Estimates of both flushing times and nitrogen source inputs were calculated under the assumption that nitrogen was not transported beyond watershed boundaries (either via import or

export), leaving future studies to quantify nitrogen budgets, manure contributions, and terrestrial-aquatic coupling coefficients.

Results

Monie Bay and its tributaries were degraded overall based nutrient, chlorophyll *a*, and dissolved oxygen concentrations. Concentrations of total nitrogen (57 ± 1 μM to 85 ± 6 μM) and total phosphorus (1.6 ± 0.1 μM to 3.8 ± 0.5 μM) exceeded threshold values (46 μM for total nitrogen and 1.2 μM for total phosphorus) over the summer (Table 3.1). Mid-reach and upstream Monie Creek (stations 9 and 8, respectively) had low dissolved oxygen concentrations (3.9 ± 0.6 mg L^{-1} and 4.8 ± 0.3 mg L^{-1}, respectively, compared to a threshold value of 5.0 mg L^{-1}) and high chlorophyll *a* concentrations (19 ± 3 μg L^{-1} and 15 ± 2 μg L^{-1}, respectively, compared to a threshold value of > 15 μg L^{-1}) while in upstream Little Monie Creek (station 5) dissolved oxygen concentrations were also low (4.5 ± 0.4 mg L^{-1}; Table 3.1). In summary, all stations were degraded (Table 3.1). Total nitrogen and oyster $\delta^{15}N$ had different patterns in different creeks, with an inverse relationship in Little Monie Creek and Little Creek, and a positive relationship in Monie Creek, and no correlation when compared across all stations in creeks (Figure 3.2). Nitrate $\delta^{15}N$ values exponentially decreased with increasing nitrate concentrations (Figure 3.3a), while ammonium $\delta^{15}N$ values increased exponentially with concentration (Figure 3.3b). This contrasting pattern indicated opposite fractionation patterns.

Potential nitrogen removal from the tributaries of Monie Bay by denitrification was low in Little Creek and Little Monie Creek (15.1%, 16.7%, respectively, Table 3.2) due in part to quick flushing times (3.5 days, 5.7 days, respectively) and high non-advective exchange (1.2 to 1.3 × 10^5 m^3 d^{-1}; Table 3.2). Monie Creek had both the highest total nutrient load and estimated nitrogen removal, both of which were influenced by watershed area (4.5 × 10^7 m^2), water volume (nearly 2.5 × 10^6 m^3), freshwater input (45,323 m^3 day^{-1}), slow flushing time (37.2 days; Table 3.2), the small non-advective exchange with Monie Bay (almost half of that of Little Monie Creek) and land use (including rural residences and poultry feeding operations; Figure 3.1). In comparison to its creeks, Monie Bay was larger than any of its tributaries (nearly 13.5 × 10^6 m^3) and was more saline (11.7 ppt) even though it received the highest mean daily precipitation (218,459 m^3 d^{-1}) since its watershed area (7.2 × 10^7 m^2) was the sum of that of its three tributaries, but nitrogen removal could not be calculated for this region as calculations relied upon non-advective exchange with Monie Bay.

Spatial patterns of water quality and oyster $\delta^{15}N$ data showed that nutrients entered upstream Monie Creek and the mouth of Monie Bay. Total nitrogen, total phosphorus, and chlorophyll *a* concentrations were highest in upstream Monie Creek (Figure 3.4a-c) where terrestrial sources of nitrogen such as rural residential and poultry inputs were available from within the watershed (station 8: 5.1 ± 3.0 ppt salinity, 85.48 ± 6.25 μM total nitrogen, 3.8 ± 0.5 μM total phosphorus, 19 ± 3 μg L^{-1} chlorophyll *a*). Concentrations decreased downstream with lowest concentrations of

total nitrogen at the mouth of Monie Bay (station 1: 12.4 ± 1.3 ppt salinity, 57.26 ± 1.28 μM total nitrogen) and lowest concentrations for total phosphorus and chlorophyll *a* in Little Creek (station 3: 10.4 ± 2.0 ppt salinity, 1.6 ± 0.1 μM total phosphorus, 8 ± 1 μg L^{-1} chlorophyll *a*, respectively; Figure 3.4a-c). Monie Creek had higher total nitrogen concentrations than Little Monie Creek ($p < 0.01$), and both creeks had higher total nitrogen and total phosphorus concentrations than Little Creek or Monie Bay ($p < 0.01$; Table 3.1; Figure 3.4a,b). Dissolved oxygen concentrations were negatively correlated with total nitrogen ($r = -0.51$, $p < 0.0001$, Figure 3.5a) and total phosphorus ($r = -0.54$, $p < 0.0001$, Figure 3.5b), but not chlorophyll *a*, and were lowest in upstream Monie Creek (station 8: 3.9 ± 0.6 mg L^{-1} dissolved oxygen; Table 3.1), and decreased with distance from the mouth of Monie Bay (Figure 3.4d). Oyster $\delta^{15}N$ values were slightly enriched in upstream Monie Creek (station 8, 12.71 ± 0.21 ‰) and the mouth of Monie Bay (station 1: 13.72 ± 0.09 ‰) and decreased towards upstream areas of Monie Bay, Little Monie Creek and Little Creek (Figure 3.4e). The range in seston $\delta^{15}N$ and oyster $\delta^{15}N$ values was not large (Figure 3.4e,f), though they were significantly different between most stations.

Due to patterns of water circulation and flushing, Monie Bay acted as both a nutrient source to its tributaries (transporting nutrients among its creeks) and as a nutrient sink for other watersheds (receiving septic and wastewater nitrogen at its mouth). Enrichment of oyster $\delta^{15}N$ in downstream areas compared to upstream areas in Monie Bay, Little Creek, and Little Monie Creek (Figure 3.4e) indicated human and/or animal waste sources of nitrogen was transported upstream. The mouths of

Monie Bay and Wicomico River mix, and enriched oyster $\delta^{15}N$ values there (station 1, 13.7 ± 0.1 ‰; Figure 3.4e) were likely influenced by Wicomico River watershed nitrogen sources such as septic (6,543 systems, MD DNR 1999), wastewater effluents (2002 nitrogen loads in Salisbury: ~ 1.8×10^5 kg TN y^{-1}, Fruitland: ~ 9.1×10^3 kg TN y^{-1}, and Delmar: ~ 5.9×10^3 kg TN y^{-1}) or poultry (~3.7×10^6 kg TN yr^{-1}; Table 3.3). Monie Bay, as an extension of Chesapeake Bay, ultimately received a portion of its anthropogenic inputs from the ~166,500 km^2 Chesapeake Bay watershed (Figure 3.6).

Discussion

Elevated allochthonous nutrient concentrations from multiple nitrogen sources were found in the upper reaches of Monie Bay's tributaries (Figure 3.4a,b; Jones et al. 1997), which led to degraded water quality (Table 3.1) correlated with agricultural practices (Cornwell et al. 1994) and land use (Figure 3.1). Measured water quality in the NERRS site (Figure 3.4a-f) was similar to data used to compile the Chesapeake Bay Health Index for the Lower Eastern Shore reporting region, which received a score of score of 34 in 2007 and a score of 45 in 2006 (Eco-Check 2007). Bay Health Index scores were degraded due to high chlorophyll *a*, low water clarity, and low benthic community index. Overall, Chesapeake Bay Health Index scores negatively correlated to land development and agricultural usage (Williams et al. 2008).

Regardless of which metric lowered index scores, the inverse relationship between oyster $\delta^{15}N$ and water quality metrics (Figure 3.4a-f) related living resources, septic and poultry nitrogen sources, and water quality. An exception was in upstream Little Monie Creek (station 5, Figure 3.1; 9.1 ppt salinity) explained by agricultural runoff entering from the watershed (Figure 3.1) at concentrations above threshold values (Table 3.1). Nitrogen sources at this station were likely different (crop fertilizers) from the remainder of the creek sites (influenced by human and/or animal wastes entering at the creek mouth from Monie Bay).

Animal and/or human wastes (likely poultry manures) were inferred to be an important nitrogen source to Monie Bay and its creeks. While only 19 poultry houses in the Monie Creek watershed were counted by digital ortho-imagery (USDA 2005), these houses contained an estimated effective year-round population of 23,661 chickens house^{-1}. Poultry population in Monie Bay's watershed was assumed proportional to the number of chickens sold (USDA 2002) and chicken houses (USDA 2005) in Somerset County (Figure 3.7a), accounting for an average of 4.7 flocks per year (USDA 2002). Poultry production in the Monie Bay watershed generated ~8.1×10^5 kg total nitrogen (untreated) yr^{-1} (Table 3.3), based on an estimated 58 kg manure chicken^{-1} yr^{-1} (containing 1.9 kg total nitrogen yr^{-1}; Naber and Bermudez 1990), roughly equivalent to that defecated by 1.9×10^5 people (assuming 4.3 kg total nitrogen generated person^{-1} yr^{-1}). Additionally, residential septic systems (699 throughout Monie Bay's watershed) along Monie Creek likely enriched the oyster $\delta^{15}N$ signal (MD DNR 1999). Nitrate from agricultural fields

likely dominated terrestrial inputs to the outlier in Little Monie Creek, as indicated by oyster $\delta^{15}N$ values (11.7 ± 0.2‰; Figure 3.4e) which were near the expected value (12.0 ‰; Harrington et al. 1998; Bateman and Kelly 2007) for this source (Choi et al. 2007) based upon combining nitrate $\delta^{15}N$ values (6.0 ‰) in nearby watersheds with similar hydric soils and flat topography (T. Fisher and T. Jordan, pers. com.) with two trophic level shifts (plankton assimilation and oyster consumption) of 3.0 ‰ each (Minagawa and Wada 1984; Adams and Sterner 2000; Fry 2006). To improve water quality, nutrient concentrations could be reduced by focusing on poultry sources due to the relationship between oyster $\delta^{15}N$ and water quality (Figure 3.4a-f).

In addition to Monie Bay, poultry sources also deliver large nitrogen inputs to the Wicomico River watershed, and other areas across Delmarva Peninsula. Animal (particularly poultry) manure in Somerset County, MD was spread for fertilizer locally during the spring (Fykes, pers. com.; Figure 3.5), which likely contributed to the elevated $\delta^{15}N$ signal along upstream portions of Monie Creek (station 8, 12.71 ± 0.21‰; Figure 3.4e). Poultry litter applications generally increase soil total nitrogen (Kingery et al. 1994) and phosphorus (Griffin et al. 2003) content and adversely impacted water quality (Woli et al. 2002). County wide, nearly 63.9×10^6 broilers and other meat-type chickens from Somerset County, MD (24^{th} in the nation) were sold in 2002 (Figure 3.7a; USDA 2002), produced from approximately 300 poultry houses (counted from ortho-imagery), while only ~2.5×10^4 people resided in this county in 2002 (Figure 3.7b; MD Department of Planning 2000). Over the long term, nearby water quality (station EE3.1 in Tangier Sound, 38° 11' 48.6744" N,

75° 58' 23.5416" W) fluctuated (Figure 3.7c) while county chicken sales generally increased (Figure 3.7a; Chesapeake Bay Program 2008). By 2002, Delmarva Peninsula hosted an effective chicken population of ~1.1×10^8, with an estimated input of 2.1×10^8 kg total nitrogen yr^{-1}, more than that generated by 4.8×10^7 people (Table 3.3), while the estimated 2002 human population on Delmarva Peninsula was only 1.2×10^6 people (Figure 3.7b).

Consistent with the quantities of nitrogen inputs from animal and human wastes, the alternative hypothesis that isotopic signatures of nitrogen source were modified by denitrification (Fry, 2006; Kendall, 1998) was rejected because estimations of potential nitrogen loss via denitrification (Seitzinger et al. 2006) was not generally dominant at this NERRS site (15 to 25 %) compared to 40% to 50% in other estuaries (Seitzinger et al. 2006). Less than 20% of nitrogen was calculated to be potentially lost via denitrification in each of the tributary creeks (Table 3.2) based on the relationship between nitrogen removal by denitrification and residence time of various aquatic ecosystems (Seitzinger et al. 2006). Rapid flushing times and low denitrification rates estimated by the simple conservative box model for this NERRS site (Table 3.2) indicated that alternative interpretations of $\delta^{15}N$ values to nitrogen sources could be rejected. While larger regions like Tomales Bay also receive multiple sources of nutrients, these ecosystems often exhibit slower flushing and more extensive nutrient recycling, resulting in nitrogen bioavailability largely controlled by denitrification (Smith et al. 1989), which influenced spatial patterns of seagrass $\delta^{15}N$ values (Fourqurean et al. 1997).

Tidal advection likely transported nitrogen from Monie Bay into its tributary creeks, with more impact on Little Monie Creek and Little Creek than on Monie Creek. This pattern is consistent with localized physical processes, including tidal scouring, which formed these creeks. Since it took roughly twice as long to flush Monie Creek (24.5 days) than Little Creek or Little Monie Creek (15.1 days and 16.7 days, respectively, Table 3.2) nitrogen sources from Monie Creek's watershed (poultry and septic; Figure 3.4e) had a greater impact on water quality there than did the respective watersheds of Little Monie Creek and Little Creek (Figure 3.6) because terrestrially-derived nitrogen remained in the tributary for a longer period of time. Likewise, poultry, septic, and wastewater nitrogen sources that entered the mouth of Monie Bay (Figure 3.4e) encroached more upon Little Monie Creek and Little Creek than Monie Creek. Monie Bay itself was likely influenced by allochthonous nitrogen sources (i.e. the Wicomico River) by bottom layer circulation patterns typical of Chesapeake Bay tributaries (Figure 3.6). For example, both wastewater effluent from the Patuxent River watershed (Fisher et al. 2006) and nutrients in Chesapeake Bay from other tributary watersheds (Testa et al. 2008) act as nutrient sources to the Patuxent River through this circulation and transport pattern.

Due to the small watershed area of Monie Bay (72.3 km^2), anthropogenic activities with associated nitrogen inputs generally occurred within 6 km of its creeks, compared to larger ecosystems (e.g. Jordan et al. 1997; Brawley et al. 2000; Turner and Rabelais 2003), and atmospheric deposition may be important to ecosystems with a high surface area to volume ratio (Giblin and Gaines 1990; Paerl 1995). Nitrogen

inputs from multiple locations occurred in other ecosystems such as Californian estuaries (Cohen and Fong 2006), the Wadden Sea (van Beusekom and de Jonge 2002, Weston et al. 2004) and the Oregon coast (Frick et al. 2007), e.g. from inflowing river and mid-estuary sources (Cohen and Fong 2006), from upwelling (van Beusekom and de Jonge 2002), or from a groundwatershed not aligned with the topographically defined watershed (Winter et al. 2003; Kasper 2006). Ecosystems around the world receive nutrient inputs from both inside and outside their watersheds, and oyster $\delta^{15}N$ values provided a powerful tool to elucidate interactions between the watersheds of Monie Bay and Wicomico River. Nutrient concentration reductions in the NERRS site will require holistic management and input reductions from poultry, septic, and wastewater sources of nitrogen derived from inside and outside its topographically defined watershed.

Chapter 3 Tables

Table 3.1: Water quality monitoring data and index

Monitoring data measurements for total nitrogen, total phosphorus, dissolved oxygen, and chlorophyll *a* measurements and the threshold values used in calculation of the Water Quality Index. Mean values (n = 6) are presented with standard errors in parentheses.

Monitoring Stations	Distance from Monie Bay mouth (km)	Surface Temperature (°C)	Surface Salinity	Secchi depth (m)	Total Nitrogen (µM)	Total Phosphorus (µM)	Dissolved Oxygen ($mg\ L^{-1}$)	Chlorophyll *a* ($\mu g\ L^{-1}$)	Water Quality Index
Threshold value (Dennison et al. 1993, Stevenson et al. 1993, Ritter and Montagna 1999, Wazniak et al. 2007, Williams et al. 2008)					*> 46*	*> 1.2*	*< 5.0*	*> 15*	
Monie Bay									
1	0.00	25.1 (1.3)	12.2 (0.5)	0.6 (0.1)	57 (1)	1.7 (0.1)	7.8 (0.3)	14 (2)	38 (6)
2	3.64	25.7 (1.3)	11.2 (0.7)	0.5 (0.1)	60 (4)	1.8 (0.2)	7.2 (0.3)	13 (2)	54 (10)
Little Creek (Wetlands)									
4	4.96	25.4 (1.5)	11.2 (0.7)	0.7 (0.1)	58 (3)	1.5 (0.1)	6.5 (0.5)	11 (2)	42 (8)
3	6.49	25.1 (1.5)	10.4 (0.8)	0.7 (0.1)	61 (3)	1.5 (0.1)	5.7 (0.3)	8 (1)	50 (0)
Little Monie Creek (Crop agriculture)									
7	5.02	25.4 (1.4)	11.2 (0.7)	0.5 (0.1)	59 (3)	1.8 (0.1)	6.4 (0.5)	11 (2)	42 (5)
6	6.22	25.3 (1.4)	10.5 (0.9)	0.6 (0.1)	64 (2)	2.1 (0.1)	5.6 (0.5)	12 (1)	42 (5)
5	7.77	25.7 (1.5)	9.1 (1.2)	0.6 (0.1)	72 (4)	3.1 (0.4)	4.5 (0.4)	13 (1)	21 (4)
Monie Creek (Septic/ manures)									
10	8.05	25.1 (1.4)	9.5 (0.8)	0.7 (0.1)	63 (3)	1.9 (0.1)	5.8 (0.5)	11 (1)	42 (5)
9	11.00	25.1 (1.5)	6.8 (1.0)	0.6 (0.4)	74 (5)	2.6 (0.2)	4.8 (0.3)	15 (2)	17 (8)
8	12.90	25.6 (1.3)	4.5 (1.3)	0.5 (0.0)	85 (6)	3.7 (0.5)	3.9 (0.6)	19 (3)	13 (6)

Table 3.2: Box model calculations of physical water characteristics

Results from a simple conservative box model of flushing time, non-advective exchange (E) and potential nitrogen removal in Monie Bay and its three tributary creeks. Salinity was measured in 2006 while daily precipitation was averaged over 1971-2000 (Office of Maryland State Climatologist, 2008).

Creek	Volume (m^3)	Mean Salinity (ppt)	Mean Daily Precipitation (m^3 d^{-1})	Watershed area (m^2)	E (m^3 d^{-1})	Flushing time (d)	Expected N removal (%)
Monie Bay	13,495,457	11.7	218,459	7.2E+07			
Little Creek (wetlands)	418,984	10.8	28,403	9.4E+06	118,071	3.5	15.1
Little Monie Creek (crop agriculture)	726,748	10.2	54,086	1.8E+07	127,972	5.7	16.7
Monie Creek (septic/manures)	2,481,109	7.0	135,970	4.5E+07	66,666	37.2	24.5

Table 3.3: Nitrogen generation in sewage, septic, and poultry manures

Nitrogen in sewage, septic, and poultry manure sources generated in the watersheds of Monie Bay, Wicomico River, and Delmarva Peninsula. Poultry manure 'People Equivalents' are estimated based on manure (wet: 1.32 kg TN chicken^{-1} year^{-1}, dry: 0.35 kg TN chicken^{-1} year^{-1}, and a conservative estimate: 0.04 kg TN chicken^{-1} year^{-1}) sewage (3.18 kg TN person-1 year-1), and septic systems (4.32 kg TN person^{-1} year^{-1}) (Naber and Bermudez 1990, Lichtenberg et al. 2002). Data sources: Delaware Department of Natural Resources and Environmental Control (DNREC), Maryland Department of the Environment (MDE), Maryland Department of Natural Resources (MD DNR), Maryland Department of Planning (MDP), U.S. Census Bureau (U.S. Census), U.S. Department of Agriculture (USDA), U.S. Environmental Protection Agency (US EPA), Virginia Department of Environmental Quality (VDEQ), Virginia Department of Health, Eastern Shore District (VDHES).

		Monie Bay	Wicomico River	Delmarva Peninsula	References
Human Population (2002)		2,576	28,028	1,172,776	MDP 2000 U.S. Census 2000 MD DNR 2009
Average Annual Chicken Population (2002)		763,560	1,788,912	101,008,080	Naber and Bermudez 1990 USDA 2002 USDA 2005
Chicken Manure 'People Equivalents'	*Wet : septic*	233,542	547,156	30,894,305	Naber and Bermudez 1990 US EPA 2002
	Dry : sewage	83,378	195,343	11,029,751	
	Dry : septic	61,398	143,848	8,122,131	
	Conservative : sewage	9,207	21,570	1,217,916	
	Conservative : septic	6,780	15,884	896,654	
Sewage Systems (2002)		0	3	27	A. Brockenbrough, VDEQ, pers. comm. P. Hansen, DNREC, pers. comm. MDE 2009
Sewage Inputs (kg TN yr^{-1})		0	196,212	556,090	Crites and Tchobanoglous 1998 MD DNR 2009 US EPA 2002 Tchobanoglous et al. 2003
Septic Systems		699	7,233	181,953	A. Butler, MDP, pers. comm. J. Davis, VDHES, pers. comm. J. Volk, DNREC, pers. comm.
Septic Inputs (kg TN yr^{-1})		10,304	112,112	3,133,864	US EPA 2002 Tchobanoglous et al. 2003
Manure Inputs (kg TN yr^{-1})	*Wet*	1,008,478	2,362,720	133,407,228	Lichtenberg et al. 2002
	Dry	265,129	621,160	35,072,840	Parker and Li 2006
	Conservative	29,276	68,589	3,872,777	USDA 2005

Chapter 3 Figures

Figure 3.1: Monie Bay, National Estuarine Research Reserve study sites

Map of Monie Bay National Estuarine Research Reserve within Delmarva Peninsula (a). Land use in Monie Bay and Wicomico River watersheds (b). Sampling stations within Monie Bay and its tributary creeks are noted (c).

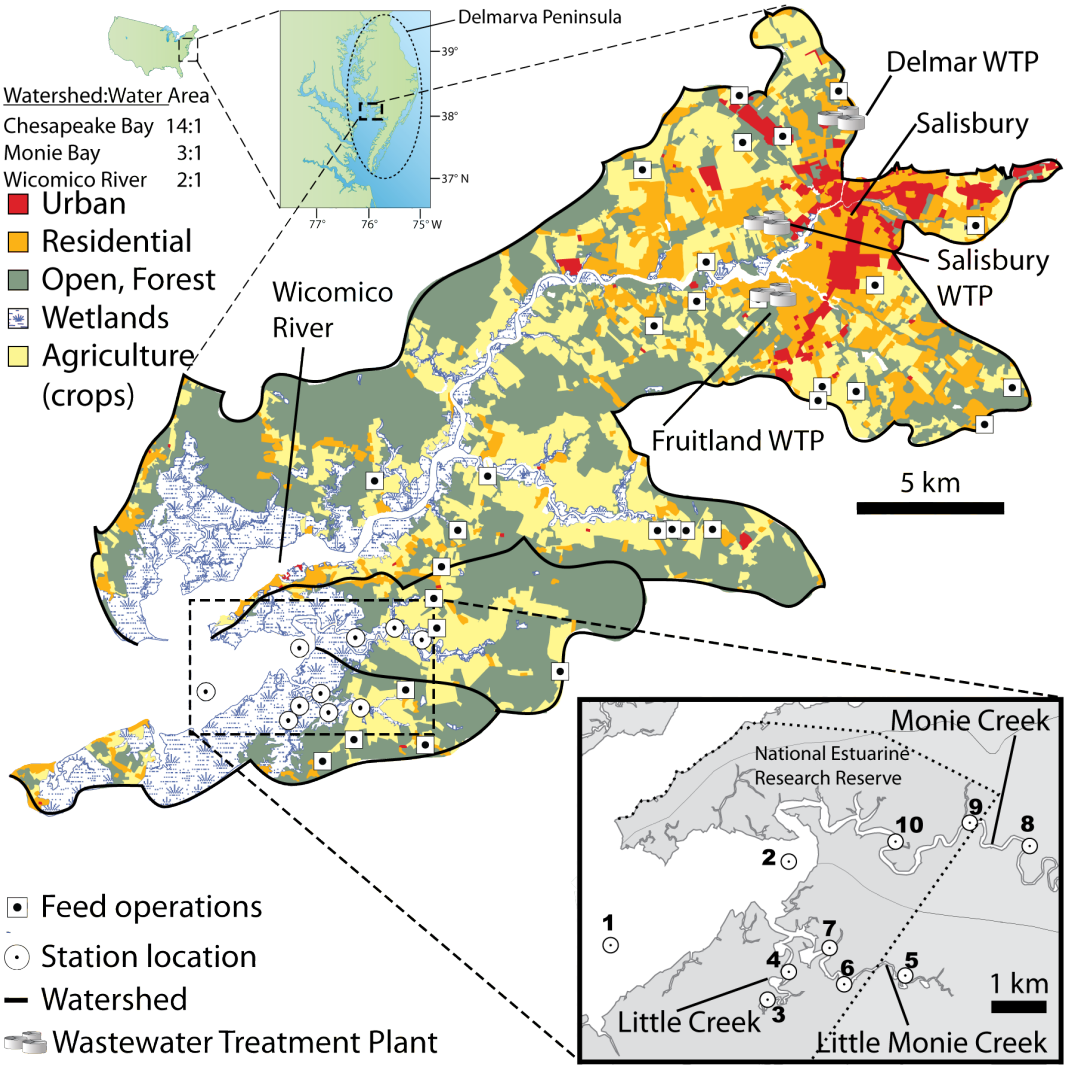


Figure 3.2: Oyster $\delta^{15}N$ vs total nitrogen and Water Quality Index

Relationships between oyster muscle $\delta^{15}N$ values and total nitrogen (µM). Stations 4 and 7 (downstream Little Creek and Little Monie Creek, respectively) both had a Water Quality Index score of 42 and oyster $\delta^{15}N$ value of 12.1 ‰.

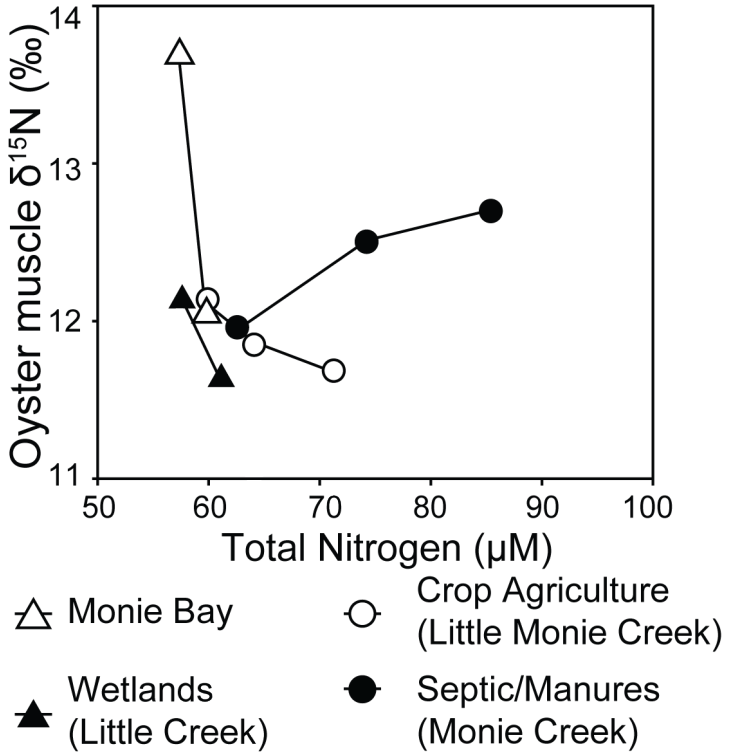


Figure 3.3: Dissolved inorganic nitrogen $\delta^{15}N$ vs. concentrations

Nitrate (a) and ammonium (b) $\delta^{15}N$ values vs. concentrations in Monie Bay and its tributary creeks.

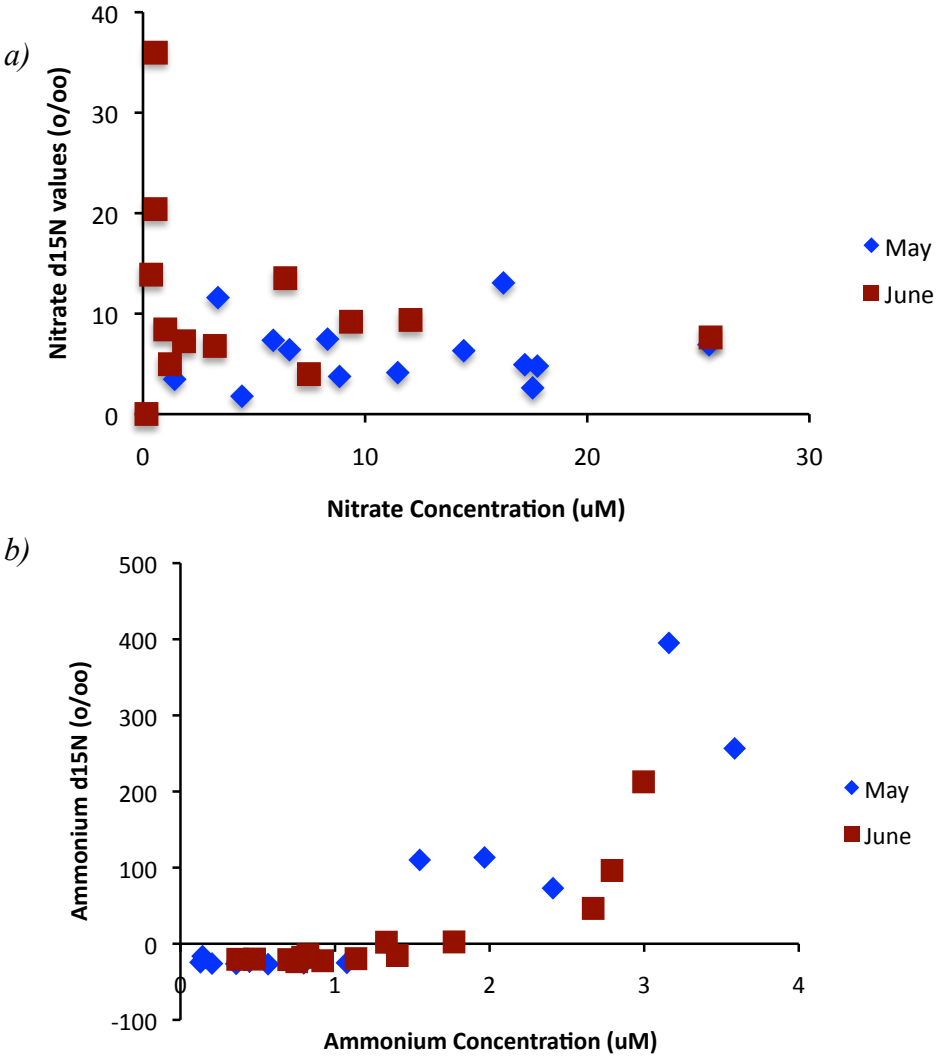


Figure 3.4. Water quality metrics and oyster $\delta^{15}N$

Water quality metrics vs. salinity for (a) total nitrogen (µM), (b) total phosphorus (µM), (c) chlorophyll *a* (µg L^{-1}), (d) dissolved oxygen (mg L^{-1}), (e) oyster muscle $\delta^{15}N$ (‰) and (f) seston $\delta^{15}N$. Means and standard error bars are plotted.

Sample sizes (n): Monie Creek (n=8 all stations), Monie Bay (n=7 both stations), Little Monie Creek (n=7 at salinity 11.0, 10.3; n=6 at salinity 9.1), Little Creek (n=7 at salinity 10.9; n=6 at salinity 10.4).

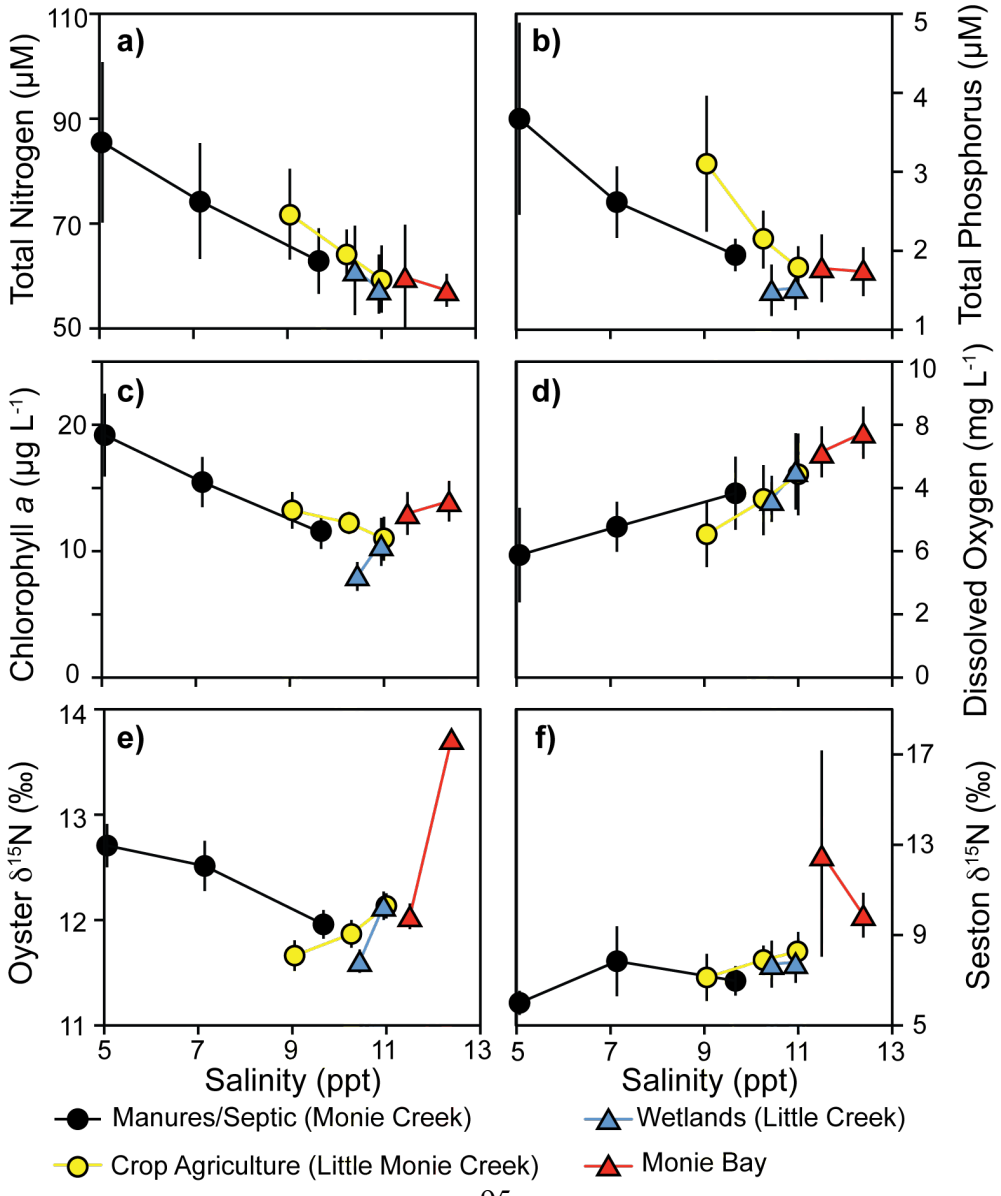


Figure 3.5: Dissolved oxygen correlations with total nutrients

Dissolved oxygen (mg L^{-1}) plotted vs. a) total nitrogen (µM) and b) total phosphorus (µM). Regression equations for all data points are reported, though data have been color coded by creek. Correlation coefficient (r) and p value (p) are reported.

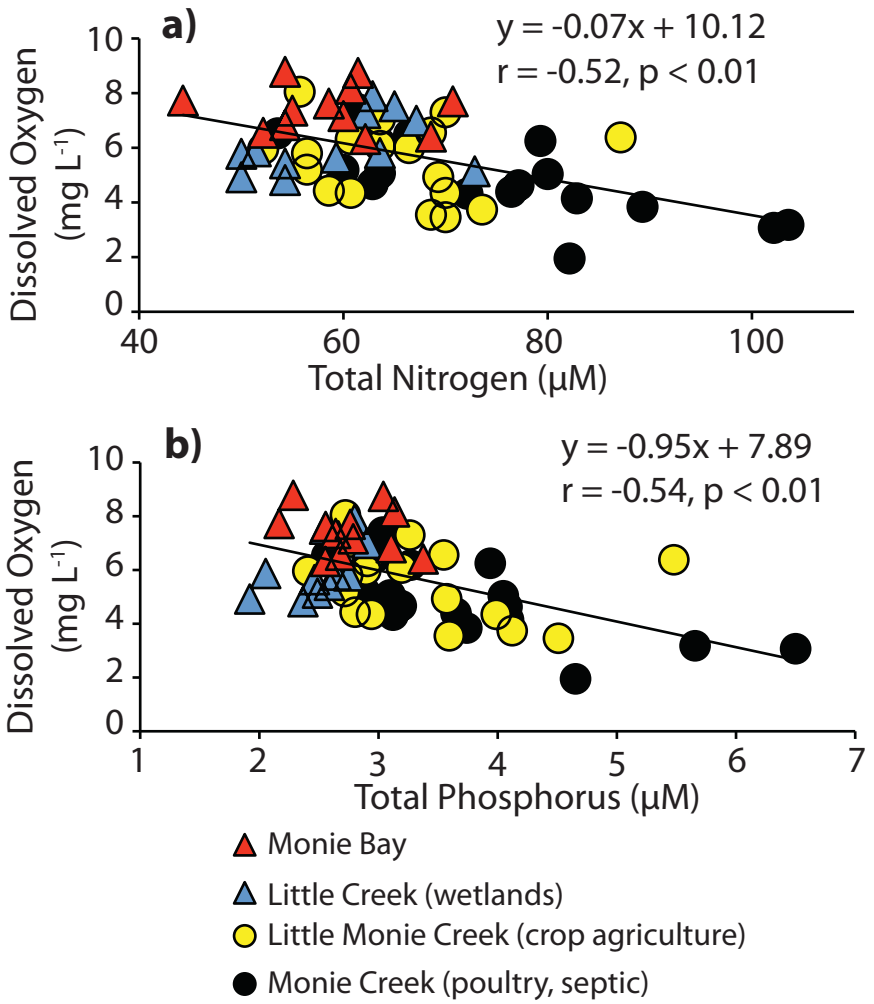


Figure 3.6: Conceptual diagram of oyster $\delta^{15}N$ and Water Quality Index

Conceptual diagram depicting spatial patterns of Water Quality Index, oyster $\delta^{15}N$ values, and land use in Monie Bay sub-watersheds and the Wicomico River watershed.

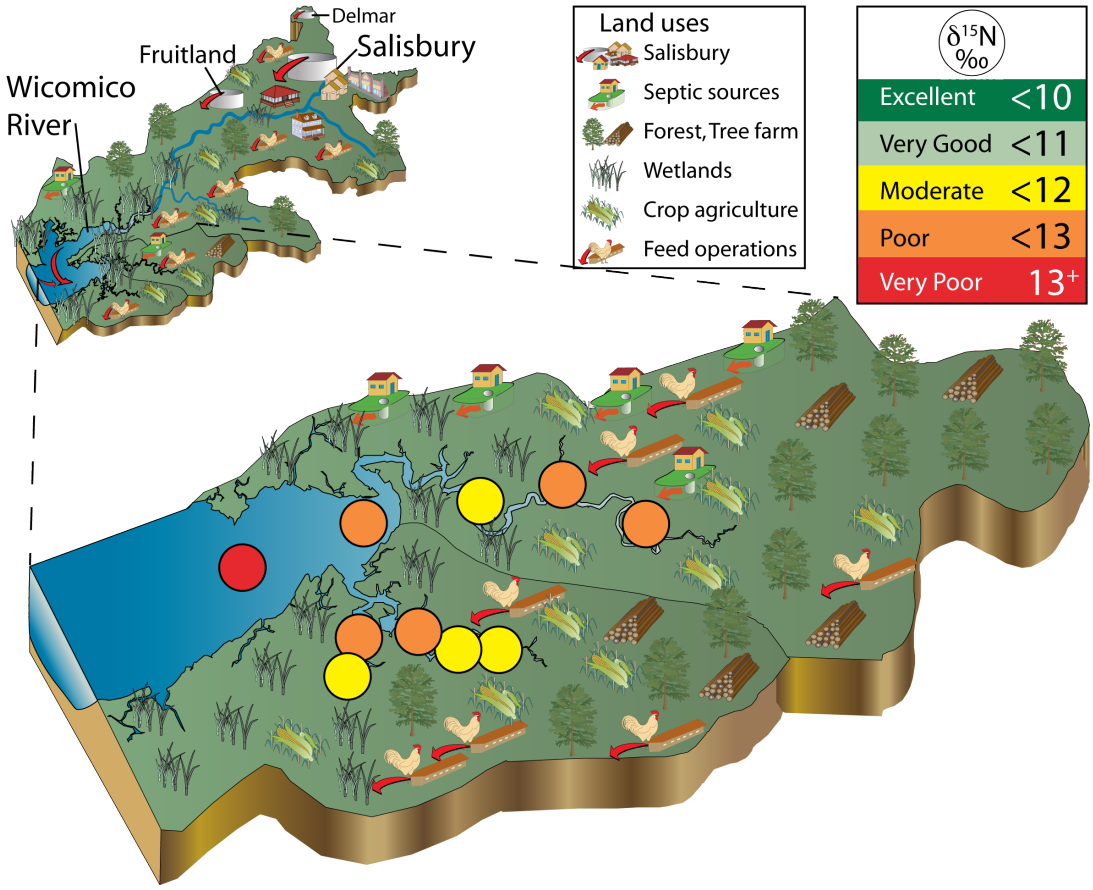
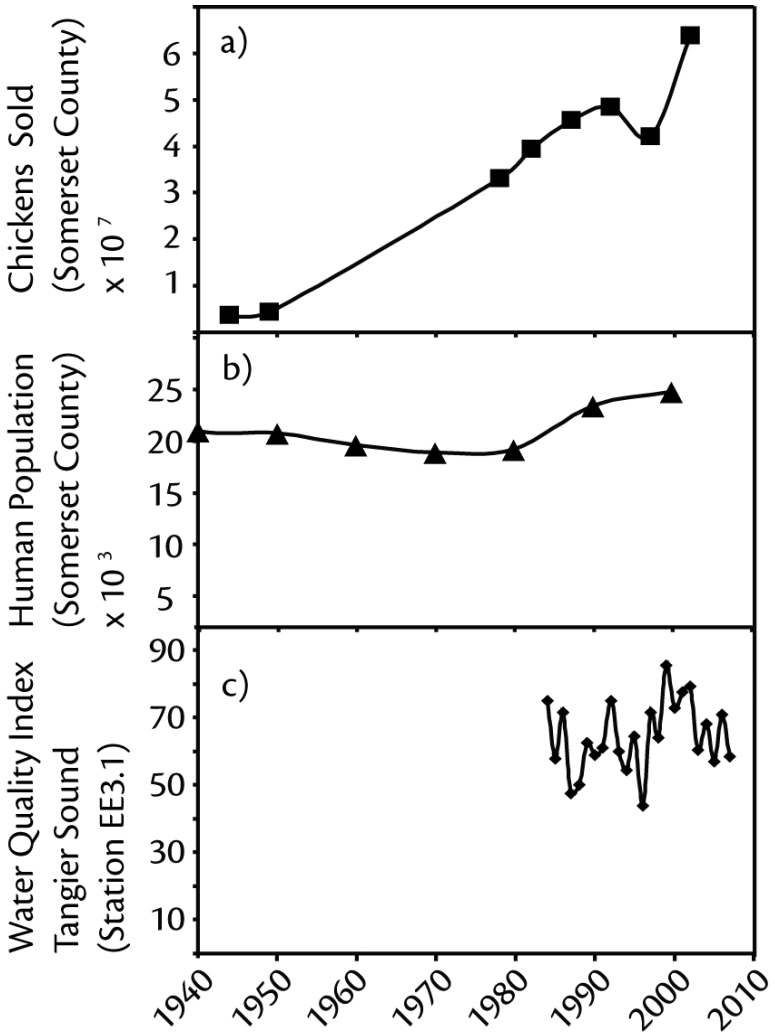


Figure 3.7: Historical populations of chicken and people, and water quality

Historical records of chickens sold (a) in Somerset County (USDA 2002), human population (b) in Somerset County (Maryland Department of Planning 2000), and Water Quality Index (c) in Tangier Sound (connecting Monie Bay to Chesapeake Bay) measured at Chesapeake Bay Program's monitoring station EE3.1 (38° 11' 48.6744" N, 75° 58' 23.5416" W; Chesapeake Bay Program 2008).



CHAPTER 4: OYSTER AND MACROALGAE BIOINDICATORS DETECT ELEVATED $\delta^{15}N$ IN MARYLAND'S COASTAL BAYS

Chapter Citation:

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Abstract

Nitrogen loading from anthropogenic sources, including fertilizer, manure, and sewage effluents has been linked with declining water quality in coastal lagoons worldwide. Freshwater inputs to mid-Atlantic coastal lagoons of the United States are from terrestrially influenced sources: groundwater and overland flow via streams and agricultural ditches, with occasional precipitation events. Stable nitrogen isotope ratios ($\delta^{15}N$) in bioindicator species combined with conventional water quality monitoring were used to assess nitrogen sources and provide insights into their origins. Water quality data revealed that nutrient concentrations derived from terrestrial sources increased after precipitation events. Tissues from two bioindicator species, a macroalgae (*Gracilaria* sp.) and the eastern oyster (*Crassostrea virginica*) were analyzed for $\delta^{15}N$ to determine spatial and temporal patterns of nitrogen sources. A broad-scale survey of deployed macroalgae (June 2004) detected regions of elevated $\delta^{15}N$. Macroalgal $\delta^{15}N$ (7.33 ± 1.15 ‰ in May 2006, and 6.76 ± 1.15 ‰ in July 2006) responded quickly to a sustained June 2006 nutrient pulse, but did not detect spatial patterns at the fine scale. Oyster $\delta^{15}N$ (8.51 ± 0.89 ‰) responded slowly over longer time periods, and exhibited a slight gradient at the finer spatial scale. Overall elevated $\delta^{15}N$ values in macroalgae and oysters were used to infer that human and animal wastes were important nitrogen sources in some areas of Maryland's Coastal Bays. Different nitrogen integration periods across multiple organisms may

be used to indicate nitrogen sources at various spatial and temporal scales, which will help focus nutrient management.

Introduction

Physical, chemical, and biological indicators are routinely used for monitoring the spatial and temporal extent of eutrophication. In addition, chemical indicators commonly used to measure eutrophication (e.g. total nitrogen or total phosphorus; Nixon 1995; Cloern 2001; Kemp et al. 2005; Bricker et al. 2008) do not detect biologically incorporated nitrogen (Costanzo et al. 2001). Analyzing stable nitrogen isotopes ($\delta^{15}N$) in bioindicator species can be used to address these limitations, as the approach has been shown to identify sources of human and animal wastes (Costanzo et al. 2001; Cohen and Fong 2005). Standard water chemistry measurements of eutrophic symptoms can be complemented with $\delta^{15}N$ in bioindicator species to increase understanding of the location and potentially infer the sources of nitrogen.

Comparison of $\delta^{15}N$ values in bioindicator species has been used to distinguish between chemically synthesized nitrogen fertilizer and human and animal waste sources (McClelland and Valiela 1998). Fertilizer production fixes atmospheric N_2 (defined as 0 ‰); as a result, nitrogen runoff from agricultural areas potentially has lower values of $\delta^{15}N$. Human and animal wastes entering groundwater have $\delta^{15}N$ values that are elevated (e.g. Sweeny and Kaplan 1980; Tucker et al. 1999) due to a combination of volatilization of ammonia and denitrification, which leave the

remaining nitrogen pool enriched with ^{15}N (McClelland and Valiela 1998; Fry 2006). Many wastewater treatment plants employ microbial processing to remove nitrogen at rates higher than in natural ecosystems. Microbial nitrogen removal processes, particularly denitrification, favor the isotopically light ^{14}N and enrich the remaining nitrate pool with ^{15}N (Cline and Kaplan 1975; Kendall 1998). Additionally, ammonia from human and animal waste fractionates during volatilization, leaving the non-volatile portion further enriched with ^{15}N (McClelland and Valiela 1998, Fry 2006). Since multiple processes enrich $\delta^{15}N$ values in biological indicator species, interpretations need to be balanced against a set of alternative hypotheses. Measurements of $\delta^{15}N$ in biological indicator species are advantageous as a complement to direct measurements that can be made on groundwater (Aravena et al. 1993), the water column, or sediments (Tucker et al. 1999), as biota minimize temporal and spatial variability. In particular, this study focused on nitrogen incorporated into macroalgae and filter feeders.

Integration of nitrogen sources occurs over varying time scales in different organisms (Gartner et al. 2002; Dattagupta et al. 2004). While $\delta^{15}N$ integration in diets has been examined across taxonomic groups (including mollusks) and diets (Vanderklift and Ponsard 2003), the temporal integration of $\delta^{15}N$ over various time scales by different organisms, due to species-specific turnover rates, has not been fully explored. Macroalgae uptake nitrogen directly from the water column and have rapid nitrogen turnover rates; therefore those organisms can provide information about available nitrogen over a period of days (Costanzo et al. 2001). Assuming

nitrogen limitation, fractionation during assimilation will be minimal (Fry 2006). Oysters are sessile, euryhaline filter feeders that derive nitrogen from a variety of sources, e.g. microorganisms, phytoplankton, detritus and inorganic particles (Langdon and Newell 1996) and have tissue nitrogen turnover rates in the order of weeks to months depending on the tissue type (Chapter 2). Temporal integration of nitrogen suggested that $\delta^{15}N$ in zebra mussels was appropriate to monitor watershed development and downstream effects despite seasonal variations (Fry and Allen 2003). Feeding over multiple trophic levels in field studies may complicate interpretation of $\delta^{15}N$, which is enriched 3-4 ‰ for each trophic level (Fry 2006). In certain cases, spatial gradients in $\delta^{15}N$ could reflect variability in available diets. Nevertheless, biological indicators such as macroalgae and oysters allow an assortment of questions to be addressed through manipulative field experiments that provide long-term integration on different time scales, which is missed by water chemistry measurements alone without highly detailed sampling.

Multiple sources of anthropogenic nitrogen affect mid-Atlantic coastal bays. Collectively, agricultural fertilizers as well as human and animal wastes have been directly linked to downstream eutrophication (Kennish 2002; Kiddon et al. 2003; Bricker et al. 2007; Wazniak et al. 2007). Long-term water quality monitoring reported recent degradation and increases in total nitrogen, despite historical improvements and decreases in total nitrogen, signaling a need to better understand the driving forces for trend shifts in this region and to identify sources of anthropogenic nitrogen (Wazniak et al. 2007). Symptoms of degradation include an

approximate doubling of dissolved organic nitrogen, increasing frequency of harmful algal blooms, e.g. brown tide (Glibert et al. 2007), and adverse effects on seagrass distribution and density (Harris et al. 2005; Wazniak et al. 2007). Human population in Maryland's Coastal Bays watersheds doubled between 1980 and 2000 to ~35,000 people, and is expected to double again by 2020 (Hager 1996). Septic and wastewater nitrogen inputs have also increased during this period (MCBP 2005). Identifying and differentiating sources of anthropogenic nitrogen can help target management efforts to reduce inputs.

This paper develops a framework for interpreting $\delta^{15}N$ from macroalgae (*Gracilaria* sp.) and oyster (*Crassostrea virginica*) tissue by addressing three questions: 1) What are the relative capabilities of macroalgae and oysters to detect nitrogen from human and animal wastes? 2) What are the broad scale spatial patterns of nitrogen from wastes spanning these coastal bays (~600 km^2)? 3) What are the fine-scale spatial patterns of influence by nitrogen from human and animal wastes within regions (ranging from ~10 to 50 km^2) of Maryland's Coastal Bays?

Methods

Study Location

This study was conducted in a series of coastal lagoons located on the mid-Atlantic coast of the United States (Figure 4.1). These coastal lagoons, including Chincoteague Bay (extending from 38°15'14" N, 75°11'57" W in the north to

37°54’14” N, 75°24’38” W in the south), cover the full length of Maryland’s and some of Virginia’s Atlantic coastline. The bays comprise a series of shallow (2 m mean depth), well-mixed, estuary lagoons behind barrier islands (Fenwick and Assateague Islands).

Due to small watershed areas (totaling 452 km^2) of Maryland’s Coastal Bays, freshwater inputs and activities that result in anthropogenic nitrogen inputs generally occur within 6 km of shore, as compared to larger ecosystems (e.g., Jordan et al. 1997; Brawley et al. 2000; Turner and Rabalais 2003). Freshwater overland sources, transporting nitrate from terrestrial recharge areas, enters Delmarva Peninsula’s coastal lagoons via both groundwater (Andres 1992; Bratton et al. 2004; Krantz et al. 2004; Manheim et al. 2004) and base flow that include riverine (Lung 1994; Schwartz 2003) and agricultural ditches (Schmidt et al. 2007). Seasonal precipitation was variable across these coastal bays, and spring 2006 was drier than average while summer precipitation events were larger than average (Figure 4.2). Salinities range from fresh in some tributaries to polyhaline (30-35‰) in the bays. There is oceanic flushing through two small channels; one near Ocean City (38°19’31” N, 75°05’33” W) toward the northern end of the bays, and the other is south of Chincoteague Bay (37°52’36” N, 75°25’04” W; Figure 4.1). Flushing rates are around 12 d in St. Martin River and 63 d in Chincoteague Bay (Pritchard 1960; Lung 1994). Land cover in the watersheds of these coastal lagoons is dominated by forests (39.5%) and crop agriculture (31.8%), although industrial poultry feeding operations (1.1%) are also located within the watersheds (Table 4.1). The region has a high

occurrence of septic systems for the residential towns of Berlin, MD and Chincoteague, VA (Souza et al. 1993). Poor water quality has been reported in the northern portion of Chincoteague Bay, which is the receiving waters for the town of Berlin, MD (Boynton 1993; Boynton et al. 1996).

Experimental Design

Macroalgae were used for both broad and fine scale surveys. Macroalgae (*Gracilaria* sp.) were deployed at 248 randomly distributed sites throughout all regions of Maryland's Coastal Bays from 7 to 12 June 2004 (Figure 4.1). Finer scale surveys were conducted from 22 to 27 May 2006 and again from 13 to 18 July 2006. Survey dates were not selected *a priori* for association with precipitation events, yet twelve precipitation events occurred between fine scale surveys in June 2006 (0.3 – 53.3 mm; Figure 4.2), making this year unusual compared to the long-term average. During the finer scale surveys, macroalgae were deployed at 100 sites randomly distributed across these coastal bays in St. Martin River (21 sites), Chincoteague Bay at Public Landing (22 sites), Johnson Bay (28 sites), and southern Chincoteague Bay (29 sites; see Figure 4.1).

Macroalgae surveys followed the deployment methods described by Costanzo et al. (2001). The macroalgae used for deployment were initially collected in Greenbackville, VA, near southern Chincoteague Bay 1 d in advance of deployment. Three sub-samples (~1.0 g dry weight each) provided an initial $\delta^{15}N$ value (10.0 ± 0.1 ‰ in June 2004, 5.2 ± 0.2 ‰ in May 2006, and 9.5 ± 0.6 ‰ in July 2006).

The remaining macroalgae were sub-sampled (~1.0 g dry weight) for deployment and placed in transparent 130 ml containers with 35 perforations of ~1.0 cm diameter distributed across the side and bottom to allow light, water, and nutrient exchange. For each site, containers (one per site) were attached to anchored surface buoys at a depth of 0.5 of the Secchi depth (rounded to nearest 10 cm).

Oysters (*Crassostrea virginica*) were deployed in the fine scale survey (2006) in a similar manner as macroalgae. Oysters were originally hatchery-reared without shell substrate (cultchless) < 1 yr old (29.8 – 95.8 mm shell height), grown in two locations in St. Martin River, and had initial $\delta^{15}N$ values of 8.2 ± 0.3 ‰. Oysters were deployed in Johnson Bay and St. Martin River from 21 May to 13 July 2006, and in southern Chincoteague Bay and Public Landing from 22 May to 14 July 2006. Oyster deployments overlapped the June precipitation events. Three oysters from a randomly selected growth location were placed in a mesh (1.9 cm holes) cage, anchored by bricks and suspended 0.5 m above bottom by surface buoys. The oysters were deployed at the same 100 sites as the macroalgae (Figure 4.1).

Data Collection and Analysis

After the deployment period, tissues from both macroalgae and oysters were analyzed for stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$). Upon collection, samples were kept on ice in the field and frozen at the laboratory (-20° C) until processing. Of the surviving oysters from each site, one was selected at random and dissected to recover the adductor muscle for $\delta^{15}N$ analysis. Tissues from both organisms were thawed,

rinsed, and oven dried at 60°C for 48 h or until thoroughly dry. Dried macroalgae tissue was finely ground using a grinding mill (Crescent 3110B Wig-L-Bug), while a mortar and pestle was used for oysters. Sub-samples (2.0 ± 0.2 mg dry weight of macroalgae, 1.0 ± 0.2 mg dry weight of oyster) were placed in tin capsules (pressed, standard weight 8 × 5 mm, Elemental Microanalysis). Nitrogen and carbon content (μg N and μg C) and natural abundance of stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) were analyzed at University of California Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Molecular %N and C:N ratio were calculated. Both $\delta^{15}N$ and $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 10^3$, where R was defined as either the $^{15}N/^{14}N$ or $^{13}C/^{12}C$ ratio. The standard reference was atmospheric N_2 (air), with 0.3663 atom % ^{15}N, defined as 0‰ (e.g. Fry 2006), while the PDB standard was used for $\delta^{13}C$. PDB refers to the Cretaceous belemnite formation at Peedee in South Carolina, USA

Data on physical parameters and nutrient concentrations were collected and analyzed in conjunction with biological data. Physical (e.g. temperature and salinity) parameters were measured with a WTW Multi 197i water quality probe and Secchi depth was also recorded. Water samples (20 ml) for nutrient analyses (total nitrogen (TN) and total phosphorus (TP)) were collected and kept on ice in the field 21 May 2006 (before precipitation events) and 13 July 2006 (after precipitation events) until freezing (-20 °C) at the laboratory for analysis. Total nutrients, rather than inorganic species, were analyzed according to standard methods (D'Elia et al. 1977; Kerouel

and Aminot 1987). Long-term nitrogen increases and recycling in these bays have been driven by the dominant dissolved organic fraction (Glibert et al. 2001; Glibert et al. 2007), and locally are at least moderately bioavailable (Seitzinger and Sanders 1999; Seitzinger et al. 2002; Mulholland et al. 2004; Glibert et al. 2006; Wiegner et al. 2006). In culture, *Gracilaria cornea* efficiently grows on organic (urea) or inorganic (NH_4^+-N, NO_3^--N, or NO_3NH_4) nitrogen (Navarro-Angulo and Robledo 1999). Therefore total, rather than dissolved inorganic, nutrients were deemed a better indicator of relative nutrient availability. Water samples (60 ml) for chlorophyll *a* were filtered onto GF/F filter paper (25 mm diameter) in the field, and kept on ice until freezing (-20 °C) at the laboratory until spectrophotometric analysis, which was conducted according to standard methods (Arar, 1997). Data from two statistical outliers (defined as $> \pm 3\sigma$ from mean, verified by Grubb's test, Barnett and Lewis 1998) were removed. Precipitation data was collected by National Park Service, Assateague Island National Seashore. Spatial patterns for all parameters were plotted with ArcMap 8.0 geographical information system. The Spatial Analyst functionality of the ArcGIS package was used to Krige raster interpolations for measured variables and their variances. If spatial autocorrelation was not confirmed, the interpolation was removed. Correlations were calculated for physical and nutrient parameters for both months. Assumptions of normality and homogeneity of variances were verified with SAS 9.1.2 (Proc Univariate), and no data transformations were required. Statistical analysis testing for differences between means using two-way ANOVAs (region, month) was also performed with SAS 9.1.2 (Proc Mixed) for all parameters, except

for those involving oysters. Oyster data was analyzed with one-way ANOVAs run on regions since only one deployment was conducted; from May to July.

Physical (including nutrients), and biological (chlorophyll *a*, macroalgae %N, macroalgae $\delta^{15}N$, macroalgae $\delta^{13}C$, and macroalgae C:N) parameters were analyzed with non-parametric multidimensional scaling (non-metric MDS) to assess spatial and temporal patterns. Separate analyses were conducted on range standardized physical/nutrient metrics and on biological metrics for each month. A Bray-Curtis similarity matrix produced a distance matrix for each set of variables, which was ordinated by non-metric MDS using PATN (Belbin, 1993). Each analysis was conducted in two dimensions with 10 random starts. Ordinations had acceptable (0.14 and 0.17, respectively) stress levels (Clarke and Warwick, 1994).

Results

Pulse of freshwater transported terrestrially-derived nutrients to the lagoons

Freshwater inputs were variable across the study period and altered salinity and nutrient concentrations. In 2004, precipitation was consistently low (0.0 – 15.0 mm; Figure 4.2) in the spring months (March to May) preceding the broad scale survey. However there were twelve precipitation events in June 2006 (0.3 – 53.3 mm; Figure 4.2). While total nitrogen was positively correlated with temperature, both total nitrogen and total phosphorus were negatively correlated with salinity (Table 4.2). Salinity decreased from May 2006 (30.1) to July 2006 (27.7), as

precipitation induced a pulse of runoff and diluted the bays. Salinity decreased towards shore at Johnson Bay and upstream at St. Martin River, while salinities at Public Landing and Chincoteague were more homogenous (Figure 4.3a-d). Higher concentrations of water column total nitrogen and total phosphorus were found in July 2006 (51.6 ± 15.8 μM N, 4.42 ± 1.04 μM P) than May 2006 (44.6 ± 3.7 μM N, 2.59 ± 0.77 μM P), except for total nitrogen at Johnson Bay. Interpolation of total nitrogen indicated a gradient decreasing offshore (Figure 4.3e-h).

Both temporal and regional differences were found in biological parameters in 2006. Chlorophyll *a* in Chincoteague and St. Martin River increased with total nitrogen and total phosphorus, temporally (Table 4.3). Yet all variables had a significant interaction between region and month (Table 4.4). Nutrients pulsed by precipitation events were also incorporated into macroalgae. Macroalgae %N increased from May (1.5%) to July (2.2%). Non-metric MDS indicated biological parameters grouped temporally but not regionally (Figure 4.4a). Chlorophyll *a* was inversely related to macroalgae $\delta^{15}N$ and $\delta^{13}C$ values and was not related to macroalgae %N or C:N (Fig. 4.4b). Macroalgae $\delta^{13}C$ was more enriched in July than in May in all regions (Table 4.3).

Broad spatial scale comparisons (2004)

The broad survey in 2004 showed distinct spatial patterns of total nitrogen concentrations across Maryland's Coastal Bays. Nutrient concentrations were highest in small creeks and lowest closest to the channels, where bay water exchanges with

oceanic water (Figure 4.5a). Concentrations of total nitrogen were lowest (0.7 to 38.8 μM) in Isle of Wight Bay, by the channel near Ocean City. Southern Chincoteague Bay, near the other channel, also tended to have low concentrations of total nitrogen (15.8 to 35.0 μM) compared to Public Landing and Johnson Bay (41.2 to 68.8 μM). St. Martin also exhibited moderate concentrations of total nitrogen (42.7 to 72.2 μM). Highest values of total nitrogen were found in Newport Bay (53.7 to 82.1 μM; Figure 4.5a). Total nitrogen and total phosphorus concentrations correlated positively with temperature and salinity but inversely with macroalgal $\delta^{15}N$ values (Table 4.2).

Macroalgal $\delta^{15}N$ and %N values varied broadly in 2004 across Maryland's Coastal Bays, and spatial patterns differed from that of total nitrogen concentrations. Highest $\delta^{15}N$ values were found in southern Chincoteague Bay (10.8‰ to 26.4‰), and then in St. Martin River (12.1‰ to 22.6‰), but were moderate in Public Landing (12.4‰ to 13.2‰). While macroalgal $\delta^{15}N$ values in Johnson Bay were moderate (9.5‰ to 17.8 ‰), the higher values tended to lie to the west of islands in Chincoteague Bay (Figure 4.5b). Broad spatial patterns of macroalgae %N were similar to that of $\delta^{15}N$. Macroalgae %N was high in Sinepuxent and Newport Bays in addition to St. Martin River and Isle of Wight Bay. Both macroalgae $\delta^{15}N$ and %N were low in Chincoteague Bay, although somewhat elevated around Chincoteague Island (Figure 4.5c). Macroalgae %N negatively correlated to total nitrogen ($r = -0.15$, $p < 0.03$) and total phosphorus (-0.35, $p < 0.01$; Table 4.2). Spatial patterns of total nitrogen concentrations, macroalgae $\delta^{15}N$, and macroalgae %N did not match (Figure 4.5a-c). In St. Martin River, total nitrogen concentrations

(55.6 ± 3.0 μM), macroalgae $\delta^{15}N$ (15.9 ± 1.2 ‰), and macroalgae %N (1.5 ± 0.1 %) were elevated, but in southern Chincoteague Bay, total nitrogen concentrations (24.1 ± 1.3 μM) and macroalgae %N (1.2 ± 0.1 %) were low while macroalgae $\delta^{15}N$ was elevated (17.3 ± 1.3 ‰).

Fine spatial scale comparisons (2006)

Regional variations in total nitrogen concentrations were detectable in the finer spatial scale sampling data (Figure 4.3e-h). During both fine-scale samplings in 2006, St. Martin River had the highest total nitrogen (54.6 ± 1.2 μM N in May, 71.3 ± 3.6 μM N in July), and Johnson Bay had the highest total phosphorus (3.25 ± 0.10 μM P in May, 5.14 ± 0.17 μM P in July), while southern Chincoteague Bay had the lowest total nitrogen (24.8 ± 1.2 μM N in May, 33.9 ± 0.6 μM N in July) and total phosphorus (1.62 ± 0.05 μM P in May, 3.26 ± 0.05 μM P in July; Table 4.3). Non-parametric MDS showed that physical parameters grouped regionally, and that total nitrogen and total phosphorus were inversely correlated with Secchi depth (Figure 4.4c,d). Southern Chincoteague Bay tended to have low total nutrients and increased Secchi depth, while St. Martin River and Public Landing exhibited gradients of nutrients (Figure 4.3e-h). While spatial patterns of macroalgal $\delta^{15}N$ were recognizable at the broad scale, they were undetectable at the finer spatial scale within regions, in both May and July. The range of macroalgal $\delta^{15}N$ values was bigger at the broad spatial scale in 2004 (8.9 to 26.4 ‰) than at the finer spatial scale in 2006 (5.5 to 8.8 ‰ in May and 2.5 to 9.1 ‰ in July). A slight north south gradient

of oyster $\delta^{15}N$ emerged within these regions (Figure 4.6a,b), particularly at Johnson Bay (7.8 to 10.3‰) and southern Chincoteague Bay (6.5 to 10.0‰).

Bioindicator species comparison

Macroalgae and oyster biological indicators both responded, but in different ways, to nutrient concentrations and sources. At the broad scale (2004), macroalgal $\delta^{15}N$ values and total nutrients (total nitrogen and total phosphorus) were inversely related (Table 4.2). At the finer spatial scale (2006), neither total nitrogen nor total phosphorus significantly correlated to macroalgae $\delta^{15}N$ (in either May or July, Table 4.2). Absolute change in macroalgae $\delta^{15}N$ from initial values were greatest in June 2004, and were negative in July 2006, while changes in oyster $\delta^{15}N$ were much smaller than macroalgae, often $< \pm 1.0$ ‰ (Figure 4.7a). Meanwhile, macroalgae %N decreased from initial values in June 2004 and changed minimally from initial values in either May or July 2006, while oyster %N values exhibited the greatest absolute change in %N, often > 1.5% (Figure 4.7b). Except in Chincoteague, macroalgae %N was higher in July than May, while macroalgae $\delta^{15}N$ decreased from May to July (Table 4.3). Macroalgae $\delta^{15}N$ and %N were positively correlated in May (0.26, $p < 0.01$, $n = 95$), but not in July (-0.14, $p = 0.17$, $n = 99$). Oyster %N values varied only slightly regionally and exhibited the least increase (1.4%) above initial values at southern Chincoteague Bay. Oyster tissue %N values were spatially consistent with total nitrogen (Table 4.3). Initial values of macroalgae $\delta^{15}N$ (5.2‰ in May and 9.5‰ in July) and oyster $\delta^{15}N$ (8.2‰) were lower than final measurements after

deployment, except at southern Chincoteague Bay (8.1 ± 0.2‰). Spatially, oyster $\delta^{15}N$ values more closely resembled macroalgae $\delta^{15}N$ in May 2006 (Johnson Bay > St. Martin River > Public Landing > southern Chincoteague Bay) than those of July 2006. Overall, both macroalgae and oysters had high isotopic values inshore, indicating anthropogenic sources of nitrogen.

Discussion

Pulse of freshwater transported terrestrially-derived nutrients to the lagoons

Freshwater inputs in June 2006 were abnormally large and frequent, compared to the long-term average, and these inputs rapidly pulsed nutrients into the coastal lagoons, resulting in changes to the macroalgae nutrient status and phytoplankton abundance. Salinity and nutrient gradients along St. Martin River (Figure 4.3a,e) in conjunction with salinity decreases and spatial patterns of nutrients in Public Landing (Figure 4.3b,f) and Johnson Bay (Figure 4.3c,g) which emanated from shore implicated transport of total nitrogen from terrestrial sources, either via groundwater or overland flow through streams or agricultural ditches. Similar nutrient pulses (e.g. dissolved nitrate) are common in other comparable coastal ecosystems (Valiela et al. 1990; Ullman et al. 2002). Temporal grouping of biological parameters (total chlorophyll *a* and macroalgae %N, C:N, $\delta^{13}C$, and $\delta^{15}N$) in the non-metric multidimensional scaling analysis indicated the biological response to nutrients was driven by precipitation (Figure 4.4a,b). For example, enrichment of macroalgae $\delta^{13}C$

occurred across all regions after the June 2006 unusually large precipitation events (Table 4.3). Typical of shallow coastal ecosystems, nutrients increased primary production, which contributed to reduced water clarity via elevated levels of phytoplankton (Figure 4.4c,d; Nixon et al. 2001). Macroalgae nitrogen incorporation was inferred from increased %N after freshwater inputs (Table 4.3).

Patterns at broad spatial scale identified by macroalgae

Across these coastal lagoons, total nitrogen concentrations and macroalgae $\delta^{15}N$ values provided different information. Spatial patterns of total nitrogen concentrations (Figure 4.5a) reflected physical ecosystem processes such as oceanic exchange, as concentrations were low near the two inlets near both ends of Assateague Island and higher in areas with poor flushing, such as Johnson Bay. Elevated macroalgae $\delta^{15}N$ in St. Martin River and southern Chincoteague Bay potentially indicated nitrogen sources, possibly from human or animal wastes, even though the concentrations of total nitrogen varied (Figs. 5a, 5b). This highlights the ability to interpret measurements of $\delta^{15}N$ in bioindicators as inputs of human or animal wastes, even when total nitrogen concentrations are low. Experimental evidence, such as that with the macroalgae *Enteromorpha*, suggests that $\delta^{15}N$ values are independent of total nitrogen concentration, though the rate of ^{15}N incorporation varies by the form of inorganic nitrogen (Cohen and Fong 2005).

Isotopic values can provide evidence of nitrogen source, particularly in conjunction with land use. Macroalgae were enriched with $\delta^{15}N$ in St. Martin River

and near Chincoteague Island, areas where land uses (e.g., residential development, poultry production) suggested a possible presence of septic and manure sources of nitrogen. Examples include St. Martin River (17.2% residentially developed watershed largely reliant on septic systems) and the adjacent Assawoman Bay (18.9% residentially developed watershed, Table 4.1, Maryland Department of Planning 2002). These results align with quantitative linkages that have been made between urban development and enriched $\delta^{15}N$ values in primary consumers (Vander Zanden et al. 2005). Animal agriculture, with isotopically enriched manure byproducts, was another comparatively prominent land use feature of these regions (1.8% and 1.4%, respectively, Table 4.1). St. Martin River, the region with the highest total nitrogen, exhibited a gradient decreasing downstream, suggesting terrestrial nitrogen inputs that diluted upon mixing with higher salinity water from ocean exchange. Yet macroalgae $\delta^{15}N$ values in these regions were elevated in the broad (June 2004) survey (Figure 4.5b) and in the fine scale survey prior to rain events (May 2006). While a total nitrogen concentration gradient suggested terrestrially derived nitrogen inputs, septic and/or manure sources were inferred to be important nitrogen sources for St. Martin River and Isle of Wight based upon enriched macroalgae $\delta^{15}N$ values.

The town of Chincoteague, VA (population 4,317; 173.1 people km^{-1}; U. S. Census Bureau 2000) is situated atop sandy soils and potentially contributes nitrogen via septic systems, as evidenced by enriched $\delta^{15}N$ values in macroalgae in the surrounding estuarine waters. This town comprises much of the residential development (1.5% of the total watershed area) in the Chincoteague Bay watershed

and relies entirely on septic systems (Maryland Department of the Environment). In addition to elevated $\delta^{15}N$, increased concentrations of total nitrogen would be expected from this potential nitrogen source; however, southern Chincoteague Bay had the lowest total nitrogen at both broad (Fig. 5a) and fine spatial scales (Fig. 3h), likely due to processes including dilution and ocean flushing, but also to some extent to small watershed size, and expansive intact wetlands (Wazniak et al. 2007).

Fine spatial scale potentially indicates sources despite lack of spatial patterns

At the fine spatial scale, patterns emerged from oyster $\delta^{15}N$ values, but not from macroalgae $\delta^{15}N$ values. North-south gradient patterns in Johnson Bay (Fig. 6a) and southern Chincoteague Bay (Fig. 6b) were detectable in oyster muscle $\delta^{15}N$. These gradients agreed with broad patterns from June 2004 macroalgae $\delta^{15}N$ (Fig. 5b). Though oyster muscle $\delta^{15}N$ gradients were slight, homogeneously elevated values implicated septic sources of nitrogen in southern Chincoteague Bay, likely from the town of Chincoteague, VA. In a similar study, spatial homogeneity of elevated $\delta^{15}N$ among hard clam tissues (*Mercenaria mercenaria*) along a eutrophication gradient has been attributed to anthropogenic sources, suggesting that elevated $\delta^{15}N$ in mollusks can still indicate nitrogen source, despite a lack of spatial pattern (Ockowski et al. 2008). Furthermore, oyster $\delta^{15}N$ values in this study were similar to, though somewhat lower than (8.5 ± 0.1‰) muscle tissue (9.4 ± 0.2‰ and 16.0 ± 2.3‰) of an Australian oyster species (*Saccostrea glomerata*) influenced by wastewater treatment effluent within 50 m (Piola et al. 2006).

Bioindicator species comparison

The growing literature on biological indicators suggests that $\delta^{15}N$ in organisms sampled from natural communities consisting of various taxonomic groups, including macrophytes (e.g. McClelland et al 1997; Cole et al. 2004; Cohen and Fong 2006), finfish (Lake et al. 2001), and mollusks (Fila et al. 2001; McKinney et al. 2002; Vander Zanden et al. 2005), or some combination (Gartner et al. 2002; Fry et al. 2003) can identify nitrogen sources. Additionally, manipulative deployment of macroalgae has been used to interpolate spatial patterns in anthropogenic sources of nitrogen through experimental fieldwork (e.g., Udy and Dennison 1997; Costanzo et al. 2001). The current study combines the benefits of each technique, providing direct comparison between taxonomic groups of primary producers and consumers along with the ability to interpolate spatial patterns based on a manipulative field design in areas where natural communities may not be currently or readily available.

The presence or absence of spatial patterns in $\delta^{15}N$ in macroalgae and oysters at different spatial scales provides a spatial context in which each can be usefully deployed as a biological indicator. While clear spatial patterns in macroalgae $\delta^{15}N$ and %N emerged at the broad spatial scale in June 2004 (Figs. 5b, 5c), spatial patterns in $\delta^{15}N$ or %N were not obvious for macroalgae deployed at the fine spatial scale (either May or July 2006). Macroalgae $\delta^{15}N$ values were homogenously distributed < 10 ‰ throughout Johnson Bay and southern Chincoteague Bay in both May and July 2006. Therefore, macroalgae may potentially be more usefully deployed as biological

indicators of nitrogen source at a broad scale (100s of km^2) rather than at the fine spatial scale (10s of km^2). The slight gradients in Johnson Bay (Fig. 6a) and southern Chincoteague Bay (Fig. 6b) suggest oyster $\delta^{15}N$ may indicate potential nitrogen source at fine spatial scales (10s of km^2).

In this study, manipulative deployments of macroalgae over multiple years in conjunction with deployment of oysters provided a comparison of isotopic responses to water chemistry factors (i.e. the unusual nutrient pulse) in addition to the comparison between species. Macroalgae $\delta^{15}N$ and %N exhibited smaller changes from initial values after receiving more precipitation during 2006 in the fine scale survey than the drier 2004 broad scale survey (Figs. 7a, 7b). Decreased regional mean macroalgal $\delta^{15}N$ values with increased standard errors from May to July 2006 (Table 4.3) and the undefined spatial patterns in Johnson Bay can be explained by the response of a relatively short nitrogen turnover rate to the unusually large precipitation events of June 2006 and associated turbulence and mixing. Quick turnover rates in macroalgae result in rapid response by $\delta^{15}N$ to environmental conditions as compared to slower tissue turnover rates in tissues of consumers such as oysters (Moore 2003; Cohen and Fong 2005), so oyster response to the precipitation events would be dampened compared to macroalgae. Similar to other studies (e.g. Gartner et al. 2002 and Fry et al. 2003), responsiveness to nitrogen cycling, as reflected in changes to $\delta^{15}N$ and %N, was greater in macroalgae than in oysters (Figs. 7a, 7b), likely due to relative physiological turnover times; days for macroalgae and weeks for oysters. Between regions of these coastal lagoons, patterns of oyster tissue

$\delta^{15}N$ values (July 2006; Table 4.3) were more similar to previous macroalgae $\delta^{15}N$ values (May 2006; Table 4.3) than to concurrent macroalgae $\delta^{15}N$ values (July 2006; Table 4.3) and did not reflect short-term nutrient pulses from the June 2006 precipitation events.

Water chemistry factors and spatial scale may influence interpretation of $\delta^{15}N$ in macroalgae and oysters to infer nitrogen source. Isotopic signals can be influenced by physical conditions such as salinity, temperature or depth (Jennings and Warr 2003) and are variable in strength. Water chemistry measurements varied over time in these coastal bays, as evidenced by a wide range of macroalgae $\delta^{15}N$ values in 2004 (Fig. 5b) and a smaller range of macroalgae $\delta^{15}N$ values with no clear finer scale spatial pattern in 2006. Since spatial patterns were detectable in 2006 by oyster $\delta^{15}N$, perhaps these tissues are less susceptible to variability in water chemistry, due to oyster physiology (Figs. 7a, 7b), and thus can detect spatial patterns at a finer spatial scale than macroalgae (see Chapter 6). This conclusion is counter-intuitive because while deployments of both macroalgae and oysters were stationary, the phytoplankton consumed by oysters (which directly uptake dissolved nitrogen) may have been transported horizontally by water circulation or tides and thus oysters might be integrate a mixture of $\delta^{15}N$ signatures from a larger spatial area than macroalgae. Nevertheless, the spatial evidence presented here and in Chapter 5 suggests that oyster $\delta^{15}N$ may be best suited for interpretation of nitrogen sources at spatial scales of 10s to 100s of km^2.

A combination of indicator species responsiveness and ecosystem features may affect the success of indicating nitrogen source. For example, oceanic mixing and short residence times in deep waters offshore southwestern Australia may have dispersed $\delta^{15}N$ signals before transmission from organic sources to filter feeders via food sources, though more responsive macroalgae reflected sewage effluent sources (Gartner et al. 2002). In another study in the northeast Atlantic, Jennings and Warr (2003) found that most spatial $\delta^{15}N$ variability in scallops is related to physical conditions (salinity, depth, and temperature). Comparatively, the shallow, coastal lagoons of the present study are characterized by residence times on the order of weeks (Pritchard 1960; Lung 1994); potentially enough time for $\delta^{15}N$ signals to persist and be incorporated into oysters, provided a sufficient time period for oyster uptake and assimilation.

Variation in responsiveness based on physiological differences between primary producers and filter feeders may potentially introduce a lag time in oyster $\delta^{15}N$ values compared those of macroalgae. The greater absolute changes found in macroalgae $\delta^{15}N$ compared to oyster $\delta^{15}N$ (Fig. 7a) suggest a more rapid response to nitrogen source, likely due to relative physiological turnover times, though oyster %N increase (Figure 4.7b) suggested new growth that would reflect phytoplankton $\delta^{15}N$ signatures during deployment. For example, when comparing across regions, oyster $\delta^{15}N$ values (July 2006) more closely resemble macroalgae $\delta^{15}N$ values from May 2006 (Johnson Bay > St. Martin River > Public Landing > southern Chincoteague Bay) than macroalgae $\delta^{15}N$ values from July 2006 (Table 4.3). In addition to different

trophic levels, this discrepancy between macroalgal and oyster $\delta^{15}N$ values may have been magnified by a time lag due to different rates or modes of nitrogen assimilation. As described in Chapter 1, variations in trophic shifts are also associated with the processes of nitrogen assimilation and excretion (Vanderklift and Ponsard 2003). Macroalgae assimilate nitrogen directly from the water column (e.g., Cohen and Fong 2006), while oysters receive their nitrogen indirectly from the water column (via consumption of a variety of nitrogen sources, for example microorganisms, phytoplankton, detritus and inorganic particles) to reflect ambient $\delta^{15}N$ (Newell and Langdon 1996; Cohen and Fong 2005). Due to the rate and timing of nitrogen assimilation, oysters integrate nitrogen in the muscle over longer time (4 months, Chapter 2) periods than macroalgae (4 days). Future studies could investigate the possibility of a lag time in oyster $\delta^{15}N$ response as compared to macroalgae due to variations in length of nitrogen incorporation.

In addition to differences between macroalgae and oysters, different species within a functional group may provide different temporal integrations, based on species-specific turnover rates. Muscle tissues in different species of filter feeding bivalves vary, e.g. ~2 mo for the eastern oyster (*Crassostrea virginica*), >3 mo for Sydney rock oyster (*Saccostrea commericalis*; Moore 2003), and > 1 y for a methanotrophic hydrocarbon seep mussel (*Bathymodiolus childressi*; Dattagupta et al. 2004). Because the diet of filter feeders includes multiple trophic levels (e.g. primary producers, detritus, etc.), which are separated by 3 to 4 ‰ (Fry 2006), mixtures of trophic levels may confound interpretation of $\delta^{15}N$. In certain cases, spatial gradients

in $\delta^{15}N$ could reflect variability in available diets. Yet identifying human and animal waste as potential nitrogen source to these bays fits with recent degradation of water quality and increases in total nitrogen identified by long-term monitoring datasets (Wazniak et al. 2007). Multiple temporal integrations among species allow different monitoring questions to be addressed by different biological indicators. Macroalgae and oysters may be suited for different roles as biological indicators, but they both may have the potential to indicate nitrogen sources at various spatial and temporal scales, which will help focus nutrient management.

Chapter 4 Tables

Table 4.1: Land use in Maryland Coastal Bays watersheds

Percent of each sub-watershed of Maryland's Coastal Bays devoted to various land uses. Data from Maryland Department of Planning, 2002.

Land use	Assawoman Bay (%)	Chincoteague Bay (%)	St. Martin River/ Isle of Wight (%)	Newport Bay (%)	Sinepuxent Bay (%)
Residential	18.9	1.5	17.2	6.9	9.4
Urban	6.6	0.2	5.5	2.0	5.9
Crop Agriculture	22.5	32.5	34.1	34.4	11.4
Animal Agriculture	1.4	0.7	1.8	0.8	0.2
Forest	27.9	40.3	37.7	43.5	38.6
Wetlands	21.5	22.9	3.4	12.0	23.1
Bare/Other	1.2	1.9	0.3	0.4	11.4
Total (ha)	2,791	17,340	13,605	11,005	3,080

Table 4.2: Correlations between nutrient, physical, and biological parameters

Correlations between nutrients (total nitrogen and total phosphorus) and physical parameters (temperature, salinity) or biological parameters (chlorophyll *a*, and macroalgal or oyster $\delta^{15}N$, %N, $\delta^{13}C$, %C, C:N) for broad spatial scale (2004) and fine spatial scale (2006) surveys. Number of measurements (n), correlation value (r) and significance (p) are reported. Significant relationships are noted in bold.

Nutrient	Physical parameter	n	r	p
June 2004				
Total nitrogen	Salinity	222	–0.75	<0.001
	Temperature	224	0.59	<0.001
	Chlorophyll a	237	0.72	<0.001
	Macroalgae $\delta^{15}N$	231	–0.30	<0.001
	Macroalgae %N	231	–0.15	0.025
Total phosphorus	Salinity	222	–0.24	<0.001
	Temperature	224	0.51	<0.001
	Chlorophyll a	237	0.84	<0.001
	Macroalgae $\delta^{15}N$	231	–0.19	0.003
	Macroalgae %N	231	–0.35	<0.001
May 2006				
Total nitrogen	Salinity	98	–0.43	<0.001
	Temperature	97	0.30	0.003
	Chlorophyll a	93	0.19	0.067
	Macroalgae $\delta^{15}N$	95	0.14	0.162
	Macroalgae %N	95	–0.05	0.646
	Macroalgae $\delta^{13}C$	94	0.15	0.146
	Macroalgae %C	94	–0.10	0.336
	Macroalgae C/N	94	0.04	0.718
Total phosphorus	Salinity	98	0.03	0.763
	Temperature	97	0.10	0.312
	Chlorophyll a	93	0.08	0.433
	Macroalgae $\delta^{15}N$	95	0.17	0.105
	Macroalgae %N	95	–0.26	0.011
	Macroalgae $\delta^{13}C$	94	0.24	0.020
	Macroalgae %C	94	–0.16	0.116
	Macroalgae C/N	94	0.22	0.035
July 2006				
Total nitrogen	Salinity	100	–0.81	<0.001
	Temperature	100	0.41	<0.001
	Chlorophyll a	100	0.55	<0.001
	Macroalgae $\delta^{15}N$	99	–0.14	0.158
	Macroalgae %N	99	0.63	<0.001
	Macroalgae $\delta^{13}C$	99	–0.44	<0.001
	Macroalgae %C	99	0.51	<0.001
	Macroalgae C:N	99	–0.57	<0.001
	Oyster $\delta^{15}N$	47	0.17	0.265
	Oyster %N	47	0.50	<0.001
	Oyster $\delta^{13}C$	47	0.19	0.206
	Oyster %C	47	0.68	<0.001
	Oyster C/N	47	0.17	0.249
Total phosphorus	Salinity	100	–0.80	<0.001
	Temperature	100	0.06	0.532
	Chlorophyll a	100	0.39	<0.001
	Macroalgae $\delta^{15}N$	99	0.02	0.853
	Macroalgae %N	99	0.56	<0.001
	Macroalgae $\delta^{13}C$	99	–0.21	0.033
	Macroalgae %C	99	0.52	<0.001
	Macroalgae C/N	99	–0.48	<0.001
	Oyster $\delta^{15}N$	47	0.27	0.068
	Oyster %N	47	0.50	<0.001
	Oyster $\delta^{13}C$	47	0.27	0.071
	Oyster %C	47	0.71	<0.001
	Oyster C/N	47	0.24	0.110

Table 4.3: Means of nutrient, physical, and biological parameters (2006)

Means of physical, nutrient, and biological parameters measured during the fine-scale survey (2006). Standard error is reported in parentheses. Sample size (n) is reported for each month in each region, except as noted by superscript: [a]: n = 8, [b]: n = 9, [c]: n = 10, [d]: n = 20, [e]: n = 21, [f]: n = 22, [g]: n = 25, [h]: n = 27, [i]: n = 28.

Parameter	Units	St. Martin River		Public Landing		Johnson Bay		Chincoteague Bay	
		May *n*=2	July *n*=19	May *n*=21	July *n*=27	May *n*=22	July *n*=28	May *n*=26	July *n*=29
Salinity		26.9 (0.3)	25.8 (0.4)	30.4 (0.0)[f]	28.5 (0.0)	31.7 (0.1)[i]	26.6 (0.2)	31.3 (0.0)[h]	29.7 (0.0)
Temperature	°C	22.5 (0.2)	31.8 (0.3)	23.1 (0.1)	29.8 (0.1)	20.3 (0.2)[i]	29.3 (0.2)	21.3 (0.3)[h]	30.6 (0.2)
Dissolved oxygen	mg L^{-1}		6.93 (0.31)	8.10 (0.06)[f]	4.47 (0.08)		5.04 (0.15)	8.17 (0.42)[g]	5.28 (0.07)
Secchi	M	0.6 (0.0)	0.3 (0.0)	0.4 (0.0)	0.4 (0.0)	0.4 (0.0)[i]	0.4 (0.0)	0.9 (0.0)	0.5 (0.0)
Total nitrogen	μM	54.6 (1.2)	71.3 (3.6)	51.0 (0.7)	58.7 (0.8)	51.2 (1.5)	50.7 (1.6)	24.8 (1.2)[g]	33.9 (0.6)
Total phosphorus	μM	2.38 (0.07)	4.79 (0.25)	3.11 (0.04)	4.79 (0.08)	3.25 (0.10)	5.14 (0.17)	1.62 (0.05)[g]	3.26 (0.05)
Total chlorophyll *a*	μg L^{-1}	38.7 (2.9)	66.0 (7.6)	46.9 (4.7)	44.9 (2.0)	41.1 (2.7)	45.4 (3.1)	19.0 (3.0)	37.1 (2.0)
Macroalgae %N	%	1.8 (0.1)	3.1 (0.1)	1.5 (0.0)	1.9 (0.1)	1.5 (0.1)	2.8 (0.1)	1.5 (0.1)	1.4 (0.0)
Macroalgae $\delta^{15}N$	‰	7.4 (0.1)	6.5 (0.2)	7.0 (0.1)	6.8 (0.2)	7.5 (0.1)	6.8 (0.2)	7.0 (0.1)	7.0 (0.3)
Macroalgae $\delta^{13}C$	‰	−20.0 (1.2)[d]	−17.1 (0.2)	−18.8 (0.2)	−16.3 (0.2)	−19.5 (0.3)	−16.1 (0.1)	−19.9 (0.3)	−15.7 (0.2)
Oyster %N	%		13.0 (0.2)[b]		12.1 (0.3)[a]		12.7 (0.3)[c]		11.6 (0.2)[e]
Oyster $\delta^{15}N$	‰		8.8 (0.2)[b]		8.4 (0.2)[a]		9.4 (0.3)[b]		8.1 (0.2)[e]
Oyster $\delta^{13}C$	‰		−21.2 (0.1)[a]		−20.6 (0.1)[a]		−21.0 (0.2)[b]		−21.1 (0.1)[e]

Table 4.4: ANOVAs between nutrient and biological parameters over years

ANOVAs run on nutrient and biological parameters (2006 data) identify regional and temporal differences and interactions. Number (n), degrees of freedom (df), mean square error (MSE), F value (F), and significance value (p) are displayed. Significant relationships are reported in bold.

Parameter	Variation	*n*	*df*	MSE	*F*	*p*
Total nitrogen	Region	192	3, 184	54.35	184.39	**<0.001**
	Month		3, 184		58.86	**<0.001**
	Region × Month		3, 184		10.84	**<0.001**
Total phosphorus	Region	192	3, 184	0.32	98.61	**<0.001**
	Month		3, 184		527.03	**<0.001**
	Region × Month		3, 184		4.09	**0.008**
Chlorophyll *a*	Region193	193	3, 185	305.76	17.03	**<0.001**
	Month		1, 185		21.93	**<0.001**
	Region × Month		3, 185		6.30	**<0.001**
Macroalgae %N	Region	193	3, 185	0.10	94.02	**<0.001**
	Month		1, 185		245.68	**<0.001**
	Region × Month		3, 185		65.44	**<0.001**
Macroalgae $\delta^{15}N$	Region	193	3, 185	0.72	1.25	0.292
	Month		1, 185		13.01	**<0.001**
	Region × Month		3, 185		3.09	**0.029**
Oyster muscle %N	Region	48	3, 44	0.56	8.86	**<0.001**
Oyster muscle $\delta^{15}N$	Region	48	3, 44	0.61	6.09	**0.002**

Chapter 4 Figures

Figure 4.1: Sampling scheme in Maryland's Coastal Bays

In 2004, macroalgae was deployed at 248 sites (triangles) across Maryland's Coastal Bays (a). The 2006 deployment of macroalgae and oyster (circles) spanned 100 randomly distributed sites across four regions of interest: b) St. Martin River c) Public Landing d) Johnson Bay and e) southern Chincoteague Bay.

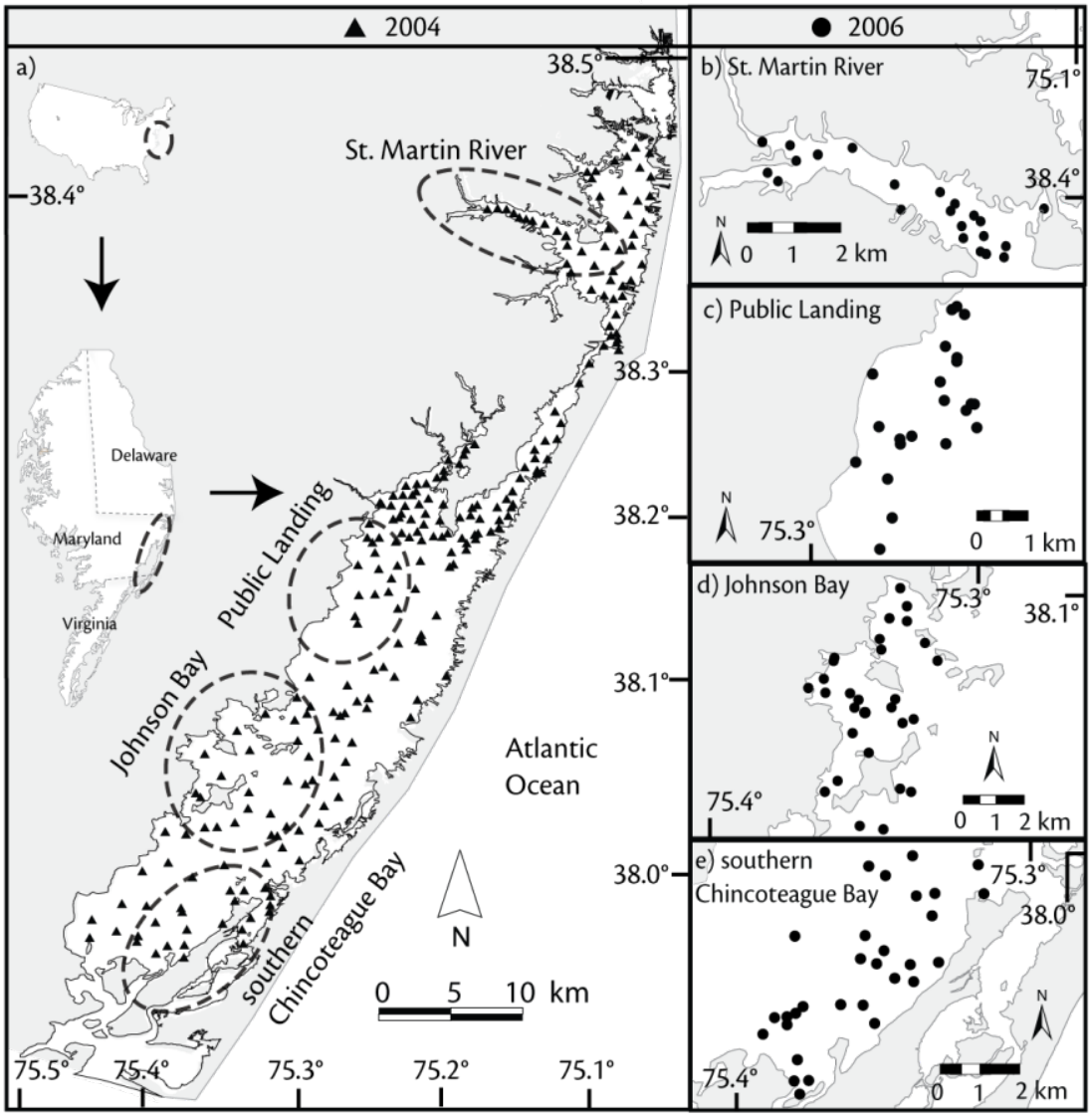


Figure 4.2: Deployment timing and weather

Precipitation (mm) and air temperature (°C) between macroalgae deployments and during oyster deployment.

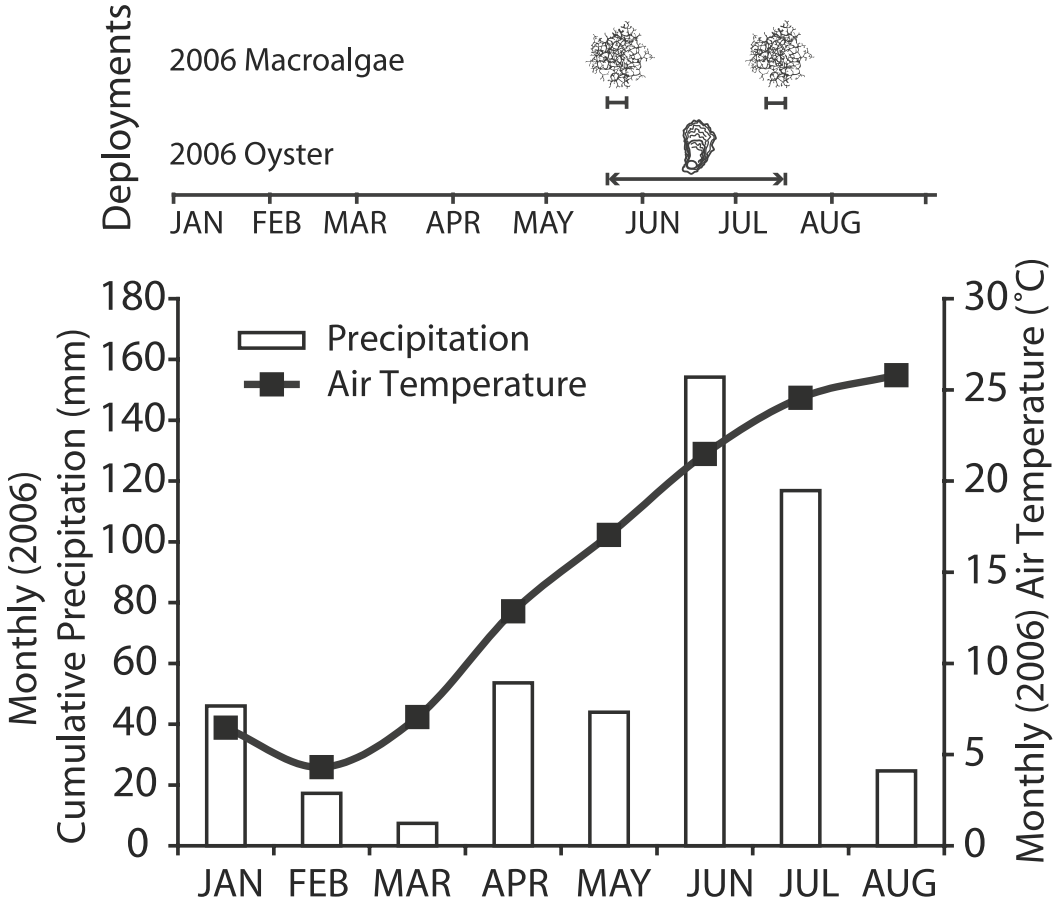


Figure 4.3 Chincoteague Bay continuous monitoring data (courtesy B. Sturgis, National Park Service)

Temperature, salinity, dissolved oxygen, turbidity, and chlorophyll a data from continuous monitoring probes in Chincoteague Bay, May and July 2006.

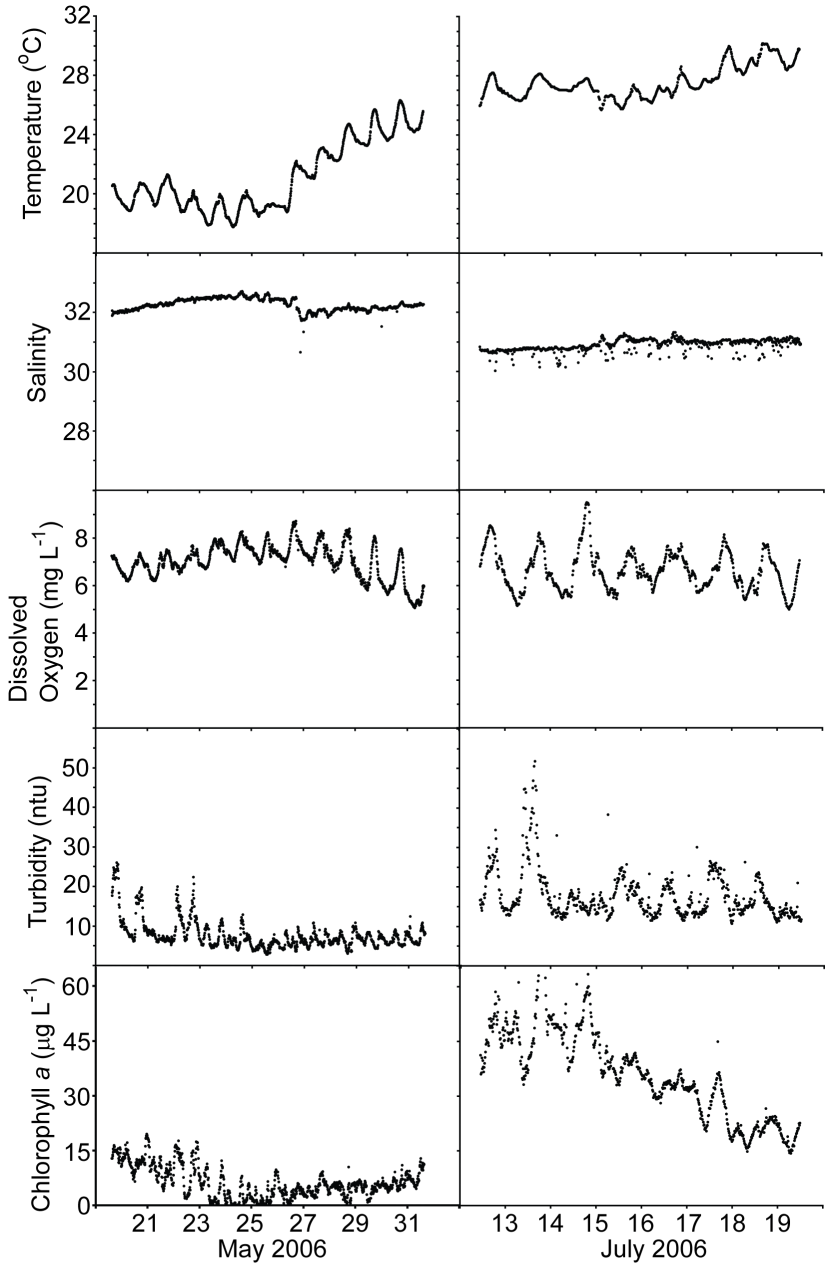


Figure 4.4: Spatial patterns of freshwater and total nitrogen

Spatial patterns of freshwater and total nitrogen in Maryland's Coastal Bays were observed May and July 2006. Salinities are reported for St. Martin River (a), Public Landing (b), Johnson Bay (c), and southern Chincoteague Bay (d). Total nitrogen is reported for St. Martin River (e), Public Landing (f), Johnson Bay (g), and southern Chincoteague Bay (h).

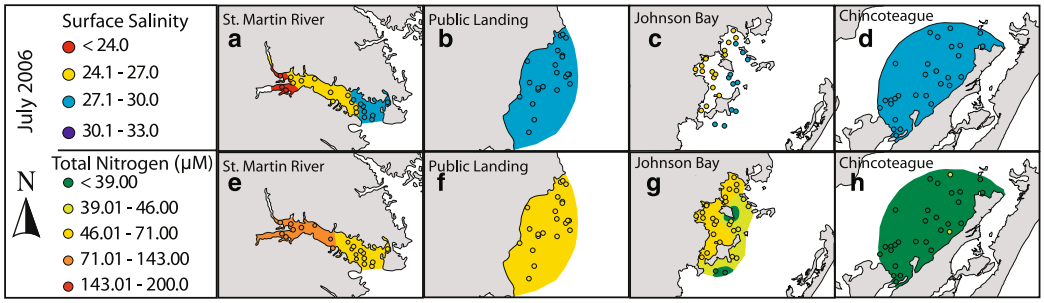


Figure 4.4. Multidimensional scaling analysis

Non-parametric multidimensional scaling plot for biological parameters (total chlorophyll *a* (the sum of chlorophyll *a* and phaeophytin), macroalgae %N, macroalgae C:N, macroalgae $\delta^{15}N$ values, and macroalgae $\delta^{13}C$ values (a). Principal axis correlation plot for biological parameters (b). Non-parametric multidimensional scaling plot for physical parameters (Secchi depth, temperature, salinity, total nitrogen, and total phosphorus) (c). Principal axis correlation plot for physical parameters (d).

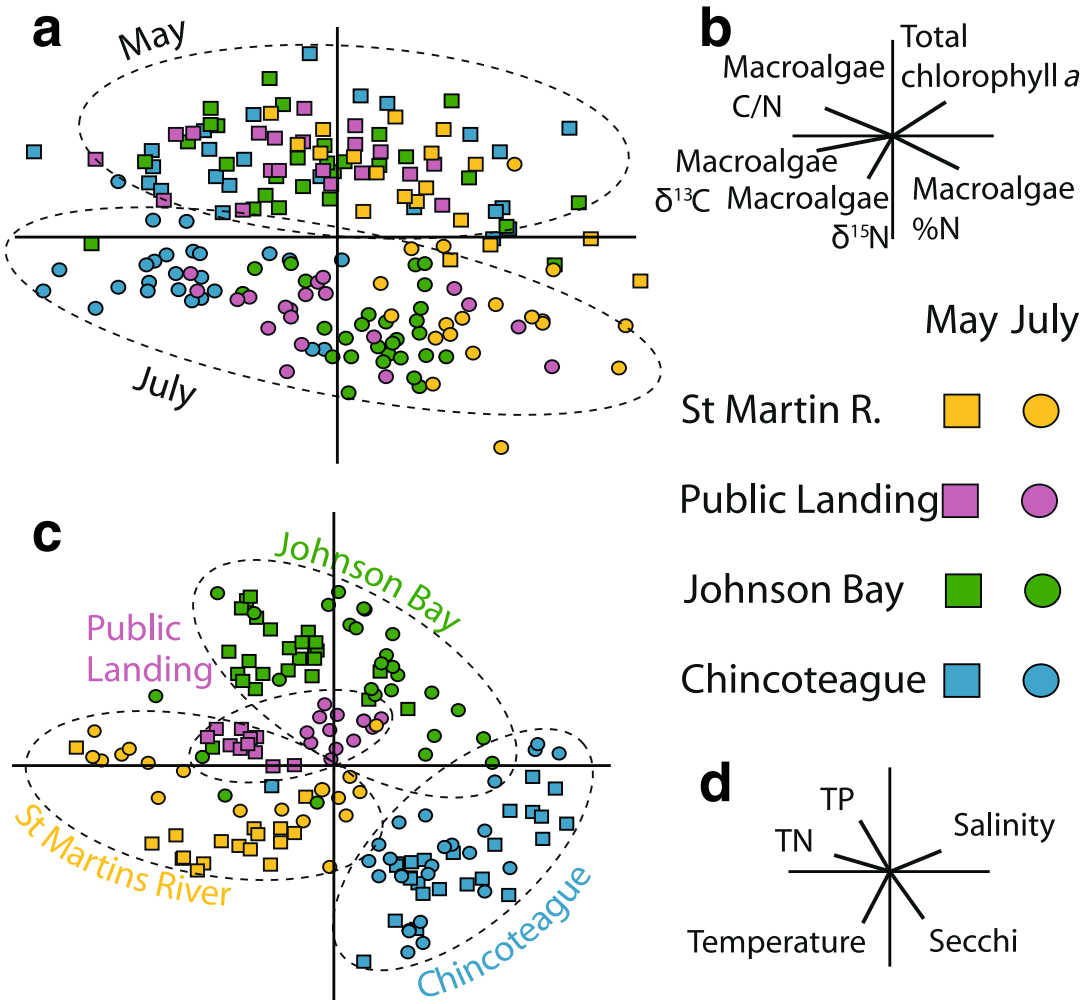


Figure 4.5: Spatial analysis of total nitrogen, and macroalgae $\delta^{15}N$ and %N

Measured total nitrogen (a), deployed macroalgae $\delta^{15}N$ (b) and %N (c) values from the broad spatial survey (June 2004).

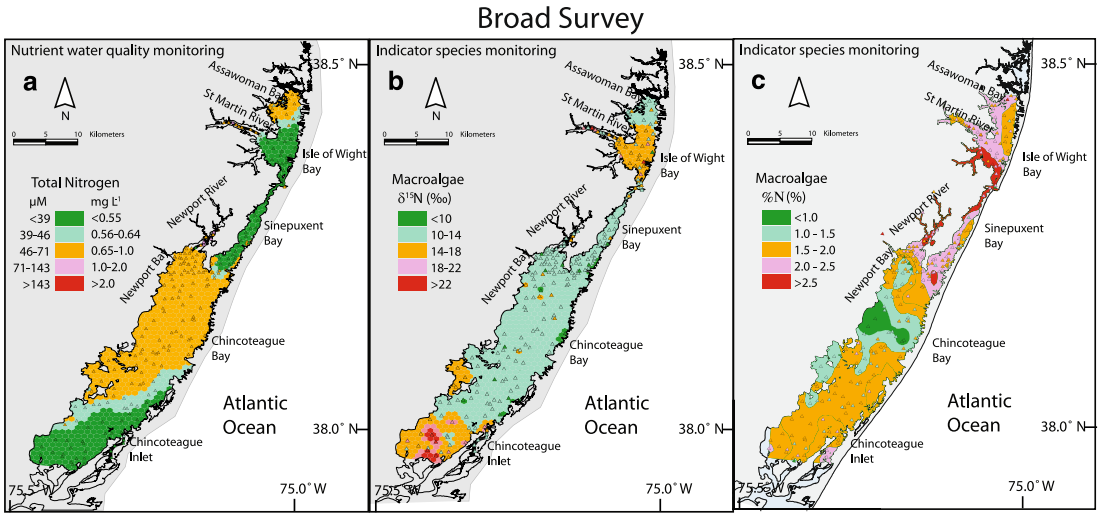


Figure 4.6: Fine spatial scale survey (2006) of oyster $\delta^{15}N$ values.

Fine spatial scale survey (2006) of oyster $\delta^{15}N$ values. Spatial patterns within Johnson Bay (e) and southern Chincoteague Bay (f) detected with oyster $\delta^{15}N$ values.

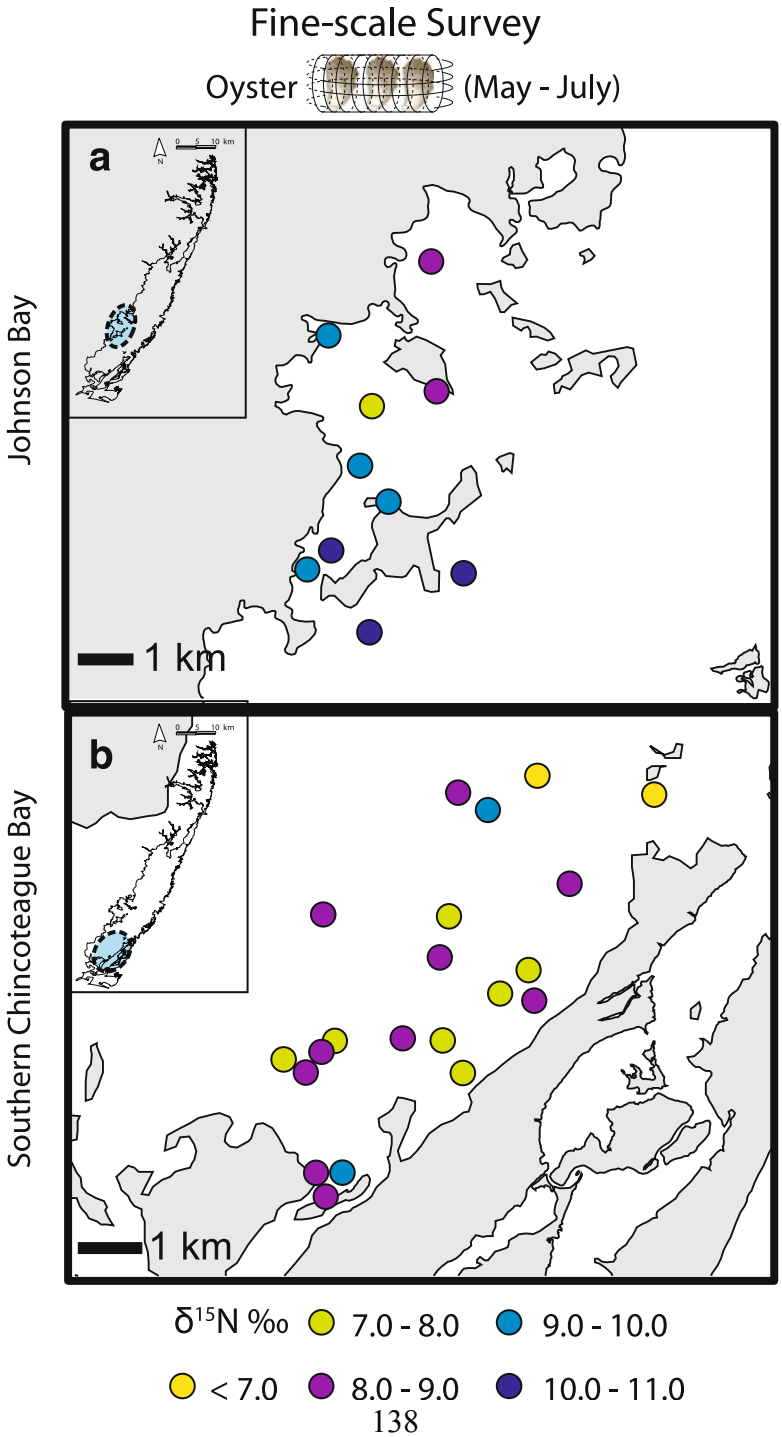
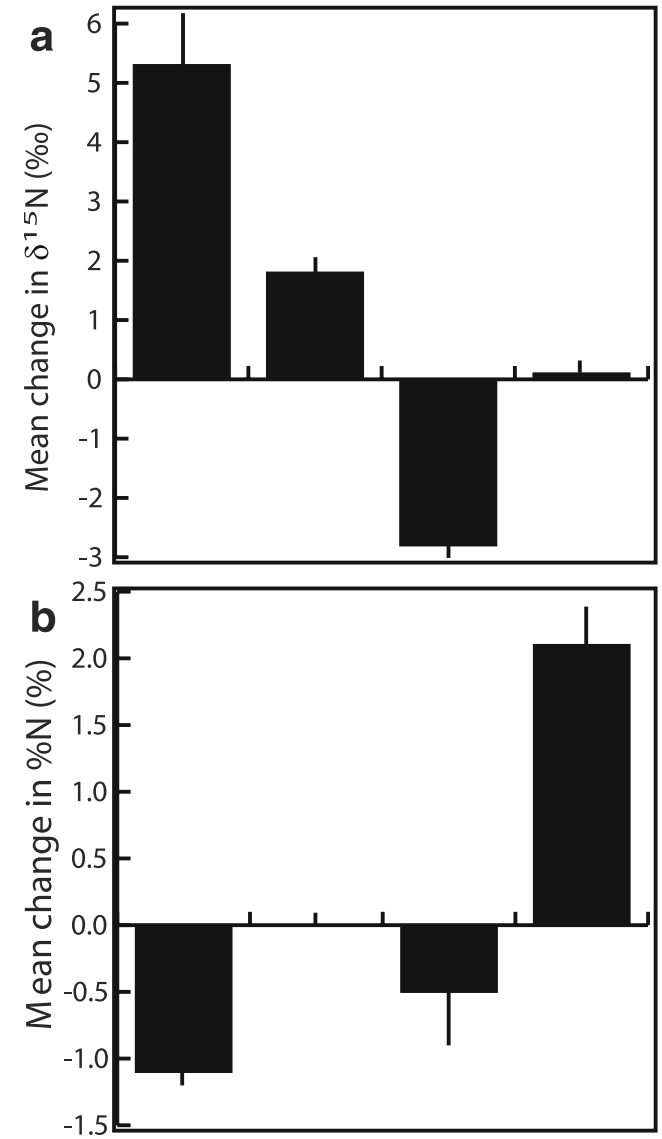


Figure 4.7: Change in $\delta^{15}N$ and %N after deployment

Change in $\delta^{15}N$ (a) and %N (b) of macroalgae and oyster from mean initial values in June 2004 and May and July 2006.



CHAPTER 5: OYSTER (*CRASSOSTREA VIRGINICA*) δ^{15}N GRADIENT LINKS LAND USE AND WATER QUALITY TO NITROGEN SOURCE IN CHESAPEAKE BAY

Abstract

Eutrophication due to excess nitrogen from multiple sources has been well documented in Chesapeake Bay. Stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopes in bioindicators such as oysters can identify nitrogen derived from human and/or animal waste (e.g. sewage, septic, animal manures) and integrate it over time (3-4 months in muscle tissue). This technique was applied broadly (11,600 km^2) and densely (87.9 km^2/site) across Chesapeake Bay tributaries to test effects of land use, salinity, flushing time, and oyster size on $\delta^{15}N$ values and to identify spatial patterns of human and/or animal wastes. Oysters grown by a network of citizen-scientists were sampled and compared to land use (areas 4% to 63% developed, 0.00% to 0.27% animal agriculture), water quality in freshwater streams and tributaries, and flushing time (0.7 days to 6.2 days). Oyster $\delta^{15}N$ decreased towards Chesapeake Bay's mouth (ranging from 16.0 ‰ in Eastern Bay to 8.3 ‰ in Lynnhaven River), and was related to flushing time ($y = 1.7\ln(x) + 10.2$, $R^2 = 0.62$, $p < 0.01$), salinity ($y = -0.91x + 22.29$, $R^2 = 0.36$, $p < 0.05$), and shell height ($y = -0.14x + 21$, $R^2 = 0.83$, $p < 0.01$). Therefore, oyster $\delta^{15}N$ may not be appropriate for the inference of nitrogen sources at the 1000s of km^2 spatial scale. Nevertheless, wastewater (transported via many mechanisms) was inferred to impact multiple tributaries. Furthermore, oyster $\delta^{15}N$ responded to stream and tributary nutrients, so it may also indicate overall water quality and the tributary scale (100s of km^2) may be most appropriate to infer nitrogen source from oyster $\delta^{15}N$ values.

Introduction

Enriched $\delta^{15}N$ signatures in sewage or animal waste arise from isotopic discrimination due to a combination of ammonia volatilization and denitrification at the source or by microbial processing employed by wastewater treatment facilities (Fry 2006; McClelland and Valiela, 1998; Sweeny and Kaplan, 1980; Tucker et al., 1999). In areas degraded by anthropogenic wastes, $\delta^{15}N$ in living resource bioindicators has inversely related with water quality parameters (Chapter 3). In contrast, synthetic fertilizers 'fixed' from atmospheric N_2 (0‰) have $\delta^{15}N$ values generally -4 to +4 ‰ (Hübner, 1986; Macko and Ostrom, 1994; Vitoria et al., 2004). Oyster stable nitrogen ($\delta^{15}N$) and carbon isotope ($\delta^{13}C$) values have been utilized as an indicator of anthropogenic waste nitrogen sources (e.g. sewage, septic, and animal wastes) in Chesapeake (Fertig et al. 2010), mid-Atlantic Coastal Bays (Fertig et al. 2009), and worldwide (Piola et al. 2006).

Denitrification rates have decreased in Chesapeake Bay with eutrophication due to anoxia-induced loss of benthic habitat (Cornwell et al. 1999), with estimates of nitrogen removal of 39.55 kg N × 10^6 $year^{-1}$ (only 26.0% of the overall nitrogen budget) in mesohaline Chesapeake Bay sediments and is thought to be controlled by coupled nitrification/denitrification rates (Kemp et al. 1990, Boynton et al. 1995). Further, denitrification is inherently spatially (Scala and Kerkhof 2000) and seasonally (lowest in spring and summer; Kemp et al. 1990, Chen et al. 2009) variable beyond the resolution of this study and is influenced by water residence time

due to a rate-limiting step of nitrate diffusion across the water-sediment interface (Sebilo et al. 2003, Seitzinger et al. 2006, Klocker et al. 2009).

Carbon sources can be inferred to be terrestrially or marine derived by interpreting $\delta^{13}C$ (Fry 2006) in biological integrators e.g. organic carbon (Guo and Santschi 1997; Ogrinc et al. 2005) and organic components of sediments (Bratton et al. 2003). Heavier (less negative) signals originating in marine sources (-14 ‰) and lighter (more negative) originating from terrestrial sources (-22 ‰; Valiela 1995) have been frequently observed in a variety of organisms including bacteria (Coffin et al. 1989) and zooplankton (Hoffman et al. 2008). In general, dissolved and particulate organic carbon is derived from old allochthonous terrestrial sources in upper Chesapeake Bay and young marine sources near its mouth (Loh et al. 2006). Oyster $\delta^{13}C$ and C/N ratio can be influenced by several factors. Marine and terrestrial carbon sources can have different signatures in part due to marine phytoplankton with a C3 photosynthetic pathway (trees and shrubs are more negative) vs. terrestrial C4 grasses such as corn and salt marsh vegetation (-7 to -13 ‰; McMillan et al. 1980, Haddad and Martens, 1987, Goñi et al. 1997). Mixing of these signatures can reflect relative marine and freshwater contributions to carbon sources. Furthermore, CO_2 limitation can result in fractionation (less negative values, Fogel et al. 1992) during photosynthesis and phytoplankton bicarbonate (HCO_3^-) assimilation. Post-depositional diagenesis is another factor to consider (Cornwell et al. 1996).

Water circulation may also influence oyster $\delta^{15}N$ and $\delta^{13}C$ patterns. Spatial patterns of nitrogen and carbon sources have been identified by oysters over small (10s of km^2; Piola et al. 2006; Daskin et al. 2008) and mid-sized (100s of km^2; Fertig et al. 2009), but have not yet been demonstrated for large-scale (10,000s of km^2) ecosystems subject to a gradient of water circulation rates. Shorter exposure times to anthropogenic nitrogen sources due to rapid circulation may limit nutrient assimilation by bioindicators, which integrate $\delta^{15}N$ signatures over time (Fertig et al. 2009, 2010). To assess the effect of water circulation, this dissertation employs flushing time estimates (Wazniak et al. 2009). For reference, 'flushing time' was defined as a measure of how fast circulation processes exchange water and water-borne materials between a confined water body and the adjacent water body with which it communicates. Wazniak et al. (2009) reported calculations of flushing time for small tributaries directly applicable to regions considered in this dissertation.

Therefore, the current study addressed the following four questions. 1) Are oyster $\delta^{15}N$ and $\delta^{13}C$ values significantly related to flushing time? 2) How do observed patterns in $\delta^{15}N$ relate to nitrogen source and water quality over broad (1,000s of km^2) and regional (100s of km^2) scales? 3) What are the dominant nitrogen and carbon sources available to oysters in Chesapeake Bay? 4) What are the relative contributions of direct vs. indirect inputs of anthropogenic nutrients to Chesapeake Bay tributaries?

Methods and Materials

Oysters were sampled in summer (June 2005, 2006) after growth for nine months (by 'oyster gardeners', citizen-scientists participating in oyster restoration). Oysters were grown in bags or cages tied to docks in shallow water at 132 sites (37 sites in 2005, 95 sites in 2006) located from Annapolis, MD (38°58'38"N 76°29'31"W) in the north to Virginia Beach, VA (36°5'24"N, 76°2'32"W) in the south. Specific locations and sample availability were a result of the locations of volunteer 'oyster gardeners'. Nevertheless, spatial coverage represented major Chesapeake Bay tributaries and sub-watersheds with sufficient salinities (5 to 30 ppt).

Oysters were sampled, handled, and analyzed for $\delta^{15}N$ as described previously (Chapter 2, Fertig et al. 2010). Modifications including analyzing five pools of five individuals for oysters collected in 2005, and only five individuals in 2006. Additionally, the University of California Davis Stable Isotope Facility analyzed $\delta^{13}C$ values and carbon content (μg C) enabling calculations of %C, and C/N ratio. For definition, $\delta^{15}N$ or $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 10^3$, where R was defined as either the $^{15}N/^{14}N$ or $^{13}C/^{12}C$ ratio and instrumental error was ± 0.2 ‰. Standard analytical references were atmospheric N_2 (air) for nitrogen, and PeeDee Belemnite for carbon.

Oyster data ($\delta^{15}N$, $\delta^{13}C$, C/N ratio, %N, and %C values) were regressed against flushing time. Estimates of tributary flushing time were taken from Wazniak et al. (2009): Elizabeth River (5.0 days), Lynnhaven River (0.7 days), Great Wicomico River (5.6 days), and Monie Bay (2.1 days). Flushing time in the

Nansemond River (1.5 days) was used as an estimate for the James River. The St. Mary's River-MD (6.2 days) was used as an estimate for the Potomac River. Broad Creek (4.1 days) and Harris Creek (4.3 days) were averaged for an estimate of the Choptank River (4.2 days). Hungars Creek (0.9 days), Nassawadox Creek (1.1 days), and Onancock Creek (1.6 days) were averaged to derive an estimate of the Lower Western Delmarva Peninsula (1.2 days). Flushing times for the Magothy (6.0 days), Rhode (3.8 days), Severn-MD (8.5 days), South (6.0 days), and West (3.2 days) rivers were averaged for an estimate for the 'Maryland Lower Western Shore' (5.5 days). Mobjack Bay (3.7 days) and the Severn River-VA (2.3 days) were averaged for an estimate of the York River.

Mean oyster $\delta^{15}N$ and $\delta^{13}C$ were mapped (ESRI Arc Map 9.3) for each sampling location in relation to locations of wastewater treatment plant point sources (Chesapeake Bay Program 2009) and regressed (Proc Reg, SAS 9.2) against land use (% Developed, % Animal Agriculture, Maryland Department of Planning, 2002; Virginia Department of Forestry 2005). 'Developed' area aggregates 'Extractive', 'Residential', and 'Urban' land use codes for Maryland and Virginia datasets.

Dissolved oxygen, total nitrogen, total phosphorus and dissolved oxygen data were downloaded from the Chesapeake Bay Program Water Quality Database (1984-present, http://www.chesapeakebay.net/). Monitoring stations were selected for proximity to oyster locations in tributaries of interest (Figure 5.1) and detailed discussion of spatial segmentation for this long-term dataset is described elsewhere

(Chesapeake Bay Program 2004). Water quality monitoring and oyster data were explored with multidimensional scaling (Proc MDS, SAS 9.2) to decrease variables and explain variability. Non-tidal freshwater stream water quality and the Benthic Integrated Biotic Index (B-IBI) data from the watersheds of the tributaries of interest were obtained from the Chesapeake Bay Program (www.chesapeakebay.net, Katie Foreman and Jacqueline Johnson, personal communication).

Results

Oyster $\delta^{15}N$ values exhibited a broad gradient decreasing north to south towards Chesapeake Bay's mouth (Figure 5.1), with values in Choptank River and Eastern Bay (13.9 ± 0.1 ‰; Table 5.1) significantly different ($p < 0.05$) than all other regions (Table 5.1) while Lynnhaven River (9.5 ± 0.1 ‰) was significantly different ($p < 0.05$) than all but Potomac River (Table 5.1). Oyster $\delta^{15}N$ was significantly related to flushing time ($y = 1.7\ln(x) + 10.2$, $R^2 = 0.62$, $p < 0.01$, Figure 5.2a), salinity ($y = -0.91x + 22.29$, $R^2 = 0.36$, $p < 0.05$, Figure 5.2b), and shell height ($y = -0.14x + 21$, $R^2 = 0.83$, $p < 0.01$, Figure 5.2c), but not other oyster parameters ($\delta^{13}C$, %N, %C, C/N ratio). Oyster nutritional condition, as indicated by tissue %N (6.7% to 16.0%), oyster %C (39% to 46%) and C/N ratio (3.0 to 4.4) had little spatial variation at either Chesapeake or tributary scales (Table 5.1). Oysters had distinct $\delta^{15}N$ and $\delta^{13}C$ signatures for each tributary (Figure 5.3). Higher oyster $\delta^{15}N$ values and lower

oyster $\delta^{13}C$ values were observed in mesohaline regions while lower $\delta^{15}N$ values and higher $\delta^{13}C$ values were observed in polyhaline regions (Table 5.1, Figure 5.4a-f).

Oyster $\delta^{15}N$ values displayed a negative quadratic relationship with land use devoted to development (urban, residential, and extraction uses; Figure 5.5a, b). At the mouths of the Severn, South, & West Rivers and the Elizabeth River, oyster $\delta^{15}N$ was highest in areas surrounding wastewater treatment plants (Figure 5.4a,e).

Oyster $\delta^{15}N$ significantly ($p < 0.05$) correlated with land use ($r = -0.20$, $r = -0.14$ for % watershed developed or devoted to animal agriculture, respectively, Table 5.2a, Figure 5.5a-d). Further, oyster $\delta^{15}N$ significantly correlated ($p < 0.05$) with water quality (e.g., total nitrogen, $r = 0.52$, $r = 0.41$, and dissolved oxygen, $r = -0.31$, $r = -0.28$) in tributaries and streams respectively (Table 5.2b,c) as well as most other water quality metrics (except water temperature in tributaries and total phosphorus in streams).

Lynnhaven and York Rivers and the Western Lower Delmarva Peninsula had lower nitrogen (total and dissolved) and chlorophyll *a* concentrations and higher salinities and Secchi depths than most other tributaries (Table 5.2b,c, Figure 5.2). Oysters in southern tributaries (Lynnhaven, Elizabeth Rivers) were bigger and had lower $\delta^{15}N$ values than elsewhere, and these metrics were inversely related, but not related to %N (Figure 5.2c, Table 5.1, Table 5.2d).

Discussion

Human/animal waste nitrogen sources inferred from oyster $\delta^{15}N$ values

Oyster $\delta^{15}N$ grand mean values in Chesapeake Bay (12.9 ± 0.1 ‰) were similar to other reported shellfish $\delta^{15}N$ values used to infer human or animal waste nitrogen sources. Example of other studies inferring human or animal wastes from enriched bivalve $\delta^{15}N$ values include: 10.4 ± 0.8 ‰ in Pamlico Sound, North Carolina (Bucci et al. 2007), 6.9 ± 0.4 ‰ in Mobile Bay, Alabama (Daskin et al. 2008) and 8.5 ± 0.9 ‰ and mid-Atlantic coastal bays of Maryland and Virginia (Fertig et al. 2009) in USA, 8.0 ± 0.9 ‰ in Marenne-Oléron Bay, France (Kang et al. 1999), and 16.0 ± 2.3 ‰ in the Manning River of New South Wales, Australia (Piola et al. 2006). Within Chesapeake Bay, oyster $\delta^{15}N$ and $\delta^{13}C$ values identified distinct isotopic signatures for tributaries (Figure 5.3). At present, it is difficult to fully account for the geographic distinction for these dual-isotope signatures. Perhaps unique combinations of nitrogen sources and oceanic mixing (Pritchard 1952, 1956) or marine/terrestrial nitrogen (Fry 1999, Loh et al. 2006) contribute to these variations.

Spatial patterns of oyster $\delta^{15}N$ values (Figure 5.4a-f) indicated that in some cases human and/or animal wastes entered tributaries from their watersheds, and in some cases from Chesapeake Bay's mainstem. Wastewater effluents were inferred to flow downstream Potomac River, for example, due to decreasing $\delta^{15}N$ values towards its mouth (Figure 5.4c). Similarly, the Upper Eastern Shore and the Choptank River

likely received diffuse sources of human and/or animal waste transported downstream from the watershed (Figure 5.4b). The opposite spatial pattern and proximity of wastewater treatment plants near tributary mouths led to the inference that sewage effluents were transported upstream into tributaries of the Lower Western Shore, Maryland (Figure 5.4a) and the Lynnhaven River (Figure 5.4f). These tributaries may receive nitrogen from Chesapeake Bay through bottom layer circulation (Testa et al. 2008) or tidal advection (Granger et al. 2000). A third situation was evident in Monie Bay: nitrogen entered from Chesapeake Bay and from its watershed (Figure 5.4d, Chapter 3), consistent with previous findings in this region (Stribling and Cornwell 1997). Nitrogen inputs from multiple locations are common globally (Granger et al. 2000, Weston et al. 2004, Frick et al. 2007, Testa et al. 2008). Spatial patterns of oyster $\delta^{15}N$ at the tributary scale (100s of km^2) can thus elucidate nitrogen transport.

In addition to nitrogen source, oyster $\delta^{15}N$ reflected land use (Figure 5.5) and overall water quality in tributaries and freshwater streams (Figure 5.6a,b) due to numerous significant correlations (Table 5.2a-d). Therefore, oysters served as a simple and powerful bioindicator that provided a link between multiple monitoring programs (Figure 5.6c). In contrast, water quality metrics were generally not significantly correlated between streams and tributaries (Table 5.2b,c) due to localized sampling (i.e. shallow streams vs. tributary channels). The ability of oyster $\delta^{15}N$ values to integrate overall water quality may be useful if bioindicators are deployed in areas lacking spatially/temporally dense quality datasets.

Consideration of physical and biological factors that influence $\delta^{15}N$ values

Interpretation of oyster $\delta^{15}N$ values must consider physical and biological factors (salinity, oyster size, flushing time) that influenced oyster $\delta^{15}N$ (Figure 5.2a,b,c, 5.7) at the large spatial scale (1,000s km^2) throughout Chesapeake Bay. Increased salinity could influence oyster $\delta^{15}N$ via increased growth (Figure 5.2b,c, Dame 1972, Paynter and DiMichele 1990), and mollusk $\delta^{15}N$ values have been found to vary with salinity in European oceans (Jennings and Warr 2003). Potentially, growth rates might also indicate overall water quality and presence/absence of human and/or animal wastes; this could be further investigated in the future.

Flushing times also affected the ability of oyster $\delta^{15}N$ to accurately reflect anthropogenic nitrogen sources (Figure 5.2a) due to a mismatch between nitrogen source availability and the rate of nitrogen incorporation by bioindicators. Tributaries with slow flushing times (e.g. ~7 days in St. Mary's River - a tributary of the Potomac River near oyster deployments) can provide sufficient time for bioindicators to completely integrate a nitrogen source and its isotopic signature (Figure 5.2a). In contrast, tributaries with rapid flushing times and/or proximity to oceanic exchange (e.g. ~1 day in Lynnhaven River) may rapidly export nitrogen and/or phytoplankton. In such tributaries, oysters may incompletely integrate nitrogen source isotopic signatures due to slow integration (3 to 4 months, Chapter 2) by oyster muscle tissue. Flushing time could therefore affect successful detection of an enriched signal, as

occurred in coastal Australia (Gartner et al. 2002) and potentially in Lynnhaven River (Figures 5.1, 5.4f). Therefore, flushing times may influence sensitivity of tributaries to anthropogenic pressures, and so should be considered when comparing oyster $\delta^{15}N$ values across tributaries.

Chesapeake Bay may be more resilient near its mouth than other areas, as exemplified by rapid oceanic exchange, low total nitrogen concentrations (Table 5.2a−d), and Bay Health Index scores consistently higher than the overall bay (1986 to 2009; www.eco-check.org). Ecosystem resilience could explain low oyster $\delta^{15}N$ values in the Lower Bay (9.5 ± 0.1 ‰ to 11 ± 0.1 ‰, Figure 5.1, Table 5.1) despite dense human population and wastewater treatment plants (25 to 50 MGD). These oyster $\delta^{15}N$ values (Figure 5.1) are similar to estimated pre-industrial values of ~8 to 9 ‰, given a + 3 to 4 ‰ trophic shift (Minagawa and Wada 1984) above pre-industrial sediment core organic matter $\delta^{15}N$ values (~5.0 ‰, Bratton et al. 2003).

Consideration of appropriate spatial scale for interpreting oyster $\delta^{15}N$ values

Sampling oysters after distribution and growth by citizen-scientist Oyster Gardeners in Chesapeake Bay provided an efficient, cost-effective mechanism to rapidly generate a large-scale (11,600 km^2) study with numerous sites (37 in 2005 and 97 sites in 2006), yielding high spatial density of sites (87.9 km^2/site). For comparison to other studies covering comparable areas (e.g. Jennings and Warr, 2003) the current study collected up to 39 times more sites per area. This dataset enabled consideration of the appropriate spatial scale for deployment of oysters as a

bioindicator of nitrogen source. The large range of oyster $\delta^{15}N$ values (Figure 5.1), gradients of salinity, oyster size, and flushing times (Figure 5.2) meant nitrogen sources could be inferred within tributaries (100s of km^2, Figure 5.4a-f) but not across Chesapeake Bay (1,000s of km^2, Figure 5.7), thereby quantifying the upper extent of spatial integration of oyster $\delta^{15}N$ bioindicators.

Chapter 5 Tables

Table 5.1: Oyster metrics in Chesapeake Bay tributaries

Mean (± standard error) oyster shell height (length, mm), $\delta^{15}N$, $\delta^{13}C$, %N, %C, C/N ratio for relevant Chesapeake Bay tributaries. Data is averaged over 2005 and 2006, except in Monie Bay (2005 data unavailable). Sample size (n), degrees of freedom (df), mean squared error (MSE), F value (F), and p value (p) are reported for ANOVA between regions.

Tributary		**n**	**Shell height** mm	**$\delta^{15}N$** per mille	**$\delta^{13}C$** per mille	**%N**	**%C**	**C:N**
Choptank River		53	54 (1)	13.4 (0.1)	-23.5 (0.1)	12.3 (0.2)	43.8 (0.2)	4.3 (0.1)
Eastern Bay		38	57 (2)	14.6 (0.1)	-22.6 (0.2)	12.6 (0.3)	44.0 (0.1)	4.2 (0.1)
Elizabeth River		19	67 (3)	11.0 (0.2)	-20.5 (0.2)	12.3 (0.4)	42.1 (1.1)	4.0 (0.1)
Great Wicomico River		5	71 (5)	10.6 (0.2)	-23.2 (0.1)	12.0 (0.3)	42.4 (0.9)	4.1 (0.0)
Lynnhave River		33	77 (2)	9.5 (0.1)	-22.4 (0.2)	12.5 (0.4)	43.5 (1.3)	4.1 (0.0)
Magothy, Severn, South, West, Rhode Rivers		157	48 (1)	13.7 (0.1)	-23.3 (0.1)	12.6 (0.1)	44.1 (0.4)	4.1 (0.0)
Monie Bay		100	56 (1)	12.2 (0.1)	-24.9 (0.3)	13.4 (0.1)	42.3 (0.2)	3.7 (0.0)
Patuxent River		88	54 (1)	13.2 (0.1)	-22.2 (0.1)	12.3 (0.2)	43.6 (0.2)	4.2 (0.1)
Potomac River		27	51 (2)	13.5 (0.2)	-21.4 (0.2)	12.0 (0.3)	43.0 (0.4)	4.3 (0.1)
Western Lower Delmarva Peninsula		5	76 (7)	10.2 (0.2)	-21.0 (0.4)	12.7 (0.1)	43.9 (0.5)	4.0 (0.1)
	n		425	545	425	425	425	425
	df		5, 419	10, 534	5, 419	4, 419	5, 419	5, 419
ANOVA	*MSE*		85.9678	0.692	3.1368	2.6491	15.0917	0.3042
	F		65.35	118.81	33.56	5.59	3.37	11.41
	p		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0054**	**<0.0001**

Table 5.2: Correlations between land use, water quality, and oyster metrics

Correlations between a) land use (Maryland Department of Planning 2002), b) stream water quality (Chesapeake Bay Program 2010), c) tributary water quality (Chesapeake Bay Program 2010), and d) oyster metrics. Significant relationships ($p < 0.05$) are asterisked.

a) Land Use

			Land Use	
	Variable		% Developed	% Animal Agriculture
	Sample size (n)		454	454
Land Use	% Developed	454	**1.00**	**-0.78***
	% Animal Agriculture	454	**-0.78***	**1.00**
Streams	Dissolved Oxygen	129	0.21	-0.21
	ssolved Organic Carbon	85	**-0.30***	**0.32***
	Total Nitrogen	85	**-0.45***	0.23
	Total Phosphorus	85	**-0.29***	0.10
	Water Temperature	141	**-0.42***	**0.36***
Tributaries	Chlorophyll a	379	-0.03	0.00
	Dissolved Oxygen	386	0.09	-0.01
	Salinity	382	**0.56***	**-0.28***
	Secchi Depth	382	0.00	**-0.16***
	Total Nitrogen	384	**-0.39***	**0.18***
	Total Phosphorus	386	**0.23***	**-0.24**
	Total Suspended Solids	386	**0.29***	0.00
	Water Temperature	387	0.09	-0.02
	Flushing Time	425	**0.36***	**-0.65***
Oysters	$\delta^{15}N$	545	**-0.20***	**-0.14***
	$\delta^{13}C$	545	**0.30***	**-0.44***
	% Nitrogen	545	**-0.11***	**0.22***
	% Carbon	545	0.09	**-0.15***
	C/N ratio	545	**0.14***	**-0.30***
	Shell height	545	0.08	0.03

b) Streams

	Variable	Sample size (n)	Dissolved Oxygen (Streams)	Dissolved Organic Carbon (Streams)	Total Nitrogen (Streams)	Total Phosphorus (Streams)	Water Temperature (Streams)
			129	85	85	85	141
Land Use	% Developed	454	0.21	**-0.30***	**-0.45***	**-0.29***	**-0.42***
Land Use	% Animal Agriculture	454	-0.21	**0.32***	0.23	0.10	**0.36***
Streams	Dissolved Oxygen	129	**1.00**	-0.10	**-0.31***	-0.13	**-0.55***
Streams	ssolved Organic Carbon	85	-0.10	**1.00**	-0.09	**0.34***	0.09
Streams	Total Nitrogen	85	**-0.31***	-0.09	**1.00**	-0.05	**0.50***
Streams	Total Phosphorus	85	-0.13	**0.34***	-0.05	**1.00**	**0.26***
Streams	Water Temperature	141	**-0.55***	0.09	**0.50***	**0.26***	**1.00**
Tribtaries	Chlorophyll a	379	0.07	0.05	-0.01	-0.01	**-0.17***
Tribtaries	Dissolved Oxygen	386	**0.22***	0.10	-0.19	**-0.23***	**-0.24***
Tribtaries	Salinity	382	-0.12	0.10	**0.30***	**0.24***	0.09
Tribtaries	Secchi Depth	382	-0.03	**-0.22***	0.19	-0.08	0.16
Tribtaries	Total Nitrogen	384	-0.07	0.17	-0.06	-0.14	-0.13
Tribtaries	Total Phosphorus	386	-0.10	**-0.21***	-0.05	0.14	0.15
Tribtaries	Total Suspended Solids	386	0.04	-0.04	-0.03	-0.16	-0.09
Tribtaries	Water Temperature	387	-0.05	**-0.26***	0.03	0.13	**0.23***
Tribtaries	Flushing Time	425	**0.31***	**-0.32***	**-0.41***	-0.24	**-0.54***
Oysters	$\delta^{15}N$	545	**-0.28***	**0.24***	**0.41***	0.17	**0.30***
Oysters	$\delta^{13}C$	545	0.16	-0.05	-0.02	-0.02	**-0.25***
Oysters	% Nitrogen	545	**-0.23***	0.01	**0.32***	-0.13	**0.31***
Oysters	% Carbon	545	0.06	-0.04	0.20	0.05	-0.06
Oysters	C/N ratio	545	**0.23***	0.02	**-0.26***	0.17	**-0.31***
Oysters	Shell height	545	0.02	0.04	0.13	0.08	-0.02

c) Tributaries

	Variable	Sample size (n)	Chlorophyll a (Tribtaries)	Dissolved Oxygen (Tribtaries)	Salinity (Tribtaries)	Secchi Depth (Tribtaries)	Total Nitrogen (Tribtaries)	Total Phosphorus (Tribtaries)	Total Suspended Solids (Tribtaries)	Water Temperature (Tribtaries)	Flushing Time (Tribtaries)
			379	386	382	382	384	386	386	387	425
Land Use	% Developed	454	-0.03	0.09	**0.56***	0.00	**-0.39***	**0.23***	**0.29***	0.09	**0.36***
Land Use	% Animal Agriculture	454	0.00	-0.01	**-0.28***	**-0.16***	**0.18***	**-0.24**	0.00	-0.02	**-0.65***
Streams	Dissolved Oxygen	129	0.07	**0.22***	-0.12	-0.03	-0.07	-0.10	0.04	-0.05	**0.31***
Streams	ssolved Organic Carbon	85	0.05	0.10	0.10	**-0.22***	0.17	**-0.21***	-0.04	**-0.26***	**-0.32***
Streams	Total Nitrogen	85	-0.01	-0.19	**0.30***	0.19	-0.06	-0.05	-0.03	0.03	**-0.41***
Streams	Total Phosphorus	85	-0.01	**-0.23***	**0.24***	-0.08	-0.14	0.14	-0.16	0.13	-0.24
Streams	Water Temperature	141	**-0.17***	**-0.24***	0.09	0.16	-0.13	0.15	-0.09	**0.23***	**-0.54***
Tribtaries	Chlorophyll a	379	**1.00**	**0.21***	**-0.42***	**-0.39***	**0.52***	**0.17***	0.07	**-0.21***	**0.35***
Tribtaries	Dissolved Oxygen	386	**0.21***	**1.00**	0.04	**0.10***	0.02	**-0.47***	0.03	**-0.77***	-0.13
Tribtaries	Salinity	382	**-0.42***	0.04	**1.00**	**0.35***	**-0.76***	**-0.12***	**0.17***	**0.11***	**-0.64***
Tribtaries	Secchi Depth	382	**-0.39***	**0.10***	**0.35***	**1.00**	**-0.48***	**-0.37***	**-0.37***	-0.05	**-0.26***
Tribtaries	Total Nitrogen	384	**0.52***	0.02	**-0.76***	**-0.48***	**1.00**	**0.28***	0.05	**-0.24***	**0.50***
Tribtaries	Total Phosphorus	386	**0.17***	**-0.47***	**-0.12***	**-0.37***	**0.28***	**1.00**	**0.37***	**0.46***	**0.39***
Tribtaries	Total Suspended Solids	386	0.07	0.03	**0.17***	**-0.37***	0.05	**0.37***	**1.00**	**0.11***	-0.05
Tribtaries	Water Temperature	387	**-0.21***	**-0.77***	**0.11***	-0.05	**-0.24***	**0.46***	**0.11***	**1.00**	0.04
Tribtaries	Flushing Time	425	**0.35***	-0.13	**-0.64***	**-0.26***	**0.50***	**0.39***	-0.05	0.04	**1.00**
Oysters	$\delta^{15}N$	545	**0.22***	**-0.31***	**-0.65***	**-0.22***	**0.52***	**0.20***	**-0.24***	0.08	**0.61***
Oysters	$\delta^{13}C$	545	0.00	0.04	**0.30***	0.12	**-0.20***	0.08	**0.15***	-0.05	**0.24***
Oysters	% Nitrogen	545	0.09	**0.12***	-0.11	**-0.12***	0.05	-0.06	0.10	-0.06	**-0.15***
Oysters	% Carbon	545	**0.13***	-0.07	**-0.26***	-0.02	0.12	0.01	**-0.14***	-0.02	**0.12***
Oysters	C/N ratio	545	-0.05	**-0.14***	0.00	0.10	-0.01	0.05	**-0.15***	0.05	**0.24***
Oysters	Shell height	545	**-0.16***	**0.13***	**0.55***	**0.18***	**-0.42***	-0.10	**0.14***	-0.01	**-0.49***

d) Oysters

	Variable	Sample size (n)	Oysters $\delta^{15}N$ 545	Oysters $\delta^{13}C$ 545	Oysters % Nitrogen 545	Oysters % Carbon 545	Oysters C/N ratio 545	Oysters Shell height 545
Land Use	% Developed	454	**-0.20***	**0.30***	**-0.11***	0.09	**0.14***	0.08
	% Animal Agriculture	454	**-0.14***	**-0.44***	**0.22***	**-0.15***	**-0.30***	0.03
Streams	Dissolved Oxygen	129	**-0.28***	0.16	**-0.23***	0.06	**0.23***	0.02
	ssolved Organic Carbon	85	**0.24***	-0.05	0.01	-0.04	0.02	0.04
	Total Nitrogen	85	**0.41***	-0.02	**0.32***	0.20	**-0.26***	0.13
	Total Phosphorus	85	0.17	-0.02	-0.13	0.05	0.17	0.08
	Water Temperature	141	**0.30***	**-0.25***	**0.31***	-0.06	**-0.31***	-0.02
Tributaries	Chlorophyll a	379	**0.22***	0.00	0.09	**0.13***	-0.05	**-0.16***
	Dissolved Oxygen	386	**-0.31***	0.04	**0.12***	-0.07	**-0.14***	**0.13***
	Salinity	382	**-0.65***	**0.30***	-0.11	**-0.26***	0.00	**0.55***
	Secchi Depth	382	**-0.22***	0.12	**-0.12***	-0.02	0.10	**0.18***
	Total Nitrogen	384	**0.52***	**-0.20***	0.05	0.12	-0.01	**-0.42***
	Total Phosphorus	386	**0.20***	0.08	-0.06	0.01	0.05	-0.10
	Total Suspended Solids	386	**-0.24***	**0.15***	0.10	**-0.14***	**-0.15***	**0.14***
	Water Temperature	387	0.08	-0.05	-0.06	-0.02	0.05	-0.01
	Flushing Time	425	**0.61***	**0.24***	**-0.15***	**0.12***	**0.24***	**-0.49***
Oysters	$\delta^{15}N$	545	**1.00**	0.04	-0.02	0.04	0.07	**-0.46***
	$\delta^{13}C$	545	0.04	**1.00**	**-0.11***	**0.13***	**0.19***	**0.18***
	% Nitrogen	545	-0.02	**-0.11***	**1.00**	**0.42***	**-0.81***	0.02
	% Carbon	545	0.04	**0.13***	**0.42***	**1.00**	**0.15***	0.01
	C/N ratio	545	0.07	**0.19***	**-0.81***	**0.15***	**1.00**	-0.02
	Shell height	545	**-0.46***	**0.18***	0.02	0.01	-0.02	**1.00**

Chapter 5 Figures

Figure 5.1. Map of mean oyster $\delta^{15}N$ values in Chesapeake Bay

Mean measured oyster $\delta^{15}N$ values in Chesapeake Bay collected at 37 sites in 2005 (circles) and 95 sites in 2006 (triangles) among 11 tributaries (watersheds in black, sub-watersheds in grey) and their streams (blue lines). Wastewater treatment plants (squares) are sized relative to flow. The locations of water quality monitoring stations are also charted for streams (diamonds) and tributaries (stars).

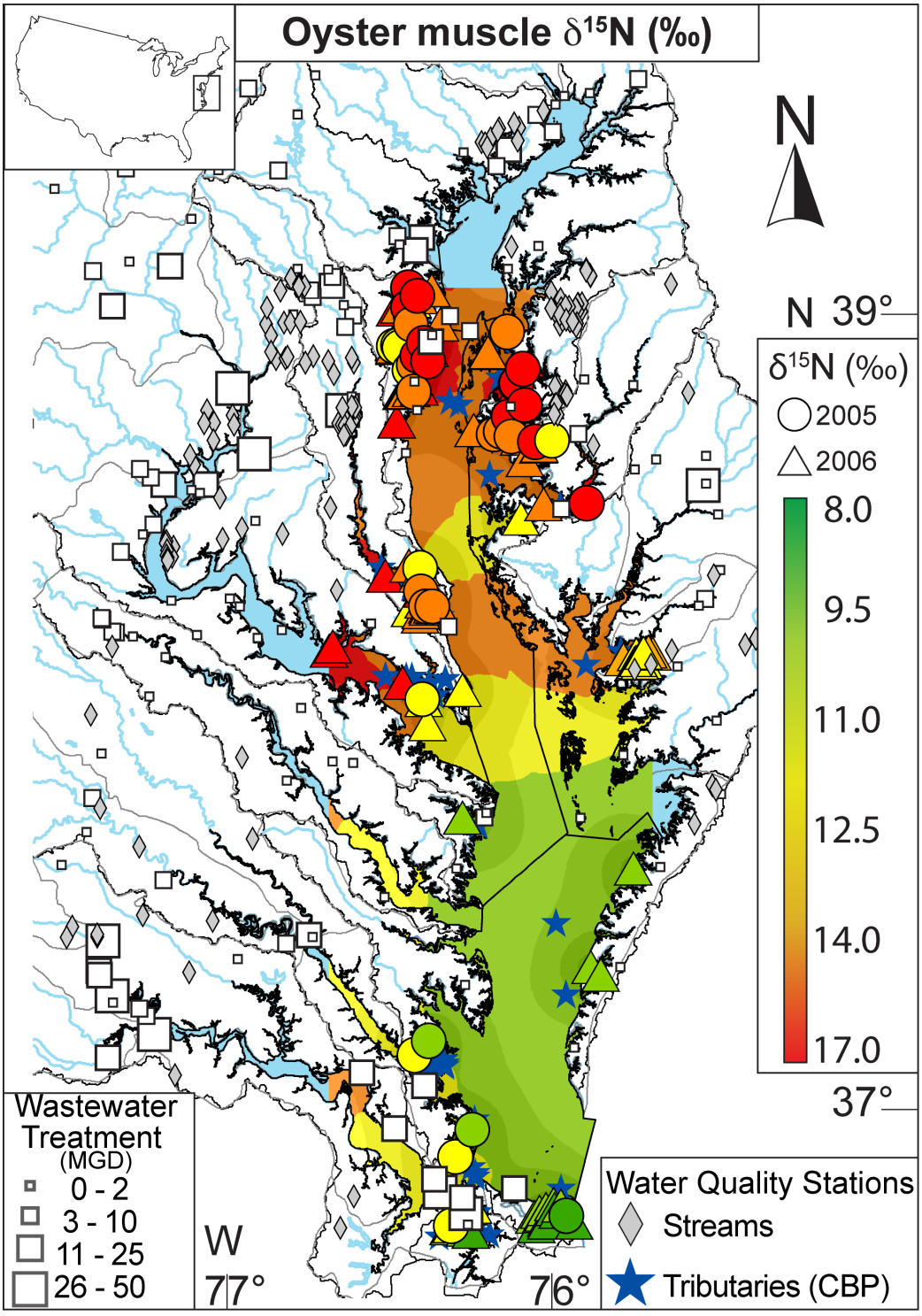

Oyster muscle δ15N (‰)
N
N 39°
δ15N (‰)
2005
2006
8.0
9.5
11.0
12.5
14.0
17.0
37°
Wastewater Treatment (MGD)
0 - 2
3 - 10
11 - 25
26 - 50
W
77°
76°
Water Quality Stations
Streams
Tributaries (CBP)

Figure 5.2: Regressions of oyster $\delta^{15}N$ vs physical and biological factors

Regressions of oyster $\delta^{15}N$ vs. flushing time (a), salinity (b), and shell height (c). Adjusted tidal prism flushing time was only available for small tributaries, so values for Broad Creek (4.1 days) and Harris Creek (4.3 days) were averaged for the Choptank River. Flushing times for Hungars Creek (0.9 days), Nassawadox Creek (1.07 days) and Onancock Creek (1.56 days) were averaged for the Lower Western Delmarva Peninsula. Also, flushing times were averaged for the Magothy River (6.0 days), Rhode River (3.8 days), Severn River, MD (8.5 days), South River (6.0 days), and West River (3.2 days). Mobjack Bay (3.7 days) and Severn River, VA (2.3 days) were averaged to represent the York River. St. Mary River, MD (6.2 days) was considered to be a proxy for the Potomac River. When averaged, standard errors of flushing times are plotted. Standard errors for salinity and shell height reflect variations across sites within that region, as variability over time (e.g. daily measurements) was averaged.

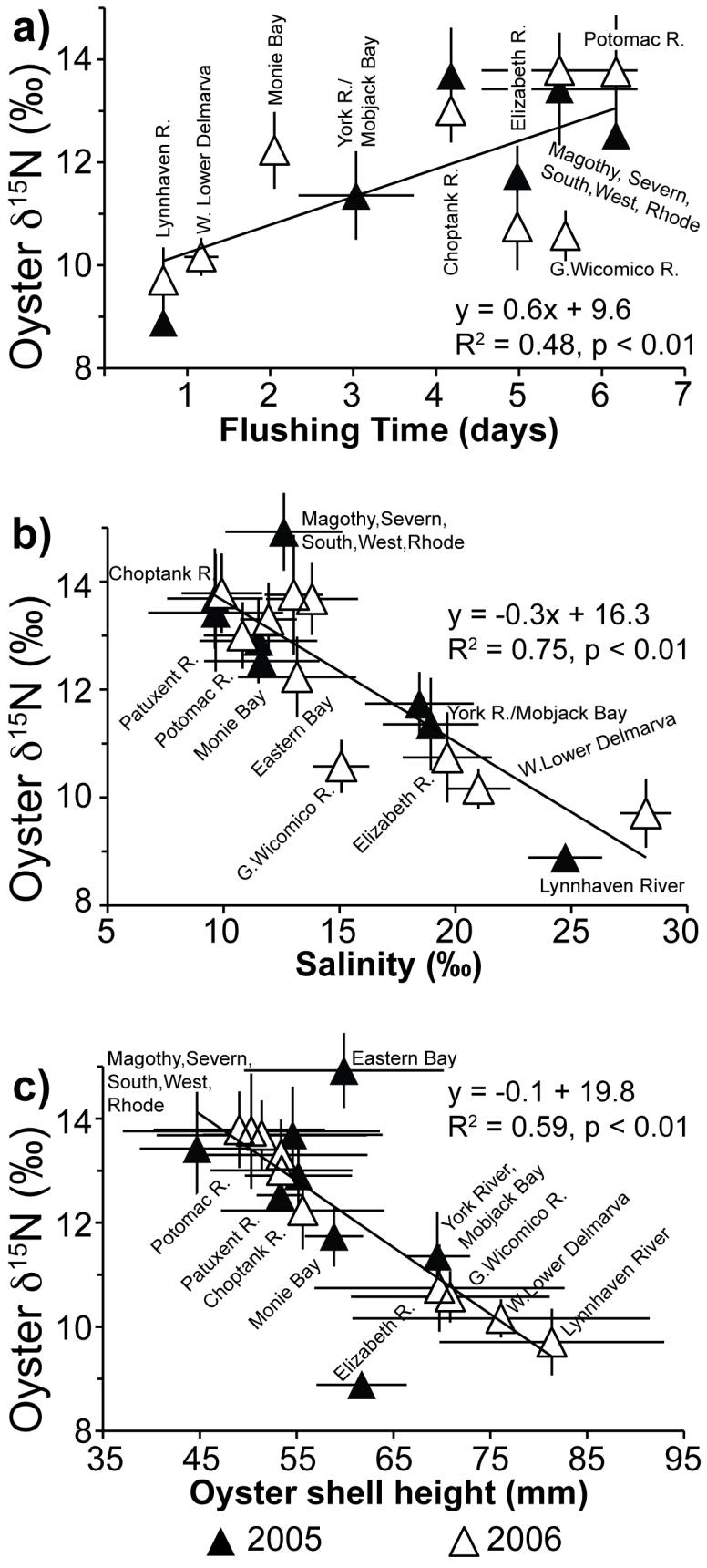

a)
Oyster δ15N (‰)
Flushing Time (days)
Lynnhaven R.
W. Lower Delmarva
Monie Bay
York R./
Mobjack Bay
Choptank R.
Elizabeth R.
Potomac R.
Magothy, Severn,
South,West, Rhode
G.Wicomico R.
y = 0.6x + 9.6
R2 = 0.48, p < 0.01
b)
Oyster δ15N (‰)
Salinity (‰)
Magothy,Severn,
South,West,Rhode
Choptank R.
Patuxent R.
Potomac R.
Monie Bay
Eastern Bay
G.Wicomico R.
York R./Mobjack Bay
W.Lower Delmarva
Elizabeth R.
Lynnhaven River
y = -0.3x + 16.3
R2 = 0.75, p < 0.01
c)
Oyster δ15N (‰)
Oyster shell height (mm)
Magothy,Severn,
South,West,
Rhode
Eastern Bay
Potomac R.
Patuxent R.
Choptank R.
Monie Bay
York River,
Mobjack Bay
G.Wicomico R.
W.Lower Delmarva
Lynnhaven River
Elizabeth R.
y = -0.1 + 19.8
R2 = 0.59, p < 0.01
2005
2006

Figure 5.3: Mean oyster $\delta^{15}N$ vs. $\delta^{13}C$

Mean $\delta^{15}N$ vs. mean $\delta^{13}C$ for each sub-watershed. Standard errors are plotted.

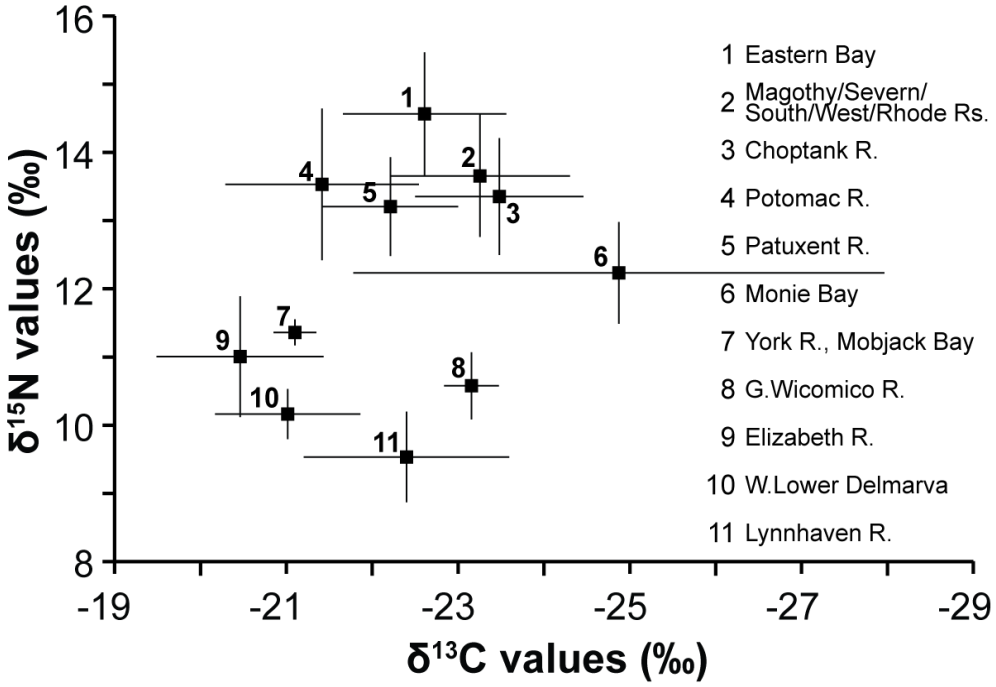


Figure 5.4: Map of oyster $\delta^{15}N$ at tributary scale

Oyster $\delta^{15}N$ values for the Lower Western Shore, Maryland (a), the Upper Eastern Shore (b), Potomac River (c), Monie Bay (d), Elizabeth River (e), and Lynnhaven River (f) from 2005 (circles) and 2006 (triangles). Chesapeake Bay Program water quality monitoring stations (grey squares) are mapped. Wastewater treatment plants (WTP) scaled according to flow (millions of gallons per day, MGD).

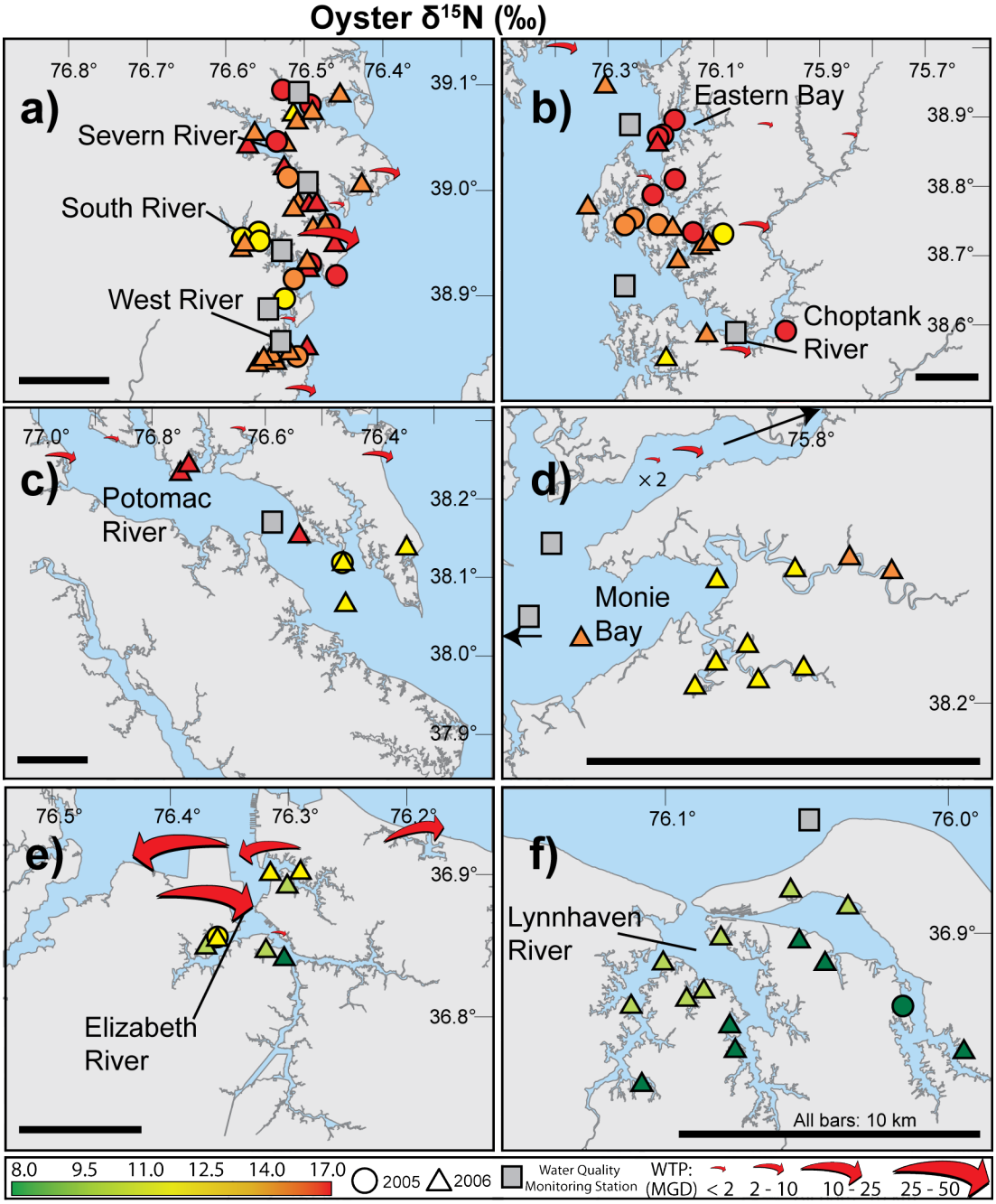

Oyster δ15N (‰)
a)
Severn River
South River
West River
b)
Eastern Bay
Choptank River
c)
Potomac River
d)
Monie Bay
×2
e)
Elizabeth River
f)
Lynnhaven River
All bars: 10 km
8.0 9.5 11.0 12.5 14.0 17.0
2005
2006
Water Quality Monitoring Station
WTP: (MGD)
< 2
2 - 10
10 - 25
25 - 50

Figure 5.5: Oyster $\delta^{15}N$ vs. land use

Oyster $\delta^{15}N$ vs. % of sub-watershed devoted to development (a) and animal agriculture (b).

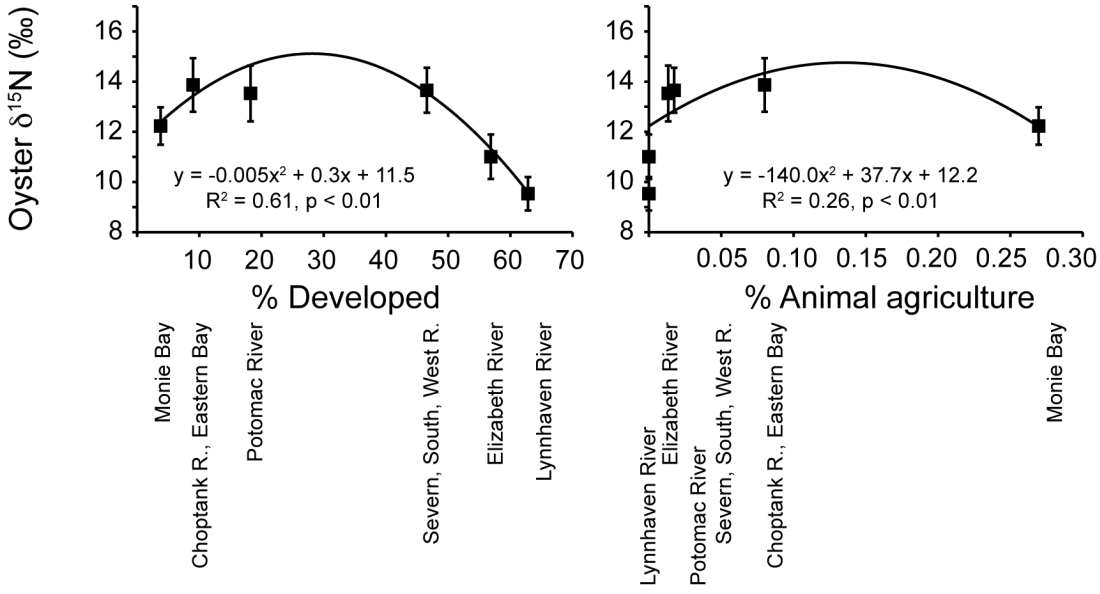


Figure 5.6: Chesapeake Bay Health vs. land use, streams, and oyster $\delta^{15}N$

Bay Health Index scores averaged over various years and compared to land use, i.e. % watershed area devoted to development and agriculture (a), the Stream Health Index (b), and oyster $\delta^{15}N$ values (c) for 2005 (closed circles) and 2006 (open triangles).

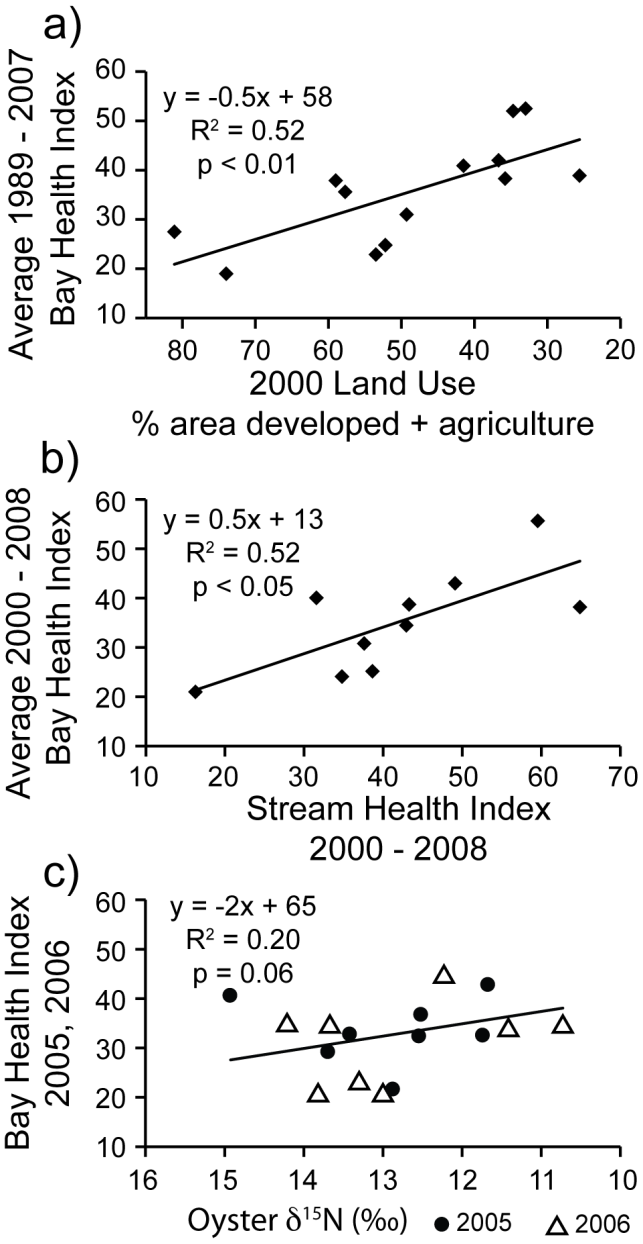
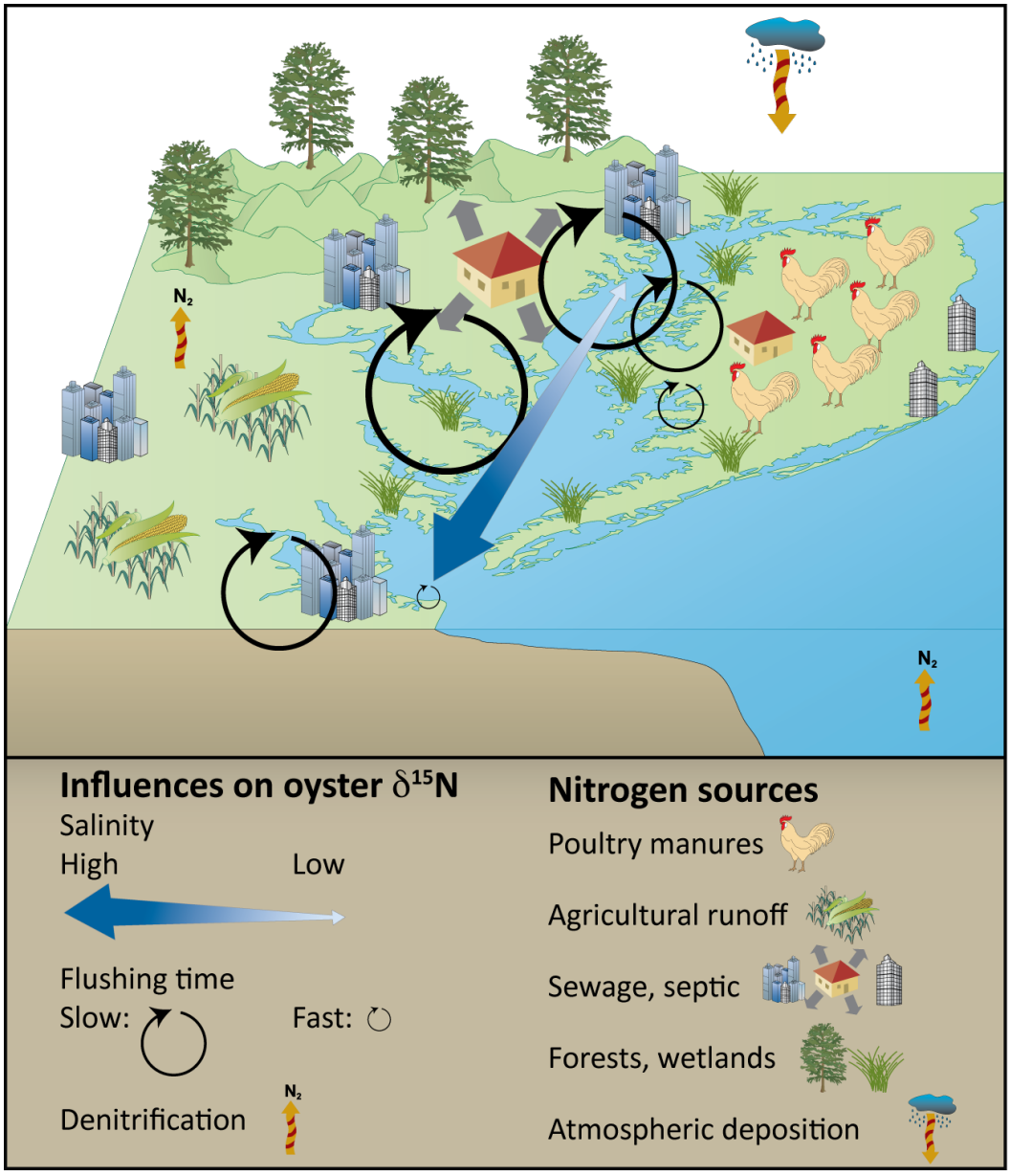


Figure 5.7: Conceptual diagram of factors potentially affecting oyster $\delta^{15}N$

Conceptual diagram depicting factors potentially influencing oyster $\delta^{15}N$. Removal of associated trends could enable comparisons of nitrogen sources at this broad spatial scale.



CHAPTER 6: DISSERTATION SUMMARY, CONTEXT, AND FUTURE RESEARCH

This dissertation establishes oyster $\delta^{15}N$ as a bioindicator of nitrogen sources, exploring spatial and temporal integration over multiple spatial scales. Together, the studies showed that $\delta^{15}N$ values in oyster tissues deployed for at least three to four months in summer are related to water quality metrics (in both estuarine and freshwater streams), integrate nitrogen sources over time (longer than macroalgae), and enable inferences of septic, sewage, or poultry manure nitrogen sources at scales ranging from 10s of km^2 to 1,000s of km^2, provided appropriate influential factors (e.g. flushing time, salinity, etc.) are considered. In this chapter, 1) research conclusions are summarized, 2) the context of the dissertation is discussed, and 3) potential avenues for future research are suggested.

Summary of major findings

Eastern oyster (Crassostrea virginica) $\delta^{15}N$ as a bioindicators of nitrogen sources: observations and modeling:

Multiple biological characteristics including a sedentary and filter feeding lifestyle make oysters appropriate $\delta^{15}N$ biological indicators of nitrogen sources. Sample size can be optimized at five individuals, which can be achieved with 7 to 8 oysters (accounting for mortality) collected from multiple locations for deployment

lasting four months (or two to three months, if only analyzing mantle or gill) at new locations to identify nitrogen sources. Manipulative field deployments can provide spatial and temporal integrations (e.g. Costanzo et al., 2001; Fertig et al., 2009). Staggering deployments would enable nitrogen source detection at desired time intervals to provide inferences of changes in nitrogen source over weeks, months, or even a year (Dattagupta et al., 2004; Fila et al., 2001; McKinney et al., 2002; Moore, 2003). Due to variations in integration periods and seasonal fluctuations in primary producers, deployment in springtime would optimize integration over summer months. For studies concerned with chronic or long-term nitrogen sources, muscle tissues best provided temporal integration but required a longer exposure time than gills or mantle. Since mantle and gill tissue $\delta^{15}N$ values responded similarly, the mantle would suffice for short-term studies due to slightly quicker response times than gills. This simple and straightforward method may aid detection and monitoring of nitrogen sources over multiple spatial and temporal scales.

Oyster $\delta^{15}N$ as a bioindicator of human and animal nitrogen and degraded water quality in a sub-estuary of Chesapeake Bay

Oyster $\delta^{15}N$ and estimates of nitrogen availability from several anthropogenic sources associated with Monie Bay National Estuary Research Reserve led to the inference that poultry manures are potentially dominant for Monie Bay, the adjacent Wicomico River, and Delmarva Peninsula. Rapid tributary flushing times and low estimates of nitrogen removal by denitrification suggested that that denitrification was

not generally responsible for elevated oyster $\delta^{15}N$ values. Oyster $\delta^{15}N$ enrichment, particularly in upstream Monie Creek and at the mouth of Monie Bay indicated degraded water quality related to human and/or animal waste sources. These discrete trends suggested spatially distinct inputs of human and/or animal waste nitrogen inputs from multiple sources and watersheds. Specifically, septic and/or animal manures entered Monie Creek from its watershed while these sources entered Monie Bay, Little Creek and Little Monie Creek likely from the adjacent Wicomico River via tidal advection and bottom layer circulation. The average annual chicken population in the Monie Bay watershed (7.6×10^5 birds) generated 2.9×10^4 kg TN $year^{-1}$ (conservative estimate) to 1.0×10^6 kg TN $year^{-1}$ (wet estimate), the equivalent of 6.7×10^3 people (conservative manure compared to septic inputs) to 2.3×10^5 (wet estimate compared to septic inputs), more than the actual human population (2.6×10^3 people). The small scale of Monie Bay (~50 km^2) and its watershed (~75 km^2) make it particularly susceptible to such anthropogenic pressures, but similar effects may be extrapolated across Delmarva Peninsula: the chicken population (1.0×10^8 birds) generated an equivalent amount of nitrogen to that generated by 8.9×10^5 people (conservative manure estimate compared to septic inputs) to 3.1×10^7 people (wet manure estimate compared to septic inputs), while 1.2×10^6 people live on the peninsula. Though nitrogen generated as manures may have multiple fates, some portion likely enters Chesapeake Bay, underscoring the critical importance of mitigating this source to successfully manage nutrients and improve water quality.

Oyster and macroalgae bioindicators detect elevated $\delta^{15}N$ in Maryland's Coastal Bays

Deployments of macroalgae and oysters provided direct comparison of $\delta^{15}N$ bioindicators at an intermediate spatial scale (100s of km^2) with nested fine spatial scales (10s of km^2) in Maryland's Coastal Bays. Enriched $\delta^{15}N$ values during spatial surveys at both the 100s km^2 and 10s km^2 scales provided evidence of potential human and/or animal waste nitrogen sources available to regions of St. Martin River (at the northern end of these bays) and the southern portion of Chincoteague Bay (both relatively developed areas reliant on septic systems and featuring poultry feeding operations). Anthropogenic nitrogen sources are of greater concern to St. Martin River due to higher total nitrogen concentrations, longer residence times, and limited access to oceanic flushing compared to the intact wetlands near the southern inlet of Chincoteague Bay. Interestingly, enriched macroalgae $\delta^{15}N$ and a north-south gradient of oyster $\delta^{15}N$ were observed in the fine-scale region of Johnson Bay but its watershed land uses largely consist of wetlands, forest, and agricultural easements which leaves multiple alternative hypotheses for this enrichment, including transport of anthropogenic nitrogen (septic or poultry sources) from southern Chincoteague Bay, denitrification, nutrient recycling from marsh erosion, or sediment re-suspension. These hypotheses remain to be tested in the near future.

Spatial limitations of these bioindicators were defined by response at different temporal scales. Macroalgae $\delta^{15}N$ and %N clearly responded spatially across

Maryland's Coastal Bays (100s of km^2) and thus this may be an appropriate spatial scale. In contrast, gradients of oyster $\delta^{15}N$ at 10s of km^2 suggested that oyster $\delta^{15}N$ might be suitable at this spatial scale. Water chemistry, signal strength, and physical factors (e.g. water residence time, degree of oceanic mixing) may be important to consider in addition to spatial scale as these variables can describe the degree to which $\delta^{15}N$ bioindicators are exposed to nitrogen sources. Overall, this demonstrates $\delta^{15}N$ bioindicators can indicate nitrogen sources independent of nitrogen concentrations and that conventional water quality metrics generally reflected physical processes (oceanic exchange) while bioindicators complementarily indicated potential nitrogen sources, augmenting conventional water quality monitoring.

Combining species and deployment techniques provided multiple time integrations of nitrogen sources while avoiding reliance on currently existing populations (particularly important for oysters in the mid-Atlantic region). Staggering deployments of multiple species within a functional group may yield temporal integration over desired time intervals due to species-specific nitrogen turnover rates while controlling for trophic level and mode of nitrogen assimilation. Temporally, macroalgae and oyster $\delta^{15}N$ responded differently to a nitrogen pulse associated with heavy precipitation events between macroalgae deployments and during oyster deployment. Quick turnover rates in macroalgae (days) resulted in rapid response with greater change in $\delta^{15}N$ to environmental conditions as compared to slower tissue turnover rates and dampened $\delta^{15}N$ responses in oyster tissues (weeks or months) that did not reflect short-term nutrient pulses. This lag may be due to physiology or modes

of nitrogen assimilation (direct uptake by macroalgae, indirect assimilation by oysters via phytoplankton).

Chesapeake Bay oyster $\delta^{15}N$ gradient links nitrogen source, land use and water quality

Oysters sampled after distribution throughout Chesapeake Bay by the Chesapeake Bay Foundation Oyster Gardening Program documented a large-scale (11,600 km^2) and spatially intense (79.5 km^2/site) oyster $\delta^{15}N$ gradient decreasing towards the estuary mouth and correlated with flushing time, salinity, and shell height. Oyster $\delta^{15}N$ related to sub-watershed land use, freshwater stream and tributary water quality, flushing time, and ultimately nitrogen source, and is among few studies globally at the scale. Salinity could influence oyster $\delta^{15}N$ via growth while quicker flushing times might limit food availability, potentially incompletely integrating isotopic signals. Quicker flushing and proximity to oceanic exchange export nitrogen more rapidly, increasing the chances an enriched signal from wastewater effluent could be missed, but these characteristics could also dampen susceptibility to anthropogenic pressures and therefore suggest differences in ecosystem resilience by varying by tributary. For example, the Lower Bay region rapidly undergoes oceanic exchange at the estuary mouth and consistently (1986 to 2009) had better water quality than other regions of Chesapeake Bay. Thus, human and/or animal waste sources were generally interpreted to be locally important but broadly influenced by physical and biological factors (flushing time, salinity, shell height). Isotopic

enrichment by denitrification (Kendall 1998) might also be confounding, but is estimated be low in estuaries compared to other ecosystems, has decreased in Chesapeake Bay with eutrophication, and is spatial variable beyond the current resolution or scope of this study, and therefore not considered.

Oyster $\delta^{15}N$ correlated to land use and water quality in freshwater streams and tributaries when measured close to oyster locations and thus links multiple monitoring programs. Nevertheless, linkages suggest that at appropriate scales, general water quality can also be inferred from oyster $\delta^{15}N$. As a simple and powerful bioindicator, oyster $\delta^{15}N$ could augment existing monitoring programs, e.g. the Mussel Watch Program, the System Wide Monitoring Program of the National Estuarine Research Reserve System, or the National Estuarine Eutrophication Assessment. This study quantifies the upper extent of spatial integration by oysters at individual locations (1,000s of km^2).

General contributions of the dissertation

The chapters herein, along with associated publications (Table 6.1, Figure 6.1, Figure 6.2), broaden our understanding of the utility of $\delta^{15}N$ bioindicators (Chapter 2), their linkages with land use (Chapter 3), the relative role for different bioindicator species (Chapter 4), and the factors which affect $\delta^{15}N$ at broad spatial scales (Chapter 5) in addition to elucidating patterns of specific ecosystems (Monie Bay National Estuarine Research Reserve in Chapter 3, Maryland's Coastal Bays in Chapter 4,

Chesapeake Bay in Chapter 5) or temporal events (summer precipitation events, seasonality). Many previous studies selected from a variety of $\delta^{15}N$ bioindicator taxa (e.g. McClelland et al. 1997, Costanzo et al. 2001, Fila et al. 2001, Lake et al. 2001, McKinney et al. 2001, 2002, Schlacher et al. 2001, 2005, Gartner et al. 2002, Fry et al. 2003, Cole et al. 2004, Cohen and Fong 2006, Benson et al. 2008), but this dissertation combines aspects of a variety of these studies by combining multiple bioindicator species and deploying samples for spatial analysis rather than opportunistically sampling. Further, it analyzes $\delta^{15}N$ data in the context of spatial scale, land use, physical factors, water quality/ overall ecosystem health, and so synthesizes across the land-water margin associated with estuarine processes.

Chesapeake Bay provides an appropriate testing ground for oyster $\delta^{15}N$ bioindicators because it contains nested watersheds with mixed land uses. Within Chesapeake Bay, the small (72.3 km^2), rural, largely forested watershed of Monie Bay National Estuarine Research Reserve served ideally as a natural laboratory to test effects of land use on oyster $\delta^{15}N$. The sub-watersheds of this embayment's tributaries Monie Creek and Little Monie Creek vary by the presence or absence of rural residential and poultry feeding operations while Little Creek provided a reference surrounded by wetlands (Chapter 3). There, oyster $\delta^{15}N$ in Monie Creek and Little Monie Creek reflected localized land uses, with elevated $\delta^{15}N$ indicative of human and/or animal wastes in the former (particularly upstream) compared to the latter (which decreased towards upstream). In contrast, Chesapeake Bay's vast watershed (164,200 km^2) and those of its major tributaries contain mixed land uses

(Chapter 5). Potentially, multiple nitrogen sources from a watershed could mix en route to assimilation by the bioindicator thus distorting waste signatures. Nevertheless, clear spatial patterns of oyster $\delta^{15}N$ emerged both at Chesapeake Bay and tributary scales, particularly in areas with wastewater treatment plants, suggesting that oyster $\delta^{15}N$ is appropriate for areas with watersheds containing mixed land uses. This enables wide applications of oyster $\delta^{15}N$ bioindicators in diverse ecosystems globally, which is important because watersheds with (approximately) a single land use are rare, and bioindicators provide limited value in such scenarios. Nitrogen sources from a watershed built out 100% (for a hypothetical, hyperbolic example) are self-evident and $\delta^{15}N$ bioindicators are superfluous.

Inferences of nitrogen sources must, of course, be tempered against alternative hypotheses including processes (denitrification, volatilization, isotopic signature mixing) and patterns (seasonality) that enrich $\delta^{15}N$ values as well as other factors (precipitation, flushing time) that may influence exposure to nitrogen source (thus potentially missing an enriched signal). Nevertheless, this body of work demonstrates that oyster $\delta^{15}N$ can be a simple yet powerful tool to examine spatial and temporal patterns of nitrogen source. As such, oyster $\delta^{15}N$ can augment established water quality monitoring, and may help target nutrient reduction efforts, thereby mitigating eutrophication more efficiently.

Both the Maryland Coastal Bays (Chapter 4) and the Chesapeake Bay (Chapter 5) studies yielded extensive datasets that reflect, to differing degrees, the

utility of working with a network of individuals for sample collection. Oyster Gardening programs in each of these ecosystems provisioned oysters, which were deployed manipulatively in Maryland's Coastal Bays. In Chesapeake Bay, however, oysters were sampled after growth minded by Oyster Gardeners – citizens actively interested in participating in oyster and Chesapeake Bay restoration. With minimal effort, sampling from this large network of individuals during redistribution to restoration reefs (Figure 6.3a-d) yielded a sizeable dataset (609 samples distributed among 147 sites) grown from Annapolis, MD (38°58'38"N 76°29'31"W) in the north to Virginia Beach, VA (36°5'24"N, 76°2'32"W) in the south (a distance of ~250 km).

Unfortunately neither specific locations nor sample availability were controllable, driven by social factors e.g. population density, voluntary participation, waterfront property ownership, geographic extent of the network, etc. Clusters were observed proximal to locations of transfer between Chesapeake Bay Foundation and individual Oyster Gardeners; specifically near 1) Annapolis, MD, 2) Virginia Beach, VA 3) Solomons Island, MD) and 4) St. Michaels, MD. Despite spatial biases, sufficient spatial coverage represented most major Chesapeake Bay tributaries and sub-watersheds with suitable salinities. Future projects could deploy oysters to fill gaps and further define the extent of spatial integration by oyster $\delta^{15}N$ by examining areas required for spatial independence and avoid spatial autocorrelation given variability of anthropogenic nitrogen signal strengths.

Incomplete control over experimental design is balanced by maximizing spatial scope with minimal effort, and still yields statistically significant relationships with land use, freshwater stream health, and tributary water quality. The technique is cheap ($8 per natural abundance $\delta^{15}N$ and $\delta^{13}C$ sample at UC Davis Stable Isotope Facility). Existing networks (i.e. http://www.cbf.org/oysters, Chesapeake Bay Foundation) provided infrastructure, quick generation of large datasets, and externalized organism care. Datasets from oysters grown by citizens are not 'garbage in garbage out' (as citizen-scientist projects are often criticized) because Oyster Gardeners only grow oysters, do not make measurements, and oyster $\delta^{15}N$ temporal integration minimizes handling variation effects. Mortalities (up to 100%) may occur at some locations but do not significantly detract from spatially intense networks.

Identifying nitrogen sources is also important to state/federal entities (Maryland Department of Natural Resources, Maryland Coastal Bays Program), fitting into management plans to improve water quality/ ecosystem health. This priority is exemplified by multiple fellowships and grants awarded from these entities to conduct research in Monie Bay National Estuarine Research Reserve (included in Chapters 2, 3, 5) and Maryland's Coastal Bays (Chapter 4). Clearly, $\delta^{15}N$ bioindicator development and application research aligned with respective missions and management plans (Table 6.1). Estuarine eutrophication continues to be prevalent (Bricker et al. 2008) and intractable (www.eco-check.org), so this priority will likely persist, necessitating additional research.

Potential future research avenues opened by this body of work

Numerous avenues exist for future research of oyster $\delta^{15}N$ as a bioindicator. Three example avenues, each with multiple possibilities, include 1) Advanced understanding of $\delta^{15}N$ physiology in bioindicator species, 2) National and global bioindicator applications, and 3) Predictive spatial modeling of human and/or animal waste hotspots.

Advanced understanding of $\delta^{15}N$ physiology in bioindicator species

A controlled laboratory experiment could build upon the manipulative fieldwork at multiple spatial scales described in this dissertation. This will better constrain interpretation of oyster $\delta^{15}N$ and better enable trend removal for variables already identified to potentially have effects. Specifically, the effects of salinity, flushing time (water flow), and organism size (oyster shell height) on oyster $\delta^{15}N$ values will be examined. Oysters of several sizes (see below) could be grown in experimental tanks adjusted to control salinity and flow through rates. A factorial design (Table 6.2) would test each combination of levels for salinity (10‰, 20‰, and 30‰), fast or slow water flow (scaled to reflect flushing times in Lynnhaven River and St. Mary's/Potomac River), and oyster size (classified as small: 35 to 55 mm, medium: 55 to 75 mm, or large: 75 to 95 mm) would help describe the relative effects of these factors on $\delta^{15}N$ values, providing a spectrum of outcomes. The experimental unit would be an individual oyster tissue (muscle, gill, and/or mantle), or sample of

seston (60 ml) or dissolved nitrogen (125 ml), all measured for $\delta^{15}N$ values. Concentrations of seston and dissolved nitrogen would also be measured. Experimental error would be assessed by replicates (3 for nitrogen and seston samples, 5 for oyster samples) and instrumental measurement error quantified with blanks and by randomly selecting samples for measurement replication. Control tanks will not include oysters but seston and dissolved nitrogen $\delta^{15}N$ values would be measured. A linear statistical or additive model generated from results would describe these relationships. This experiment will benefit deployments of oyster and other bioindicators by enabling a correction factor for comparing across regimes of physical features or across ecosystems useful for removing trends due to these variables. It will further add value to the literature on $\delta^{15}N$ physiology because while an increasing number of field studies of $\delta^{15}N$ in various organisms are reported, few include controlled experiments, and most experiments focus on fractionation effects associated with diets, which could also be influenced by these physical features.

Additionally, our understanding of $\delta^{15}N$ bioindicators as nitrogen source identification tools would benefit from a direct examination of other potentially confounding factors such as denitrification. Currently an active area of research, spatial and temporal variations of denitrification rates, along with improving rate measurement technology, would help constrain or quantify the extent this process might influence bioindicators of nitrogen sources. Though labor intensive, selective concurrent measurements of denitrification along a spatially stratified bioindicator sampling scheme (including transects across the gradient of aquatic to watershed

ecosystems) could provide some indication of the extent (if any) of this effect despite high spatial variability. This approach unfortunately may be impractical for large-scale efforts and cannot be incorporated into approaches that rely upon citizen networks.

Concurrent measurements of other variables may also yield important patterns. Additional physiological effects (such as starvation, stress, life stage, gestation, etc.,) have been shown to enrich $\delta^{15}N$ in organisms, but mechanisms and other factors remain untested, while much of the $\delta^{15}N$ bioindicator literature ignores these sources of variation and resulting biases could be examined. Multiple isotopic analyses (e.g. of carbon, oxygen, sulfur) could produce a matrix of isotopic signatures that could be used to further distinguish important eutrophication sources. Alternatively, conjunction of stable isotope analysis with measurements of other compounds indicative of human and/or animal wastes (e.g. pharmaceuticals, artificial hormones, caffeine) or comparative libraries of antimicrobial resistance (Frana and Venso 2009) or DNA may yield fruitful results. Ultimately, distinguishing nitrogen sources as derived from 'human and/or waste sources' is sometimes not specific enough evidence to irrefutably prove responsibility on the part of an individual entity (i.e. a residence, farm, poultry feeding operation, etc.) and thus mandate a behavior change or regulatory enforcement because blame could be placed upon pets, migratory birds or waterfowl, wildlife, etc. in addition to humans or domestic animals since the biochemical mechanisms resulting in enriched $\delta^{15}N$ are the same for all. Ideally, identification of nitrogen source would be species-specific.

Both current and future advanced understanding of employing $\delta^{15}N$ bioindicators can be synthesized into a handbook or manual with the aim of transferring this technology, its methods, caveats, and interpretation steps from research to practice. Gathering this data together would also identify current knowledge gaps and address issues associated with localized interpretation. This type of manual or handbook would be aimed at those charged with improving water quality and reducing eutrophication. Included sections would be factors that influence isotope cycling, bioindicator selection, methodologies, data analysis, interpretation steps, and communication of results to decision makers, stakeholders, and the public. Practical examples would be included, as well as a synthesis of the literature and examples from across the world. Utility of such a handbook would be measured by increasing utilization of $\delta^{15}N$ bioindicators at relevant spatial and temporal scales to target important nitrogen sources over time.

Scaling up to national and global bioindicator applications

Oyster $\delta^{15}N$ is a simple and powerful bioindicator (Fertig et al. 2010) that can be scaled up to national or global studies, and augment existing monitoring programs at the national scale with nested spatial units. Examples include partnering or including oyster $\delta^{15}N$ in programs such as the Mussel Watch Program (O'Connor and Lauenstein 2006), the System Wide Monitoring Program (Figure 6.4) of the National Estuarine Research Reserve System (Wenner and Geist 2001, Kennish 2004, Brush et al. 2007, NERRS 2007), or the National Estuarine Eutrophication Assessment

(Bricker et al. 2008), each of which would provide comparisons between multiple biogeographic regions. For example, the Mussel Watch program would be a particularly appropriate partner as it already has established numerous locations for sampling a variety of mollusks and currently measures a wide variety of contaminants including heavy metals, persistent organic pollutants, and other detrimental compounds accumulated in these filter feeders.

Oyster restoration activities similar to the Chesapeake Bay Foundation Oyster Gardening Program would also be appropriate networks to include, as these programs are increasingly popular and are available along much of the Atlantic seaboard enabling further extension to a national scope. Examples programs include Virginia Department of Environmental Quality[1], Maryland Department of Natural Resources[2], Maryland Coastal Bays Program[3], Delaware Center for the Inland Bays[4], New York and New Jersey[5] with another program in Mobile Bay, Alabama[6] providing a

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[1] http://www.deq.state.va.us/coastal/gardening.html

[2] http://www.oysters.maryland.gov/

[3] www.mdcoastalbays.org

[4] http://darc.cms.udel.edu/ibog/

[5] http://www.nynjbaykeeper.org/index.php?option=com_content&view=article&id=72&Itemid=68

[6] http://www.aces.edu/pubs/docs/A/ANR-1207/

foothold into the Gulf of Mexico and a potential to further extend this network to New England through the Massachusetts Bays Program[7] (Jason Baker, Massachusetts Bays Program, personal communication). Future iterations could include training selected Oyster Gardeners in sample preparation; saving additionally on otherwise time consuming tasks. With experience, an individual can dissect tissues from 50 oysters in a full day, grind 30 samples per hour once dried thoroughly (at least two to three days at 60°C), and prepare more than 12 samples per hour for isotopic analysis by weighing out 1.0 ± 0.2 mg and packaging into tin capsules. In addition to providing ecosystem, regional, and national scopes, partnering with monitoring and oyster restoration networks that have dovetailed missions with research aims leverages both and facilitates direct communication of nitrogen sources with interested communities, stakeholders, environmental managers, and decision makers.

Predictive spatial modeling of human and/or animal waste hotspots

Relationships identified between oyster $\delta^{15}N$ and land use, water quality in streams and tributaries, and tributary residence time, salinity, and oyster size could be modeled to provide a more holistic and synthetic approach. Spatial models could efficiently generate interpolated spatial predictions of anthropogenic nitrogen 'hotspots'. Specific hot spots could be verified with limited and rapid bioindicator

[7] http://www.mass.gov/envir/massbays/default.htm

deployment (Figure 6.5). Once identified and verified, ‘hotspots’ could be targeted for nutrient reduction efforts, including upgrading septic systems to wastewater treatment, or rapid implementation of agricultural best management practices, potentially leading to improved water quality and decreased eutrophication. Improved ecosystem health and function is the overarching goal for each of these potential future research and application avenues, and has been the motivating principle behind the research presented in this dissertation.

Chapter 6 Tables

Table 6.1: Publications and deliverables associated with this dissertation

Publication	Year	Citation	Availability
Journal Articles	2010	**Fertig, B.**, Carruthers, T.J.B., Dennison, W.C., Fertig, E.J., Altabet, M.A. In Press. Eastern oyster (*Crassostrea virginica*) $\delta^{15}N$ as a bioindicator of nitrogen sources: observations and modeling. Marine Pollution Bulletin	http://dx.doi.org/10.1016/j.marpolbul.2010.03.013
	2009	**Fertig, B.**, Carruthers, T.J.B., Dennison, W.C., Jones, A., Pantus, F., and Longstaff, B. 2009. Oyster and macroalgae bioindicators detect elevated $\delta^{15}N$ in Maryland's Coastal Bays. Estuaries and Coasts 32: 773-786	http://dx.doi.org/10.1007/s12237-009-9148-x
	Submitted	**Fertig, B.,** Carruthers, T.J.B., Dennison, W.C. Submitted. Oyster $\delta^{15}N$ as a bioindicator of human and animal nitrogen and degraded water quality in a sub-estuary of Chesapeake Bay. Submitted to Ecological Applications.	
	Preparation	**Fertig, B.,** Carruthers, T.J.B., Dennison, W.C., Williams, M.R. In preparation. Oyster (*Crassostrea virginica*) $\delta^{15}N$ gradient links land use and water quality to nitrogen source in Chesapeake Bay. Targeted to Marine Pollution Bulletin or Estuaries and Coasts.	
Reports	2009	**Fertig, B.**, Carruthers, T.J.B., Dennison, W.C. 2009. Final Report: Connecting monitoring, long-term, and broad-scale water quality datasets through an estuarine biological indicator of nitrogen source: $\delta^{15}N$ in *Crassostrea virginica* tissues. Prepared for National Estuarine Research Reserve System. 81 pp.	
	2008	**Fertig, B.**, Carruthers, T.J.B., Dennison, W.C. 2008. Final Report: 2007 Water Quality Monitoring in Monie Bay. Prepared for National Estuarine Research Reserve System. 46 pp.	
	2008	Beckert, K., **Fertig, B.,** O'Neil, J.M., Carruthers, T.J.B., Wazniak, C., Sturgis, B., Hall, M., Jones, A., and Dennison, W. C. 2008. Fine scale patterns of water quality in three regions of Maryland's Coastal Bays: assessing nitrogen source in relation to land use. Prepared for Maryland's Coastal Bays Program. 50 pp.	http://ian.umces.edu/press/reports/publication/100/fine_scale_patterns_of_water_quality_in_three_regions_of_marylands_coastal_bays_assessing_nitrogen_source_in_relation_to_land_use_2008-04-02/
	2007	**Fertig, B.**, Carruthers, T.J.B., Dennison, W.C. 2007. Linking Monie Bay watershed land use to nitrogen stable isotopes in tissues of the native eastern oyster, *Crassostrea virginica*. Prepared for National Estuarine Research Reserve System. 76 pp.	http://ian.umces.edu/press/reports/publication/83/linking_monie_bay_watershed_land_use_to_nitrogen_stable_isotopes_in_tissues_of_the_native_eastern_oyster_crassostrea_virginica_2007-08-24/
	2006	**Fertig, B**., Carruthers, T.J.B. Wazniak, C., Sturgiss, B., Hall, M., Dennison, W.C. 2006. Water quality in four regions of the Maryland Coastal Bays: assessing nitrogen source in relation to rainfall and brown tide. Prepared for Maryland Coastal Bays Program. 49 pp.	http://ian.umces.edu/press/reports/publication/69/water_quality_in_four_regions_of_the_maryland_coastal_bays_assessing_nitrogen_source_in_relation_to_rainfall_and_brown_tide_2006-11-01/

Publication	Year	Citation	Availability
Book Chapters	2010	Kimmel, D.G., Townsend, H., Carruthers, T.J.B., and **Fertig B.** 2010. Environmental Statistics: Balancing simplicity and explanatory power. In: Longstaff, B.J., Carruthers, T.J.B., Dennison, W.C., Lookingbill, T.R., Hawkey, J.M., Thomas, J.E., Wicks, E.C., and Woerner, J. (eds.) Integrating and Applying Science: A practical handbook for effective coastal ecosystem assessment. IAN Press, Cambridge, MD, p113-132	http://ian.umces.edu/press/books/publication/259/integrating_and_applying_science_a_handbook_for_effective_coastal_ecosystem_assessment_2010-05-10/
	2010	Wicks, E.C., Longstaff, B.J**., Fertig, B.,** and Dennison, W.C. 2010. Ecological Indicators: Assessing ecosystem health using metrics. In: Longstaff, B.J., Carruthers, T.J.B., Dennison, W.C., Lookingbill, T.R., Hawkey, J.M., Thomas, J.E., Wicks, E.C., and Woerner, J. (eds.) Integrating and Applying Science: A practical handbook for effective coastal ecosystem assessment. IAN Press, Cambridge, MD, p 61-77	http://ian.umces.edu/press/books/publication/259/integrating_and_applying_science_a_handbook_for_effective_coastal_ecosystem_assessment_2010-05-10/
	2009	Wazniak C.E., Hall M.R., Bailey E.M., Boward D.M., Boynton W.R., Bratton J.F., Carruthers T.J.B., Chalmers R.J., Cole L.W., Cornwell J.C., **Fertig B.**, Glibert P.M., Jones A.B., Jordan T.E., McCoy J., McGinty M., Shedlock R.J., Sherwell J., Sturgis R.B., Thomas J.E., Trice T.M., Wells D.V. 2009. Water Quality Reponses to Nutrients. In: Dennison W.C., Thomas J.E., Cain C.J., Carruthers T.J.B., Hall M.R., Jesien R.V., Wazniak C.E., Wilson D.E. (eds.) Shifting Sands: Environmental and cultural change in Maryland's Coastal Bays. IAN Press, Cambridge, MD, p 249–292	http://ian.umces.edu/press/books/publication/93/shifting_sands_environmental_and_cultural_change_in_maryland_s_coastal_bays_2009-06-08/
Posters	2008	**Fertig, B.,** Carruthers, T.J.B., Dennison, W.C. 2008. Oyster $\delta^{15}N$ as a bioindicator of waste nitrogen and degraded water quality in a sub-estuary of Chesapeake Bay. Poster at NOAA National Estuarine Research Reserve Annual Meeting, Monterey, CA	http://ian.umces.edu/press/posters/publication/205/oyster_delta_15n_as_a_bioindicator_of_waste_nitrogen_and_degraded_water_quality_in_a_sub_estuary_of_chesapeake_bay_2008-11-01/
	2007	**Fertig, B.**, Carruthers, T.J.B., Dennison, W.C. 2007. Developing the eastern oyster, *Crassostrea virginica*, as a bioindicator of nitrogen source over multiple spatial scales. *Poster at 2007 University of Maryland Marine, Estuarine Environmental Science Program Colloquium*	
Newsletters	2008	Beckert, K. Fertig, B., O'Neil, J., Carruthers, T.J.B., Dennison, W.C., Fisher, T.R. 2008. Upstream land use affects water quality in Maryland's Coastal Bays. IAN Press.	http://ian.umces.edu/press/newsletters/publication/192/upstream_land_use_affects_water_quality_in_maryland_s_coastal_bays_2008-08-01/
	2007	Fertig, B. Carruthers, T.J.B., Dennison, W.C. 2007. Biological indicators enhance water quality monitoring in Maryland's Coastal Bays. IAN Press.	http://ian.umces.edu/press/newsletters/publication/77/biological_indicators_enhance_water_quality_monitoring_in_maryland_s_coastal_bays_2007-03-02/

Publication	Year	Citation	Availability
Grants and Awards	2010	Maryland Coastal Bays Program "Where does nitrogen in Johnson Bay come from? Relationships between land use, nitrogen cycling, and denitrification." Co-PI with Drs. J.M. O'Neil and R.H. Kelsey. ($25,000)	
	2010	Atlantic Estuarine Research Society Student Travel Award ($125)	
	2009	Coastal and Estuarine Research Federation Student Travel Award ($350)	
	2008	NERRS Graduate Research Fellowship "Connecting monitoring, long-term, and broad-scale water quality datasets through an estuarine biological indicator of nitrogen source" ($29,500)	
	2007	NERRS Graduate Research Fellowship "Establishing a link between $\delta^{15}N$ in *Crassostrea virginica* tissues to land use: spatial analysis and modeling approaches" ($29,500)	
	2006	NERRS Graduate Research Fellowship "Developing the eastern oyster, *Crassostrea virginica*, as a biological indicator of nitrogen sources" ($29,500)	
Presentation	2010	**Fertig, B.,** Carruthers, T.J.B., Dennison, W.C. (March 2010) "Oyster $\delta^{15}N$ as a bioindicator of human and animal nitrogen and degraded water quality in a sub-estuary of Chesapeake Bay." *Atlantic Estuarine Research Society. Atlantic City, NJ.*	
	2009	**Fertig, B.,** Carruthers, T.J.B., Dennison, W.C., Altabet, M.A., Fertig, E.J. (November 2009) "Variations of $\delta^{15}N$ in eastern oysters (*Crassostrea virginica*) as a baseline to assess waste nitrogen sources" *Student speaker at MEES Colloquium, Annapolis, MD*	
	2009	**Fertig, B.,** Carruthers, T.J.B., Dennison, W.C., Altabet, M.A., Fertig, E.J. (November 2009) "Variations of $\delta^{15}N$ in eastern oysters (*Crassostrea virginica*) as a baseline to assess waste nitrogen sources" *CERF 20th Biennial International Conference, Portland, OR*	http://ian.umces.edu/press/presentations/publication/245/variations_of_delta_15n_in_eastern_oysters_crassostrea_virginica_as_a_baseline_to_assess_waste_nitrogen_sources_2009-11-24/
	2009	Beckert, K., **Fertig, B.,** Carruthers, T.J.B., Dennison, W.C. (March 2009) "Oyster $\delta^{15}N$ as a downstream bioindicator of water quality declines linked to land use in a watershed of Maryland's Coastal Bays." *Atlantic Estuarine Research Society. Ocean City, MD*	
	2008	**Fertig, B.** (September 2008) "What oysters can tell us about the sources of pollution in Chesapeake Bay" *Brownbag Lunch Seminar Series, Chesapeake College*	
	2008	**Fertig, B.,** Carruthers, T.J.B., Dennison, W.C., Jones, A.B., Pantus F., and Longstaff, B. (March 2008) "Identifying anthropogenic nitrogen loading in Maryland's Coastal Bays using stable isotope analysis of biological indicators" *Atlantic Estuarine Research Society. Lewes, DE*	

Publication	Year	Citation	Availability
Presentations (continued)	2008	**Beckert, K., Fertig, B.**, O'Neil, J., Carruthers, T.J.B., and Dennison, W.C. (February 2008) "Fine scale patterns of water quality in three regions of Maryland's Coastal Bays: assessing nitrogen source in relation to land use" *Maryland Coastal Bays Program Science and Technology Advisory Committee Meeting, Cambridge, MD*	
	2007	**Fertig, B.**, Carruthers, T.J.B., and Dennison, W.C. (November 2007) "Identifying anthropogenic nitrogen sources in Monie Bay (Chesapeake Bay National Estuarine Research Reserve) using stable isotopes of the oyster, *Crassostrea virginica*" *ERF 19th Biennial International Conference, Providence, RI*	
	2007	**Fertig, B.**, Carruthers, T.J.B., and Dennison, W.C. (May 2007) "Developing the eastern oyster, *Crassostrea virginica*, as a biological indicator of nitrogen source: short- and long-term bioindicators in Maryland's Coastal Bays and linking land use to stable isotopes in Monie Bay" *Maryland Department of Natural Resources, lunchtime seminar series*	
	2007	**Benson, E**., **Fertig, B.**, and **Florkowski, L.** (March 2007) "From land to lake to sea: Ecological monitoring over various spatial scales and complexities" (Co-Invited speakers, split between authors) at Integration and Application Network Seminar Series	http://ian.umces.edu/seminarseries/seminar/44/from_land_to_lake_to_sea_ecological_monitoring_over_various_spatial_scales_and_complexities_2007-03-08/
	2007	**Fertig, B.** (February 2007) "Developing the eastern oyster, Crassostrea virginica, as a biological indicator of nitrogen source in Chesapeake Bay". *Chesapeake Bay Foundation Oyster Gardening Program*	
	2006	**Fertig, B.**, Carruthers, T.J.B., and Dennison, W.C. (July 2006) "Developing the eastern oyster, *Crassostrea virginica*, as a biological indicator of nitrogen source" *Cooperative Oxford Laboratory*	
		Horn Point Laboratory Student Seminar Series:	
	2010	March 2010: "Oyster $\delta^{15}N$ as a bioindicator of human and animal nitrogen and degraded water quality in a sub-estuary of Chesapeake Bay."	
	2009	January 2009: "Stable nitrogen isotopes in the native eastern oyster (*Crassostrea virginica*) as an indicator of nitrogen source."	
	2007	October 2007: "Identifying anthropogenic nutrient loading in Maryland's Coastal Bays using stable isotope analysis of biological indicators"	
	2006	December 2006: "Comparing the eastern oyster, *Crassostrea virginica*, to the macroalgae *Gracilaria* sp. as a biological indicator of nitrogen source in Maryland's Coastal Bays"	
	2006	June 2006: "Developing the eastern oyster, *Crassostrea virginica* as a biological indicator of nitrogen source"	

Table 6.2: Factorial experiment testing effects of salinity, flushing, and oyster size

Factorial experimental design to test effects of salinity, flushing time, and oyster size on $\delta^{15}N$ values. Experimental unit is an individual oyster tissue (muscle, gill, or mantle), or sample of seston (60 ml) or dissolved nitrogen (125 ml). Experimental error is assessed with replicates (3 for nitrogen and seston samples, 5 for oyster samples) and instrumental measurement error will also be assessed with blanks and by randomly selecting samples for measurement replication. Controls will not include oysters in the experimental setup.

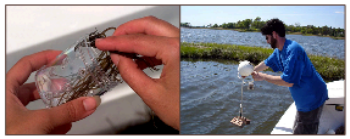
		Salinity			
		10	20	30	Control
Flushing time (flow rate)	Rapid	Nitrate x 3	Nitrate x 3	Nitrate x 3	Nitrate x 3
		Ammonium x 3	Ammonium x 3	Ammonium x 3	Ammonium x 3
		Seston x 3	Seston x 3	Seston x 3	Seston x 3
		Small x 5	Small x 5	Small x 5	No oysters
		Medium x 5	Medium x 5	Medium x 5	
		Large x 5	Large x 5	Large x 5	
	Slow	Nitrate x 3	Nitrate x 3	Nitrate x 3	Nitrate x 3
		Ammonium x 3	Ammonium x 3	Ammonium x 3	Ammonium x 3
		Seston x 3	Seston x 3	Seston x 3	Seston x 3
		Small x 5	Small x 5	Small x 5	No oysters
		Medium x 5	Medium x 5	Medium x 5	
		Large x 5	Large x 5	Large x 5	

Chapter 6 Figures

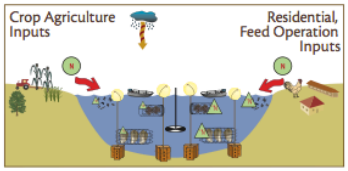
Figure 6.1: Newsletter showing utility of macroalgae and oyster bioindicators

BIOINDICATORS PROVIDE ADDITIONAL INSIGHT

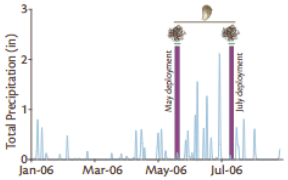
Deploying bioindicators



Deploying Gracilaria.



To deploy both bioindicators, they were suspended by buoys and anchored with bricks. *Gracilaria* gathered from Greenbackville was deployed in perforated cups at half Secchi depth in May and July 2006. Young oysters from the Maryland Coastal Bays Oyster Gardening Program were deployed in mesh cages just above the bottom from May until July 2006. After deployment these bioindicators were measured for heavy stable nitrogen isotopes, which indicate nitrogen sources. Atmospheric nitrogen, crop agriculture fertilization, and forests have lighter nitrogen isotopes than poultry manure runoff and septic sources. When nitrogen inputs from these sources reach aquatic systems and are taken up by plankton and the bioindicators, it is possible to measure variations in the isotopes and infer sources.

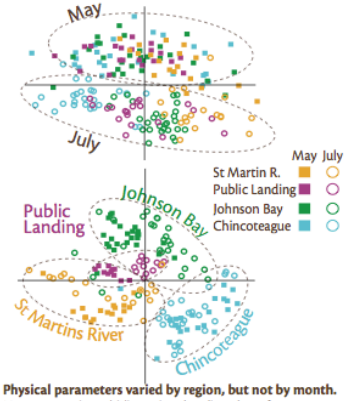


Heavy rains in June 2006 occurred between Gracilaria *deployments and during the* Crassostrea *deployment period. Deployment timing is marked with lines.*

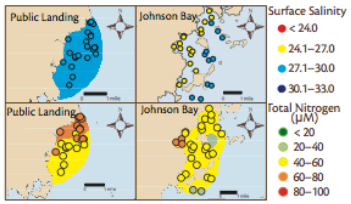
June rain effects

Precipitation during June pulsed nutrients from terrestrial sources downstream, which affected biological parameters.

Biological parameters varied by month, not region. *Nonparametric multidimensional scaling plots of chlorophyll a, Gracilaria %N, Gracilaria $\delta^{15}N$, Gracilaria $\delta^{13}C$, and Gracilaria C:N. This pattern would conventionally have been missed.*



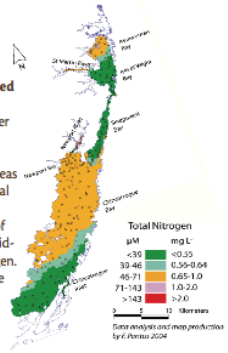
Physical parameters varied by region, but not by month. *Nonparametric multidimensional scaling plots of temperature, salinity, Secchi depth, total nitrogen and phosphorus.*



Public Landing and western Johnson Bay freshened by July. Either groundwater or overland flow input freshwater. **Localized total nitrogen was found in these areas.**

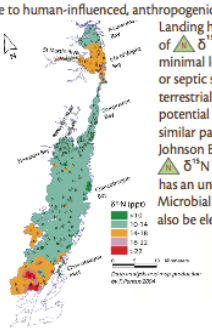
BIOINDICATORS REVEAL PATTERNS AT DIFFERENT SCALES

Macroalgae: regional patterns



Total nitrogen varied across regions in 2004. St Martin River in the north had high levels of total nitrogen. Middle areas of Maryland's Coastal Bays, such as Public Landing and parts of Johnson Bay, had mid-levels of total nitrogen. Lower Chincoteague Bay had low total nitrogen loads.

***Gracilaria* detects broad regional patterns well.** $\delta^{15}N$ in *Gracilaria* was high in St. Martin River and Chincoteague. While their total nutrient loads differed, they both are likely due to human-influenced, anthropogenic sources. Public Landing had low values of $\delta^{15}N$, suggesting minimal loads from sewage or septic sources, leaving terrestrial agriculture as a potential nutrient source. A similar pattern was found in Johnson Bay. A spike of $\delta^{15}N$ inside Mills Island has an unknown source. Microbial processing could also be elevating $\delta^{15}N$.

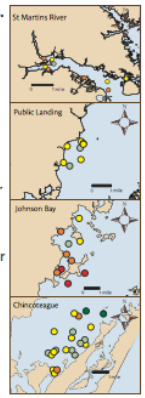


Broad and intensive spatial surveys of total nitrogen and Gracilaria *$\delta^{15}N$ in Maryland's Coastal Bays indicated nitrogen sources in 2004.*

Oysters: local patterns

Deploying oysters

Oysters reveal patterns in regions. In *C. virginica* tissues, short-term nutrient fluctuations or pulses are either not incorporated or averaged out. Due to longer nutrient integration periods, patterns within regions can be found. This is particularly apparent in southward gradients at Johnson Bay and around Chincoteague Island, indicating increasing human-influenced, anthropogenic effects. Challenges include sample loss as deployment duration is lengthened and mortality. At Public Landing, for example, 9% of buoys were lost and 50% of the collected oysters died.



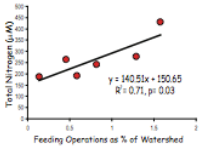
Crassostrea $\delta^{15}N$

Lighter < 7 (‰)
7–8
8–9
9–10
Heavier > 10

Both indicator species provide information about nitrogen sources. *Macroalgae provides a good estimate of broad patterns over short periods of time, while the oyster takes longer to incorporate nitrogen. As a result, each benefit water quality monitoring programs differently.*

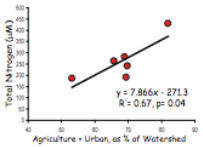
Retrieving oysters

RELATING LAND USE TO NITROGEN



Significant positive relationship between area of feeding operations and stream total nitrogen concentrations

Crop agriculture is the dominant land use in St. Martin River watershed.



Significant positive relationship between anthropogenic land use (agricultural and urban) and total nitrogen concentrations

An assessment of total nitrogen concentrations in six streams of the St. Martin River watershed during high-flow months (2006-2008) revealed distinct relationships to land use. Although feeding operations comprised less than 2% of the sub-watershed areas of the streams, total nitrogen displayed a significant positive relationship with this form of land use. Similarly, agricultural and urban land use had a significant positive relationship with total nitrogen, indicating that human landscape modification is increasing nutrient concentrations and degrading overall water quality of the St. Martin River watershed.

CONCLUSIONS

- Land use pressures contribute to water quality degradation in St. Martin River.
- Spatial patterns reveal high nutrient concentrations in upstream areas.
- High chlorophyll and low dissolved oxygen result from high nutrient inputs.
- The Bishopville dam may enhance denitrification and accumulate nutrients.
- Land use, especially feeding operations, is linked to high nitrogen concentrations.

RECOMMENDATIONS

- *Focus* on ameliorating land use runoff to improve water quality.
- *Target* upstream areas for monitoring nutrient loading.
- *Examine* links between nutrients, feeding operations, and nutrient management.
- *Consider* consequences of removing the Bishopville dam, especially nutrient release.
- *Preserve* natural land cover (wetlands and forest) as critical in reducing nutrient inputs.

References:

Hager, P. 1996. *Worcester County, MD. Pages 20-24 in Beidler, K., P. Gant, M. Ramsay, and G. Schultz, eds. Proceedings-Delmarva's Coastal Bay watersheds: not yet up the creek. EPA/600/R-95/052. U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, Narragansett, RI, USA.*

U.S. Department of Agriculture. 2002 *Census of Agriculture. Accessed 7 July 2008. Available online: http:www.nass.usda.gov/Census/Create_Census_US_CNTY.jsp*

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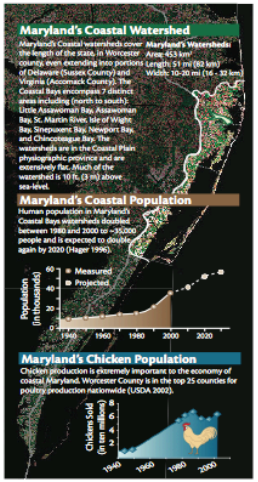
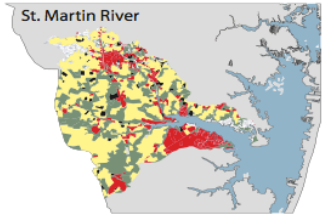
SCIENCE COMMUNICATION Graphics, design and layout by Kris Beckert, Ben Fertig, Judy O'Neil, Tim Carruthers, Bill Dennsion and Thomas Fisher. Field assisstance by Mike Malpezzi, Emily Nauman, & David Needham.

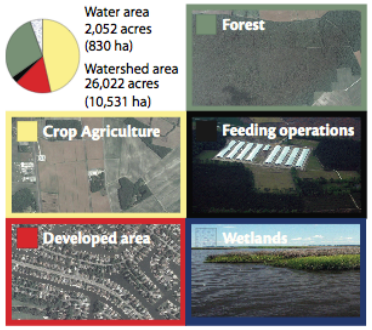
Integration & Application Network

UPSTREAM LAND USE AFFECTS WATER QUALITY IN MARYLAND'S COASTAL BAYS

Coastal lagoon ecosystems across the Delmarva Peninsula are rapidly evolving due to changing land use patterns and shifts towards intensive agriculture, particularly poultry production, and rural-residential development. These changes in the coastal lagoon seascape are especially evident in the northern Coastal Bays watershed of the St. Martin River. This region is intensely developed in areas (e.g. Ocean Pines canal community), is composed of a high percentage of crop agriculture, and contains a number of poultry feeding operations. Water quality degradation continues to be an important issue in this region.





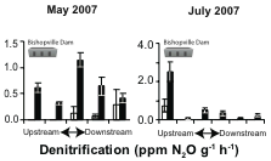


Feature	Description
Land:water ratio	12.6
Residence time (days)	20-30
Average depth (m)	1.2
Year-round population (year 2000)	9080
Bottom sediment type	clay and silt
Land soil type (from coast to interior)	well-drained to hydric

Figure 6.2: Newsletter relating land use effects on water quality

STRONG WATER QUALITY GRADIENTS IN ST. MARTIN RIVER

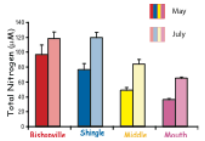
Bishopville Dam increases denitrification



Denitrification, the release of nitrogen to the atmosphere, decreases downstream.

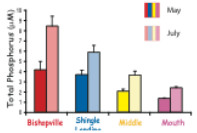
The Bishopville dam marks the uppermost intrusion of saltwater in St. Martin River. Nutrients and sediments from agriculture and residential areas above the dam impact water quality. High denitrification, which converts accumulated nitrogen in sediments to innocuous N_2 gas, was observed at the dam. Rates of denitrification throughout St. Martin River remained low in May, but were significantly increased in July. In the near future, the dam will be removed, which will likely affect downstream water quality.

The mouth of St. Martin River, looking out towards the barrier islands.

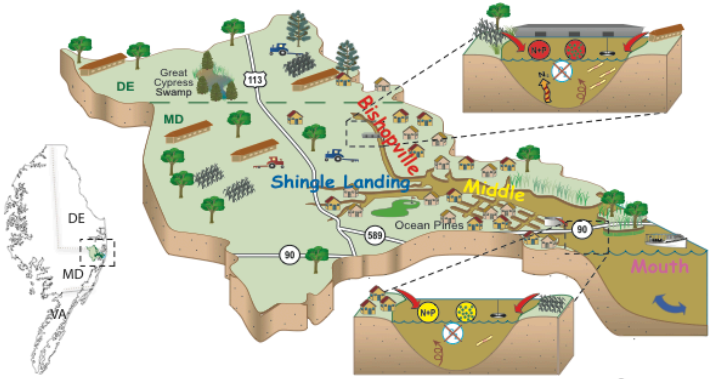


Total nitrogen decreases downstream from the freshwater prongs.

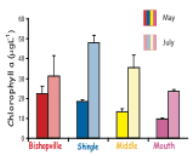
Measurements of total nitrogen and total phosphorus in both upstream prongs of the river were significantly higher than downstream locations in both May and July 2007. Overall concentrations increased from May to July, possibly as the result of regional drought conditions. Nutrient loads were mostly organic, and dissolved inorganic nutrients comprised a very small percentage of both concentrations.



Phosphorus concentrations are highest in upstream sections of the river.

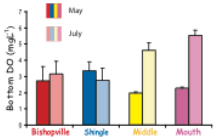


St. Martin River is freshwater-fed by the Shingle Landing and Bishopville Prongs. Inputs of nitrogen and phosphorus from feeding operations and crop agriculture above the Bishopville dam lead to high concentrations of bacteria, chlorophyll a, decreased Secchi depth, sediment resuspension, low dissolved oxygen, and high rates of denitrification. These measurements decrease through the Middle of the River towards the Mouth, where inputs come mainly from urban and crop land and are diluted by



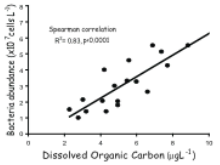
Chlorophyll concentrations were highest in upstream sections.

Both freshwater prongs of St. Martin River had high concentrations of chlorophyll *a*, especially in July. Chlorophyll *a* showed a trend of decreasing concentration downstream. Low dissolved oxygen, resulting from a combination of the degradation of phytoplankton, increased temperature, and limited circulation, was also observed upstream. Dissolved oxygen was higher towards the mouth of the river. These patterns were most likely observed as the result of tidal flushing and lower nutrient inputs in the downstream reaches.



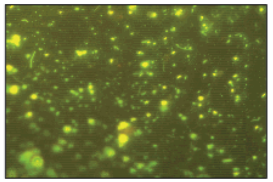
Dissolved oxygen remained low throughout 2007.

Filtering water for dissolved nutrients in the coastal bays.



Bacterial abundance and dissolved organic carbon are strongly correlated.

Naturally occuring bacteria in the St. Martin River provide an important link in the food web and impact nutrient cycling. Samples taken in different sections of the river revealed that high organic nutrient concentrations are concurrent with bacteria, decreasing towards the mouth. Abundances are similar to those in nutrient-enriched systems such as the Chesapeake Bay. Bacteria abundance displayed a strong correlation with dissolved organic carbon.



Bacteria and viruses of St. Martin River, stained using the SYBER Green method

Figure 6.3: Scaling up to Chesapeake Bay with Oyster Gardeners

Partnering with Oyster Gardeners maximizes spatial scope, connects with interested communities, and facilitates science communication. Oyster spat on shell (a) are distributed to Oyster Gardeners for growth, after which they are returned and samples are collected for $\delta^{15}N$ analysis (b) before redistribution to restoration reefs (c and d).

a)  b) 

c)  d) 

Figure 6.4: Application in the National Estuarine Research Reserve System

National Estuarine Research Reserves are establishes in most coastal states (left) and could provide one of several models for scaling up to a national scope across multiple biogeographic regions, with four along the Atlantic coast highlighted. The System Wide Monitoring Program provides detailed water quality data within each research reserve providing a nested hierarchy, with Monie Bay, a component of Chesapeake Bay, MD Research Reserve presented (right).

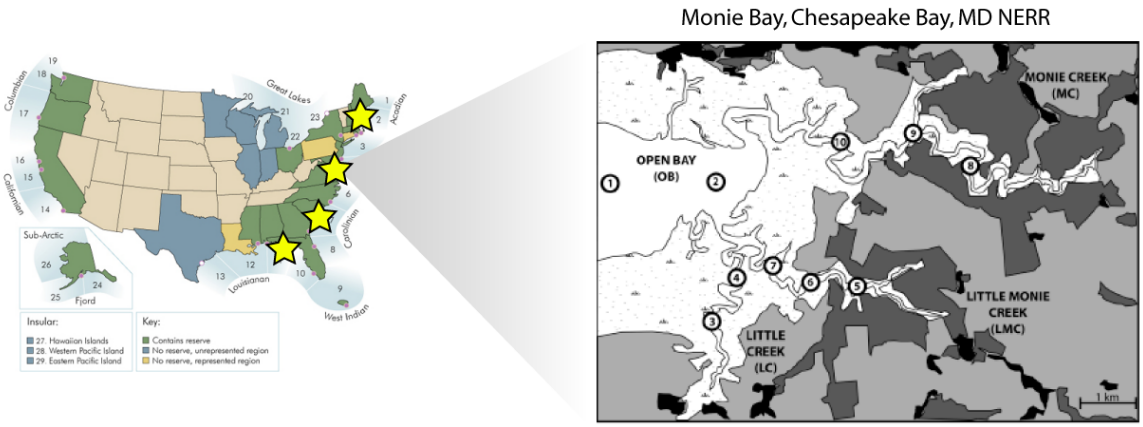
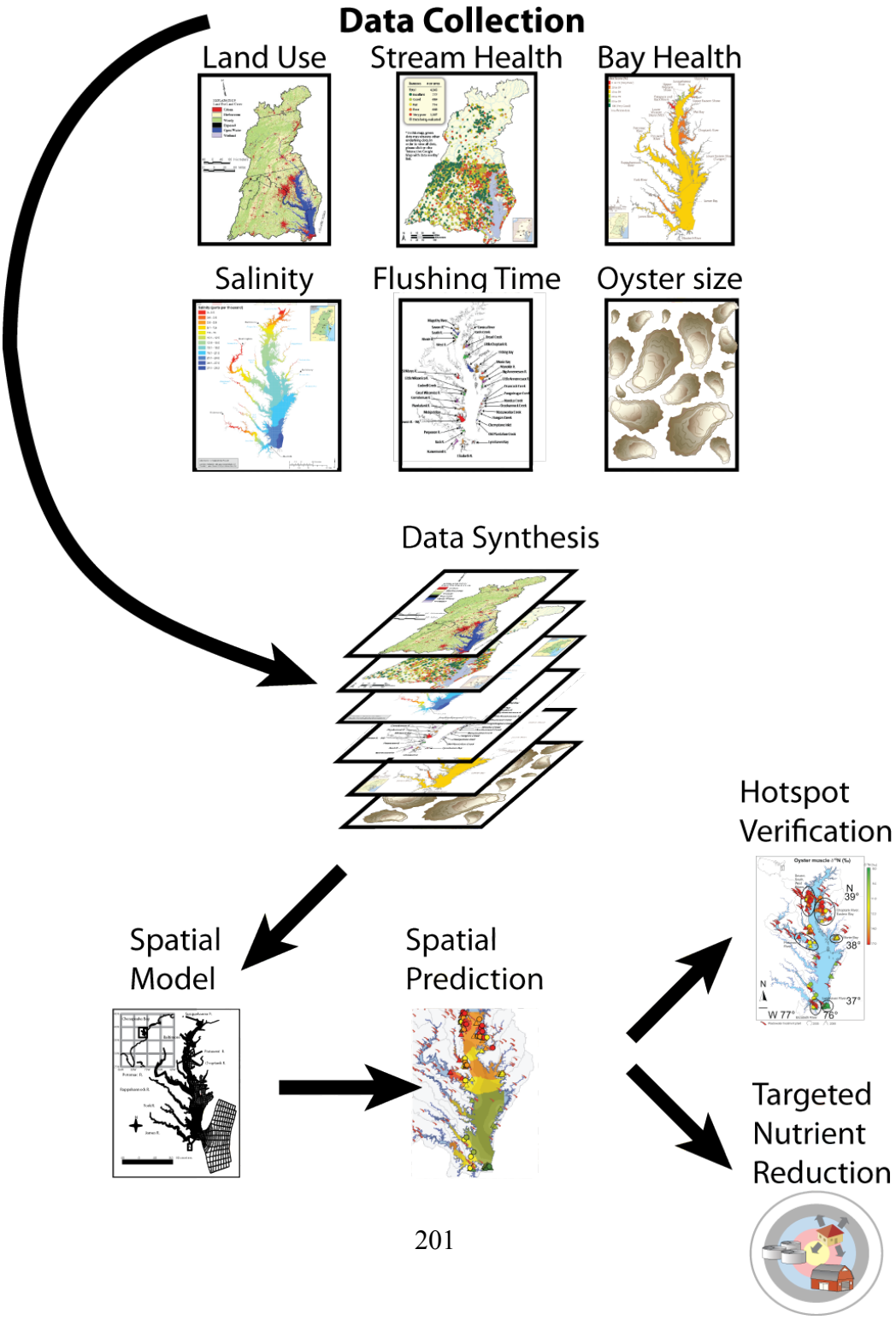


Figure 6.5: Inputs and outputs of spatially predictive models of $\delta^{15}N$ bioindicators



COMPLETE REFERENCE LIST

Adams, T.S., and R.W. Sterner. 2000. The effect of dietary nitrogen content on trophic level N-15 enrichment. Limnology and Oceanography. 45: 601–607.

Adelson, J.M., G.R. Helz, C.V. Miller. 2001. Reconstructing the rise of recent coastal anoxia: molybdenum in Chesapeake Bay sediments. Geochimica et Cosmochimica Acta. 65: 237–252

Altabet, M. 2006. Isotopic tracers of the marine nitrogen cycle: present and past. *In:* Volkman, J.K. (Ed.), Handbook of Environmental Chemistry. Marine Organic Matter: Biomarkers, Isotopes and DNA, Vol. 2N. Springer-Verlag, Berlin, Heidelberg, New York, pp. 251–293.

Anderson D.A., P.M. Glibert, J.M. Burkholder. 2002. Harmful algal blooms and eutrophication: Nutrient sources, composition and consequences. Estuaries 25:562–584

Anderson, W.T. and Fourqurean, J.W., 2003. Intra- and interannual variability in seagrass carbon and nitrogen stable isotopes from south Florida, a preliminary study. Organic Geochemistry. 34, 185-194.

Andres, A. S. 1992. Estimate of nitrate flux to Rehoboth and Indian River Bays, Delaware, through direct discharge of ground water. Open File Report No. 35, Delaware Geological Survey, Newark DE. 39p.

Apple, J. K., P. A. Del Giorgio, and R. I. E. Newell. 2004. The effects of system-level

nutrient enrichment on bacterioplankton production in a tidally influenced estuary. Journal of Coastal Research. SI (45), 110 – 133.

Apple, J.K. 2005. The regulation of bacterioplankton carbon metabolism in a temperature salt-marsh system. Ph.D. Dissertation. University of Maryland, College Park. 345 pp.

Apple, J.K., P.A. del Giorgio, and W.M. Kemp. 2006. Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary. Aquatic Microbial Ecology. 43:243-254.

Arar, E. J. 1997. Method 446.0 *In Vitro* determination of chlorophylls *a*, *b*, $c_1 + c_2$ and pheopigments in marine and freshwater algae by visible spectrophotometry. Revision 1.2 National Exposure Research Laboratory Office of Research and Development, U.S. Environmental Protection Agency. Cincinnati, Ohio

Aravena, R., M. L. Evans, and J. A. Cherry. 1993. Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems. Ground Water 31:180-186.

Baden, S. P., L. Loo, L. Pihl, and R. Rosenberg. 1990. Effects of eutrophication on benthic communities including fish: Swedish west coast. Ambio 19:113-122

Bateman, A. S. and S. D. Kelly. 2007. Fertilizer nitrogen isotope signatures. Isotopes in Environmental and Health Studies. 43:237-247.

Bebianno, M.J., Geret, F., Hoarau, P., Serafim, M.A., Coelho, M.R., Gnassia-Barelli, M. and Romeo, M., 2004. Biomarkers in Ruditapes decussatus: a potential bioindicator

species. Biomarkers. 9, 305-330.

Belbin, L. 1993. PATN Pattern Analysis Package: User's Guide. Commonwealth Scientific and Industrial Research Organisation, Division of Wildlife and ecology.

Benson, E.R., J.M. O'Neil, W.C. Dennison. 2008. Using the aquatic macrophyte *Vallisneria americana* (wild celery) as a nutrient bioindicator. Hydrobiologia. 596: 187–196.

Boag, B., Neilson, R. and Scrimgeour, C.M., 2006. The effect of starvation on the planarian Arthurdendyus triangulatus (Tricladida : Terricola) as measured by stable isotopes. Biology and Fertility of Soils. 43, 267-270.

Boesch D.F., R.B. Brinsfield, R.E. Magnien. 2001. Chesapeake Bay eutrophication: scientific understanding, ecosystem restoration, and challenges for agriculture. Journal of Environmental Quality. 30: 303 – 320

Borja A., A. Ranasinghe, S.B. Weisberg. 2009. Assessing ecological integrity in marine waters, using multiple indices and ecosystem components: Challenges for the future. Marine Pollution Bulletin. 59: 1-4

Borja, A., V. Valencia, J. Franco, I. Muxika, J. Bald, M. J. Belzunce, and O. Solaun. 2004. The water framework directive: Water alone, or in association with sediment and biota, in determining quality standards? Marine Pollution Bulletin 49:8-11

Borum J. 1996. Shallow waters and land/sea boundaries. *In:* Jørgensen B.B., Richardson K. (eds.) Eutrophication in coastal marine ecosystems. American Geophysical Union, Washington, DC, p 179–203

Bosley, K.L., Witting, D.A., Chambers, R.C. and Wainright, S.C., 2002. Estimating turnover rates of carbon and nitrogen in recently metamorphosed winter flounder Pseudopleuronectes americanus with stable isotopes. Marine Ecology-Progress Series. 236, 233-240.

Boynton W.R., J.H. Garber, R. Summers, W.M. Kemp. 1995. Inputs, transformations, and transport of nitrogen and phosphorus in Chesapeake Bay and selected tributaries. Estuaries 18: 285–314

Boynton, W. R. (ed.). 1993. Maryland's Coastal Bays: An assessment of aquatic organisms, pollutant loadings and management options. Chesapeake Biological Laboratory, Solomons, Maryland. Ref. No. [UMCEES] CBL 93-053.

Boynton, W. R., L. Murray, J. D. Hagy, C. Stokes, and W. M. Kemp. 1996. A comparative analysis of eutrophication patterns in a temperate coastal lagoon. Estuaries 19:408-421.

Bratton, J. F., J. K. Böhlke, F. T. Manheim, and D. E. Krantz. 2004. Ground water beneath coastal bays of the Delmarva Peninsula: ages and nutrients. Ground Water 42:1021-1034

Bratton, J.F., S.M. Colman, and R.R Seal. 2003. Eutrophication and carbon sources in Chesapeake Bay over the last 2700 yr: Human impacts in context. Geochimica et Cosmochimica Acta. 67:3385–3402

Brawley, J. W., G. Collins, J. N. Kremer, C. H. Sham, and I. Valiela. 2000. A time-

dependent model of nitrogen loading to estuaries from coastal watersheds. Journal of Environmental Quality 29:1448-1461

Breitburg D.L. 2002. Effects of hypoxia and the balance between hypoxia and enrichment, on coastal fishers and fisheries. Estuaries 25:767–781

Bricker, S., B. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2007. Effects of nutrient enrichment in the nation's estuaries: A decade of change. NOAA Coastal Ocean Program Decision Analysis Series No. 26. National Centers for Coastal Ocean Science, Silver Spring, MD. 322 pp.

Bricker, S.B., B. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2008. Effects of nutrient enrichment in the nation's estuaries: A decade of change. Harmful Algae. 8:21– 32. doi:10.1016/j.hal.2008.08.028.

Bros, W.E. and Cowell, B.C., 1987. A technique for optimizing sample size (replication). Journal of Experimental Marine Biology and Ecology. 114, 63-71.

Brush G.S., C. Lenk, J. Smith. 1980. The natural forests of Maryland: an explanation of the vegetation map of Maryland (with 1:250,000 map). Ecological Monographs. 50: 77–92

Brush G.S.1984. Stratigraphic evidence of eutrophication in an estuary. Water Resources Research 20:531–541

Brush, M.J., K.A. Moore, E.D. Condon. 2007. Synthesis of data from the National Estuarine Research Reserve System-Wide Monitoring Program for the Mid-Atlantic

Region. Final Report to NOAA/UNH Cooperative Institute for Coastal and Estuarine Environmental Technology (CICEET). 120 pp.

Bucci, J.P., S. Rebach, D. DeMaster, and W.J. Showers. 2007. A comparison of blue crab and bivalve d15N tissue enrichment in two North Carolina estuaries. Environmental Pollution 145: 299-308

Carmichael, R.H., Hattenrath, T., Valiela, I., and Michener, R.H., 2008. Nitrogen stable isotopes in the shell of *Mercenaria mercenaria* trace wastewater inputs from watersheds to estuarine ecosystems. Aquatic Biology. 4, 99-111.

Chen, F.J., G.D. Jia, and J.Y. Chen. 2009. Nitrate sources and watershed denitrification inferred from nitrate dual isotopes in the Beijiang River, south China. Biogeochemistry. 94: 163-174.

Chesapeake Bay Program. 2004. Chesapeake Bay Program Analytical Segmentation Scheme: Revisions, decisions, and rationales 1983-2003. Monitoring and Analysis Subcommittee Tidal Monitoring and Analysis Workgroup. Annapolis, Maryland.

Chesapeake Bay Program. 2008. Chesapeake Bay Program Water Quality Monitoring Database (1984 – Present). Accessed online 4 September 2008. http://www.chesapeakebay.net/data_waterquality.aspx

Chesapeake Bay Program. 2009. Point sources and Priority Agricultural Watersheds – Chesapeake Bay Watershed. Map and GIS data. www.chesapeakebay.net

Choi, W.-J., G.-H. Han, S.-M. Lee, G.-T. Lee, K.-S. Yoon, S.-M. Choi, H.-M. Ro. 2007. Impact of land-use types on nitrate concentration and $\delta^{15}N$ in unconfined groundwater in rural areas of Korea. Agriculture, Ecosystems, and Environment 120:259-268.

Clarke, K. R. and R. M. Warwick. 1994. Change in Marine Communities: An approach to statistical analysis and interpretation. Natural Environment Research Council, Plymouth, United Kingdom

Cline, J.D., and I.R. Kaplan. 1975. Isotopic fractionation of dissolved nitrate during denitrification in the eastern tropical North Pacific Ocean. Marine Chemistry. 3:271–299. doi:10.1016/0304-4203 (75)90009-2.

Cloern, J.E. 2001. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series. 210:223–253. doi:10.3354/meps210223.

Coffin, R.B., B. Fry, B.J. Peterson and R.T. Wright. 1989. Carbon isotopic compositions of estuarine bacteria. Limnology and Oceanography. 34: 1305-1310.

Cohen, R. A. and P. Fong. 2005. Experimental evidence supports the use of $\delta^{15}N$ content of the opportunistic green macroalga *Enteromorpha intestinalis* (Chlorophyta) to determine nitrogen sources to estuaries. Journal of Phycology. 41:287-293.

Cohen, R.A., and P. Fong. 2006. Using opportunistic green macro- algae as indicators of nitrogen supply and sources to estuaries. Ecological Applications. 16:1405–1420. doi:10.1890/1051-0761 (2006)016[1405:UOGMAI]2.0.CO;2.

Cole, M.L., I. Valiela, K.D. Kroeger, G.L. Tomasky, J. Cebrian, C. Wigand, R.A. McKinney, S.P. Grady, and M.H. Carvalho daSilva. 2004. Assessment of a $\delta^{15}N$ isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. Journal of Environmental Quality. 33:124–132.

Cole, M.L., Kroeger, K.D., McClelland, J.W. and Valiela, I., 2006. Effects of watershed land use on nitrogen concentrations and delta (15) nitrogen in groundwater. Biogeochemistry. 77, 199-215.

Coleman, D.C., and Fry, B., 1991. Carbon Isotope Techniques, first ed. Academic Press, San Diego.

Cooper S.R., Brush G.S. 1991. Long-term history of Chesapeake Bay anoxia. Science 254:992–996

Cooper S.R., Brush G.S. 1993. A 2500-year history of anoxia and eutrophication in Chesapeake Bay. Estuaries 16: 617–626

Cornwell, J. C., J. M. Stribling, and J. C. Stevenson. 1994. Biogeochemical studies at the Monie Bay National Estuarine Research Reserve. Washington, D. C.: Organizing for the Coast: Thirteenth International Conference of the Coastal Society, pp. 645 - 655

Cornwell, J.C., J.C. Stevenson, D.J. Conley, and M. Owens. 1996. A sediment chronology of Chesapeake Bay eutrophication. Estuaries. 19: 488-499.

Cornwell, J.C., W.M. Kemp and T.M. Kana. 1999. Denitrification in coastal ecosystems:

methods, environmental controls, and ecosystem level controls, a review. Aquatic Ecology 33: 41–54, 1999.

Costanzo, S. D., M. J. O'Donohue, W. C. Dennison, N. R. Loneragan, and M. Thomas. 2001. A new approach for detecting and mapping sewage impacts. Marine Pollution Bulletin 42:149-156.

Costanzo, S. D., M. J. O'Donohue, and W. C. Dennison. 2004. Assessing the influence and distribution of shrimp pond effluent in a tidal mangrove creek in north-east Australia. Marine Pollution Bulletin 48:514-525

Curtin P.D., Brush G.S., Fisher G.W. (eds.) 2001. Discovering the Chesapeake: the history of an ecosystem. Johns Hopkins University Press, Baltimore, MD

D'Elia, C.F., P.A. Steudler, and N. Corwin. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. Limnology and Oceanography 22: 760-764.

Dame, R.F. 1972. Comparison of various allometric relationships in intertidal and subtidal American oysters. Fishery Bulletin. 70:1121-1126.

Dame, R.F. 1972. The ecological energies of growth, respiration and assimilation in the intertidal American oyster Crassostrea virginica. Marine Biology 17:243-250.

Daskin, J. H., D. R. Calci, W. Burkhardt III, R. H. Carmichael. 2008. use of N stable isotope and microbial analyses to define wastewater influence in Mobile Bay, AL. Marine Pollution Bulletin 56:860-868.

Dattagupta, S., D.C. Bergquist, E.B. Szalai, S.A. Macko, and C.R. Fisher. 2004. Tissue carbon, nitrogen, and sulfur stable isotope turnover in transplanted *Bathymodiolus childressi* mussels: Relation to growth and physiological condition. Limnology and Oceanography. 49:1144–1151.

Davidson, E.A., S. Seitzinger. 2006. The enigma of progress in denitrification research. Ecological Applications. 16: 2057-2063

Deniro, M.J. and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta. 45: 341-351

Dennison, W. C., R. J. Orth, K. A. Moore, J. C. Stevenson, V. Carter, S. Kollar, P. W. Bergstrom, and R. A. Batiuk. 1993. Assessing water quality with submersed aquatic vegetation. BioScience 43:86-94.

Dennison, W.C., J.E. Thomas, C.J. Cain, T.J.B. Carruthers, M.R. Hall, R.V. Jesien, C.E. Wazniak, and D.E. Wilson. 2009. Shifting Sands: Environmental and cultural change in Maryland's Coastal Bays. IAN Press. Cambridge, MD.

Dettman, E.H. 2001. Effect of Water Residence Time on Annual Export and Denitrification of Nitrogen in Estuaries: A Model Analysis. Estuaries. 24: 481-490.

Dillow, J. A., W. S. L. Banks, and M. J. Smigaj. 2002. Groundwater quality and discharge to Chincoteague and Sinepuxent Bays adjacent to Assateague Island National Seashore,Maryland. Water-Resources Investigations Report 02-4029. U.S. Geological Survey, Baltimore, Maryland, USA.

Ebersole, E., M. Lane, M. Olson, E. Perry, and W. Romano. 2002. Assumptions and procedures for calculating water quality status and trends in tidal waters of the Chesapeake Bay and its tributatires: a cumulative history. Report prepared for Chesapeake BayProgram, Tidal Monitoring and Analysis Workgroup. Maryland Department of Natural Resources, Annapolis, Maryland, USA. (http://www.dnr.state.md.us/bay/tribstrat/stat_trend_hist.pdf)

Eco-Check. 2007. Chesapeake Bay 2007 Health Report Card. http://www.eco-check.org/reportcard/chesapeake/2007/

Elliott, E.M., and Brush, G.S., 2006. Sedimented organic nitrogen isotopes in freshwater wetlands record long-term changes in watershed nitrogen source and land use. Environmental Science and Technology. 40, 2910-2916.

Ferreira, J. G. 2000. Development of an estuarine quality index based on key physical and biogeochemical features. Ocean and Coastal Management 43:99–122.

Fertig, B., Carruthers, T.J.B., Dennison, W.C., Fertig, E.J., Altabet, M.A. 2010. Eastern oyster (*Crassostrea virginica*) $\delta^{15}N$ as a bioindicator of nitrogen sources: observations and modeling. Marine Pollution Bulletin In Press.

Fertig, B., Carruthers, T.J.B., Dennison, W.C., Jones, A.B., Pantus, F., and Longstaff, B., 2009. Oyster and macroalgae bioindicators detect elevated $\delta^{15}N$ in Maryland's Coastal Bays. Estuaries and Coasts. 32, 773-786.

Fielding, K. P. 2002. Differential substrate limitation in small tributaries of Chesapeake

Bay, Maryland influenced by non-point source nutrient loading. Masters. University of Maryland.

Fila, L., R.H. Carmichael, A. Shriver, and I. Valiela. 2001. Stable N isotopic signatures in bay scallop tissue, feces, and pseudofeces in Cape Cod estuaries subject to different N loads. Biological Bulletin. 201:294–296. doi:10.2307/1543374.

Finlay, J.C., and Kendall, C. 2007. Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems, in: Michener, R.H., and Lajtha, K. (Eds.), Stable isotopes in ecology and environmental science. Blackwell Publishing, pp. 283-333.

Fisher, T. R., J. D. Hagy III, W. R. Boynton, M. R. Williams. 2006. Cultural eutrophication in the Choptank and Patuxent estuaries of Chesapeake Bay. Limnology and Oceanography 51:435-447

Fogel, M.L., Cifuentes, L.A., Velinsky, D.J., and Sharp, J.H. 1992. Relationship of carbon availability in estuarine phytoplankton to isotopic composition. Marine Ecology Progress Series. 82: 291-300

Ford, S.E., and Smolowitz, R., 2007. Infection dynamics of an oyster parasite in its newly expanded range. Marine Biology. 151,119-133.

Ford, S.E., Cummings, M.J., and Powell, E.N. 2006. Estimating mortality in natural assemblages of oysters. Estuaries and Coasts. 29, 361-374.

Fourqurean, J. W., T. O. Moore, B. Fry, J. T. Hollibaugh. 1997. Spatial and temporal

variations in C:N:P ratios, $\delta^{15}N$, and $\delta^{13}C$ of eelgrass *Zostera marina* as indicators of ecosystem processes, Tomales Bay, California, USA.

Fourqurean, J.W., Escorcia, S.P., Anderson, W.T. and Zieman, J.C., 2005. Spatial and seasonal variability in elemental content $\delta^{13}C$, and $\delta^{15}N$ of *Thalassia testudinum* from South Florida and its implications for ecosystem studies. Estuaries and Coasts. 28, 447-461.

Fourqurean, J.W., Marba, N., Duarte, C.M., Diaz-Almela, E. and Ruiz-Halpern, S., 2007. Spatial and temporal variation in the elemental and stable isotopic content of the seagrasses *Posidonia oceanica* and *Cymodocea nodosa* from the Illes Balears, Spain. Marine Biology. 151, 219-232.

Frana, M.F. and E.A. Venso. 2009. Identifying sources of fecal pollution in shellfish and nontidal waters in Maryland watersheds. Final Report to Maryland Department of the Environment. MOU U00P820244. 52 pp.

Frazer, T.K., Ross, R.M., Quetin, L.B. and Montoya, J.P., 1997. Turnover of carbon and nitrogen during growth of larval krill, Euphausia superba Dana: a stable isotope approach. Journal of Experimental Marine Biology and Ecology. 212, 259-275.

Frick, W. E., T. Khangaonkar, A. C. Sigleo, and Z. Q. Yang. 2007. Estuarine-ocean exchange in a North Pacific estuary: comparison of steady state and dynamic models. Estuarine Coastal and Shelf Science 74:1-11

Fry, B. 1999. Using stable isotopes to monitor watershed influences on aquatic trophodynamics. Canadian Journal of Fisheries and Aquatic Science. 56: 2167–2171

Fry, B. 2006. Stable Isotope Ecology, 1st edition. Springer, New York, New York.

Fry, B. and Allen, Y. C., 2003. Stable isotopes in zebra mussels as bioindicators of river-watershed linkages. River Research and Applications. 19,683-696.

Fry, B., A. Gace, and J.W. McClelland. 2003. Chemical indicators of anthropogenic nitrogen-loading in four Pacific estuaries. Pacific Science. 57:77–101. doi:10.1353/psc.2003.0004.

Fuller, B.T., J.L. Fuller, N.E. Sage, D.A. Harris, T.C. O'Connell, R.E.M. Hedges. 2005. Nitrogen balance and delta N-15: why you're not what you eat during nutritional stress. Rapid Communications in Mass Spectrometry. 19: 2497–2506.

Gallegos C.L., Jordan T.E. 2002. Impact of the spring 2000 phytoplankton bloom in Chesapeake Bay on optical properties and light penetration in the Rhode River, Maryland. Estuaries 25: 508–518

Galloway, J.N., F.J. Dentener, D.G. Capone, E.W. Boyer, R.W. Howarth, S.P. Seitzinger, G.P. Asner, C.C. Cleveland, P.A. Green, E.A. Holland, D.M. Karl, A.F. Michaels, J.H. Porter, A.R. Townsend, C.J. Vorosmarty. 2004. Nitrogen cycles: past, present, and future. Biogeochemistry. 70: 153-226.

Gartner, A., P. Lavery, and A. J. Smit. 2002. Use of δ15N signatures of different functional forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in sewage dispersal. Marine Ecology-Progress Series. 235: 63-73

Garton, D.W., C.D. Payne, J.P. Montoya. 2005. Flexible diet and trophic position of

dreissenid mussels as inferred from stable isotopes of carbon and nitrogen. Canadian Journal of Fisheries and Aquatic Sciences. 62: 1119–1129.

Gaye-Siessegger, J., Focken, U., Abel, H. and Becker, K., 2004. Individual protein balance strongly influences delta N-15 and delta C-13 values in Nile tilapia, Oreochromis niloticus. Naturwissenschaften. 91, 90-93.

Giblin A. E., and A. G. Gaines. 1990. Nitrogen inputs to a marine embayment: the importance of groundwater. Biogeochemistry 10:309-328.

Glibert, P. M., C. E. Wazniak, M. R. Hall, and B. Sturgis. 2007. Seasonal and interannual trends in nitrogen and brown tide in Maryland's Coastal Bays. Ecological Applications 17(5)Supplement:S79- S87

Glibert, P. M., J. Harrison, C. Heil, and S. Seitzinger. 2006. Escalating wordwide use of urea – a global change contributing to coastal eutrophication. Biogeochemistry 77:441-463.

Glibert, P. M., R. Magnien, M. W. Lomas, J. Alexander, C. Fan, E. Haramoto, M. Trice, and T. M. Kana. 2001. Harmful algal blooms in the Chesapeake and coastal bays of Maryland, USA: comnparison of 1997, 1998, and 1999 events. Estuaries 24(6A):875-883.

Goñi M.A., K.C. Ruttenberg, and T.I. Eglinton. 1997. Sources and contributions of terrigenous organic carbon to surface sediments in the Gulf of Mexico. Nature. 389: 275–278.

Granger, S., M. Brush, B. Buckley, M. Traber, M. Richardson, and S.W. Nixon. 2000. An Assessment of Eutrophication in Greenwich Bay. Paper No. 1 in: M. Schwartz (ed.) Restoring Water Quality in Greenwich Bay: A Whitepaper Series. Rhode Island Sea Grant, Narragansett, R.I. 20pp.

Grant, M., R.V. Kopple. 2009. A stable isotope investigation into the feeding behavior of *Brychius hungerfordi* Spangler (Coleoptera: Haliplidae), a federally endangered crawling water beetle. The Coleopterists Bulletin. 63: 71–83.

Griffin, M.P.A., and Valiela, I., 2001. Delta N-15 isotope studies of life history and trophic position of Fundulus heteroclitus and Menidia menidia. Marine Ecology Progress Series. 214, 299-305.

Griffin, T. S., C. W. Honeycutt, and Z. He. 2003. Changes in soil phosphorus from manure application. Soil Science Society of America Journal 67:645-653.

Groffman, P.M., M.A. Altabet, J.K. Bohlke, K. Butterbach-Bahl, M.B. David, M.K. Firestone, A.E. Giblin, T.M. Kana, L.P. Nielson, M.A. Voytek. 2006. Methods for measuring denitrification: diverse approaches to a difficult problem. Ecological Applications. 16: 2091-2122

Guo, L. and P.H. Santschi. 1997. Isotopic and elemental characterization of colloidal organic matter from the Chesapeake Bay and Galveston Bay. Marine Chemistry 59: 1-15

Haddad R.I. and C.S. Martens. 1987. Biogeochemical cycling in an organic-rich coastal

marine basin; 9, sources and accumulation rates of vascular plant-derived organic material. Geochimica et Cosmochimica Acta. 51: 2991–3001.

Hager, P. 1996. Worcester County, MD. p. 20-24. *In* K. Beidler, P. Gant, M. Ramsay, and G. Schultz (eds.), Proceedings – Delmarva's Coastal Bay watersheds: not yet up the creek. EPA/600/R-95/052. United States Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, Narragansett, Rhode Island, USA.

Hagy J.D. 2002. Eutrophication, hypoxia and trophic transfer efficiency in Chesapeake Bay. PhD dissertation, University of Maryland, College Park, MD

Hagy J.D., W.R. Boynton, C.W. Wood, K.V. Wood. 2004. Hypoxia in Chesapeake Bay, 1950–2001: long-term changes in relation to nutrient loading and river flow. Estuaries 27:634 – 658

Hagy, J. D. 1996. Residence times and net ecosystem processes in Patuxent River Estuary. MS Thesis. UMD College Park

Harding Jr. L.W. 1994. Long-term trends in the distribution of phytoplankton in Chesapeake Bay: roles of light, nutrients and streamflow. Marine Ecology Progress Series 104:267–291

Harding Jr. L.W., E.S. Perry. 1997. Long-term increase of phytoplankton biomass in Chesapeake Bay, 1950 – 1994. Marine Ecology Progress Series 157:39–52

Harrington, R. R., B. P. Kennedy, C. P. Chamberlain, J. D. Blum, and C. L. Folt. 1998. ^{15}N

enrichment in agricultural catchments: field patterns and application to tracking Atlantic salmon (*Salmo salar*). Chemical Geology 147:281-294.

Harris, L., S. Granger, and S. Nixon. 2005. Evaluation of the health of eelgrass (*Zostera marina* L.) beds within the Maryland Coastal Bays: a report to the National Park Service. University of Rhode Island, Graduate School of Oceanography, Narragansett, Rhode Island, USA.

Haubert, D., Langel, R., Scheu, S. and Ruess, L., 2005. Effects of food quality, starvation and life stage on stable isotope fractionation in Collembola. Pedobiologia. 49, 229-237.

Heikoop, J.M., Dunn, J.J., Risk, M.J., Tomascik, T., Schwarcz, H.P., Sandeman, I.M, and Sammarco, P.W., 2000. δ15N and δ13C of coral tissue show significant inter-reef variation. Coral Reefs. 19, 189-193.

Hobson, K.A. and Clark, R.G., 1992. Assessing Avian Diets Using Stable Isotopes .2. Factors Influencing Diet-Tissue Fractionation. Condor. 94, 189-197.Heikoop

Hobson, K.A., Alisauskas, R.T. and Clark, R.G., 1993. Stable-Nitrogen Isotope Enrichment in Avian-Tissues Due to Fasting and Nutritional Stress - Implications for Isotopic Analyses of Diet. Condor. 95, 388-394.

Hoering, T.C. and H.T. Ford. 1960. The isotope effect in the fixation of nitrogen by Azotobacter. Journal of the American Chemical Society. 82: 376-378

Hoffman, J.C., D.A. Bronk, and J.E. Olney. 2008. Organic Matter Sources Supporting

Lower Food Web Production in the Tidal Freshwater Portion of the York River Estuary, Virginia. Estuaries and Coasts. 31:898–911

Holland A.F., A.T. Shaughnessy, M.H. Hiegel. 1987. Long-term variation in mesohaline Chesapeake Bay macrobenthos: spatial and temporal patterns. Estuaries 10:227–245

Horrigan, S. G., J. P. Montoya, J. L. Nevins, J. J. McCarthy. 1990. Natural isotopic composition of dissolved inorganic nitrogen in the Chesapeake Bay. Estuarine and Coastal Shelf Science. 30: 393-410.

Horton, T. 2003. Turning the Tide. Saving the Chesapeake Bay, 2nd ed. rev. and expanded edition. Island Press, Washington D.C.

Hübner, H., 1986. Isotope effects of nitrogen in the soil and biosphere, in: Fritz, P. and Fontes, J.C., (Eds.), Handbook of Environmental Isotope Geochemistry, Vol 2B, The Terrestrial Environment. Elsevier, Amsterdam, pp. 361-425.

Jennings, S. and K.J. Warr. 2003. Environmental correlates of large-scale spatial variation in the $\delta^{15}N$ of marine animals. Marine Biology. 142: 1131-1140.

Jin, Z.F., Chen, Y.X., Wang, F. and Ogura, N., 2004. Detection of nitrate sources in urban groundwater by isotopic and chemical indicators, Hangzhou City, China. Environmental Geology. 45, 1017-1024.

Jones, A., T. Carruthers, F. Pantus, J. Thomas, T. Saxby, W. Dennison. 2004. A water quality assessment of the Maryland Coastal Bays including nitrogen source identification using stable isotopes. Report to Maryland Coastal Bays Program. 60

pp.

Jones, T. W., L. Murray, and J. C. Cornwell. 1997. A two-year study of the short-term and long-term sequestering of nitrogen and phosphorus in the Maryland National Estuarine Research Reserve. Monie Bay, MD: Maryland National Estuarine Research Reserve, pp. 1-100.

Jordan, T. E., D. L. Correll, and D. E. Weller. 1997. Effects of agriculture on discharges of nutrients from coastal plain watersheds of Chesapeake Bay. Journal of Environmental Quality 26:836-848.

Kana, T.M., C. Darkangelo, M.D. Hunt, J.B. Oldham, G.E. Bennett, J.C. Cornwell. 1994. Membrane inlet mass spectrometer for rapid high-precision determination of N_2, O_2, and Ar in environmental water samples. Analytical Chemistry. 66: 4166-4170.

Kang, C.K., P.-G. Saurlau, P. Richard, and G.F. Blanchard. 1999. Food sources of the infaunal suspension-feeding bivalve *Cerastoderma edule* in a muddy sandflat of Marennes-Oléron Bay, as determined by analyses of carbon and nitrogen stable isotopes. Marine Ecology Progress Series. 187: 147-158.

Karlsen, A.W., T.M. Cronin, E.S. Ishman, D.A. Willard, C.W. Holmes, M. Marot, R. Kerhin. 2000. Historical trends in Chesapeake Bay dissolved oxygen based on benthic *Foraminifera* from sediment cores. Estuaries 23:488–508

Kasper, J.W. 2006. Simulated ground-water flow at the Fairmount Site, Sussex County, Delaware (USA) with implications for nitrate transport. MS Thesis, University of Delaware, Newark. 133 pages.

Kearney, M.S., A.S. Rogers, J.P.R.G. Townsend, E. Rizzo, D. Stutzer, J.C. Stevenson, K.L. Sundberg. 2002. Landsat imagery shows decline of coastal marshes in Chesapeake and Delaware Bays. EOS 3:173–178

Kemp W.M., E.M. Smith, M. Marvin-DiPasquale, W.R. Boynton. 1997. Organic carbon-balance and net ecosystem metabolism in Chesapeake Bay. Marine Ecology Progress Series. 150:229–248

Kemp W.M., P.Sampou, J. Caffrey, M. Mayer, K. Henriksen, W.R. Boynton. 1990. Ammonium recycling versus denitrification in Chesapeake Bay sediments. Limnology & Oceanography 35:1545 – 1563

Kemp W.M., R. Batiuk, R. Bartleson, P. Bergstrom, V. Carter, C.L. Gallegos, W. Hunley, L. Karrh, E.W. Koch, J.M. Landwehr, K.A. Moore, L. Murray, M. Naylor, N.B. Rybicki, J.C. Stevenson, D.J. Wilcox. 2004. Habitat requirements for submerged aquatic vegetation in Chesapeake Bay: Water quality, light regime, and physical-chemical factors. Estuaries. 27: 363-377.

Kemp, W. M., Boynton, W.R., Adolf, J.E., Boesch, D.F., Boicourt, W.C., Brush, G., Cornwell, J.C., Fisher, T.R., Glibert, P.M., Hagy, J.D., Harding, L.W., Houde, E.D., Kimmel, D.G., Miller, W.D., Newell, R.I.E., Roman, M.R., Smith, E.M., Stevenson. J.C., 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. Marine Ecology Progress Series. 303, 1-29.

Kendall, C., E.M. Elliott, S.D. Wankel. 2007. Tracing anthropogenic inputs of nitrogen to ecosystems. *In:* R. Michener, K. Lajtha (eds.). Stable Isotopes in Ecology and

Environmental Science (Ecological Methods and Concepts). 2nd edition. Wiley-Blackwell. 592 pp.

Kendall. C. 1998. Tracing Nitrogen Sources and Cycling in Catchments. In: Isotope Tracers in Catchment Hydrology (1998), C. Kendall and J. J. McDonnell (Eds.). Elsevier Science B.V., Amsterdam. pp. 519-576.

Kennedy, V.S., L.L. Breisch. 1983. Sixteen decades of political management of the oyster fishery in Maryland's Chesapeake Bay. Journal of Environmental Management. 164: 153-171

Kennedy, V.S., Shaw, K.S., and Newell, R.I.E., 2009. Discriminatory predation by three invertebrates on eastern oysters (Crassostrea virginica) compared with non-native Suminoe oysters (C. ariakensis). Invertebrate Biology. 128, 16-25.

Kennish, M. J. 2002. Environmental threats and environmental future of estuaries. Environmental Conservation 29:78-107

Kennish, M. J. 2004. Estuarine Research, Monitoring, and Resource Protection. Boca Raton, CRC Press. 297pp.

Kennish, M.J. 2004. NERRS research and monitoring initiatives. Journal of Coastal Research. SI 45: 1-8.

Kerouel, R. and Aminot., A., 1987. Procédure optimisée hors-contaminations pour l'analyze des éléments nutritifs dissous dans l'eau de mer. Marine Environmental Research. 22, 19-32.

Kiddon, J. A., J. F. Paul, H. W. Buffam, C. S. Strobel, S. S. Hale, D. Cobb, and B. S. Brown. 2003. Ecological condition of US Mid-Atlantic estuaries 1997-1998. Marine Pollution Bulletin 46:1224-1244

Kimbrough, K.L., Johnson, W.E., Lauenstein, G.G., Christensen, J.D. and Apeti, D.A., 2008. An assessment of two decades of contaminant monitoring in the nation's coastal zone. NOAA, Silver Spring, MD, USA.

Kimmel, D.G., Townsend, H., Carruthers, T.J.B., and Fertig B. 2010. Environmental Statistics: Balancing simplicity and explanatory power. In: Longstaff, B.J., Carruthers, T.J.B., Dennison, W.C., Lookingbill, T.R., Hawkey, J.M., Thomas, J.E., Wicks, E.C., and Woerner, J. (eds.) Integrating and Applying Science: A practical handbook for effective coastal ecosystem assessment. IAN Press, Cambridge, MD, p113-132

Kingery, W. L., C. W. Wood, D. P. Delaney, J. C. Williams, and G. L. Mullins. 1994. Impact of long-term land application of broiler litter on environmentally related soil properties. Journal of Environmental Quality 23:139-147

Klocker, C.A., S.S. Kaushal, P.M Groffman, P.M. Mayer, R.P. Morgan. 2009. Nitrogen uptake and denitrification in restored and unrestored streams in urban Maryland, USA. Aquatic Science. 71: 411-424.

Knowles, R., and Blackburn, T.H., 1993. Nitrogen Isotope Techniques. Academic Press, Inc. Harcourt Brace Jovanovich, San Diego.

Krantz, D. E., F. T. Manheim, J. F. Bratton, D. J. Phelan. 2004. Hydrogeologic setting and ground water flow beneath a section of Indian River Bay, Delaware. Ground Water 42:1035-1051

Lake, J. L., McKinney, R.A., Osterman, F.A., Pruell, R.J., Kiddon, J., Ryba, S.A., and Libby, A.D., 2001. Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems. Canadian Journal of Fisheries and Aquatic Science. 58, 870-878

Landgon, C.J., R.I.E. Newell. 1990. Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demise*. Marine Ecology Progress Series. 58: 299-310.

Langdon, C.J. and Newell, R.I.E., 1996. Digestion and nutrition in larvae and adults, in: Kennedy, V.S., Newell, R.I.E. and Eble, A.F. (Eds.), The eastern oyster Crassostrea virginica. Maryland Sea Grant, College Park, MD, USA, pp. 231-260.

Lea, C., R.L. Pratt, T.E. Wagner, E.W.Hawkes, and A.E. Almario. 2003. Use of submerged aquatic vegetation habitat requirements as targets for water quality in Maryland and Virginia Coastal Bays. Assateague Island National Seashore, Maryland and Virginia. National Parks Service Technical Report NPS/NRWRD/NRTR-2003/316. National Parks Service Water Resources Division, Fort Collins, Colorado, USA.

Leavitt, P.R., C.S. Brock, C. Ebel, and A. Patoine. 2006. Landscape-scale effects of urban nitrogen on a chain of freshwater lakes in central North America. Limnology & Oceanography. 51: 2262–2277.

Lefebvre, S., Clement, J.-C., Pinay, G., Thenail, C., Durand, P. and Marmonier, P., 2007. 15N-Nitrate signature in low-order streams: effects of land cover and agricultural practices. Ecological Applications. 17, 2333-2346.

Lichtenberg, E., D. Parker, and L. Lynch. 2002. Economic value of poultry litter supplies in alternative uses. University of Maryland Center for Agricultural and Natural Resource Policy, Policy Report No. 02-02

Loh, A.N., J.E. Bauer, E.A. Canuel. 2006. Dissolved and particulate organic matter source-age characterization in the upper and lower Chesapeake Bay: A combined isotope and biochemical approach. Limnology and Oceanography. 51:1421–1431

Lorrain, A., Paulet, Y.M., Chauvaud, L., Savoye, N., Donval, A. and Saout, C., 2002. Differential delta C-13 and delta N-15 signatures among scallop tissues: implications for ecology and physiology. Journal of Experimental Marine Biology and Ecology. 275, 47-61.

Lung, W. S. 1994. Water quality modeling of the St. Martin River, Assawoman and Isle of Wight Bays. Maryland Department of the Environment, Baltimore, Maryland.

Macko, S.A., N.E. Ostrom. 1994. Pollution studies using nitrogen isotopes. *In:* Lajtha, K., Michener, R.M. (Eds.), Stable Isotopes in Ecology and Environmental Science. Blackwell Scientific Publishers, Oxford, pp. 45–62.

Manheim, F. T., D. E. Krantz, and J. F. Bratton. 2004. Studying ground water under Delmarva Coastal Bays using electrical resistivity. Ground Water 42:1052-1068.

Mariotti, A., Germon, J.C., and Leclerc, A., 1982. Nitrogen isotope fractionation associated with the NO2- → N2O step of denitrification in soils. Canadian Journal of Soil Science. 62, 227-241.

Maryland Department of Planning, 2002. Land Use Land Cover (2002) GIS data. http://www.mdp.state.md.us/OurProducts/mapping.shtml

Maryland Department of Planning. 2000. Historical Census: Population of Maryland's Regions and Jurisdictions, 1790 – 1990. Data online. Accessed 2 Oct 2008. http://www.mdp.state.md.us/msdc/census/Historical_Census/dw_Hiscens_idx.htm

Maryland State Climatologist Office. 2008. Data Request. http://metosrv2.umd.edu/~climate/

Maryland State Climatologist. 2008. Data available online. Accessed 18 May 2007. http://www.atmos.umd.edu/~climate/

Matthews, B. and Mazumder, A., 2005. Consequences of large temporal variability of zooplankton delta N-15 for modeling fish trophic position and variation. Limnology and Oceanography. 50, 1404-1414.

MCBP [Maryland Coastal Bays Program]. 2005. About the Maryland Coastal Bays Program. Maryland Department of Natural Resources, Annapolis, Maryland.

McClelland, J.W., I. Valiela, R.H. Michener. 1997. Nitrogen-stable isotope signatures in

estuarine food webs: a record of increasing urbanization in coastal watersheds. Limnology and Oceanography. 42: 930–937.

McClelland, J.W., I. Valiela. 1998. Linking nitrogen in estuarine producers to land- derived sources. Limnology and Oceanography. 43: 577–585.

McCutchan, J.H.Jr, W.M. Lewis, C. Kendall, C.C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos. 102: 378 – 390.

McIlvin M. R., and M. A. Altabet. 2005. Chemical conversion of nitrate and nitrite to nitrous oxide for nitrogen and oxygen isotopic analysis in freshwater and seawater. Analytical Chemistry 77:5589-5585

McKinney, R. A., J. L. Lake, M. A. Charpentier, and S. Ryba. 2002. Using mussel isotope ratios to assess anthropogenic nitrogen inputs to freshwater ecosystems. Environmental Monitoring and Assessment 74:167-192.

McKinney, R. A., Nelson, W.G., Wigand, C., and Charpentier, M.A., 2001. Ribbed mussel nitrogen isotope signatures reflect nitrogen sources in coastal salt marshes. Ecological Applications 11, 203-214.

McMillan C., P.L. Parker, and B. Fry. 1980. $^{13}C/^{12}C$ ratios in seagrasses. Aquatic Botany. 9: 237–249.

MD Department of the Environment. 2009. The 66 Major Facilities Discharging to the Chesapeake Bay. www.mde.state.md.us/assets/document/The 66 Major Facilities.pdf Accessed December 2009.

MD DNR. 1999. Chesapeake and Coastal Watershed Service. Accessed 8 Aug 2008. http://www.dnr.state.md.us/watersheds/surf/indic/les/les_septic_indmap.html

MD DNR. 2009. Watershed Profiles. Online. http://mddnr.chesapeakebay.net/wsprofiles/surf/prof/prof.html Accessed December 2009.

Millero, F. J. 2005. Chemical Oceanography. 3rd Ed. CRC Press.

Mills, K., M.J. Kennish, and K.A. Moore. 2008. Research and monitoring components of the National Estuarine Research Reserve System. Journal of Coastal Research SI 55: 1-8.

Minagawa, M., E. Wada. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimca Acta. 48: 1135–1140.

Miyake, Y. and E. Wada. 1971. The isotope effect on the nitrogen in biochemical, oxidation-reduction reactions, Rec. Oceanogr. Works Jpn., 11, 1–6, 1971.

Montoya, J.P., McCarthy, J.J. 1995. Isotopic fractionation during nitrate uptake by phytoplankton grown in continuous culture. Journal of Plankton Research 17, 439-464.

Moore, K. B. 2003. Uptake dynamics and sensitivity of oysters to detect sewage-derived nitrogen. B.Sc. Honours Thesis, University of the Sunshine Coast, Sippy Downs,

Australia.

Moore, S.K. and Suthers, I.M., 2005. Can the nitrogen and carbon stable isotopes of the pygmy mussel, Xenostrobus securis, indicate catchment disturbance for estuaries in northern New South Wales, Australia? Estuaries. 28, 714-725.

Mulholland, M. R., G. Boneillo, and E. C. Minor. 2004. A comparison of N and C uptake during brown tide (*Aureococcus anophagefferens*) blooms from two coastal bays on the east coast of the USA. Harmful Algae 3:361-376

Naber, E. C. and A. J. Bermudez. 1990. Poultry manure management and utilization problems and opportunities. Ohio State University Extension Bulletin 804. http://ohioline.osu.edu/b804/index.html

National Estuarine Research Reserve System. 2007. The National Estuarine Research Reserve's System-Wide Monitoring Program (SWMP): A scientific framework and plan for detection of short-term variability and long-term change in estuaries and coastal habitats of the United States (2002; Revised 2007). 50 pp.

Navarro-Angulo, L. and D. Robledo. 1999. Effects of nitrogen source, N:P ratio and N-pulse concentration and frequency on the growth of *Gracilaria cornea* (Gracilariales, Rhodophyta) in culture. Hydrobiologia 398/399: 315-320

Newell R.I.E. 1988. Ecological changes in Chesapeake Bay: Are they the result of overharvesting the Eastern oyster (*Crassostrea virginica*)? *In:* Lynch M.P., E.C. Krome (eds.) Understanding the estuary: advances in Chesapeake Bay research. Chesapeake Research Consortium Publication 129 (CBP/TRS 24/88). Gloucester

Point, VA, p 536–546

Newell, R. I. E. and C. J. Langdon. 1996. Mechanism and Physiology of Larval and Adult Feeding, p. 185-223. *In* Kennedy, V.S., Newell, R.I.E. and Eble, A.F. (eds.) The Eastern Oyster *Crassostrea virginica*. Maryland Sea Grant.

Newell, R.I.E., Alspach, G.S., Kennedy, V.S., and Jacobs, D. 2000. Mortality of newly metamorphosed eastern oysters (Crassostrea virginica) in mesohaline Chesapeake Bay. Marine Biology. 136, 665-676.

Nixon S.W., J.W. Ammerman, L.P. Atkinson, V.M. Berounsky, G. Billen, W.C. Boicourt, W.R. Boynton, T.M. Church, D.M. Ditoro, R. Elmgren, J.H. Garber, A.E. Giblin, R.A. Jahnke, N.J.P. Owens, M.E.Q. Pilson, S.P. Seitzinger. 1996. The fate of nitrogen and phosphorus at the land sea margin of the North Atlantic Ocean. Biogeochemistry. 35: 141-180.

Nixon, S. W. 1995. Coastal marine eutrophication: a definition, social causes, and future concerns. Ophelia 41:199-219

Nixon, S. W., B. Buckley, S. Granger, and J. Bintz. 2001. Responses of very shallow marine ecosystems to nutrient enrichment. Human and Ecological Risk Assessment 75:1457-1481

O'Connor, T.P. 2002. National distribution of chemical concentrations in mussels and oysters in the US. Marine Environmental Research. 53: 117-143

O'Connor, T.P. and Lauenstein, G.G. 2006. Trends in chemical concentrations in mussels

and oysters collected along the US coast: Update to 2003. Marine Environmental Research. 62: 261-285

Oczkowski, A., S. Nixon, K. Henry, P. DiMilla, M. Pilson, S. Granger, B. Buckley, C. Thornber, R. McKinney, J. Chaves. 2008. Distribution and trophic importance of anthropogenic nitrogen in Narragansett Bay: an assessment using stable isotopes. Estuaries and Coasts. 31:53-69

Officer, C. B. 1980. Box models revisited. In P. Hamilton and R. B. Macdonald (eds.) Estuarine and Wetland Processes. Marine Science Series. Vol 11. New York: Plenum Press.

Ogrinc, N., G. Fontolan, J. Faganeli, and S. Covelli. 2005. Carbon and nitrogen isotope compositions of organic matter in coastal marine sediments (the Gulf of Trieste, N Adriatic Sea): indicators of sources and preservation. Marine Chemistry 95: 163–181

Orth, R.J., K.A. Moore. 1987. Submerged aquatic vegetation in Delaware's inland bays. *In:* Sellner, K. (ed.), Phytoplankton, Nutrients, Macroalgae, and Submerged Aquatic Vegetation in Delaware Inland Bays, 1985–1986. Final Report. Academy of Natural Sciences to the Delaware Department of Natural Resources, pp. 86–109.

Paerl, H. W. 1995. Coastal eutrophication in relation to atmospheric nitrogen deposition: current perspectives. Ophelia 41:237-259.

Paerl, H.W. 1997. Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient

sources. Limnology & Oceanography. 42: 1154-1165

Parker, D. and Q. Li. 2006. Poultry litter use and transport in Caroline, Queen Anne's Somerset and Wicomico Counties in Maryland: A Summary Report. University of Maryland, College Park: Agricultural and Resource Economics.

Pasquaud, S., J. Lobry, P. Elie. 2007. Facing the necessity of describing estuarine ecosystems: a review of food web ecology study techniques. Hydrobiologia 588:159–172 DOI 10.1007/s10750-007-0660-3

Paynter, K.T., and L. DiMichele. 1990. Growth of tray-cultured oyster (*Crassostrea virginica* Gmelin), in Chesapeake Bay. Aquaculture 87:289-297.

Pihl, L., S.P. Baden, and R.J. Diaz. 1991. Effects of periodic hypoxia on distribution of demersal fish and crustaceans. Marine Biology 108:349-360.

Piola, R. F., S. K. Moore, and I. M. Suthers. 2006. Carbon and nitrogen stable isotope analysis of three types of oyster tissue in an impacted estuary. Estuarine Coastal and Shelf Science 66:255-266

Powell, E.N., and Ashton-Alcox, K.A., 2004. A comparison between a suction dredge and a traditional oyster dredge in the transplantation of oysters in Delaware Bay. Journal of Shellfish Research. 23, 803-823.

Pritchard D.W. 1956. The dynamic structure of a coastal plain estuary. Journal of Marine Research 15:33–42

Pritchard D.W. 1967. Observations of circulation in coastal plain estuaries. *In:* Lauff G.H.

(*ed.*) Estuaries. American Association for the Advancement of Science, Washington, DC, p 37–44

Pritchard, D. W. 1960. Salt balance and exchange rate for Chincoteague Bay. Chesapeake Science 1:48-57

Pritchard, D. W. 1969. Dispersion and flushing of pollutants in estuaries. American Society of Civil Engineers. Journal of the Hydraulics Division. 95(HYI): 115-124

Pritchard, D.W. 1952. Salinity distribution and circulation in the Chesapeake Bay estuarine system. Journal of Marine Research. 11:106-123.

Ritter, M.C., and P.A. Montagna. 1999. Seasonal hypoxia and models of benthic response in a Texas bay. Estuaries 22:7-20.

Rothschild B.J., J.S. Ault, P. Goulletquer, M. Heral. 1994. Decline of the Chesapeake Bay oyster population: a century of habitat destruction and overfishing. Marine Ecology Progress Series 111:29 – 39

Sakano, H., Fujiwara, E., Nohara, S. and Ueda, H., 2005. Estimation of nitrogen stable isotope turnover rate of Oncorhynchus nerka. Environmental Biology of Fishes. 72, 13-18.

Scala, D.J. and L.J. Kerkhof. 2000. Horizontal heterogeneity of denitrifying bacterial communities in marine sediments by terminal restriction fragment length polymorphism analysis. Applied and Environmental Microbiology. 66: 1980-1986

Schlacher T.A., T.J.B. Carruthers, W.C. Dennison, J. Pocock, M. Katouli. 2001. Maroochy

Mooloolah loads and impacts study. WBM- Oceanics, Brisbane

Schlacher, T.A., B. Liddell, T.F. Gaston, M. Schlacher-Hoenlinger. 2005. Fish track wastewater pollution to estuaries. Oecologia. 144: 570–584.

Schlesinger, W.H. 1997. Biogeochemistry. 2nd edition. Academic Press. 588 pp.

Schmidt J. P., C. J. Dell, P. A. Vadas, and A. L. Allen. 2007. Nitrogen export from coastal plain field ditches. Journal of Soil and Water Conservation. 62:235-243

Schwartz, M.C. 2003. Significant groundwater input to a coastal plain estuary: Assessment from excess radon. Estuarine Coastal and Shelf Science 56:31–42. doi:10.1016/S0272-7714(02)00118-X.

Sebilo, M. G. Billen, M. Grably, and A. Mariotti. 2003. Isotopic composition of nitrate-nitrogen as a marker of riparian and benthic denitrification at the scale of the whole Seine River system. Biogeochemistry. 63: 35–51

Seitzinger, S. P. R. W. Sanders, and R. V. Styles. 2002. Bioavailability of DON from natural and anthropogenic sources to estuarine plankton. Limnology and Oceanography 47:353-366

Seitzinger, S. P., and R. W. Sanders. 1999. Atmospheric inputs of dissolved organic nitrogen stimulate estuarine bacteria and phytoplankton. Limnology and Oceanography 44:721-730

Seitzinger, S., J. A. Harrison, J. K. Böhlke, A. F. Bouman, R. Lowrance, B. Peterson, C. Tobias, and G. Van Drecht. 2006. Denitrification across landscapes and

waterscapes: a synthesis. Ecological Applications 16:2064-2090

Seitzinger, S.P., L.P. Nielson, J. Caffrey, P.B. Christensen. 1993. Denitrification measurements in aquatic sediments: A comparison of three methods. Biogeochernistry. 23: 147-167

Shearer, G. and Kohl, D.H., 1988. Nitrogen Isotopic Fractionation and O-18 Exchange in Relation to the Mechanism of Denitrification of Nitrite by Pseudomonas stutzeri. Journal of Biological Chemistry. 263, 13231-13245.

Smil, V. 2000. Enriching the Earth: Fritz Haber, Carl Bosch, and the transformation of world food production. MIT Press. 358 pp.

Smith G.F., E.B. Roach, D.G. Bruce. 2003. The location, composition, and origin of oyster bars in mesohaline Chesapeake Bay. Estuarine & Coastal Shelf Science 56:391–409

Smith, G.F., Bruce, D.G., Roach, E.B., Hansen, A., Newell, R.T.E. and McManus, A.M., 2005. Assessment of recent habitat conditions of eastern oyster Crassostrea virginica bars in mesohaline Chesapeake Bay. North American Journal of Fisheries Management. 25, 1569-1590.

Smith, M.E., and D. M. Dauer. 1994. Eutrophication and macrobenthic communities of the lower Chesapeake Bay: I. Acute effects of low dissolved oxygen in the Rappahannock River. Pages 76-84 *in* P. Hill and S. Nelson, editors. Toward a sustainable watershed: the Chesapeake experiment. Proceedings of the 1994 Chesapeake Research Conference, Chesapeake Research Consortium Publication Number 149. Norfolk, Virginia, USA.

Smith, S. V., J. T. Hollibaugh, S. J. Dollar, and S. Vink. 1989. Tomales Bay, California: a case for carbon-controlled nitrogen cycling. Limnology and Oceanography 34(1): 37-52.

Souza, S., B. Krinsky, and J. Seibel. 1993. Maryland's Coastal Bays: An assessment of aquatic ecosystems, pollutant loadings, and management options. Maryland Department of the Environment, Baltimore, Maryland

Stevenson, J.C., L.W. Staver, and K.W. Staver. 1993. Water quality associated with survival of submersed aquatic vegetation along an estuarine gradient. Estuaries 16:346-361.

Stribling, J.M. and J.C. Cornwell. 1997. Identification of Important Primary Producers in a Chesapeake Bay Tidal Creek System Using Stable Isotopes of Carbon and Sulfur. Estuaries. 20:77-85.

Strickland, J. D. H. and Parsons, T. R. 1972. A Practical Handbook of Seawater Analysis; Alger Press: Ottawa, Canada

Suter, G.W., (ed.). 1993. Ecological risk assessment. Lewis, Chelsea, Michigan, USA.

Sweeny, R.E., I.R. Kaplan. 1980. Tracing flocculent industrial and domestic sewage transport on San Pedro Shelf, southern California, by nitrogen and sulphur isotope ratios. Marine Environmental Research 3, 215–224.

Sweeting, C.J., Jennings, S., and Polunin, N.V.C. 2005. Variance in isotopic signatures as a descriptor of tissue turnover and degree of omnivory. Functional Ecology 19: 777-

784

Technicon Industrial Method No. 158-71 W/A† Tentative. 1977. Technical Industrial Systems. Tarrytown, New York, 10591.

Technicon Industrial Method No. 804-86T. August 1986. Technicon Industrial Systems. Tarrytown, New York, 10591.

Technicon Industrial Method No. 818-87T. February 1987. Technicon Industrial Systems. Tarrytown, New York, 10591.

Testa, J. M., W. M. Kemp, W. R. Boynton, and J. D. Hagy. 2008. Long-term changes in water quality and productivity in the Patuxent River estuary: 1985 to 2003. Estuaries and Coasts 31: 1021-1037

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G. and Slade, N.A., 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for del13C analysis of diet. Oecologia. 57, 32-37.

Tucker, J., N. Sheats, A. E. Giblin, C. S. Hopkinson, and J. P. Montoya. 1999. Using stable isotopes to trace sewage-derived material through Boston Harbor and Massachusetts Bay. Marine Environmental Research 48:353-375

Turner, R. E. and N. N. Rabalais. 2003. Linking landscape and water quality in the Mississippi River Basin for 200 years. BioScience 53:563-572

Tyler R.M. 2007. Effects of coverage by benthic seaweed mats on (northern quahog = hard clam) *Mercenaria mercenaria* in a eutrophic estuary. Journal of Shellfish Research

26:1021-1028

U.S. Census Bureau. 2000. Summary File 1 (SF 1) and Summary File 3 (SF 3).

Udy, J. W., and Dennison, W. C., 1997. Physiological responses of seagrasses used to identify anthropogenic nutrient inputs. Marine and freshwater research 48, 605-614

Ullman, W. J., A. S. Andres, J. R. Scudlark, and K. B. Savidge. 2002. Storm-water and base-flow sampling and analysis in the Delaware Inland Bays: preliminary report of findings 1998-2000: Open File Report No. 44, Delaware Geological Survey, Newark DE. 44p.

Ullman, W.J., B. Chang, D.C. Miller and J.A. Madsen, 2003. Groundwater mixing, nutrient diagenesis, and discharges across a sandy beachface, Cape Henlopen, Delaware (USA). Estuarine, Coastal, and Shelf Science 57:539-552.

US EPA. 1979a. Method No. 160.2 (with slight modification) in Methods for chemical analysis of water and wastes. United States Environmental Protection Agency, Office of Research and Development. Cincinnati, Ohio. Report No. EPA-600/4-79-020 March 1979. 460pp.

US EPA. 1979b. Method No. 365.2 in Methods for chemical analysis of water and wastes. United States Environmental Protection Agency, Office of Research and Development. Cincinnati, Ohio. Report No. EPA-600/4-79-020 March 1979. 460pp.

US EPA. 2002. Onsite Wastewater Treatment Systems Manual. EPA/625/R-00/008 http://www.epa.gov/nrmrl/pubs/625r00008/html/625R00008.htm

USDA. 2002. Census of Agriculture. Available online. http://www.nass.gov/Census/Create_Census_US_CNTY.jsp. Accessed 7 July 2008

USDA. 2005. FSA Aerial Photography Field Office. National Agriculture Imagery Program. Salt Lake City, Utah.

Valderrama, J.C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Marine Chemistry 10: 109-122.

Valdes-Murtha, L.M. 1997. Analysis of critical habitat requirements for restoration and growth of submerged vascular plants in the Delaware and Maryland coastal bays. Thesis. Marine Studies, University of Delaware, Newark, Delaware, USA.

Valiela, I. 1995. Marine Ecological Processes. 2nd edition. Springer Science+Business Media, Inc. New York, New York.

Valiela, I., J. Costa, K. Foreman, J. M. Teal, B. Howes, and D. Aubrey. 1990. Transport of groundwater-borne nutrients from watersheds and their effects on coastal waters. Biodegradation 10:177-197.

Van Beusekom, J. E. E., and V. N. de Jonge. 2002. Long-term changes in Wadden Sea nutrient cycles: importance of organic matter import from the North Sea. Hydrobiologia 475: 185-194

Vander Zanden, M. J., Y. Vadeboncoeur, M. W. Diebel and E. Jeppesen. 2005. Primary consumer stable nitrogen isotopes as indicators of nutrient source. Environmental

Science and Technology 39:7509-7515.

Vanderklift, M.A. and S. Ponsard. 2003. Sources of variation in consumer-diet $\delta^{15}N$ enrichment: a meta-analysis. Oecologia 136:169–182 DOI 10.1007/s00442-003-1270-z

Ventura, M. and Catalan, J., 2008. Incorporating life histories and diet quality in stable isotope interpretations of crustacean zooplankton. Freshwater Biology. 53, 1453-1469.

Virginia Department of Forestry. 2005. Virginia Land Use 2005. GIS Data. http://www.dof.virginia.gov/gis/datadownload.shtml

Vitoria, L., N. Otero, A. Soler, A. Canals. 2004. Fertilizer characterization: isotopic data (N, S, O, C, and Sr). Environmental Science and Technology 38: 3254–3262.

Vitousek, P.M., J.D. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger, and G.D. Tilman. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications. 7: 737-750

Volk, J.A., K.B. Savidge, J.R. Scudlark, A.S. Andres, and W.J. Ullman, 2006. Nitrogen loads through baseflow, stormflow, and underflow form the watershed to Rehoboth Bay, Delaware. Journal of Environmental Quality 35:1742-1755

Wada, E., T. Kadonaga, S. Matsuo. 1975. ^{15}N abundance in nitrogen of naturally occurring substances and global assessment of denitrification from isotopic viewpoint. Geochemical Journal. 9: 139–148.

Ward, L. G., M. S. Kearney, J. C. Stevenson. 1998. Variations in sedimentary environments and accretionary patters in estuarine marshes undergoing rapid submergence, Chesapeake Bay. Marine Geology. 151: 111-134

Waser, N.A.D., Harrison, P.J., Nielsen, B., Calvert, S.E. 1998. Nitrogen isotope fractionation during the uptake and assimilation of nitrate, nitrite, ammonium, and urea by a marine diatom. Limnology and Oceanography 43, 215-224.

Wazniak, C. E., M. R. Hall, T. J. B. Carruthers, B. Sturgis, W. C. Dennison, and R.J. Orth. 2007. Linking water quality to living resources in a Mid-Atlantic lagoon system, USA. Ecological Applications 17(5) Supplement:S64-S78

Wazniak, C. E., P. M. Glibert. 2004. Potential impacts of brown tide, *Aurecoccus anophagefferens*, on juvenile hard clams, *Mercenaria mercenaria*, in the coastal bays of Maryland, USA. Harmful Algae 3:321–329.

Wazniak, T., W. Boicourt, and E. North. 2009. Residence Times of Small Chesapeake Bay Tributaries. University of Maryland Center for Environmental Science, Horn Point Laboratory. 28 pp. July 16, 2009

Wellman, R.P., F.D. Cook, H.R. Krouse. 1968. Nitrogen-15 – Microbiological alteration of abundance. Science. 161: 269

Wenner, E.L. and M. Geist. 2001. The National Estuarine Research Reserves program to monitor and preserve estuarine waters. Coastal Management. 29: 1-17.

Weston, K., T. D. Jickells, L. Fernand, and E. R. Parker. 2004. Nitrogen cycling in the

southern North Sea: consequences for total nitrogen transport. Estuarine Coastal and Shelf Science. 59:559-573

Wicks, E.C., Longstaff, B.J., Fertig, B., and Dennison, W.C. 2010. Ecological Indicators: Assessing ecosystem health using metrics. In: Longstaff, B.J., Carruthers, T.J.B., Dennison, W.C., Lookingbill, T.R., Hawkey, J.M., Thomas, J.E., Wicks, E.C., and Woerner, J. (eds.) Integrating and Applying Science: A practical handbook for effective coastal ecosystem assessment. IAN Press, Cambridge, MD, p 61-77

Wiegner, T. N., S. P. Seitzinger, P. M. Glibert, and D. A. Bronk. 2006. Bioavailability of dissolved organic nitrogen and carbon from nine rivers in the eastern United States. Aquatic Microbial Ecology 43:277-287

Williams M, B. Longstaff, C. Buchanan, R. Llanso, W. Dennison. 2009. Development and evaluation of a spatially-explicit index of Chesapeake Bay health. Marine Pollution Bulletin. 59: 14-25

Winter, T.C., D.O. Rosenberry, and, J.W. LaBaugh. 2003. Where does ground water in small watersheds come from? Ground Water 41, no. 7: 989-1000.

Witting, D.A., Chambers, R.C., Bosley, K.L., Wainright, S.C. 2004. Experimental evaluation of ontogenetic diet transitions in summer flounder (Paralichthys dentatus), using stable isotopes as diet tracers. Canadian Journal of Fisheries and Aquatic Sciences. 61: 2069-2084

Woli, K. P., T. Nagumo, R. Hatano. 2002. Evaluating impact of land use and N budgets on stream water quality in Hokkaido, Japan. Nutrient Cycling in Agroecosystems.

63:175-184

York, J.K., Tomasky, G., Valiela, I. and Repeta, D.J., 2007. Stable isotopic detection of ammonium and nitrate assimilation by phytoplankton in the Waquoit Bay estuarine system. Limnology and Oceanography. 52, 144-155.

Zhang, L., M. A. Altabet, T. Wu, and O. Hadas. 2007. Sensitive measurement of NH4+ 15N/14N (δ15NH4+) at natural abundance levels in fresh and saltwaters. Analytical Chemistry 79: 5297-5303

Zimmerman A.R., E.A. Canuel. 2000. A geochemical record of eutrophication and anoxia in Chesapeake Bay sediments: anthropogenic influence on organic matter composition. Marine Chemistry 69:117–137