

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.

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Monie Bay component of Chesapeake Bay, MD National Estuarine Research Reserve

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Abstract

Deleterious effects of nitrogen loading from anthropogenic sources, e.g. fertilizer and manure runoff as well as sewage effluents, are well documented in Chesapeake Bay. The Monie Bay component of the Chesapeake Bay National Estuarine Research Reserve in Maryland acts as a 'natural laboratory' with respect to land use. This characteristic enables testing oyster $\delta^{15}\text{N}$ to infer nitrogen sources from septic systems, poultry manure, and agricultural applications. Water quality monitoring was conducted in 2008 and compared to long-term water quality monitoring in the adjacent Tangier Sound. Ecosystem health, as described by a Water Quality Index declined in the long-term, and current conditions suggest that Monie Bay is not pristine. Deployed oysters were analyzed for $\delta^{15}\text{N}$ to infer nitrogen sources, and were compared to oysters throughout Chesapeake Bay to provide context for Monie Bay. From oyster $\delta^{15}\text{N}$, human and animal wastes were inferred to enter Monie Bay from terrestrial sources to Monie Creek and from either Wicomico River or Tangier Sound to the mouth of Monie Bay. Potential nitrogen sources include the effective year-round population of ~450,000 chickens in Somerset County which produced $\sim 8.6 \times 10^5$ kg total nitrogen (untreated) yr^{-1} , roughly equivalent to that defecated by 2.0×10^5 people. Compared to Chesapeake Bay, oyster $\delta^{15}\text{N}$ values in Monie Bay were moderate, suggesting this region is well flushed. STELLA modeling described oyster isotope cycling, and results suggested deploying oysters for four months was sufficient for successful application of oyster $\delta^{15}\text{N}$ to identify nitrogen sources. Oyster $\delta^{15}\text{N}$ information can complement water quality monitoring programs as part of an ensemble of data to assess the health of ecosystems such as the National Estuarine Research Reserve System.

Keywords: water quality monitoring, long-term trends, nitrogen source, land use, eastern oyster, $\delta^{15}\text{N}$, Monie Bay, Chesapeake Bay, spatial analysis

Introduction

Chesapeake Bay water quality has declined due to excessive nutrient loading, as has been well documented (Kemp et al., 2005). Since pre-colonial times, annual terrestrial nitrogen inputs to Chesapeake Bay and its tributaries increased six- to eight-fold (Boynton et al. 1995). Almost half of the total nutrient loads to the Patuxent River and 60-70% of the total nitrogen and phosphorous loads to the mainstream Chesapeake Bay and the Potomac River are derived from non-point sources (Boynton et al. 1995). Once nutrients have entered aquatic ecosystems, it is difficult to distinguish between non-point sources such as agricultural fertilizers and point-sources such as septic systems or wastewater treatment plants. Long-term water quality monitoring datasets are critical to identifying areas most impacted by excess nutrients. This report builds upon previous water quality monitoring in the Monie Bay component of the Maryland Chesapeake Bay National Estuarine Research Reserve.

Monie Bay, Chesapeake Bay National Estuarine Research Reserve

Land use in Somerset County is largely rural, with areas of intensive poultry feeding operations in addition to large tracts of corn, soy, and pine forest. Overall, the Monie Bay watershed is ~30% farmland, ~42% forests, and ~28% undeveloped wetlands (Figure 1). Tree farms, mainly loblolly pine, are also abundant in this county (~60,000 acres are owned by the state and managed privately), but are generally not fertilized (due to economic constraints, Fykes, pers. com.). Tree farm plots are initially grown out 'naturally', (growth or species selection are not controlled) for 15 - 20 years. Plots are then 'thinned', where brush, junk, and young pines are selectively felled for

use as pulp. At this time selective herbicides are applied to remove everything but rows of loblolly pine. Anecdotal evidence suggests that wildlife populations, including deer and turkeys, increase at this stage. The rows of loblolly pine are grown for an additional ~ 20 years before clear-cut harvesting for lumber and starting at the beginning of the cycle again.

Both state and federal governments are significant landowners, as nearly 15% of the land is designated as recreation or wildlife management areas. One such area is Monie Bay, part of the National Estuarine Research Reserve System. This sub-estuary of Chesapeake Bay is a tributary of Tangier Sound and can serve as a natural laboratory to link land use to aquatic processes and downstream water quality. The Monie Bay ecosystem includes three tidal creeks differing only by the surrounding land use. Such land use configuration allows direct comparisons of nitrogen sources from poultry farm runoff (Little Monie Creek), crop agriculture (Monie Creek, near the border of the estuarine reserve), and a sub-watershed dominated by wetlands and forests (Little Creek, used as a reference). Water quality monitoring also extended into portions of the Wicomico River. Water from both Monie Bay and Wicomico River meet at their mouths. Effluents from waste water treatment plants near the towns of Salisbury, Delmar, and Fruitland are discharged into Wicomico River. Local land uses along each of Monie Bay's creeks has previously been linked to the aquatic ecology of the ecosystem, driving intra- and inter-creek environmental gradients in salinity, nutrients, and dissolved organic matter quality and quantity (Apple et al. 2004).

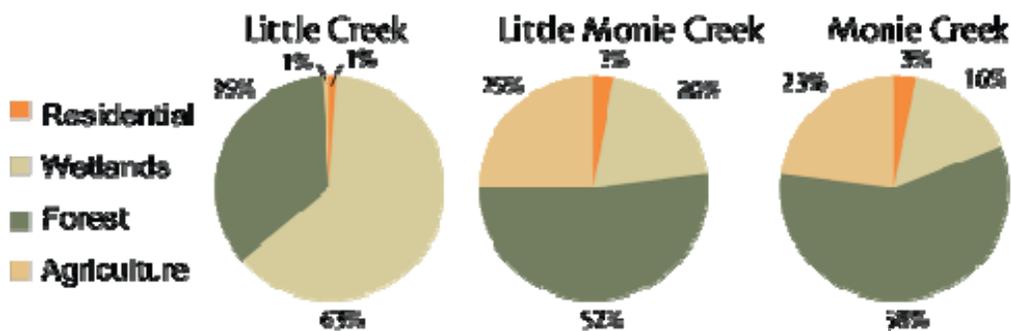
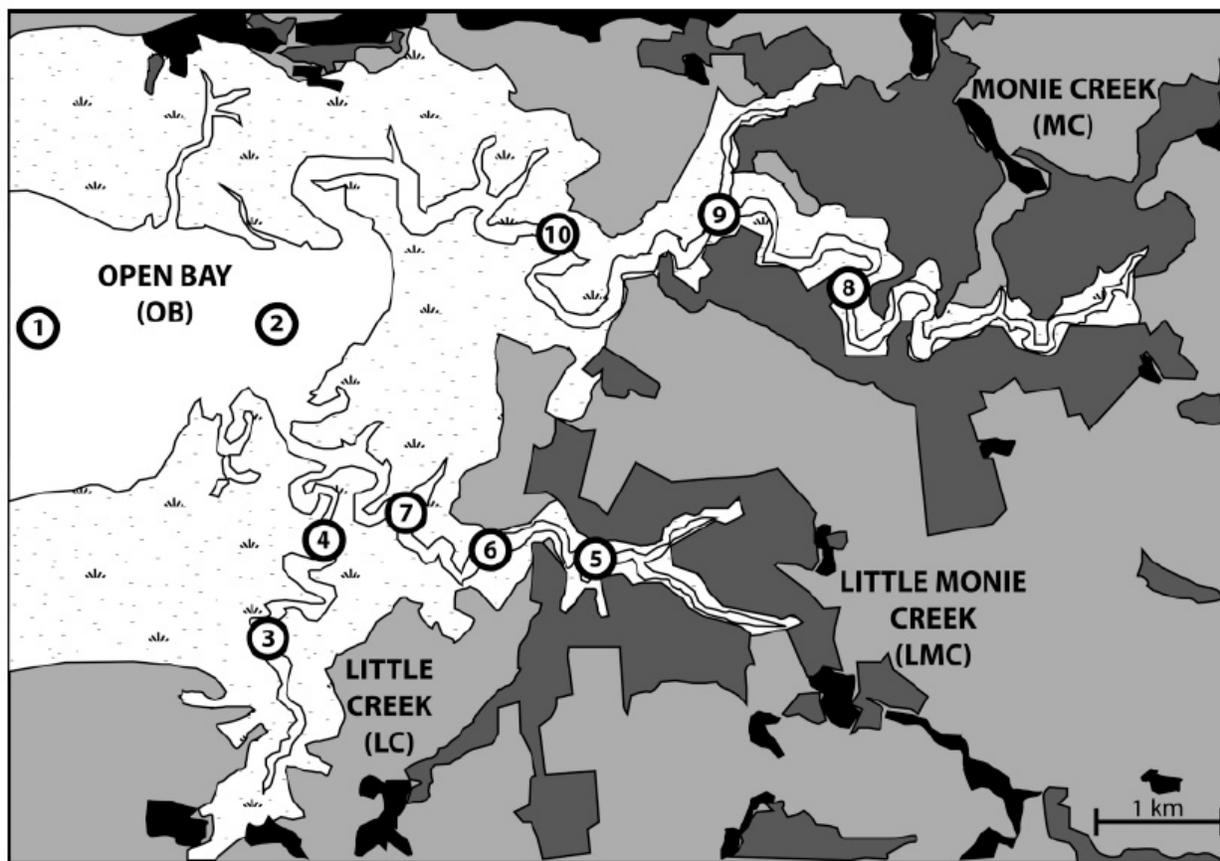


Figure 1. Map of Monie Bay component of the Chesapeake Bay National Estuarine Research Reserve in Maryland, a tributary of Tangier Sound, off of Chesapeake Bay. Land use surrounding each of Monie Bay's three tributary creeks is charted below. Monitoring stations are noted. (Apple et al. 2004)

Monie Bay's watershed lies within Somerset County, Maryland. Somerset County is home to 24,747 people, which roughly 9 acres for every person or 0.12 people/acre or 0.30 people/hectare (Figures 2a, 2b. US Department of Commerce, 2001). Due to this low population density, there are few septic systems within the watershed. Meanwhile, the nearby Lower Wicomico River watershed contains the urban center of Salisbury, and contains over twice the population density and a corresponding order of magnitude more septic systems, as well as three wastewater treatment plants, which are absent in Monie Bay watershed. Nitrogen discharge loads in 2005 from the Salisbury, Delmar, and Fruitland, wastewater treatment plants were near 500,000 lbs N year⁻¹, 20,000 lbs N year⁻¹, and 10,000 lbs N year⁻¹ respectively (Figures 3a, 3b, 3c Maryland Department of Environment, 2007a,b,c). Overall, relatively low levels of nitrogen loading from septic and wastewater treatment plants within the Monie Bay watershed potentially allow direct identification of nitrogen source in each creek.

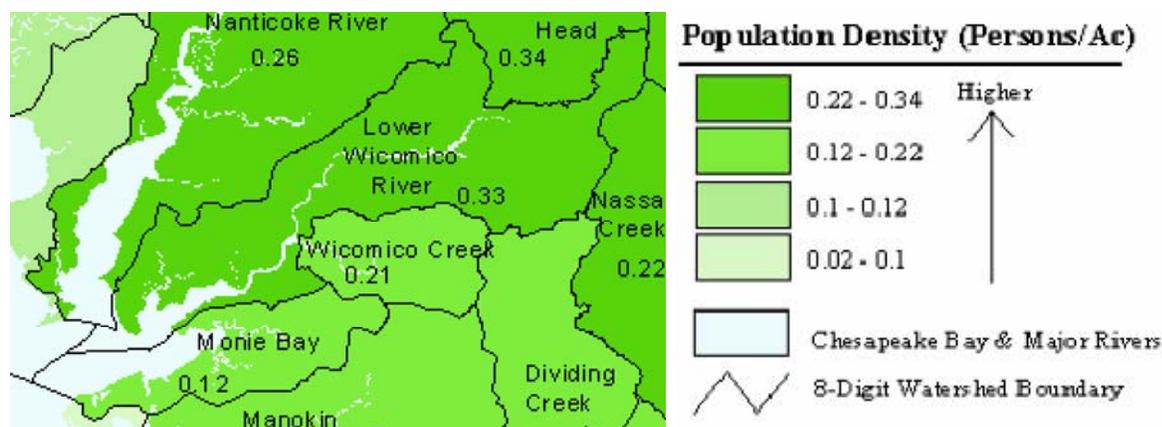


Figure 2a: Population density in Monie Bay watershed and surrounding watersheds.

While generally rural, Monie Bay watershed in Somerset County has very low population density, particularly as compared to nearby Lower Wicomico River watershed, which contains the towns of Salisbury and Fruitland. (Modified from Maryland Department of Natural Resources, 1999a)

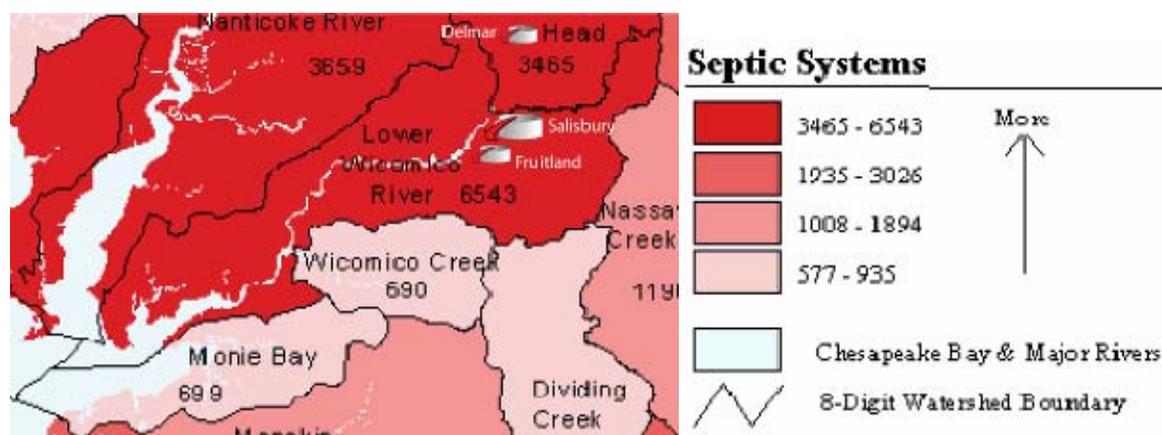


Figure 2b: Septic systems and wastewater treatment plants in Monie Bay watershed and surrounding watersheds. Due to the rural nature of the Monie Bay watershed, there are relatively few septic systems in place. The four sub-watersheds of the Wicomico River have an order of magnitude more septic systems, as well as three sewage treatment plants. (Modified from Maryland Department of Natural Resources, 1999b)

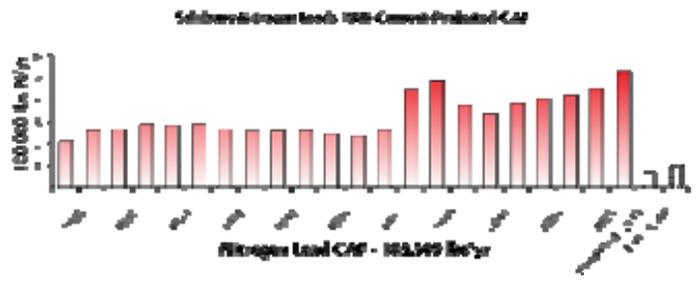


Figure 3a: Historical and projected nitrogen loads in 100,000 lbs N year⁻¹ to Wicomico River from Salisbury Wastewater Treatment Plant (Modified from Maryland Department of the Environment, 2007a).

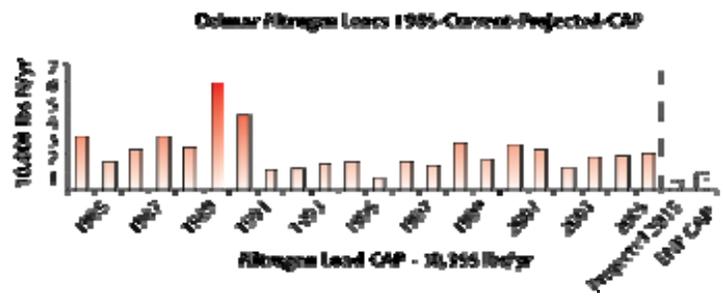


Figure 3b: Historical and projected nitrogen loads in 10,000 lbs N year⁻¹ to Wicomico River from Delmar Wastewater Treatment Plant (Modified from Maryland Department of the Environment, 2007b).

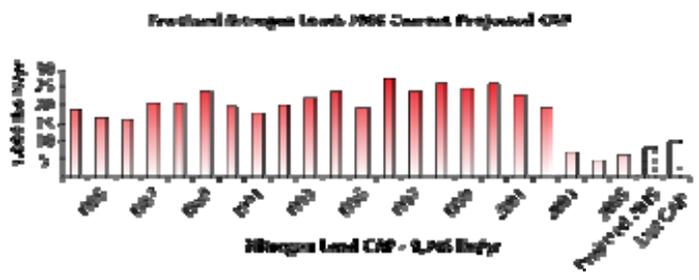


Figure 3c: Historical and projected nitrogen loads in 1,000 lbs N year⁻¹ to Wicomico River from Fruitland Wastewater Treatment Plant (Modified from Maryland Department of the Environment, 2007c).

Dominant marsh plants in Monie include *Spartina alterniflora*, *S. patens*, and *Juncus roemerianus*, in total comprised of 5-8 plant species and diversity tending to be higher around Little Creek and biomass greater around Monie Creek (Jones et al. 1997, Stribling and Cornwell 1997). Previous carbon and sulfur stable isotope studies indicate these marsh plants substantially contribute to consumers' diets (with potential temporal variation), with a balance between C4 (e.g. *Spartina alterniflora*) and C3 (e.g. *J. roemerianus*) marsh plants, phytoplankton and benthic algae (Stribling and Cornwell 1997). Consumers and macrobenthic communities include tubificid oligochaetes *Tubificoides heterochaetus* and *T. brownae*, the aorid amphipod *Leptocheirus plumulosus*, the tellinid bivalve *Macoma balthica* and the venerid bivalve *Gemma gemma*, ostracods, and nemerteans, as well as the polychaetes *Glycera dibranchiate* and *Marenzelleria viridis* (Kemp, 2006). Blue crabs, *Callinectes sapidus*, and mud crabs *Panopeus herbstii* have also been observed on occasion (personal observation).

Marsh accretion is delicately balanced with sea-level rise (Stevenson et al. 1988), with average vertical accretion rates 3.0 mm y^{-1} for the last 200 years (Kearney et al. 1994), but ultimately the marsh may be susceptible to long-term erosional forces. Monie Bay marshes are comprised of three sedimentary environments: 1) high wave energy bay bank marshes, 2) low energy tidal channel bank deposits, and 3) organic rich fine-grained black marsh sediments (Ward et al. 1988). Porewater NH_4^+ and PO_4^- concentration profiles seasonally follow plant growth patterns and are generally higher in agriculturally influenced marshes (Cornwell et al 1994, Stribling and Cornwell 2001). Sediment accretion rates and nutrient contents suggest that Monie tidal marshes may serve as sinks for N and P burial (Zelenke and Cornwell 1996), trapping 35% of nitrogen

and 81% of phosphorus inputs from the surrounding watershed. An additional 10% may be removed from the estuary via denitrification, a biogeochemical transformation, which has been measured (with high seasonal variability) at $\sim 60 \mu\text{mol N m}^{-2} \text{ h}^{-1}$ in sediments (Merrill and Cornwell, 2000). Due to this ecosystem service of nitrogen removal, Monie Bay and its tidal marsh creeks may be excellent locations to study nitrogen source with biological indicators.

Previous water quality monitoring

Water quality monitoring in 1994-1995, 2000-2002, and 2006-2007 indicated that Little Monie Creek and Little Creek were similar with respect to salinity, temperature, and water volume, while spring flows freshened the upper portions of Little Monie Creek as compared to Little Creek. Nutrient concentrations declined from the upper reaches downstream due to dilution and biogeochemical processing (Apple et al. 2004; Fertig et al 2007). Little Creek had lower nutrient and phytoplankton chlorophyll *a* concentrations due to differences in their watersheds. Little Monie Creek and Monie Creek were higher in total suspended solids, dissolved inorganic nitrogen, and dissolved inorganic phosphorus and chlorophyll *a* than Little Creek. Generally, nutrient concentrations were highest in Monie Creek, with Little Monie Creek slightly lower concentrations and Little Creek had much lower concentrations. Agricultural runoff has been implicated in nearly doubling total nitrogen and total phosphorous in Little Monie Creek. Nitrate concentrations peaked ($\sim 50 \mu\text{M}$) in February, declined by April, and were extremely low the rest of the year. The nitrogen concentrations were highest in Little Monie Creek, then Monie Creek and did not vary temporally in Little Creek. Ammonium peaked in

December and March in all creeks, but was low the rest of the year (Jones et al. 1997). Salinity varied temporally, being lowest until early spring and highest in summer and fall, and spatially in that Monie Creek was freshest.

Connecting monitoring, long-term, and broad-scale water quality datasets

To place Monie Bay water quality into the broader context of Chesapeake Bay, long-term and broad-scale water quality datasets for Monie Bay and Chesapeake Bay were compared. Further, oyster $\delta^{15}\text{N}$ from Monie Bay were compared with broad-scale datasets. Specifically, the questions asked were 1) what links exist between $\delta^{15}\text{N}$ values from deployments and long-term datasets at Monie Bay? 2) How does oyster $\delta^{15}\text{N}$ in Monie Bay relate to broad-scale water quality datasets? 3) How does Monie Bay fit into the context of Chesapeake Bay for oyster $\delta^{15}\text{N}$, water quality, and their respective relationships? My primary hypotheses are: 1) Oyster $\delta^{15}\text{N}$ values from Monie Bay relate to overall long-term nutrient and water quality trends 2) Broad-scale spatial patterns of oyster $\delta^{15}\text{N}$ will be found, and that these patterns will relate to broad patterns of water quality. 3) Monie Bay water quality, though moderate over 2006-7, is better on average than that in the Chesapeake and that oyster $\delta^{15}\text{N}$ values are lower than those found in Chesapeake Bay overall. Cutting across datasets extends the utility of the oyster as a biological indicator, and augments existing water quality monitoring programs.

Materials and Methods

Water Quality Monitoring

Fortnightly, a suite of hydrographic conditions (e.g. salinity, temperature, dissolved oxygen concentration and saturation, pH, and conductivity) were monitored using a standard YSI field probe. Secchi depth was also measured using standard techniques. Water samples were collected for total nitrogen and total phosphorus (10 mL), and were filtered (approximately 4 mL) for dissolved inorganic nitrogen and dissolved inorganic phosphorus. Filtering was also conducted in the field for chlorophyll (60 mL), total suspended solids and total volatile solids (300 mL) onto GF/F Watman filter paper (25 mm and 47 mm diameter, respectively). Chemical analyses for chlorophyll *a* were conducted at the Department of Health and Mental Hygiene, while all other samples were analyzed at Chesapeake Biological Laboratory.

Water Quality Index

Overall system health was assessed by combining several variables considered critical for seagrass and benthic communities into a single-value Water Quality Index (WQI) in a manner similar to Wazniak et al. (2007) and Fertig et al. (2006). Levels of total nitrogen (TN), total phosphorous (TP), chlorophyll *a*, and dissolved oxygen in the bottom layer were considered for assessment (Baden et al. 1990, Pihl et al. 1991, Pihl et al., 1992, Dennison et al. 1993, Stevenson et al. 1993, Smith and Dauer 1994, Valdes-Murtha 1997, Ritter and Montagna 1999, and Lea et al. 2003). For each parameter, if a site met the biologically relevant threshold value indicated in Table 1, it received a score of one; otherwise it received a score of zero. A single-value Water

Quality Index was calculated for each site within each creek and the Open Bay by averaging the resulting scores for each of the four parameters. A Water Quality Index of 1 would indicate all metrics were met, that of 0 would indicate none were, and an intermediate score would mean that some metrics were met. For example, on June 22, 2006 site MB1, in the open bay, had 0.76 mg N L^{-1} and so received a 0 for TN, had $0.0674 \text{ mg P L}^{-1}$ for TP and so received a 0, $12.0 \text{ } \mu\text{g L}^{-1}$ chlorophyll *a* earning it a 1, and 6.83 mg L^{-1} attaining a 1 for dissolved oxygen and an overall Water Quality Index score of 0.5.

Management Objective	Water Quality Indicator	Reference Value
Maintain seagrass	Chlorophyll <i>a</i>	Chl <i>a</i> < $15 \text{ } \mu\text{g L}^{-1}$
Maintain seagrass	Total phosphorus	TP < $1.2 \text{ } \mu\text{M}$ OR TP < 0.037 mg L^{-1}
Maintain seagrass	Total nitrogen	TN < $46 \text{ } \mu\text{M}$ OR TN < 0.65 mg L^{-1}
Maintain benthic communities	Bottom dissolved oxygen	DO > 5.0 mg L^{-1}

Table 1. Table of management objectives together with water quality

indicators and reference values to determine the status of the objectives

(Wazniak et al., 2007)

Long-term dataset analysis

Water quality monitoring conducted in 2008 was compared to long-term monitoring conducted in Tangier Sound as part of a larger, Chesapeake Bay monitoring program. Long-term monitoring data in Tangier Sound was downloaded from the Chesapeake Bay Program's Water Quality Monitoring Database (1983-2007). Tangier Sound, station EE3.1 ($38^{\circ} 11' 48.6744'' \text{ N}$ $75^{\circ} 58' 23.5416'' \text{ W}$) was selected because it

is the closest Chesapeake Bay Program monitoring station to Monie Bay. Total nitrogen, total phosphorus, chlorophyll a, and dissolved oxygen data were downloaded for analysis. A Water Quality Index for this station was calculated over time using the methods noted above. Dates with missing data points were removed to avoid bias in the Water Quality Index.

Deploying, sampling, and analyzing oyster biological indicators

Oysters were deployed at each of the ten stations to detect nitrogen source on 15 April 2008 and all oysters were collected on 13 October 2008 (Figure 1). At each station, a mesh (1.9 cm) bag, containing 10 oyster spat on shell, were anchored with three bricks and suspended (0.5 m) above the bottom to minimize sediment smothering by using a marked buoy. Fouling organisms, predators, and trapped sediments were removed from mesh bags as needed to maintain water flows throughout deployment.

At the end of deployment, oyster samples were collected and kept on ice in the field and frozen at the laboratory (-20° C) until processing. Five surviving oysters were dissected to recover individual adductor muscles. Tissues were thawed, rinsed, and oven dried (60 °C) for 48 h or until thoroughly dry. Dried oyster tissue was finely ground using a mortar and pestle. Sub-samples (1.0 ± 0.2 mg dry weight) were placed in tin capsules. These sub-samples were analyzed for nitrogen and carbon content ($\mu\text{g N}$ and $\mu\text{g C}$) and natural abundance of stable nitrogen isotopes ($\delta^{15}\text{N} = \left(\frac{^{15}\text{N}/^{14}\text{N}_{\text{sample}}}{^{15}\text{N}/^{14}\text{N}_{\text{standard}}} - 1 \right) \times 10^3$) at University of California Davis Stable Isotope Facility with a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Molecular %N was calculated. The

standard reference was atmospheric N₂ (air), with 0.3663 atom % ¹⁵N, defined as 0‰ (e.g. Fry 2006).

Oyster collection over a broad scale: Chesapeake Bay

Oysters grown throughout the Chesapeake Bay were sampled from the Chesapeake Bay Foundation (CBF) oyster gardening program. This program distributes *C. virginica* spat on shell to a network of citizens. Citizens cultured the oysters on private property for nine months until ultimate redistribution to reefs located throughout the Chesapeake Bay and its tributaries for oyster population restoration purposes. This study sampled oysters cultured in 121 of these locations from central collection sites before CBF redistributed the remainder of each garden to the restoration reefs. From each of the 121 selected oyster garden sites, 5 oysters were randomly sampled. Measurement of five individual oysters was previously determined to be the optimal trade-off between error and effort (Fertig et al. 2007). After collection, oysters were handled and prepared as described above for re-collected oysters in Monie Bay.

STELLA modeling of required exposure time

Oyster isotope physiology can be expressed in a model as a set of differential equations. Required exposure times were modeled using equations constructed using STELLA Research software package (STELLA). The time step model assumed oysters responded to and reflected a mixture of the seston signal encountered as well as a trophic shift, but that only a small portion of the tissue in question (muscle, gills, or mantle) responded due to some combination of new growth or tissue turnover

(Figure 4). The decay of $\delta^{15}\text{N}$ over time reflected the portion of tissue that changed (C) and its $\delta^{15}\text{N}$ value. It is assumed that C is constant. The time step model was formulated as Equation 1,

$$\delta_{O(t)} = \delta_{O(t-dt)} + (\delta_i - \delta_d) * dt \quad \text{Eq. 1}$$

Equation 2 defined the $\delta^{15}\text{N}$ of the inputs to oyster tissues (δ_i) as:

$$\delta_i = (\delta_S + \Delta) * C \quad \text{Eq. 2}$$

Equation 3 described the decay of $\delta^{15}\text{N}$ in the oyster tissue (δ_d) over time:

$$\delta_d = C * \delta_{O(t)} \quad \text{Eq. 3}$$

Where,

$\delta_{O(t)}$ = $\delta^{15}\text{N}$ of oyster at time t ,

$\delta_{O(t-dt)}$ = $\delta^{15}\text{N}$ of oyster at the previous time

δ_i = $\delta^{15}\text{N}$ of the input

δ_d = decay of $\delta^{15}\text{N}$ in the oyster tissue

δ_S = measured seston $\delta^{15}\text{N}$

Δ = change in $\delta^{15}\text{N}$ due to a trophic shift

C = the portion of oyster tissue changing $\delta^{15}\text{N}$ with time

Measured seston $\delta^{15}\text{N}$ data from Monie Bay (2006) were used as initial inputs to build and parameterize the model. The C parameter was tuned by trial and error using measured data from each of the 10 stations in Monie Bay. Literature values report a trophic shift of 3-4 ‰ per trophic level, and so both scenarios of 3 and 4 were used as Δ for model verification and error assessment. The least squared error $(\delta^{15}\text{N}_{\text{measured}} - \delta^{15}\text{N}_{\text{modeled}})^2$, a common practice, was used to assess model error and measured data was

compared to modeled predictions for each oyster tissue. For calibration and verification, the dataset from the seasonal analysis at the Horn Point Laboratory dock, including seston $\delta^{15}\text{N}$, was utilized. Model error was assessed by least squares and RMSE. A reasonable constant value of seston $\delta^{15}\text{N}$ was input to assess model reasonability and to identify oyster tissue $\delta^{15}\text{N}$ 'memory'. Reasonability was assessed by verification that oyster $\delta^{15}\text{N}$ values reached steady state consistent with the trophic shift, change in tissue, and the seston value. Oyster $\delta^{15}\text{N}$ 'memory', that is, the duration of initial $\delta^{15}\text{N}$ values, was used to identify the length of time required to reach steady state, and thus optimal deployment duration. With the tuned C and Δ parameters, oyster tissues were modeled and compared to measured data using measured seston $\delta^{15}\text{N}$ values over time in Monie Bay and at the Horn Point Laboratory dock.

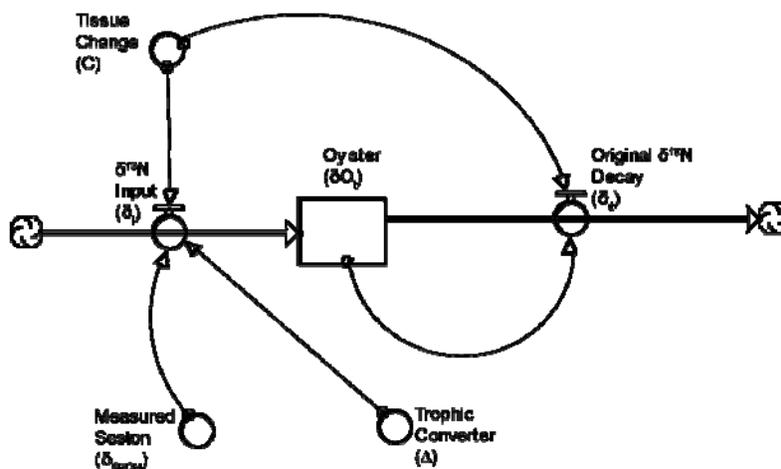


Figure 4. Graphical depiction of STELLA oyster $\delta^{15}\text{N}$ model. The $\delta^{15}\text{N}$ of the oyster tissue of interest at time t is represented by the stock (box) Oyster δ_{Ot} . The flow to the left of δ_{Ot} (the arrow with a cloud at its end intersecting a modified circle) represents the inflow – the overall $\delta^{15}\text{N}$ signature encountered by the oyster from its immediate surroundings ($\delta^{15}\text{N}$ input, δ_i). δ_i is influenced by the portion of the oyster tissue that changes (C) over time step t , the $\delta^{15}\text{N}$ of seston (δ_s), and a trophic converter (Δ) based on the literature value of 3-4 ‰ and then tuned by trial and error for the model. The flow to the right of δ_{Ot} represents the decay (δ_d) of the $\delta^{15}\text{N}$ signal in the oyster tissue over time t due to changes in the oyster tissue (C).

Results

Precipitation Record

2008 was a somewhat wetter year than average. While only six precipitation events were greater than 25.4 mm (1 inch), there were 13 events over the course of the winter, spring and summer (Figure 5). Average daily rainfall from 1971-2000 is compared to that which occurred during 2008.

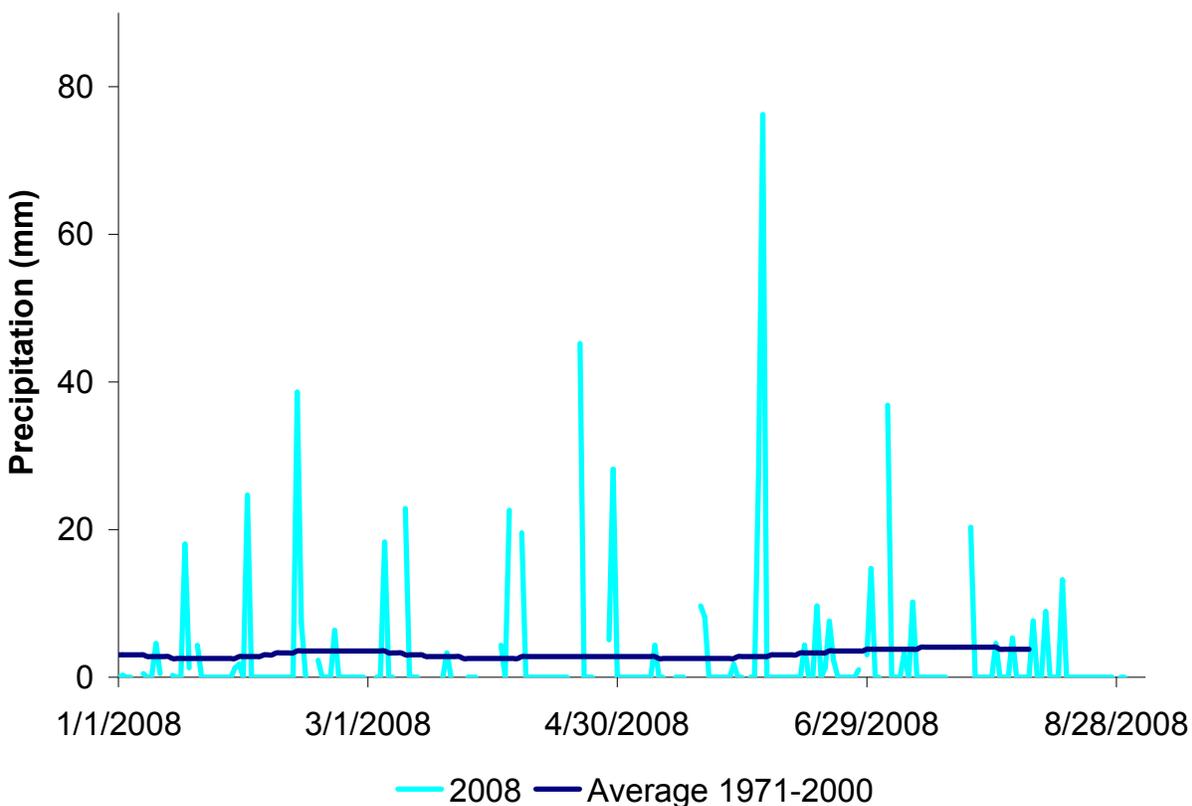


Figure 5: Daily total precipitation record for Princess Anne, MD (Data received from the Office of the State Climatologist, Department of Atmospheric and Oceanic Science, University of Maryland, College Park, 2009).

Spatial Patterns Between Creeks and Monie Bay

Spatial patterns were examined relative to the mouth of Monie Bay. Similar to 2006 and 2007, station MB1 was defined as being located at the mouth of Monie Bay, and so for Figures 6a-6e, MB1 is defined as a distance of 0 and all other stations are measured (in km) in reference to this station. Similar to previous years, both total nitrogen (Figure 6a) and total phosphorus (Figure 6b) increased with distance from the mouth of Monie Bay, suggesting that nutrient inputs are located in upstream areas, likely from terrestrial sources. This pattern extended across all tributaries and Monie Bay. Chlorophyll *a* also generally increased with distance from the mouth of Monie Bay (Figure 6c). Dissolved oxygen concentrations generally decreased with distance from the mouth of Monie Bay (Figure 6d). This follows the expected pattern of eutrophication, where excess nutrients fuel algal blooms and subsequently the bottom of the water is depleted of oxygen as algal detritus is aerobically decomposed.

Spatial patterns of Water Quality Index were similar to those of the relevant measurements. Monie Creek had the lowest overall Water Quality Index (0.16), while Monie Bay had the highest (0.52), and the other two creeks were intermediate: Little Creek had 0.44 and Little Monie Creek had 0.24. Low Water Quality Index scores in Monie Creek were attributed to rare attainments of thresholds for dissolved oxygen, total phosphorus, and total nitrogen, in that order (Table 2). Water Quality Index was highest in middle reaches, i.e. stations MB 2, MB 4, and MB 7 and lower in upstream areas than downstream areas (Figure 6e). These patterns suggest that terrestrial sources of nutrients impact the tributary creeks, while Monie Bay may be impacted from mixing with other waters such as Wicomico River and/or Tangier Sound.

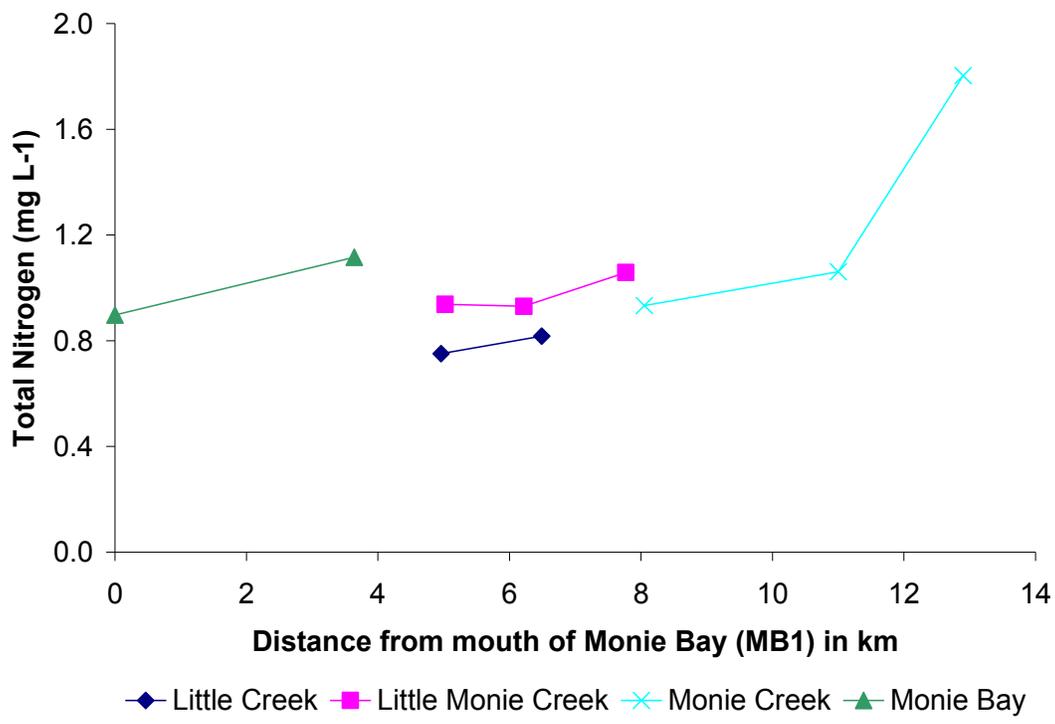


Figure 6a. Total nitrogen (mg L⁻¹) as related to distance from the mouth of Monie Bay.

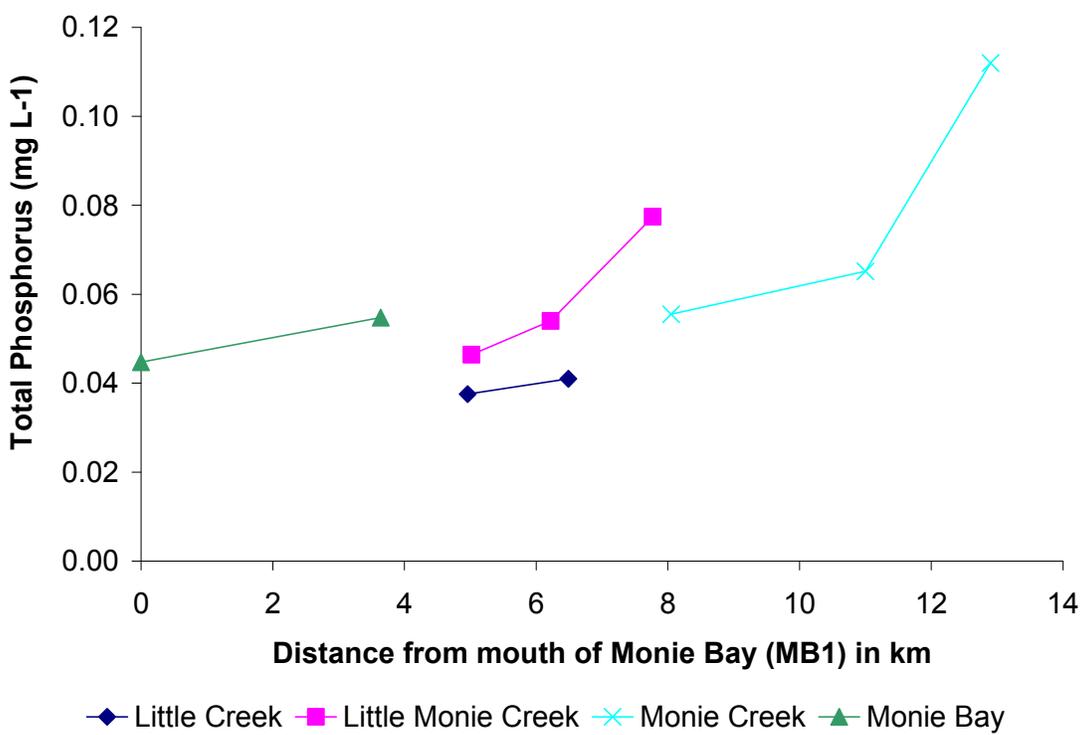


Figure 6b. Total phosphorus (mg L⁻¹) vs distance from the mouth of Monie Bay

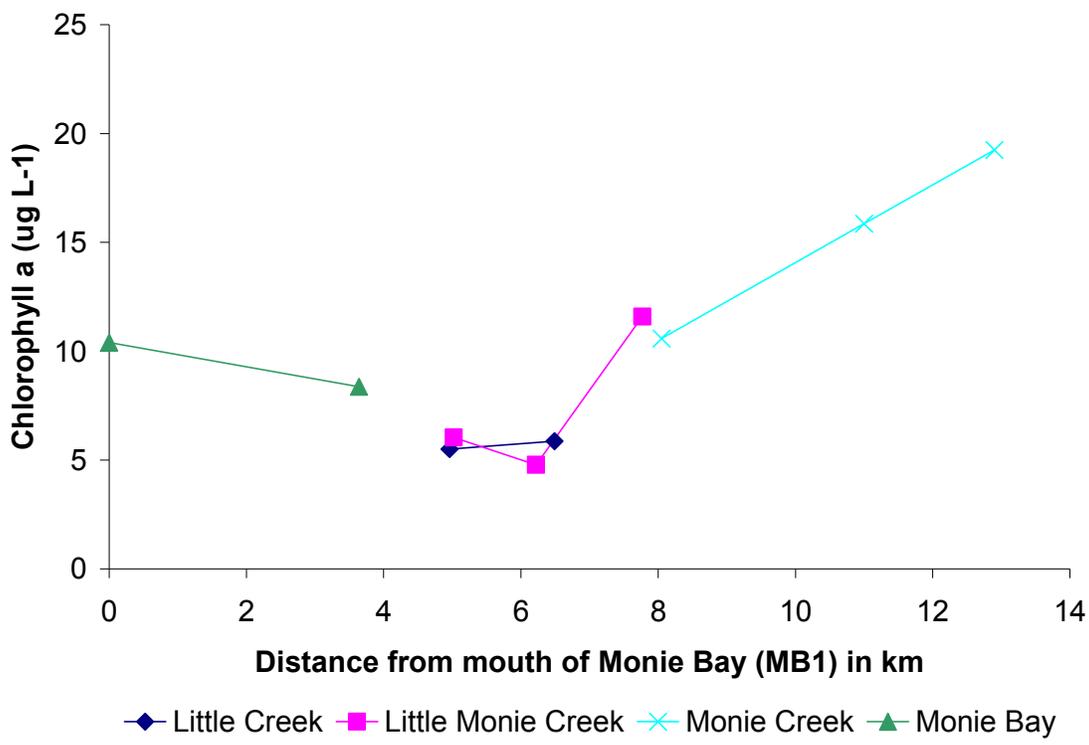


Figure 6c. Chlorophyll a ($\mu\text{g L}^{-1}$) as related to distance from the mouth of Monie Bay.

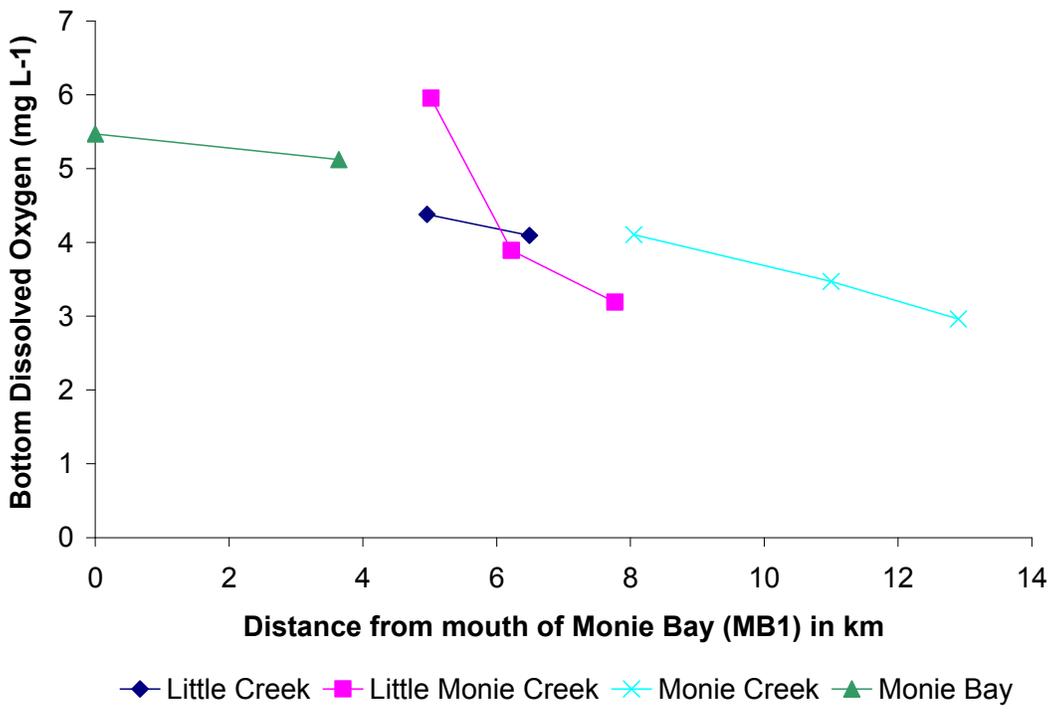


Figure 6d. Bottom dissolved oxygen (mg L^{-1}) vs. distance from the mouth of Monie Bay.

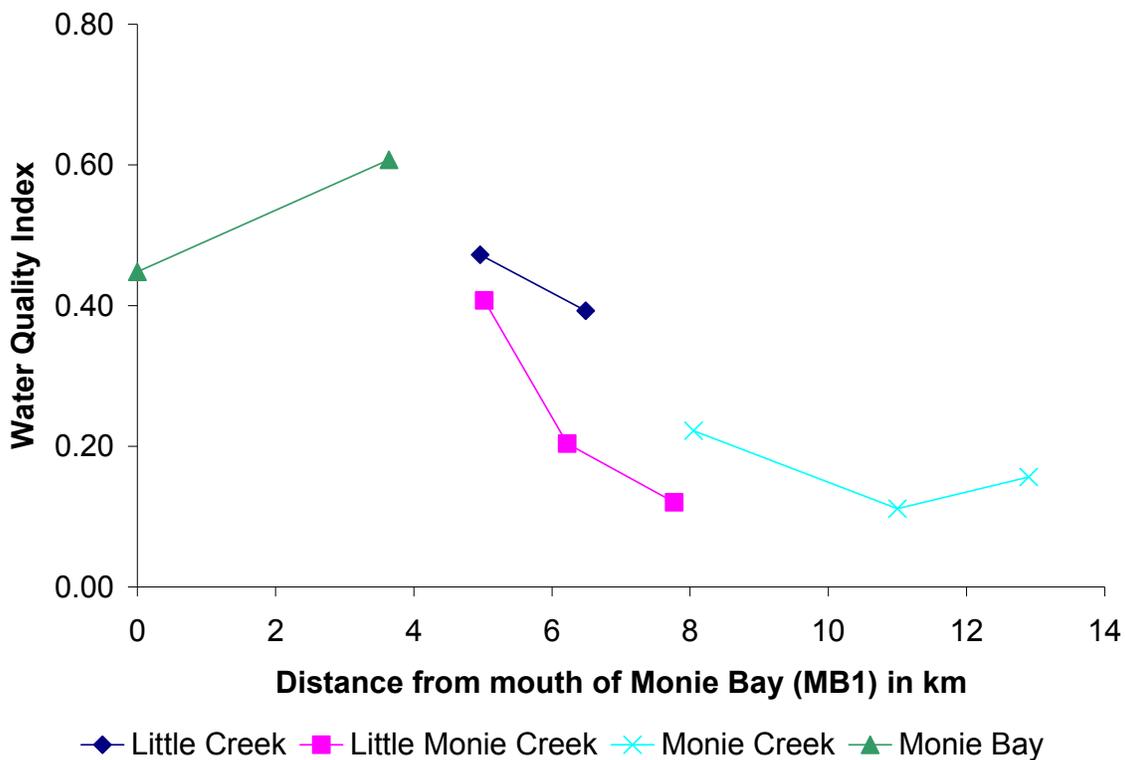


Figure 6e. Water Quality Index vs. distance from the mouth of Monie Bay (MB1)

Between creeks, spatial patterns were very similar to those previously observed in 2006-7. Patterns were generally observed between creeks, except for Secchi depth (Figure 7a) and temperature (Figure 7b). In other monitoring metrics Little Creek and Little Monie Creek were also similar over the course of the monitoring season. Monie Creek and Monie Bay tended towards the ends of the spectrum with respect to salinity (Figure 7c) and dissolved oxygen (Figures 7d and 7e). These two regions had the highest concentrations of total nitrogen (Figure 7f). Dissolved inorganic nitrogen concentrations did not generally differ spatially, though ammonia spiked in Little Creek (Figure 7g) while nitrite/nitrate spiked in Monie Creek in the middle of the monitoring period (Figure 7h). Unlike other spatial patterns, nitrite concentrations were similar

between Little Creek and Monie Bay, as well as between Little Monie Creek and Monie Creek (Figure 7i) – these pairings were uncommon in other years as well. Monie Creek had the highest total phosphorus (Figure 7j), phosphate (Figure 7k) and chlorophyll *a* (Figure 7l) concentrations while Little Creek had the lowest. Phosphate concentrations were lowest in Monie Bay (Figure 7k). Monie Bay had the highest total suspended solids and total volatile solids (Figures 7m, 7n).

Seasonal Patterns in Physical and Chemical Water Quality Monitoring

Physical and chemical water quality monitoring identified several temporal patterns throughout the 2008 monitoring period. Secchi depth (Figure 7a) was similar between creeks throughout the summer, except for the first sampling time on April 15, 2008. Secchi depth was somewhat decreased during the middle of the summer (June and July) compared to the fall (October). Surface water temperatures followed predictable seasonal patterns of summer warming and peaked during the summer at around 30 °C in August (Figure 7b). Salinity minimums were found in late May, and henceforth continual increases were observed until the end of monitoring in October, reaching nearly 18 ppt (Figure 7c). Monie Creek had lower mean salinity than the other creeks or Monie Bay, largely due to available freshwater inputs and distance from Monie Bay. Dissolved oxygen concentrations at the bottom of the creeks were variable throughout the summer, but only reached below 2.0 mg L⁻¹ in Monie Creek (Figure 7d). Peaks of oxygen concentrations were found in late May (except for Little Monie Creek) while oxygen was generally depleted in late April (except for Little Monie Creek) and September (except for Monie Bay). Likewise, similar seasonal and between-creek

patterns were observed for oxygen saturation (Figure 7e). Generally, total nitrogen concentrations were relatively constant throughout the summer, except for a large peak in Monie Creek and Monie Bay towards the end of May (Figure 7f). This apparent pulse in these regions was not apparent for longer than one sampling date. Ammonia concentrations pulsed coincident with the pulse in total nitrogen, but were generally consistent otherwise throughout the summer (Figure 7g). Potentially, this suggests that the pulse of total nitrogen was largely comprised of ammonia. Sources of nitrogen which contain large proportions of ammonia include human and animal waste. Nitrite + Nitrate (NO_{23}) concentrations were higher in spring than during the summer (Figure 7h). Possibly, these nutrients were consumed by phytoplankton or other biological activity, which increased during the summer months. Concentrations of nitrite (NO_2) did not vary consistently over the course of the summer (Figure 7i). Total phosphorus concentrations exhibited seasonal patterns similar to those of total nitrogen concentrations (Figure 7j). A pulse of phosphorus was observed in late May in Monie Creek and Monie Bay, coincident with the pulse of total nitrogen. Slightly later in the early summer, total phosphorus concentrations in Little Creek and Little Monie Creek reached their maximum, but this was muted compared to that in the other two regions. Phosphate concentrations (Figure 7k) were generally consistent over the monitoring period except for a pulse coincident with that of total phosphorus. The main component of total phosphorus that is biologically available is phosphate. Phosphates are also a component of human and animal wastes, particularly poultry manures. Chlorophyll *a* concentrations, trended upwards throughout the summer, but with the exception of Monie Creek during April 29, 2008, never reached $15 \mu\text{g L}^{-1}$, the threshold value used in

the calculation of the Water Quality Index (Figure 7l). It is possible that the measurement of chlorophyll *a* on this date ($44.3 \mu\text{g L}^{-1}$), is erroneous due to contamination. Total suspended solids (TSS) remained generally consistent throughout the summer, with a slight peak at the end of the monitoring period in the fall (Figure 7m). Meanwhile, total volatile solids generally tended to increase over the course of the monitoring period (Figure 7n). At the beginning of the monitoring period Water Quality Index was highest in Little Creek, but was later superseded by Monie Bay (Figure 8).

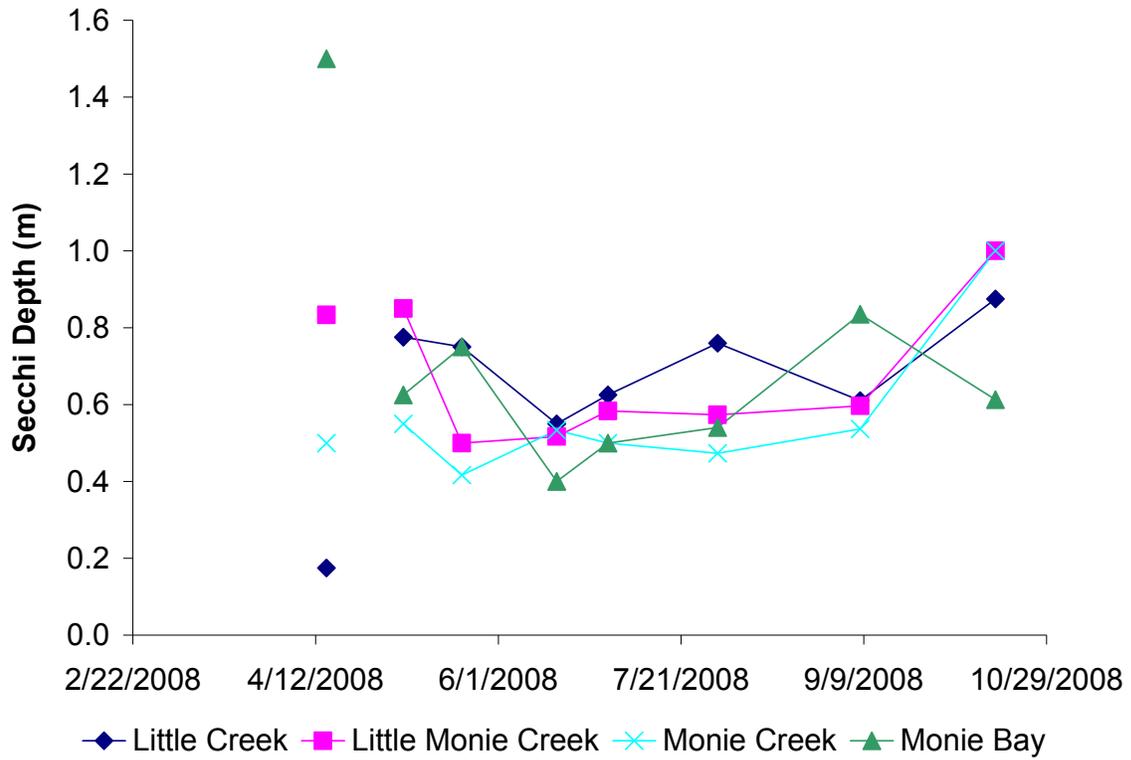


Figure 7a: Temporal patterns of Secchi depth averaged for each creek.

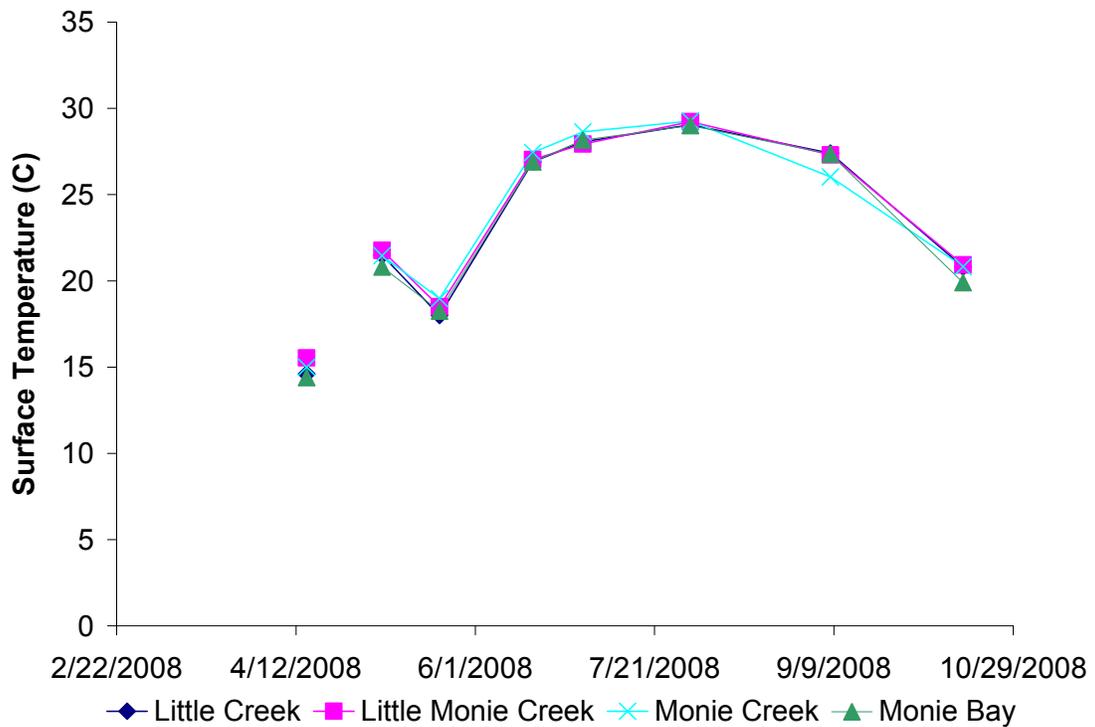


Figure 7b: Temporal patterns of surface temperature averaged for each creek.

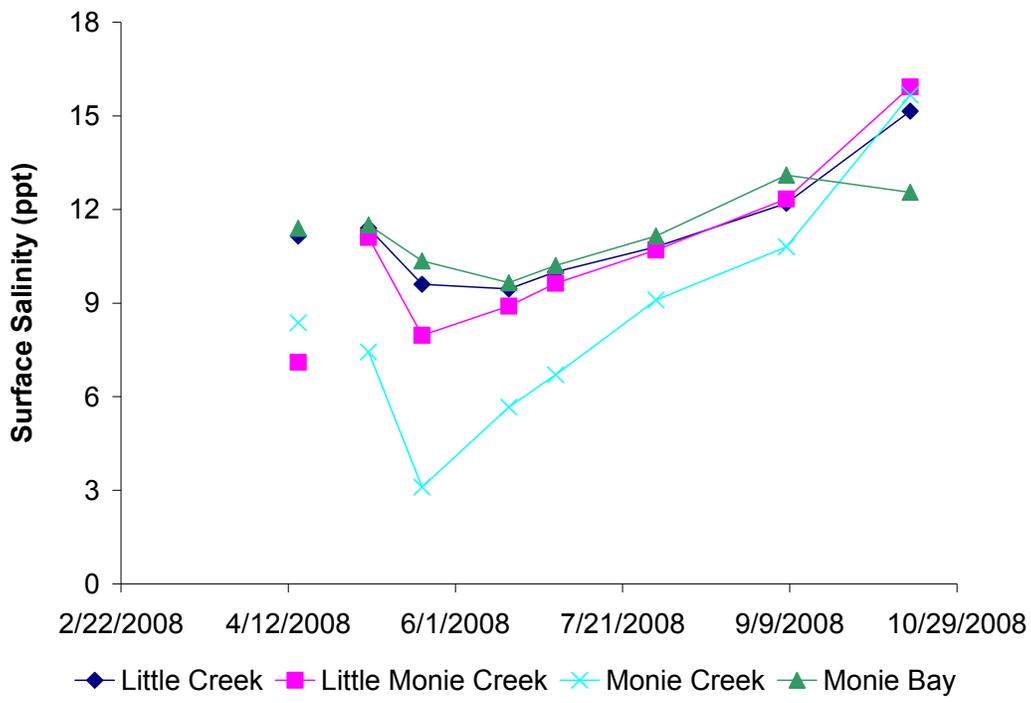


Figure 7c: Temporal patterns of surface salinity averaged for each creek.

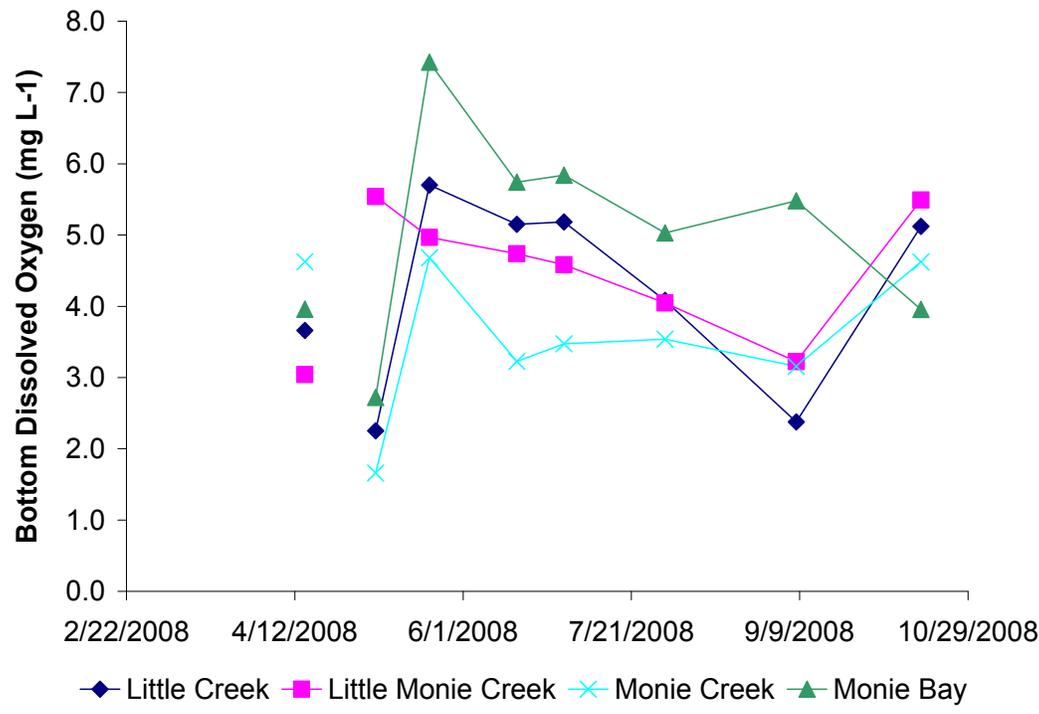


Figure 7d: Temporal patterns of bottom layer dissolved oxygen concentrations.

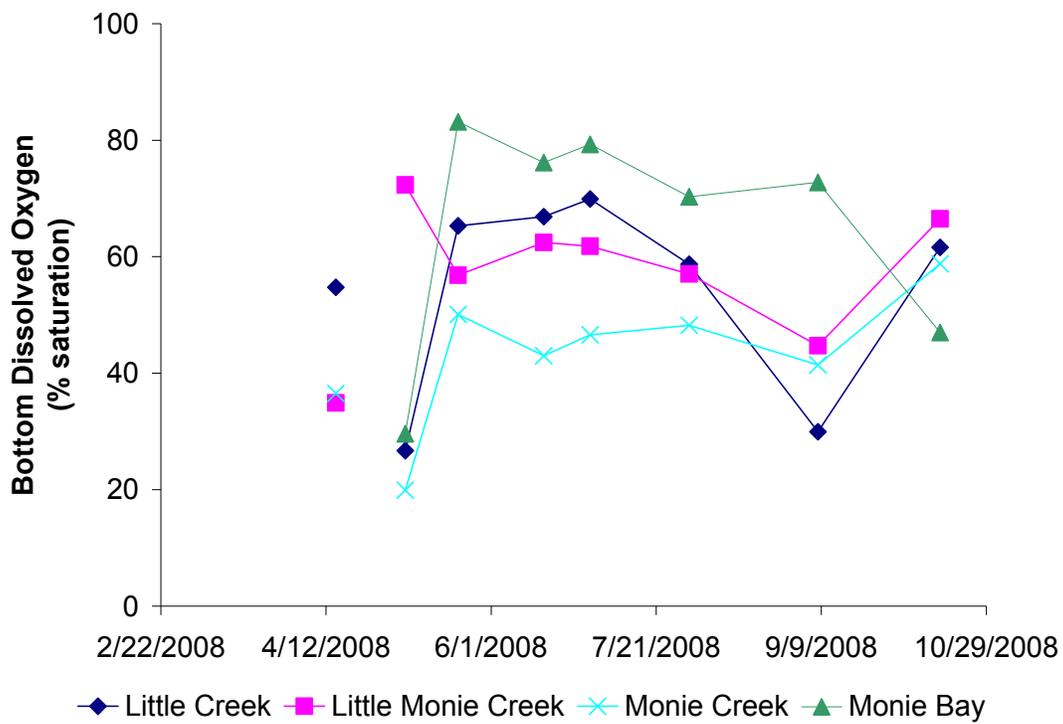


Figure 7e: Temporal patterns of bottom layer dissolved oxygen percent saturation.

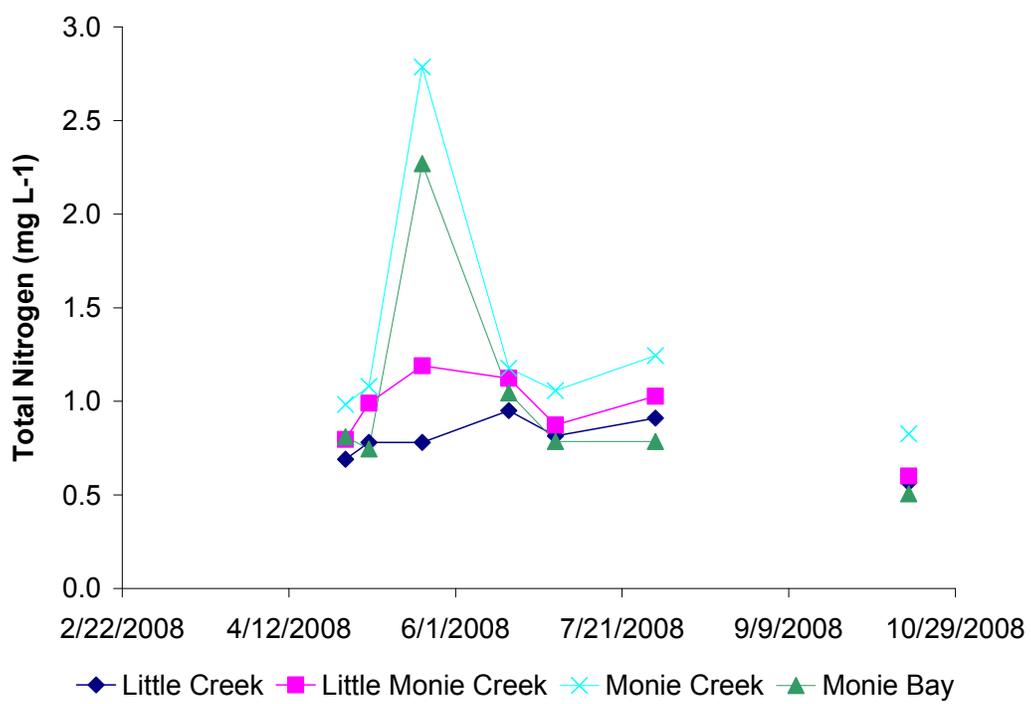


Figure 7f: Temporal patterns of total nitrogen.

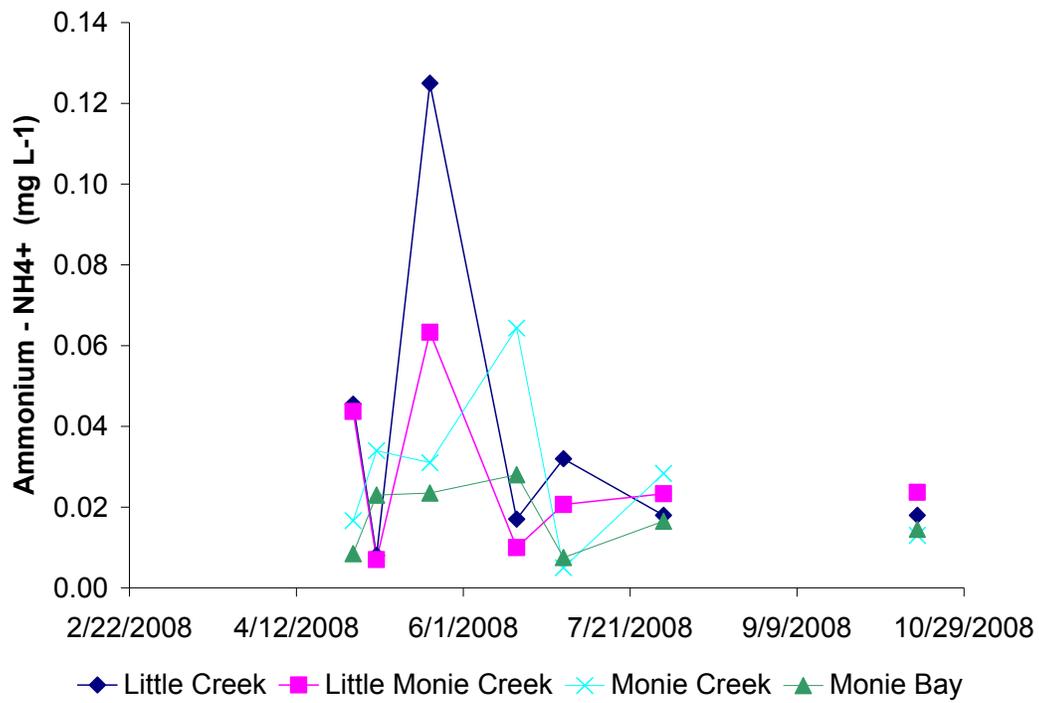


Figure 7g: Temporal patterns of ammonium.

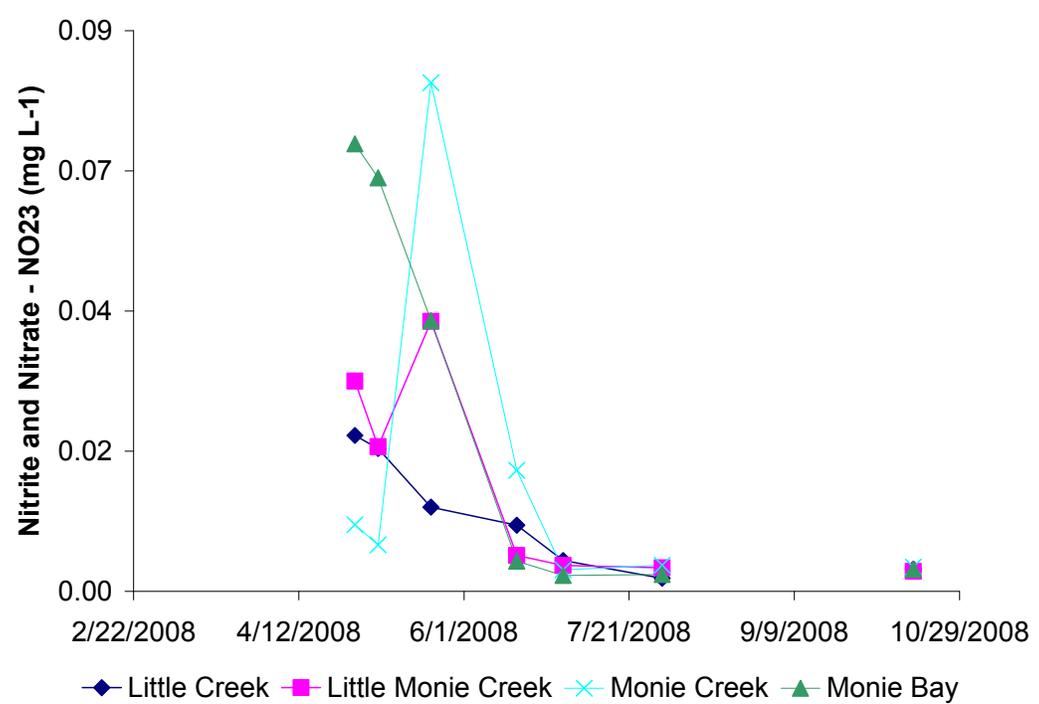


Figure 7h: Temporal patterns of combined nitrite/nitrate averaged for each creek.

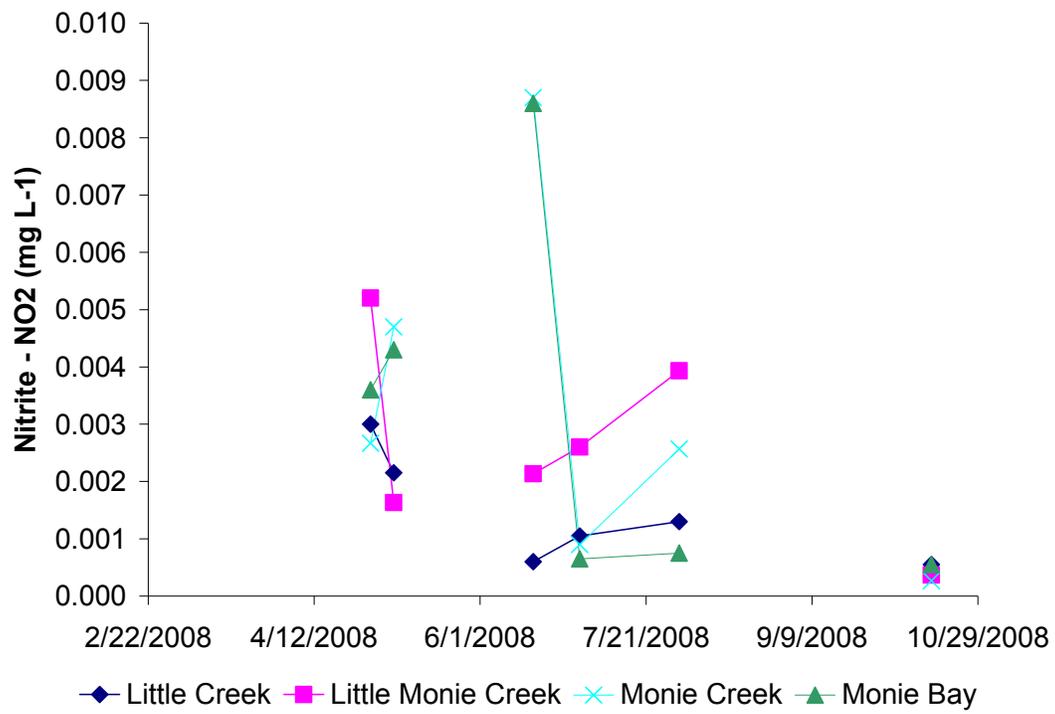


Figure 7i: Temporal patterns of nitrite averaged for each creek.

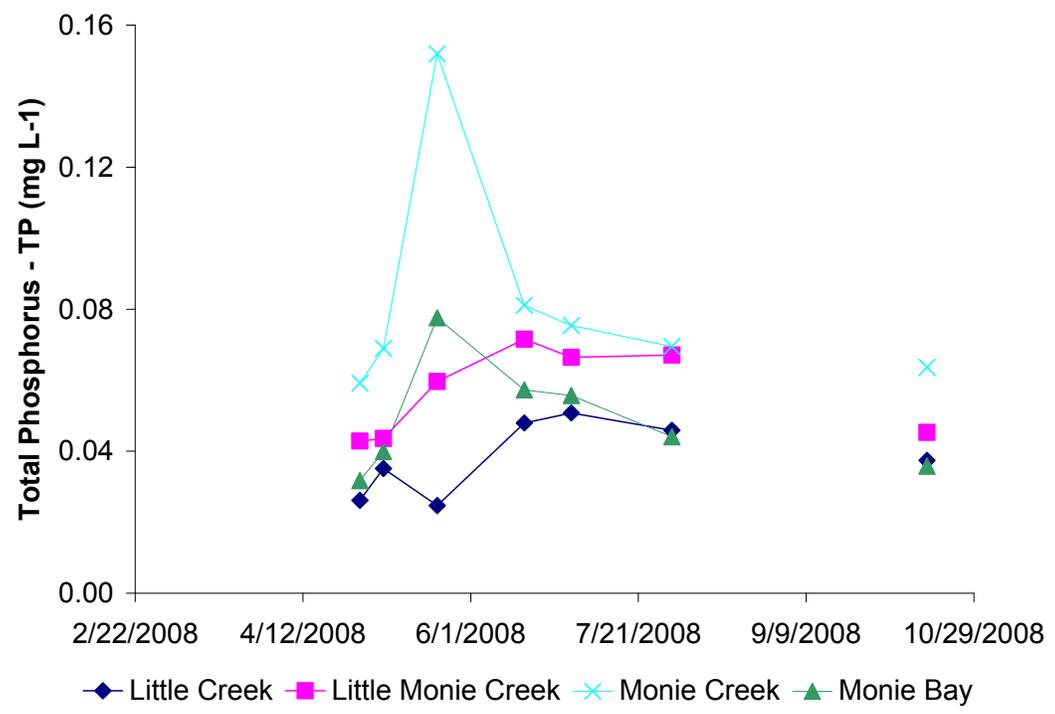


Figure 7j: Temporal patterns of total phosphorus.

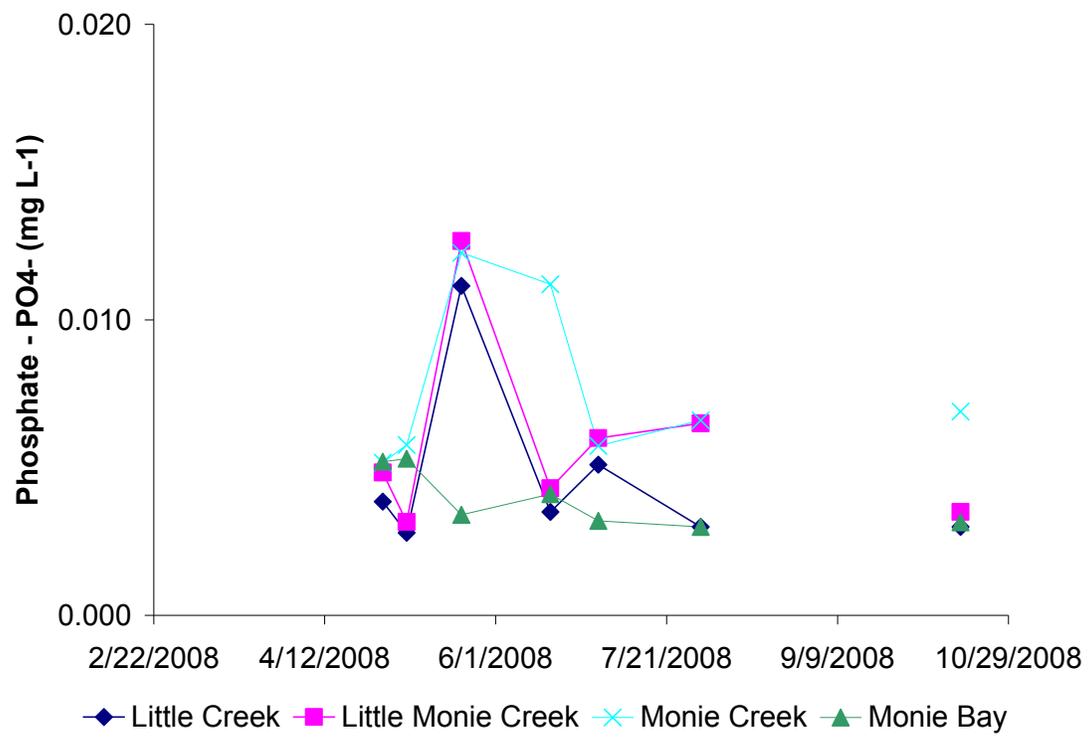


Figure 7k: Temporal patterns of phosphate averaged for each creek.

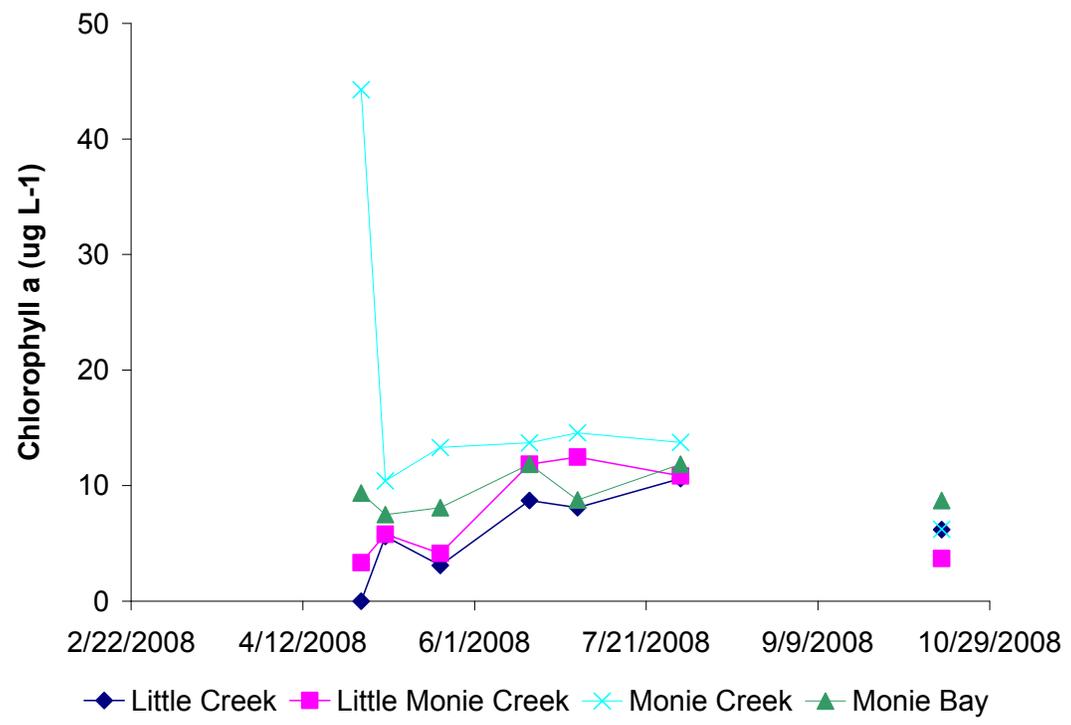


Figure 7l: Temporal patterns of chlorophyll a.

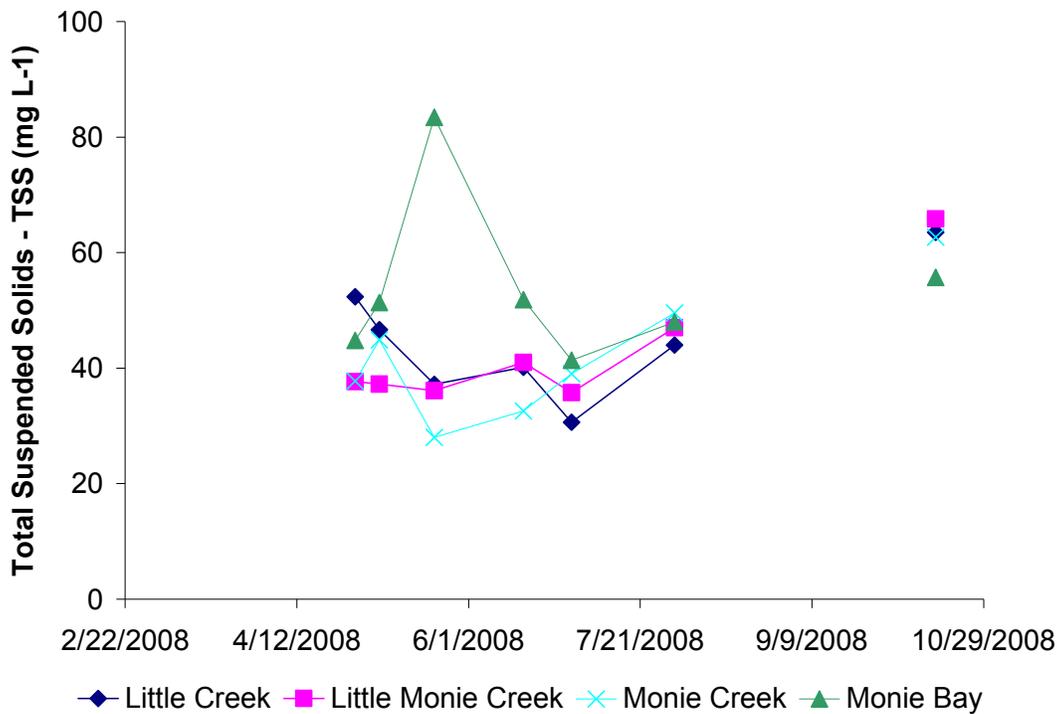


Figure 7m: Temporal patterns of total suspended solids averaged for each creek.

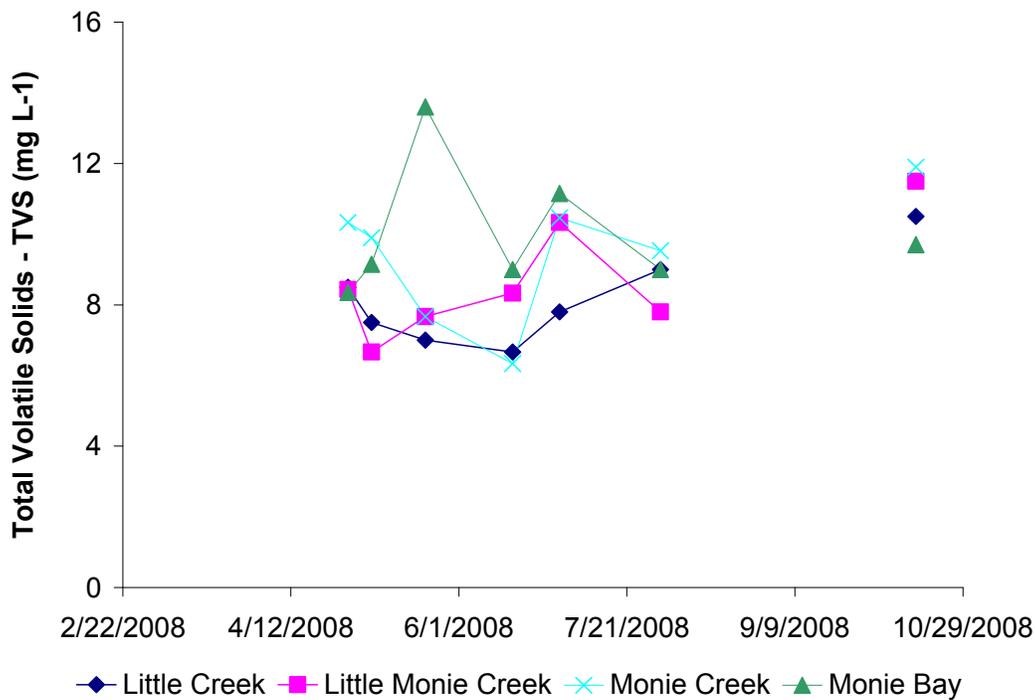


Figure 7n: Temporal patterns of total volatile solids.

Water Quality Index

Both temporal and spatial trends in the water quality index were apparent. Table 2 displays the mean attainment of threshold values for total nitrogen, total phosphorus, dissolved oxygen and chlorophyll a during the monitoring period. The mean of these was used to calculate the Water Quality Index (WQI). Variations in overall Water Quality Index were extreme, ranging from 0 to 1.0 over the course of the monitoring period in Little Creek, from 0 to approximately 0.6 in Little Monie Creek and from roughly 0.4 to 1.0 in Monie Creek. Over the beginning of the monitoring period, Water Quality Index was highest in Little Creek, but was later superseded by Monie Bay (Figure 8). Between the two, Monie Bay had a higher mean threshold attainment for dissolved oxygen, possibly due to larger surface area and higher mixing rates, being located adjacent to both Wicomico River and Tangier Sound. Temporally, Water Quality Index was generally highest in each creek at the beginning and end of the monitoring period. Generally, however, Water Quality Index was low, < 0.5, despite that Monie Bay and portions of its watershed are protected as a part of the National Estuarine Research Reserve System.

Regions	Site	Water Quality Parameter	Mean Attainment
Little Creek	MB 3	Total nitrogen	0.14
		Total phosphorus	0.43
		Dissolved oxygen	0.29
		Chlorophyll	1.00
	MB 4	Total nitrogen	0.29
		Total phosphorus	0.43
		Dissolved oxygen	0.50
		Chlorophyll	1.00
Little Monie Creek	MB 5	Total nitrogen	0.00
		Total phosphorus	0.00
		Dissolved oxygen	0.00
		Chlorophyll	0.57
	MB 6	Total nitrogen	0.00
		Total phosphorus	0.00
		Dissolved oxygen	0.13
		Chlorophyll	1.00
	MB 7	Total nitrogen	0.00
		Total phosphorus	0.29
		Dissolved oxygen	0.63
		Chlorophyll	1.00
Monie Bay	MB 1	Total nitrogen	0.00
		Total phosphorus	0.14
		Dissolved oxygen	0.75
		Chlorophyll	1.00
	MB 2	Total nitrogen	0.14
		Total phosphorus	0.43
		Dissolved oxygen	0.71
		Chlorophyll	1.00
Monie Creek	MB 10	Total nitrogen	0.14
		Total phosphorus	0.00
		Dissolved oxygen	0.13
		Chlorophyll	1.00
	MB 8	Total nitrogen	0.14
		Total phosphorus	0.14
		Dissolved oxygen	0.00
		Chlorophyll	0.43
	MB 9	Total nitrogen	0.14
		Total phosphorus	0.00
		Dissolved oxygen	0.00
		Chlorophyll	0.43

Table 2. Mean attainment of threshold values for each water quality parameter over the monitoring period for each station. Attainment is a comparison of measurements against threshold values as described earlier.

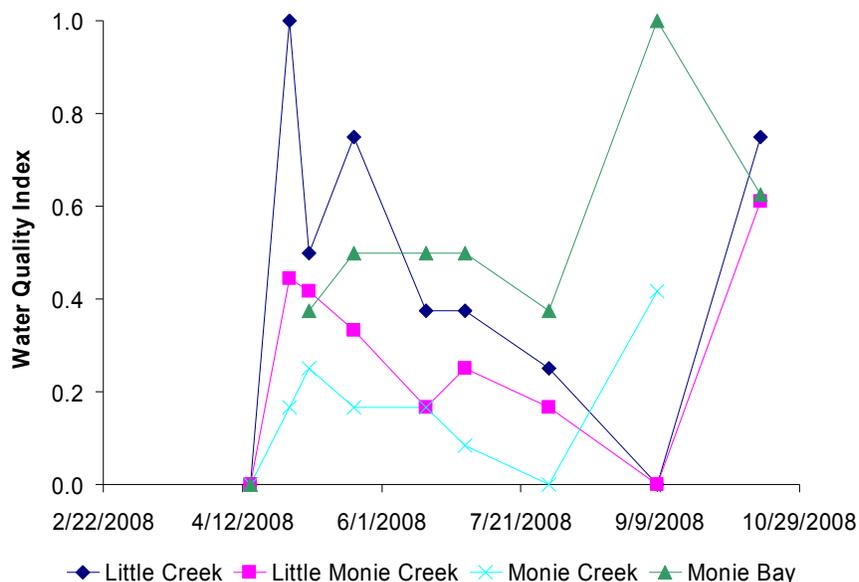


Figure 8: Temporal trends in the Water Quality Index (WQI)

Long-term trends in water quality

Tangier Sound exhibited long-term changes in water quality. Monitoring data from station EE3.1 (38° 11' 48.6744" N, 75° 58' 23.5416" W) was plotted over time. Total nitrogen, (~ 0.5 to 1.5 mg L^{-1}) trended upwards between 1986 and 2007 (Figure 9a). In 2008, total nitrogen was in the upper concentration ranges of historic data. In contrast, total phosphorus markedly decreased in Tangier Sound over time (Figure 9b). Phosphorus policy changes likely affected this by limiting phosphates in detergents and improving wastewater treatment. Relative cyclic stable range of chlorophyll *a* $5\text{-}15 \text{ } \mu\text{g L}^{-1}$ appears to occur from 1987-2002, after which concentrations increased (Figure 9c). Chlorophyll *a* patterns were fueled by increases in total nitrogen. Dissolved oxygen concentrations were stable over the long-term between 8 and 9 mg L^{-1} (Figure 9d).

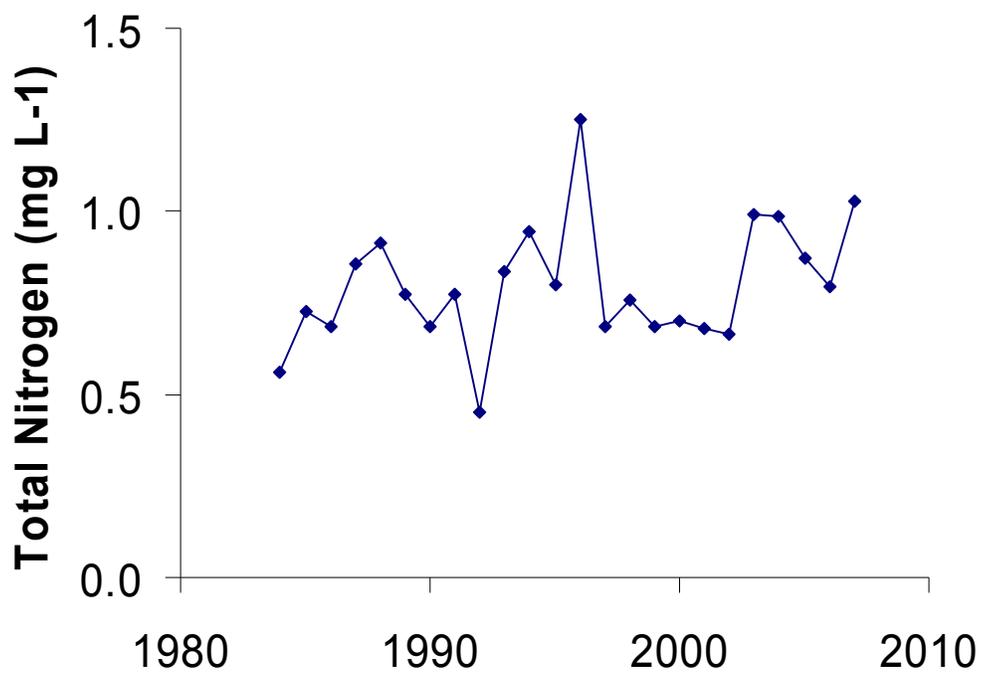


Figure 9a: Long-term trends in total nitrogen at Tangier Sound

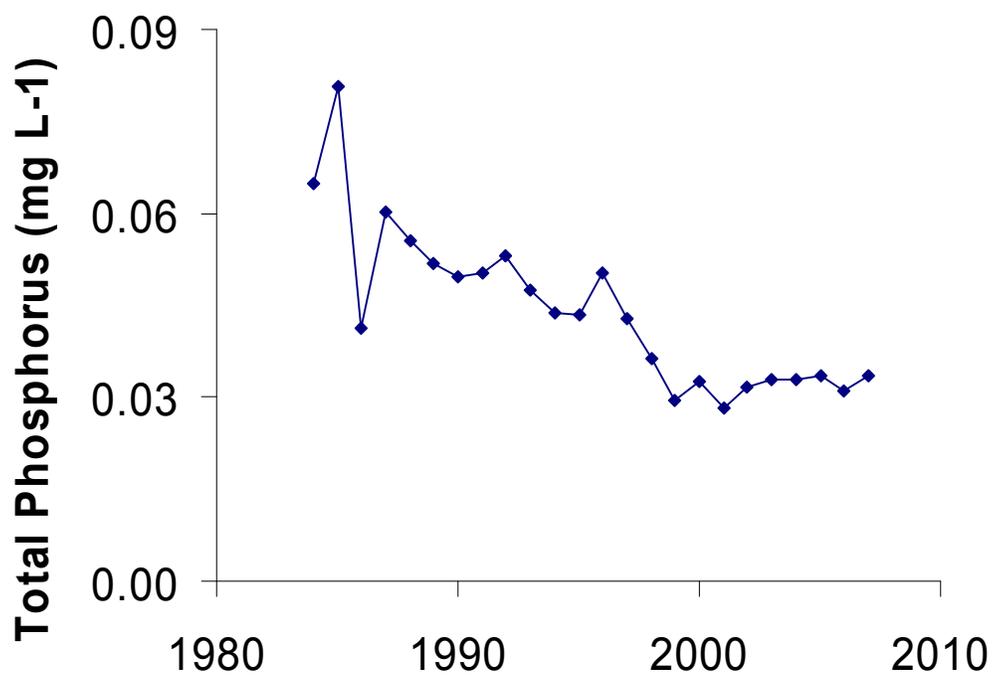


Figure 9b: Long-term trends in total phosphorus at Tangier Sound

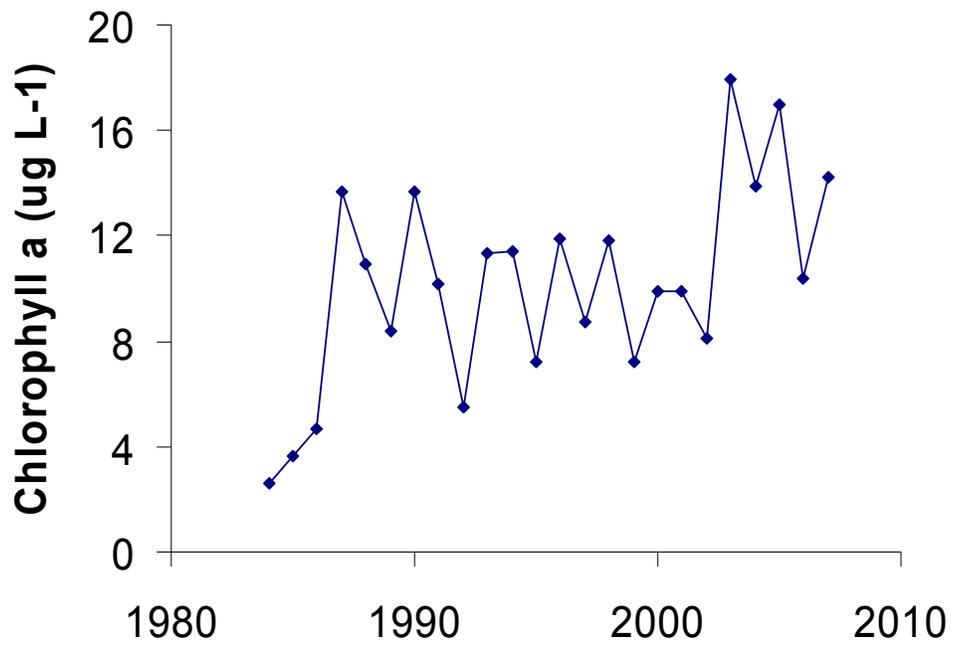


Figure 9c: Long-term trends in chlorophyll a at Tangier Sound

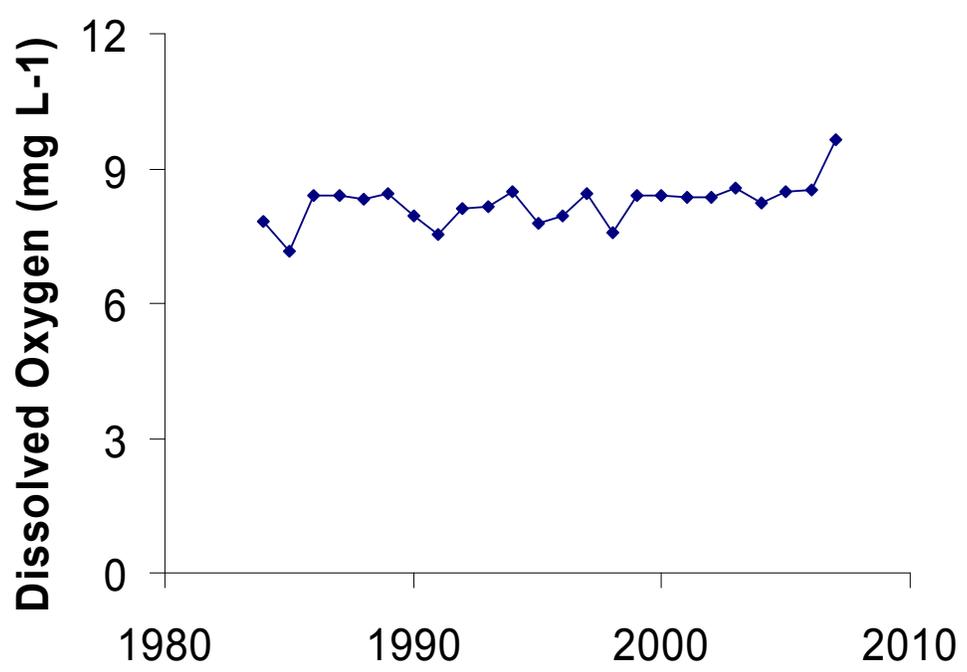


Figure 9d: Long-term trends in dissolved oxygen at Tangier Sound

Long-term changes in water quality have coincided with long-term changes in chicken production and human population. The increase in total nitrogen and chlorophyll, decrease in total phosphorus, and stability of dissolved oxygen have occurred during a period of relative decline and resurgence of chicken production and slight increases in human population (Figure 10a, 10b). These data are collected from Somerset County and the US Department of Agriculture. Chicken production (Figure 10a) and human population (Figure 10b), and are compared to overall variability in Water Quality Index (Figure 10c) in Tangier Sound (Station EE3.1). However, while the long-term trend in water quality appears to be variable with no discernable trend, there appear to be relationships over shorter time periods. Water quality appears to generally decrease between 1986 and 1995, a period of increased broiler chicken production. After a decline in chicken production, however, through 1997, there is an increase in water quality. Water quality again declines after a resurgence of broiler chicken production in Somerset County up to 2002.

Over the long-term, large-scale poultry litter application can alter soil chemical compositions, increasing total nitrogen content to 30 cm depth (Kingery et al. 1994) and has been shown to affect water quality (Woli et al. 2002). Long-term records of water quality in Tangier Sound (connecting Monie Bay with Chesapeake Bay) fluctuate, becoming degraded after periods of increases in numbers of chickens sold and improving after declines in chicken sales (Figures 10a, 10c; Chesapeake Bay Program 2008; station EE3.1). Long-term trends place the current water quality into context.

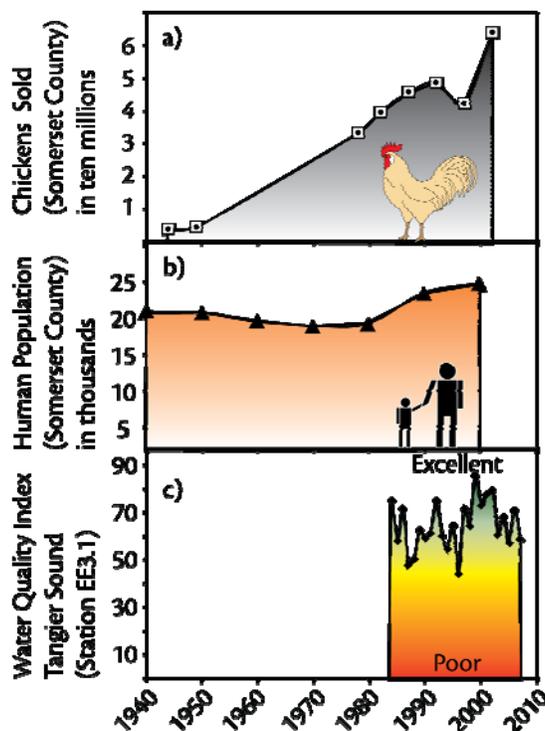


Figure 10: Comparison of historical data of a) broiler chickens sold in Somerset County, MD b) human population in Somerset County, MD and c) Water Quality Index at Tangier Sound, (station EE3.1)

Oyster $\delta^{15}\text{N}$ enables inferences to be made about human and animal waste sources

Oyster muscle $\delta^{15}\text{N}$ varied spatially and indicated nitrogen sources. Spatial patterns of $\delta^{15}\text{N}$ in muscle tissues varied by creek (Figure 11a). In general, these spatial patterns match those of land use within the watershed (Figure 12). Similar to previous years, Monie Creek had the highest $\delta^{15}\text{N}$ values. In Monie Bay, $\delta^{15}\text{N}$ values decreased away from the mouth, but then once entering Little Monie Creek, $\delta^{15}\text{N}$ values increased upstream. The spatial pattern in Little Monie Creek is different from that observed in previous years. There was little change along creek axis in $\delta^{15}\text{N}$ in oysters deployed in Little Creek. Enriched values of $\delta^{15}\text{N}$ indicated an overall influence of human and animal wastes in waters of the research reserve. However, variability was somewhat larger

than in previous years. Elevated $\delta^{15}\text{N}$ signals suggest that human and animal wastes enter Monie Creek from anthropogenic sources within the watershed, while human and/or animal wastes can be inferred to enter Monie Bay at its mouth, via either Wicomico River or Tangier Sound from $\delta^{15}\text{N}$ values in oysters.

Oyster muscle $\delta^{15}\text{N}$ values in Monie Creek were deduced to be derived from animal or human waste nitrogen. Terrestrial anthropogenic waste sources could explain the complementary patterns of increasing $\delta^{15}\text{N}$ and decreasing water quality gradients heading upstream along the creek axis (Figures 11a and 6e). Poultry manure and additional inputs from septic systems are likely contributors, based upon geographic proximity and magnitude of estimated nitrogen inputs. Animal (particularly poultry) manure, which in Somerset County, MD was spread locally during the spring (Fykes, pers. com.), likely contributed to the elevated $\delta^{15}\text{N}$ signal. Though only 19 poultry houses in the Monie Creek watershed were counted by digital ortho-imagery (USDA 2005), these contained an estimated effective year-round population of 23,661 chickens house⁻¹ and produced $\sim 8.6 \times 10^5$ kg total nitrogen (untreated) yr⁻¹, based on an estimated 58 kg manure chicken⁻¹ yr⁻¹ (containing 1.8 kg total nitrogen yr⁻¹; Naber and Bermudez 1990), roughly equivalent to that defecated by 2.0×10^5 people (assuming 4.3 kg total nitrogen generated person⁻¹ yr⁻¹). Additionally, residential septic systems along the upstream portion of Monie Creek (station 8) may have enriched the oyster $\delta^{15}\text{N}$ signal, although only 699 septic systems were in the entire Monie Bay's watershed, including that for Little Monie Creek and Little Creek (MD DNR 1999b).

Other metrics of oyster tissues also varied spatially. After deployment, oyster nitrogen content varied along creek axis though it did not vary initially. Further, oyster

muscle %N was highest towards the mouth of the creeks and Monie Bay, and nitrogen content decreased linearly upstream (Figure 11b). Muscle %N was highest in Little Monie Creek and lowest in Monie Creek.

Carbon isotopes and content varied spatially as well. Stable carbon isotopes, $\delta^{13}\text{C}$, decreased linearly with distance from the mouth of Monie Bay (Figure 11c). Likely, this is due to increasing influence of marine carbon, which is enriched compared to most forms of terrestrial sources of carbon due to differences in photosynthetic pathways that discriminate between ^{12}C and ^{13}C at different rates (Peterson et al. 1985). Meanwhile, carbon content (%C) exhibited spatial patterns identical to those of nitrogen content, though carbon content was much higher (Figure 11d). The carbon: nitrogen ratio ranged from 4.0 to nearly 4.4, and tended increase along stream axes and to be higher in upstream areas than downstream areas (Figure 11e).

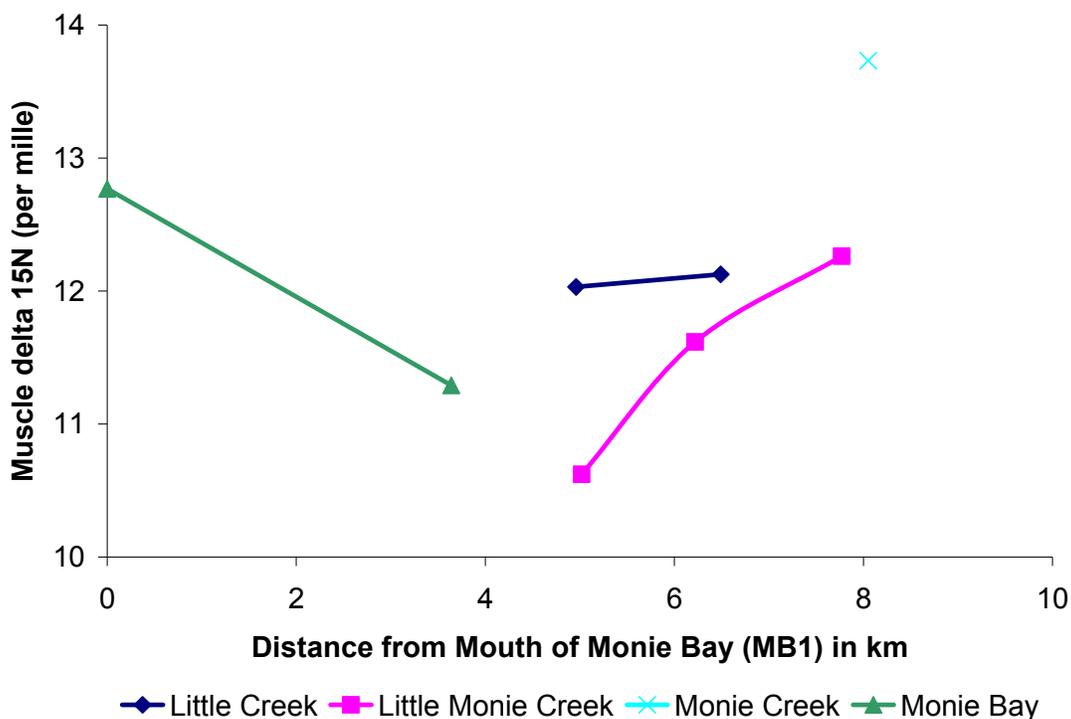


Figure 11a. Oyster muscle $\delta^{15}\text{N}$ vs distance from the mouth of Monie Bay.

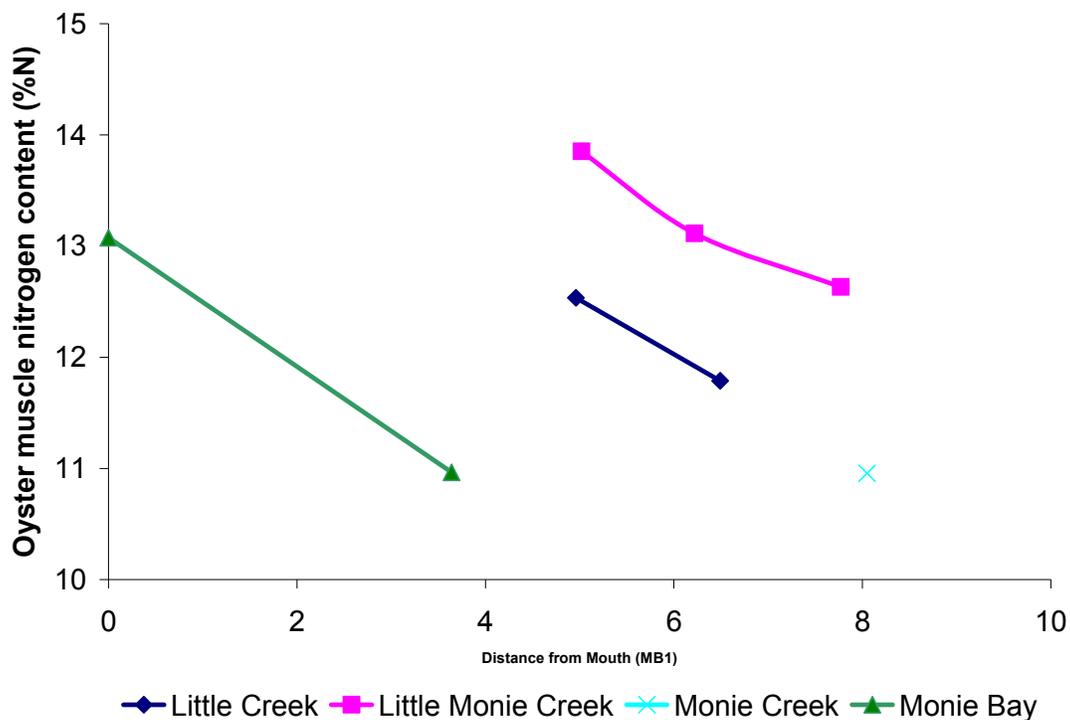


Figure 11b. Oyster muscle % nitrogen vs distance from the mouth of Monie Bay.

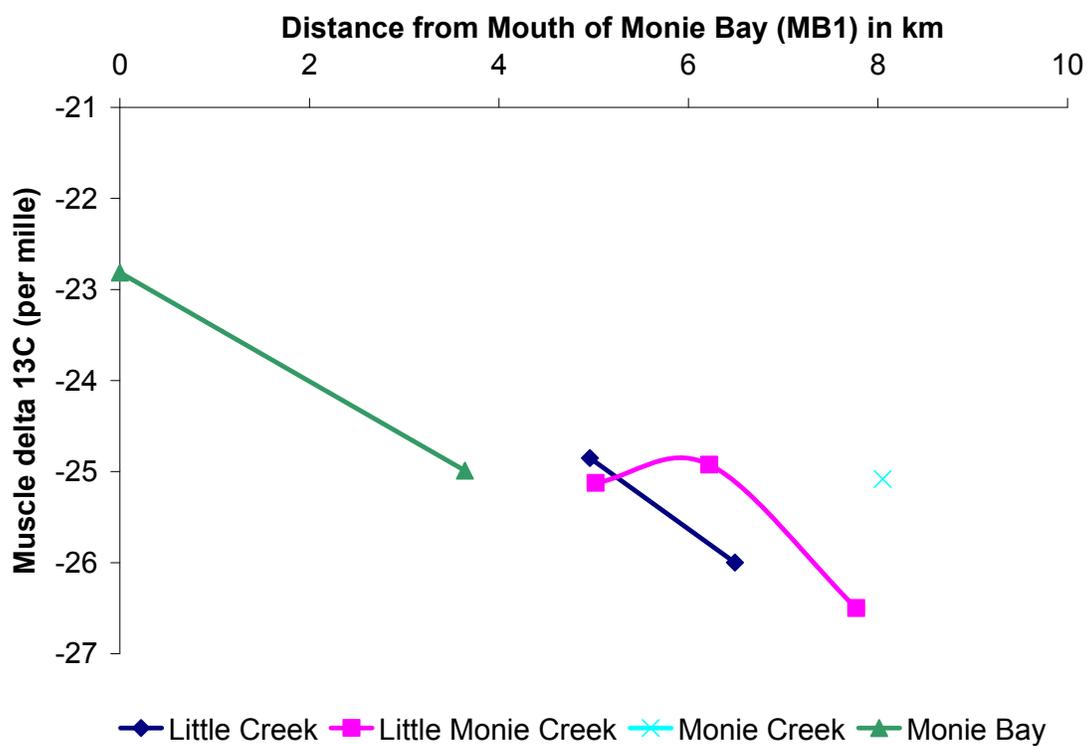


Figure 11c. Oyster muscle $\delta^{13}\text{C}$ vs distance from the mouth of Monie Bay.

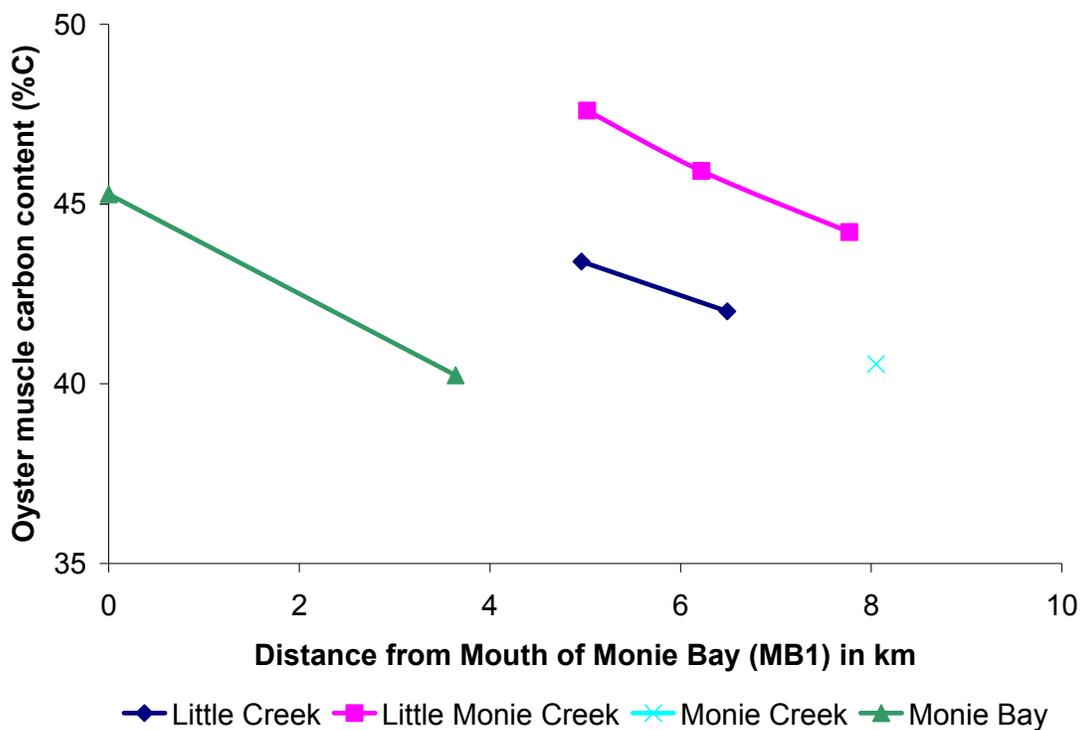


Figure 11d. Oyster muscle %C vs distance from the mouth of Monie Bay.

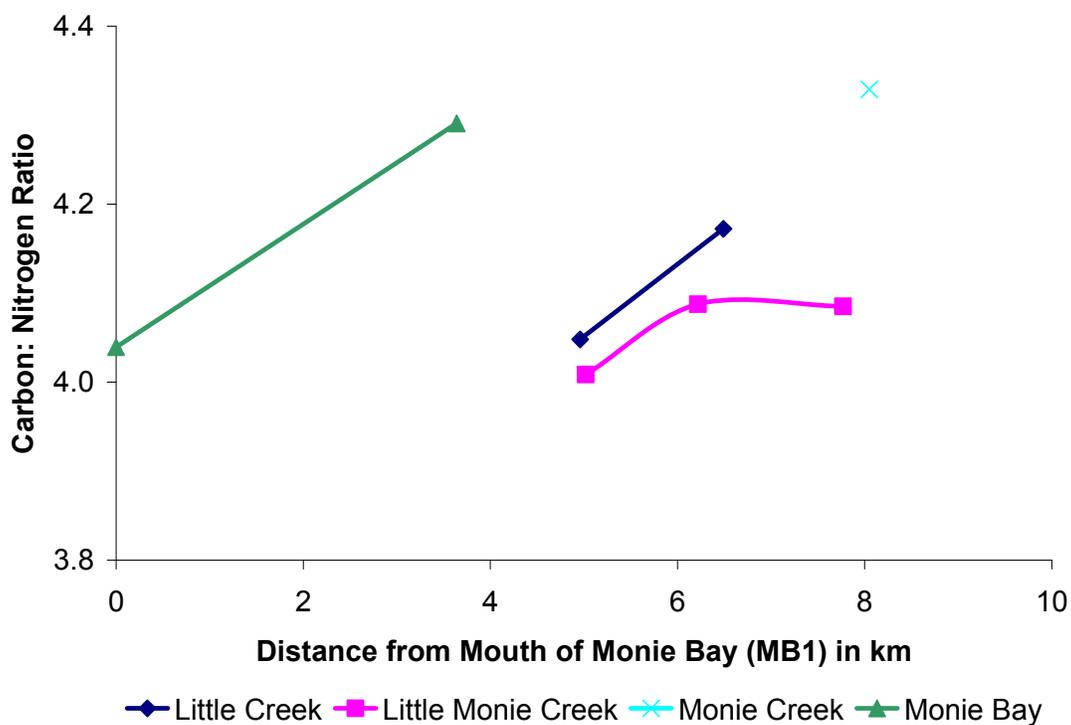


Figure 11e. Oyster muscle carbon: nitrogen ratio vs distance from Monie Bay's mouth

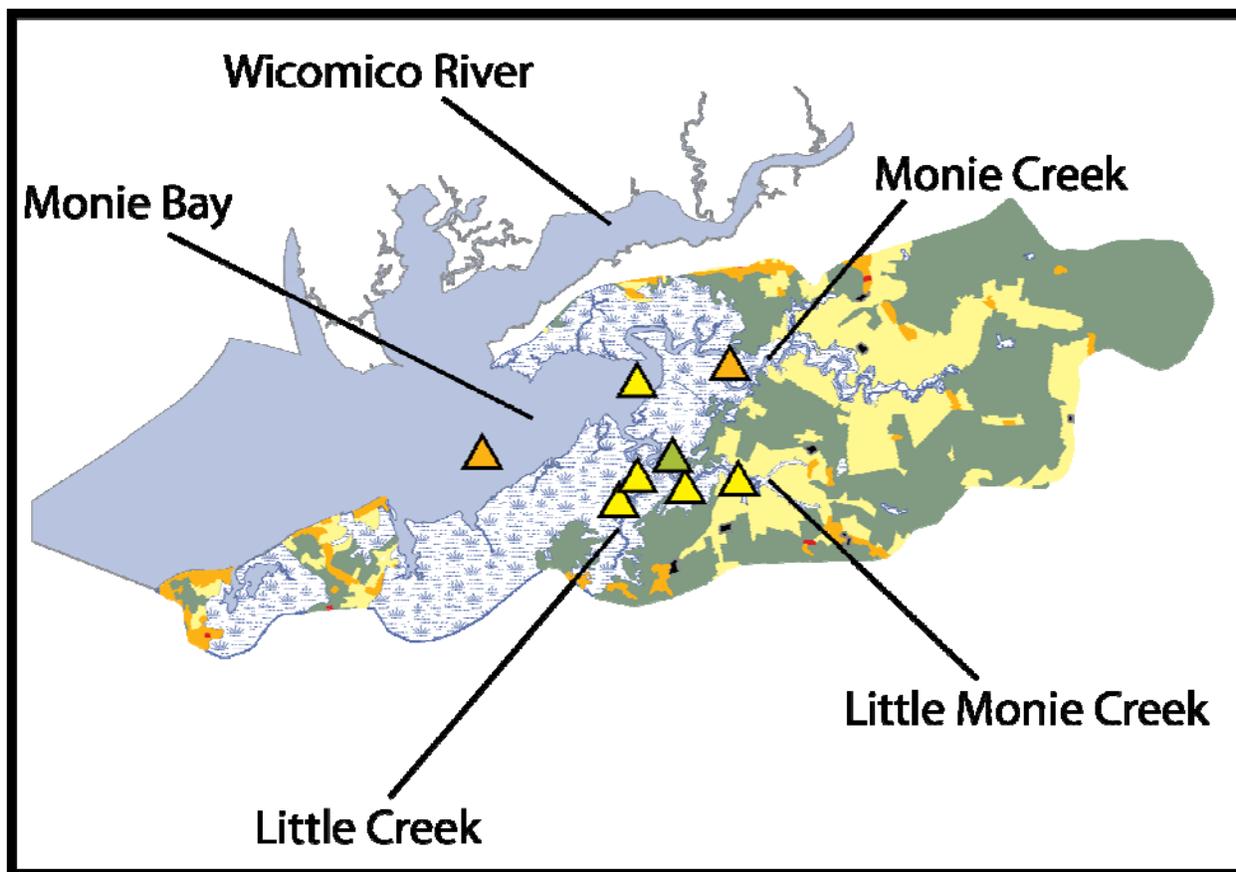


Figure 12. Map of oyster muscle $\delta^{15}\text{N}$ in Monie Bay and its creeks in the context of land use (triangles denote $\delta^{15}\text{N}$ values: Dark green 8.0 - 9.5 ‰, light green 9.6 - 11.0 ‰, yellow 11.1 - 12.5 ‰, orange 12.6 - 14.0 ‰, red 14.1 - 16.0 ‰). Land use in Monie Bay's watershed is also shown (green: forest, yellow: crop agriculture, spotted blue: wetlands, solid blue: water, orange: residential development, black: poultry feeding operations).

Contextualizing Monie Bay within Chesapeake Bay

Oyster $\delta^{15}\text{N}$ in Monie Bay fits within broader spatial patterns across Chesapeake Bay. Isotopic values in Monie bay ranged from 10.6 - 13.7 ‰ (Figure 12). Oyster isotopes in Monie Bay are in the middle of the overall range throughout Chesapeake Bay, which was 8.3 - 16.0 ‰. Oyster $\delta^{15}\text{N}$ values were lowest at the mouth of the Chesapeake and generally increased with latitude, reaching higher values in the South and Severn Rivers as well as Eastern bay and the Myles River, and intermediate values

in mesohaline portions of the Chesapeake (Figure 13). Situated in the mesohaline region, oyster $\delta^{15}\text{N}$ fit this broad spatial pattern. Compared to Chesapeake Bay, oyster $\delta^{15}\text{N}$ values in Monie Bay were moderate, suggesting this region is well flushed. Alternatively, in addition to indications of anthropogenic nitrogen sources, the broad spatial pattern could be due to relative mixing with oceanic waters, particularly at the mouth of Chesapeake Bay.

Monie Bay did not uniquely receive human and/or animal wastes from Chesapeake Bay in addition to from its watershed. The pattern of decreasing oyster isotope values, suggestive of allochthonous anthropogenic inputs, was observed in Monie Bay, but also in the Lynnhaven and Elizabeth Rivers in Virginia, near the mouth of the Chesapeake. This pattern was observed even though oyster $\delta^{15}\text{N}$ was higher in Monie Bay than either of these two rivers in Virginia (Figure 13). However, this pattern was not universal. The Potomac River, for example, exhibited the expected pattern of terrestrial anthropogenic inputs, inferred from the observation of oyster $\delta^{15}\text{N}$ decreasing towards the mouth of the Potomac River. This pattern is consistent with the location of the Blue Plains sewage treatment plant which discharges effluent into the Potomac. No discernible spatial patterns were detectable in other rivers, such as the South, Severn, or Choptank Rivers. Nevertheless, the detection of spatial patterns at multiple spatial scales by oyster $\delta^{15}\text{N}$ renders this a useful tool for identifying potential sources of anthropogenic nitrogen.

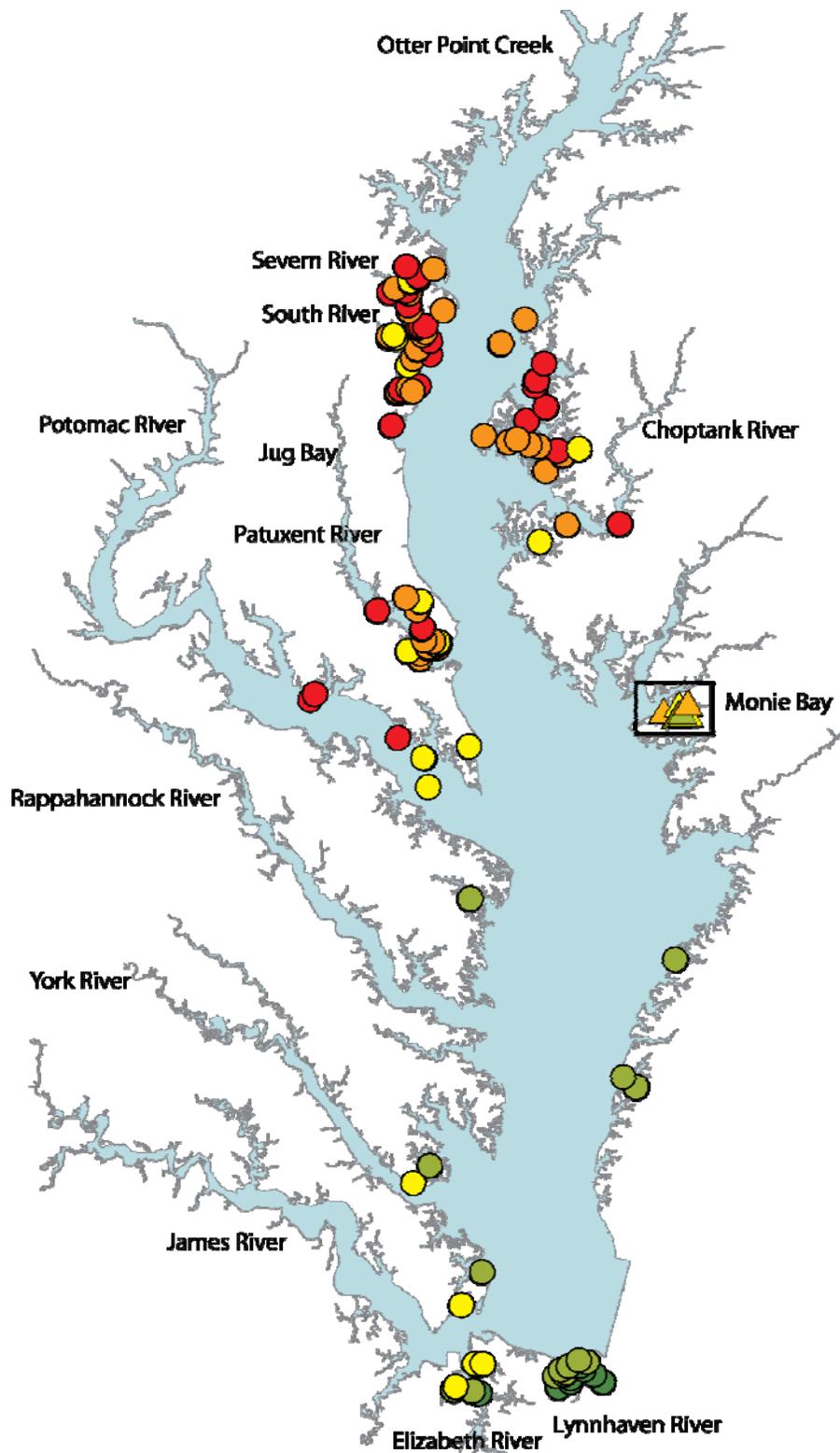


Figure 13: Oyster muscle $\delta^{15}\text{N}$ in Chesapeake Bay (circles: Dark green 8.0 - 9.5‰, light green 9.6 - 11.0‰, yellow 11.1 - 12.5‰, orange 12.6 - 14.0‰, red 14.1 - 16.0‰)

Context from the county and Delmarva Peninsula is needed for perspective on coarse-scale anthropogenic input calculations presented for Monie Bay and its watershed. Nearly 63.9×10^6 broilers and other meat-type chickens from Somerset County, MD (24th in the nation) were sold in 2002 (Figure 10a; USDA 2002) and were produced from approximately 300 poultry houses (counted from ortho-imagery, Figure 14) while only $\sim 2.5 \times 10^4$ people resided in this county in 2002 (Figure 10b; MD Department of Planning 2000). Furthermore, Delmarva Peninsula hosts an effective chicken population of $\sim 1.1 \times 10^8$, producing an estimated 2.1×10^8 kg total nitrogen yr^{-1} , more than that generated by 4.8×10^7 people, while in comparison the estimated 2002 human population on Delmarva Peninsula was only 1.2×10^6 people (Table 3, U.S. Census Bureau 2000). These estimates have been calculated assuming no net nitrogen import or export across watershed boundaries. Future studies could more precisely quantify nitrogen budgets and manure contributions for the Monie Bay ecosystem in addition to the detection of these anthropogenic sources.

Watershed	Human Population	Chicken Manure 'People Equivalents'	Sewage Systems	Sewage Inputs (kg TN yr^{-1})	Septic Systems	Septic Inputs (kg TN yr^{-1})	Average Annual Chicken Population	Manure Inputs (kg TN yr^{-1})
Monie Bay	2,576	365,050	0	0	699	10,304	828,138	1,576,353
Wicomico	28,028	855,260	3	196,212	7,233	112,112	1,940,209	3,693,170
Delmarva Peninsula	1,172,776	48,290,918	27	556,090			109,550,814	208,528,964

Table 3. Relative inputs to Monie, Wicomico, and Delmarva Peninsula watersheds from sewage, septic, and chicken manure sources. Chicken Manure 'People Equivalents' are estimated based on the assumed generation 2.0 kg total nitrogen (TN) chicken-1 year-1 and 4.3 kg TN person⁻¹ year⁻¹.

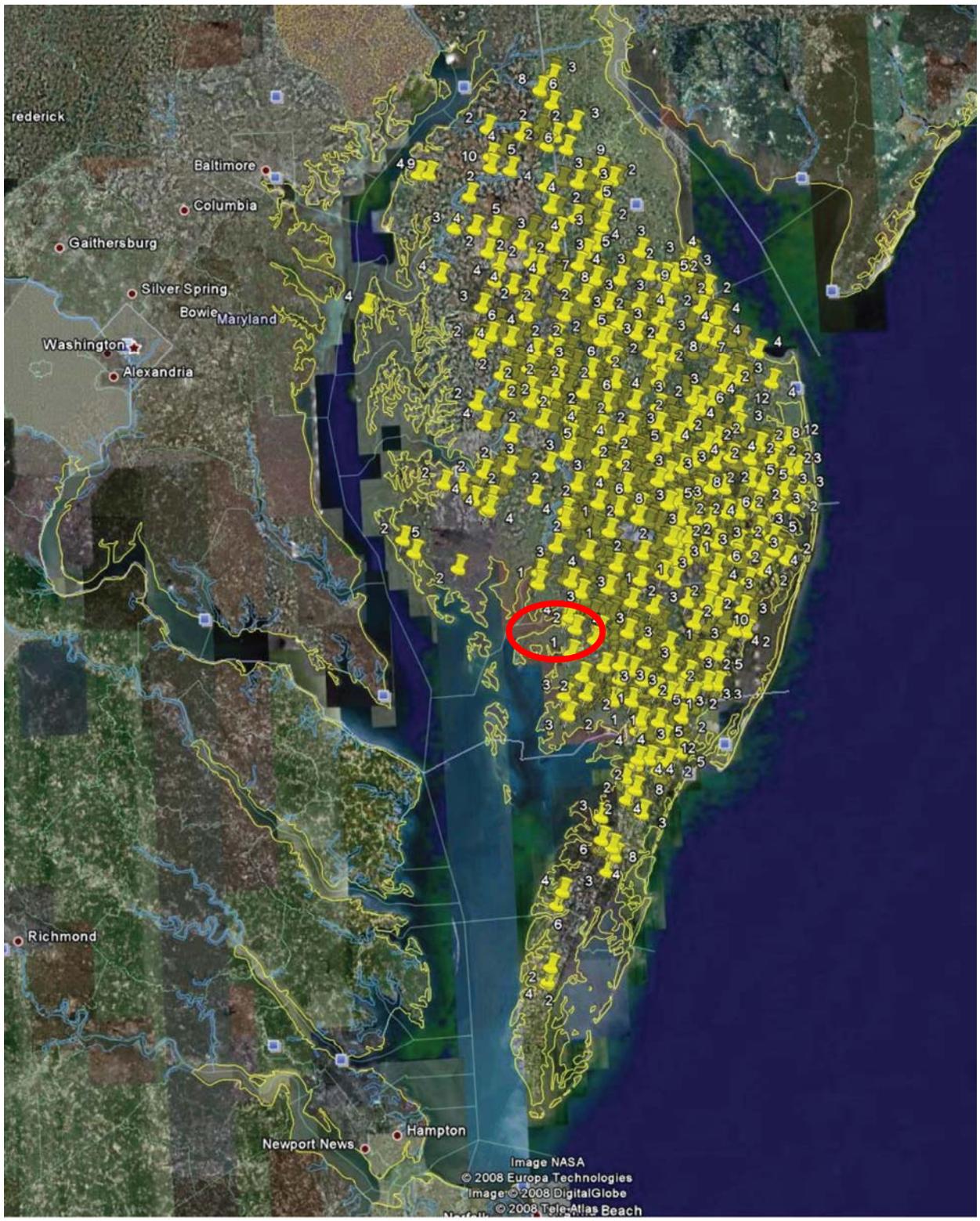


Figure 14: Poultry feeding operations in Delmarva Peninsula. Numbers atop pushpins indicate the number of feeding houses. Monie Bay and watershed circled in red.

STELLA modeling of required exposure time for oysters as bioindicator

Through STELLA modeling, additional questions regarding the utility of *Crassostrea virginica* $\delta^{15}\text{N}$ as a biological indicator can be addressed to enable the oyster to be used as a monitoring tool. First, the model was verified against previously measured oyster $\delta^{15}\text{N}$ from Monie Bay in 2006 (Figure 15). Seston $\delta^{15}\text{N}$ was incorporated into the model as well, and modeled oyster values were offset from the seston $\delta^{15}\text{N}$ by a trophic converter (Δ) of 3 ‰. A value of 3 ‰ for the trophic converter yielded smaller errors than 4 ‰, which has also been reported in the literature (Minawaga and Wada 1984). The model parameter C (the proportion of tissue changing due to new growth and tissue turnover or repair) was smallest in the muscle while similar or equal in the mantle and gills. It makes biological sense that the C term is smallest in the muscle tissue, because this tissue changes the slowest and thus integrates over the longest period of time. A C value of 0.004 for muscle yielded error of 2.412 while that of 0.009 for gills and mantle yielded errors of 1.714 and 1.889 respectively. This error is generally reasonable considering it is summed over 10 stations and thus error for one station was comparable to 0.2 ‰ instrument error.

Overall, modeled oyster $\delta^{15}\text{N}$ for all three tissues matched measured values very well. All modeled tissues follow the general trend of seston $\delta^{15}\text{N}$ and end matching the mean measured values of oyster $\delta^{15}\text{N}$ at Monie bay and its creeks (Figure 15). The modeled mantle $\delta^{15}\text{N}$ most resembles the seston $\delta^{15}\text{N}$, while the muscle $\delta^{15}\text{N}$ contains the smoothest curve, least resembling rapid changes in seston $\delta^{15}\text{N}$. At individual stations, model oyster tissues had lower model error and also resembled SPOM $\delta^{15}\text{N}$, matching measurements of oyster $\delta^{15}\text{N}$ at the end of deployment.

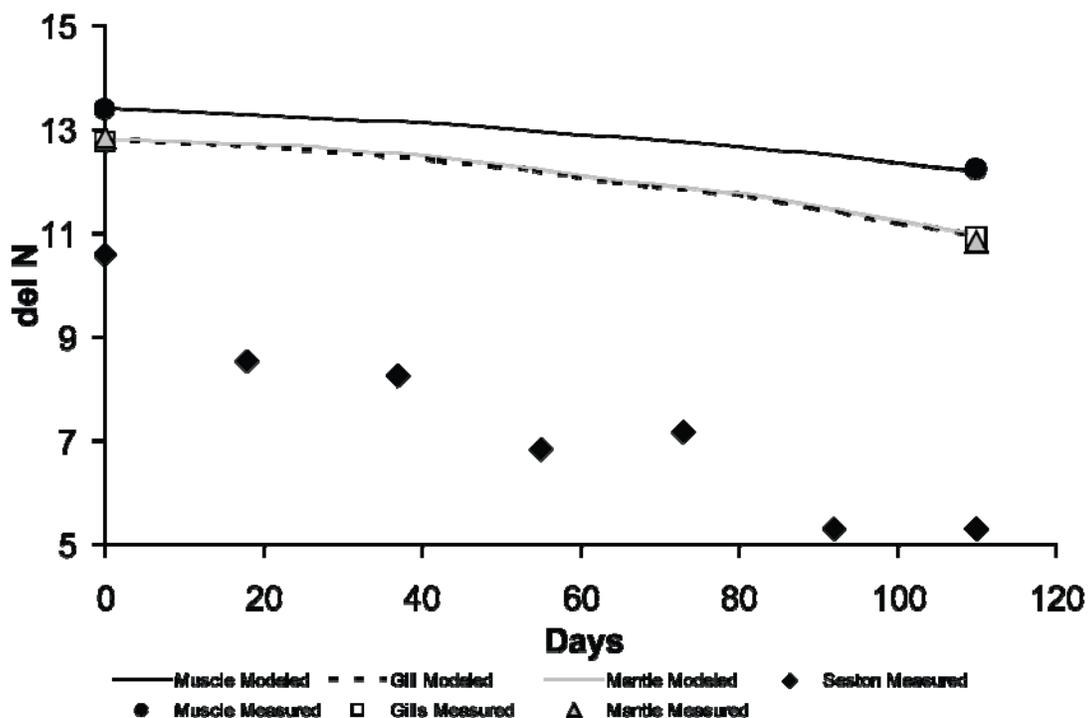


Figure 15: Modeled oyster muscle (solid black line), gill (dotted black line), and mantle (grey line) $\delta^{15}\text{N}$ compared to measured values for oyster muscle (black circle), gill (white square), and mantle (grey triangle) $\delta^{15}\text{N}$ and for seston $\delta^{15}\text{N}$ (black diamonds) in Monie Bay. Measured values for oyster tissues and seston are means across all ten stations.

STELLA also identified the required exposure time of oysters to local waters necessary for tissue $\delta^{15}\text{N}$ to converge upon local conditions. Given constant seston inputs, modeled oyster tissue decreased exponentially until reaching a steady state offset from the seston $\delta^{15}\text{N}$ by the trophic converter (Δ). Steady state occurred after 680 days by the muscle and 390 days by the gills and mantle (Figure 16). However, oyster muscle reached 90% of steady state by 120 days. This suggests that given instrumental measurement error of ± 0.2 ‰, a deployment period of 4 months could provide a reasonable estimate of ambient steady state $\delta^{15}\text{N}$ in water conditions. Therefore, oysters intended for deployment purposes can be measured after four months of feeding on plankton which have incorporated nitrogen at a desired location.

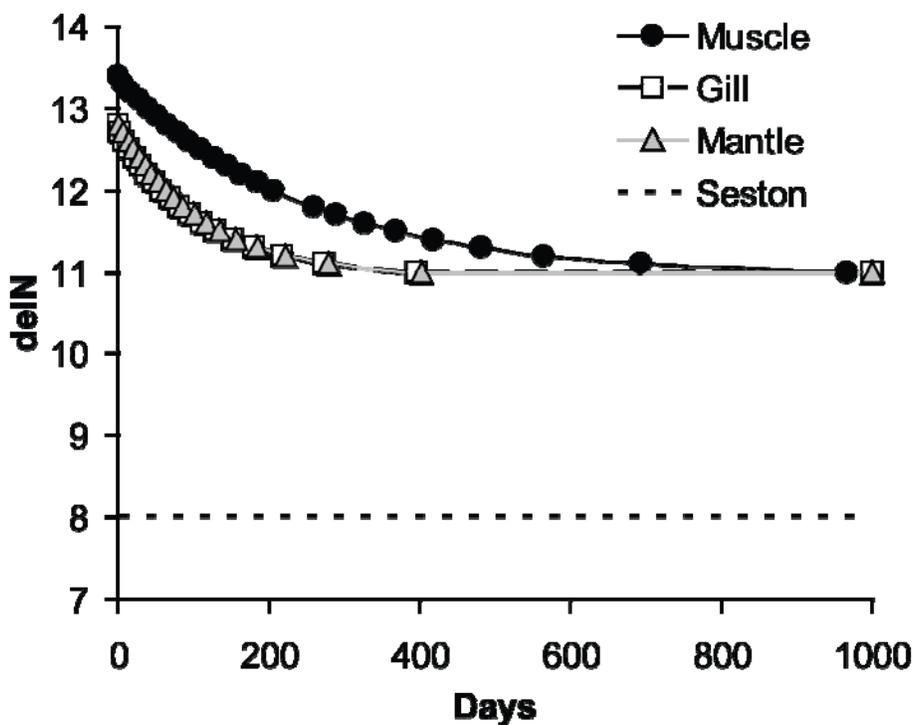


Figure 16: Modeled required exposure time to reach a constant seston $\delta^{15}\text{N}$ value. The model used a C value tuned with the 2006 Monie Bay dataset and a trophic converter $\Delta = 3$ and a constant seston $\delta^{15}\text{N}$ value of 8, a reasonable value for this dataset. Initial $\delta^{15}\text{N}$ values for each tissue were set at initial measured values.

STELLA model answers implementation questions and can extend future utility

Calibration and extension of the oyster's indicator capabilities regarding nitrogen sources may be effected by modeling oyster isotope physiology and cycling. A STELLA model based upon Monie Bay fieldwork provides vital information for the utility of the oyster as bioindicator, such as the required exposure time at a location of interest necessary to identify local isotopic signatures. The current STELLA model can refine deployment logistics such as duration and timing for other applied monitoring purposes. This information is pivotal to avoid confounding interpretations of isotopic data based on physiological rates of tissue change and corresponding isotopic signature variations. However, one of its limitations is that because it was designed based on data from

young oysters, it is only applicable to this age range (one to two years old). Future extensions of the STELLA model can incorporate land use to more quantitatively identify the importance of nitrogen sources based on watershed and water quality inputs. Similar models have been able to account for variation in chlorophyll *a* or dissolved organic carbon based on estuary characteristics and water quality monitoring (Meeuwig 1999, Cifuentes and Eldridge 1998, Carmichael et al. 2004). Improved modeling linkages with land use data would allow generalizations and predictions of nitrogen source to be made for other estuarine systems, including other components of the National Estuarine Research Reserve System. Linking STELLA directly to monitored water quality metrics, e.g. total nitrogen or dissolved nitrate concentrations, would provide a powerful tool to readily identify nitrogen sources over broad regions which are currently monitored regularly over various temporal and spatial scales.

Discussion

This research, in conjunction with two years previous research on oyster $\delta^{15}\text{N}$ in the Monie Bay component of Chesapeake Bay National Estuarine Research Reserve System, provides evidence that oysters can serve as biological indicators and that anthropogenic nitrogen sources can be inferred from $\delta^{15}\text{N}$ in their tissues. Deployment provides ability to identify nitrogen sources in areas where natural communities are not readily available or to identify spatial patterns through interpolation.

Water quality monitoring data can be used to calibrate and extend the utility of indicators of nitrogen pollution. Biological indicators, such as the eastern oyster (*Crassostrea virginica*), can be used in conjunction with water quality monitoring data to identify important nitrogen sources. Spatial patterns were very similar to those previously observed in 2006-7. Nutrient inputs were located in upstream areas, likely from terrestrial sources. Spatial patterns of Water Quality Index were similar to those of the relevant measurements. Generally, however, Water Quality Index was low, < 0.5 , despite that Monie Bay and portions of its watershed are protected as a part of the National Estuarine Research Reserve System.

Comparisons of water quality over seasonal and long-term time periods and oyster $\delta^{15}\text{N}$ over small and broad scales can be instructive for placing current status and trends into context for better understanding this ecosystem. Tangier sound has exhibited long-term changes in water quality. Long-term records of water quality in Tangier Sound (connecting Monie Bay with Chesapeake Bay) fluctuate, becoming degraded after periods of increases in numbers of chickens sold and improving after declines in chicken sales.

As hypothesized, oyster $\delta^{15}\text{N}$ values were helpful for inferring spatial sources of anthropogenic nitrogen and linking to water quality trends. Water Quality Index values indicate two sources of nitrogen to the Monie Bay system; one smaller source arriving from terrestrial sources along Monie Creek, and a larger source external to Monie Bay. Oyster muscle $\delta^{15}\text{N}$ varied spatially in a manner complementary to Water Quality Index and indicated nitrogen sources. Elevated $\delta^{15}\text{N}$ signals suggest that human and animal wastes enter Monie Creek from anthropogenic sources within the watershed, while human and/or animal wastes can be inferred to enter Monie Bay at its mouth, via either Wicomico River or Tangier Sound based on $\delta^{15}\text{N}$ values in oysters.

Oyster muscle $\delta^{15}\text{N}$ values in Monie Creek were deduced to be derived from animal or human waste nitrogen. Though only 19 poultry houses in the Monie Creek watershed were counted by digital ortho-imagery (USDA 2005), these contained an estimated effective year-round population of 23,661 chickens house⁻¹ and produced $\sim 8.6 \times 10^5$ kg total nitrogen (untreated) yr⁻¹, based on an estimated 58 kg manure chicken⁻¹ yr⁻¹ (containing 1.8 kg total nitrogen yr⁻¹; Naber and Bermudez 1990), roughly equivalent to that defecated by 2.0×10^5 people (assuming 4.3 kg total nitrogen generated person⁻¹ yr⁻¹). In addition to potential inputs from the large quantity of poultry and corresponding manures, septic sources and wastewater treatment plants are also alternative potential anthropogenic inputs. Residential septic systems along Monie Creek may have enriched the oyster $\delta^{15}\text{N}$ signal, although only 699 septic systems were in the entire Monie Bay's watershed, including that for Little Monie Creek and Little Creek (MD DNR 1999b). The nearest sewage and septic sources of nitrogen are the Salisbury, Delmar, and Fruitland wastewater treatment plants, which currently input

substantial nitrogen loads into the Wicomico River, and are slated for enhanced nitrogen removal upgrades by 2015 (Maryland Department of the Environment, 2007a,b,c). The sewage and septic nitrogen detected in the creeks of Monie Bay are potentially due to these sources of nitrogen.

Oyster $\delta^{15}\text{N}$ in Monie Bay fits within broader spatial patterns across Chesapeake Bay. Monie Bay did not uniquely receive human and/or animal wastes from Chesapeake Bay in addition to from its watershed. Context from the county and Delmarva Peninsula is needed for perspective on coarse-scale anthropogenic input calculations presented for Monie Bay and its watershed. Delmarva Peninsula hosts an effective chicken population of $\sim 1.1 \times 10^8$, producing an estimated 2.1×10^8 kg total nitrogen yr⁻¹, more than that generated by 4.8×10^7 people, while in comparison the estimated 2002 human population on Delmarva Peninsula was only 1.2×10^6 people (Table 3).

Indication of sewage and septic inputs to Monie Bay from external sources fits with results from other hazardous material monitoring. Dorabawila and Gupta (2005) observed concentrations of the endocrine disruptor estradiol (E2) in Monie Bay at 2.3 ng L^{-1} . Note that concentrations as low as 1 ng L^{-1} can induce vitellogenin production in male fish (Dorabawila and Gupta, 2005). E2 is commonly transported to estuarine ecosystems via effluents from wastewater treatment plants.

Wherever sewage and septic sources of nitrogen ultimately arrive from, results presented here suggest Monie Bay acts as a nitrogen sink, rather than a nitrogen source. In general, oligohaline marshes such as Monie Bay can act as sinks of nitrogen and phosphorous in the short term by denitrification, and in the long-term by sediment

burial of organic matter and marsh accretion. Preliminary results from mixing plots presented in this study suggest non-conservative mixing potentially due to net nitrification, in contrast to previous datasets, which indicated net denitrification. Yet sediment denitrification rates in Monie Bay and its creeks have not been reported in the literature previously. Nutrient burial, calculated by multiplying sediment burial rate ($\text{g m}^{-2} \text{y}^{-1}$) by nutrient concentration (mg g^{-1}) in a core sample, has been found to be lower in Monie Bay than the upper marshes of the Patuxent River (Merrill and Cornwell, 2000). Through STELLA modeling, additional questions regarding the utility of *Crassostrea virginica* $\delta^{15}\text{N}$ as a biological indicator can be addressed to enable the oyster to be used as a monitoring tool.

Monie Bay exemplifies that managed areas are open ecosystems

Like other ecosystems, Monie Bay is subjected to anthropogenic stressors in the form of multiple nutrient inputs from both inside and outside its topographically defined watershed. Similar patterns of anthropogenic nutrient sources are typical of Chesapeake Bay tributaries, such as the Patuxent River. Sewage discharge to the Patuxent River from its watershed is a major cause of increased nutrients over a 30 yr record (Fisher et al. 2006). Chesapeake Bay acts as an additional source of nutrients to the Patuxent River as nutrients from other portions of Chesapeake Bay's watershed enter at the mouth via bottom layer circulation (Testa et al. 2008). Examples of multiple routes of nutrient delivery, e.g. from inflowing river and mid-estuary sources to a California estuary, have been revealed through variability in macroalgae $\delta^{15}\text{N}$ values (Cohen and Fong 2006). Mixing or upwelling processes can also provide ecosystems

with additional nutrient inputs from outside its defined watershed, such as in the Wadden Sea (e.g. van Beusekom and de Jonge 2002, Weston et al. 2004) and the Oregon coast (Frick et al. 2007). Furthermore, in some cases of homogeneously low topography, nutrient delivery via groundwater may extend beyond a topographically defined watershed as watertable and surface topographies may not align (Winter et al. 2003; Kasper 2006). Such external groundwater inputs have been identified in Maryland's Coastal Bays watersheds, which are characterized by high soil permeability and a lack of river/stream freshwater inputs (Dillow et al. 2002, Ullman et al. 2003; Volk et al. 2006). Oyster biological indicators have also been successfully applied in these coastal lagoons and have detected elevated $\delta^{15}\text{N}$ values (Fertig et al. 2009). Note that in these coastal lagoon ecosystems, given a high surface area to volume ratio of coastal lagoons, atmospheric deposition may be more important than in larger, deeper ecosystems (Giblin and Gaines 1990; Paerl 1995). Therefore, in such ecosystems around the world, nutrient sources external to the associated watersheds will need to be addressed to achieve improvements in water quality conditions.

Monie Bay, as an extension of Chesapeake Bay, ultimately receives a portion of its anthropogenic inputs from the $\sim 166,500 \text{ km}^2$ Chesapeake Bay watershed. Such anthropogenic nutrient inputs may dominate those generated within the sub-watershed. Due to its small watershed area (72.3 km^2), anthropogenic activities in Monie Bay's watershed that result in downstream nitrogen inputs generally occur within 6 km of its creeks, as compared to larger ecosystems (e.g. Jordan et al. 1997; Brawley et al. 2000; Turner and Rabelais 2003). Positioned in Chesapeake Bay's mesohaline region, oceanic exchange is less important to Monie Bay than to Maryland's Coastal Bays,

where two inlets help to control residence times (Pritchard 1960). Macroalgae in these coastal lagoons may utilize bioavailable organic nitrogen, which when recycled drives nutrient cycles in Maryland's Coastal Bays (Glibert et al. 2007).

Management efforts to reduce nutrient inputs to aquatic ecosystems can be made more effective when aided by knowledge of sources. For example, macroalgae $\delta^{15}\text{N}$ have identified spatial patterns of wastewater inputs (Costanzo et al. 2001). The current study identified two distinct inputs of human or animal waste nitrogen to Monie Bay, which can be targeted for future nutrient reduction efforts. Both non-point and point-sources can be addressed, but because human or animal waste nitrogen (indicated by oyster $\delta^{15}\text{N}$) explains much of the variation of the Water Quality Index, this nitrogen source may hold priority to address, at both of its input locations. Inputs to Monie Bay suggest that this research reserve may act as a nitrogen sink for other watersheds, such as the Wicomico River. Further deployments of oysters transecting Wicomico River leading to Monie Bay could identify if septic or wastewater nitrogen from the Wicomico River enters Monie Bay. Interactions between watersheds suggest the need to holistically manage research reserves and other protected areas. In certain cases, environmental management will need to extend efforts outside topographically defined watersheds.

Management Implications

Ultimately, preserving the Monie Bay component of the National Estuarine Research Reserve and the Deale Island Wildlife Management Area are not enough to keep Monie Bay and its creeks in pristine condition. As broad spatial patterns suggest,

exchange with Chesapeake Bay and hence inputs from other watersheds, including the Lower Wicomico River sub-watershed, potentially influences and degrades water quality in Monie Bay and downstream portions of its creeks. Therefore, a broad management scope is necessary to protect this resource for recreation, research, and other management uses due to the complex ecological influences and interchanges between Monie Bay and nearby watersheds.

Within the confines of the Monie Bay component of the Chesapeake Bay National Estuarine Research Reserve, it is important to continue efforts to understand the factors driving nutrient sources, sinks, transport, and exchange mechanisms. Monitoring will be increasingly important to assess Monie Bay ecosystem health and focus pertinent management nutrient reduction efforts. Efforts must be coordinated and kept in context of external sources, which potentially add much larger amounts of nutrients to the system than internal sources.

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Appendix 1: Water Quality Monitoring Data

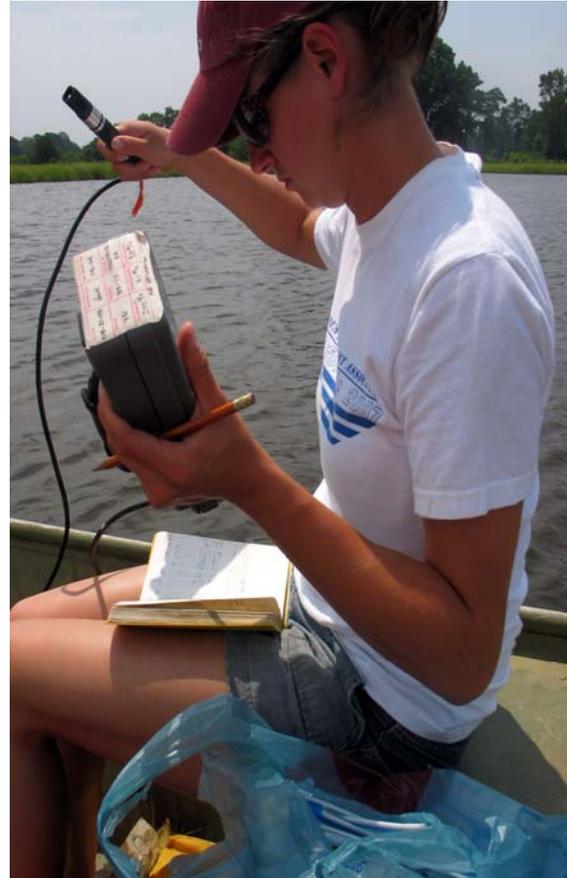
Date	Time	Regions	Site	Secchi depth (m)	Total Depth (m)	Surface Temperature (C)	Surface Salinity	Surface DO (mg L-1)	Surface DO (% saturation)	Bottom Temperature	Bottom Salinity	Bottom DO (mg L-1)	Bottom DO (% saturation)
4/15/2008	15:25	Monie Bay	MB 1	1.5	2.5	14.4	11.4	3.96	40.6	14.3	7.4	3.96	nd
4/15/2008	nd	Monie Bay	MB 2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/15/2008	13:15	Little Creek	MB 3	0.3	0.3	14.8	11.0	3.45	37.6	nd	nd	nd	77.5
4/15/2008	13:33	Little Creek	MB 4	0.1	2.0	14.4	11.3	3.60	36.7	14.2	9.0	3.66	32.0
4/15/2008	14:20	Little Monie Creek	MB 5	0.5	0.8	15.9	8.7	2.97	32.5	16.0	8.7	3.00	31.6
4/15/2008	14:41	Little Monie Creek	MB 6	1.0	2.5	15.7	10.1	3.04	32.3	15.7	10.1	3.01	32.4
4/15/2008	14:59	Little Monie Creek	MB 7	1.0	3.5	15.0	2.5	3.20	33.0	15.1	11.0	3.12	40.7
4/15/2008	11:53	Monie Creek	MB 8	nd	nd	15.0	6.4	5.66	54.8	nd	nd	nd	49.1
4/15/2008	12:24	Monie Creek	MB 9	0.5	3.5	15.0	8.4	4.93	51.2	14.8	8.5	4.79	45.9
4/15/2008	12:44	Monie Creek	MB 10	0.5	3.0	15.0	10.3	4.54	47.6	14.7	10.4	4.46	14.6
4/29/2008	nd	Monie Bay	MB 1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/29/2008	nd	Monie Bay	MB 2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/29/2008	nd	Little Creek	MB 3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/29/2008	nd	Little Creek	MB 4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/29/2008	nd	Little Monie Creek	MB 5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/29/2008	nd	Little Monie Creek	MB 6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/29/2008	nd	Little Monie Creek	MB 7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/29/2008	nd	Monie Creek	MB 8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/29/2008	nd	Monie Creek	MB 9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/29/2008	nd	Monie Creek	MB 10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
5/6/2008	2:12	Monie Bay	MB 1	0.7	2.9	20.3	11.9	2.80	32.5	20.3	11.9	2.77	32.3
5/6/2008	1:45	Monie Bay	MB 2	0.6	3.2	21.3	11.1	2.39	28.0	21.3	11.1	2.67	26.9
5/6/2008	2:38	Little Creek	MB 3	0.7	2.8	21.3	11.3	2.11	25.3	21.3	11.3	2.11	25.1
5/6/2008	2:47	Little Creek	MB 4	0.9	2.1	21.7	11.5	2.50	30.1	21.4	11.5	2.39	28.3
5/6/2008	3:10	Little Monie Creek	MB 5	0.8	1.4	21.7	10.5	1.84	21.9	21.6	9.4	1.51	21.4
5/6/2008	3:25	Little Monie Creek	MB 6	0.9	1.1	21.7	11.4	2.36	28.3	21.6	11.4	2.31	27.6
5/6/2008	3:40	Little Monie Creek	MB 7	0.9	3.2	21.9	11.4	2.45	29.4	21.8	11.4	12.80	168.0
5/6/2008	12:37	Monie Creek	MB 8	0.5	3.3	21.7	4.8	1.50	18.1	21.4	3.7	1.29	16.5
5/6/2008	1:02	Monie Creek	MB 9	0.7	3.6	21.7	7.4	1.78	21.0	21.6	7.6	1.76	20.4
5/6/2008	1:20	Monie Creek	MB 10	0.5	3.2	21.0	10.1	2.00	23.0	21.0	10.1	1.93	22.8
5/22/2008	13:40	Monie Bay	MB 1	0.5	3.0	18.1	10.8	7.75	87.5	18.0	10.9	7.81	87.4
5/22/2008	15:25	Monie Bay	MB 2	1.0	2.5	18.4	9.9	7.17	78.7	17.9	10.0	7.04	78.9
5/22/2008	14:07	Little Creek	MB 3	1.0	3.5	18.0	9.1	6.09	62.0	17.9	9.1	5.40	62.1
5/22/2008	14:25	Little Creek	MB 4	0.5	3.0	18.0	10.1	6.29	70.0	18.0	10.1	6.00	68.5
5/22/2008	14:45	Little Monie Creek	MB 5	0.5	2.0	18.9	5.0	4.37	50.0	19.0	5.0	3.80	40.7
5/22/2008	15:00	Little Monie Creek	MB 6	0.5	2.0	18.3	8.7	5.82	65.3	18.2	8.6	4.36	54.0
5/22/2008	15:10	Little Monie Creek	MB 7	0.5	3.0	18.3	10.2	6.76	78.9	18.2	10.2	6.74	75.7
5/22/2008	12:21	Monie Creek	MB 8	0.5	2.5	19.2	0.9	4.65	50.3	19.0	0.9	4.53	47.7
5/22/2008	12:45	Monie Creek	MB 9	0.5	3.0	19.2	2.5	4.36	51.3	18.0	2.5	3.97	41.5
5/22/2008	13:03	Monie Creek	MB 10	0.3	3.5	18.5	5.9	5.53	62.4	18.4	6.0	5.54	61.0
6/17/2008	13:40	Monie Bay	MB 1	0.4	3.0	26.8	10.0	6.01	79.8	26.8	10.1	6.00	79.3
6/17/2008	15:25	Monie Bay	MB 2	0.4	1.9	27.0	9.3	5.46	72.5	26.9	9.3	5.48	73.0
6/17/2008	14:10	Little Creek	MB 3	0.6	2.1	26.9	9.3	4.99	65.2	26.9	9.3	4.89	62.6
6/17/2008	14:25	Little Creek	MB 4	0.5	2.4	26.9	9.6	5.53	72.6	26.9	9.6	5.41	71.1
6/17/2008	14:45	Little Monie Creek	MB 5	0.5	1.4	27.4	7.9	4.68	61.0	27.1	7.9	4.15	54.8
6/17/2008	15:00	Little Monie Creek	MB 6	0.6	1.9	26.9	9.2	4.84	64.1	26.8	9.3	4.80	63.2
6/17/2008	15:05	Little Monie Creek	MB 7	0.5	2.7	26.8	9.6	5.34	70.5	26.8	9.6	5.26	69.3
6/17/2008	12:20	Monie Creek	MB 8	0.6	3.1	27.7	3.2	2.48	32.5	27.4	3.3	2.00	27.3
6/17/2008	12:50	Monie Creek	MB 9	0.6	2.5	27.6	5.6	3.58	46.9	27.6	5.6	3.50	46.7
6/17/2008	13:05	Monie Creek	MB 10	0.4	3.1	27.0	8.2	4.39	56.5	26.9	8.3	4.18	55.0
7/1/2008	14:18	Monie Bay	MB 1	0.5	2.7	28.0	10.7	6.10	82.9	28.0	10.7	6.04	81.9
7/1/2008	14:38	Monie Bay	MB 2	0.5	2.0	28.3	9.7	5.78	78.6	28.3	9.7	5.64	76.7
7/1/2008	13:35	Little Creek	MB 3	0.6	2.5	28.2	9.9	4.76	64.2	27.9	9.9	4.72	63.6
7/1/2008	13:50	Little Creek	MB 4	0.7	2.7	27.9	10.1	5.70	76.8	27.9	10.1	5.65	76.2
7/1/2008	12:56	Little Monie Creek	MB 5	0.6	1.2	28.3	8.9	3.76	50.8	28.3	8.9	3.74	50.5
7/1/2008	13:05	Little Monie Creek	MB 6	0.6	1.2	27.7	9.9	4.71	63.0	27.7	9.9	4.54	61.3
7/1/2008	13:19	Little Monie Creek	MB 7	0.6	2.7	27.8	10.1	5.58	75.3	27.7	10.1	5.47	73.6
7/1/2008	11:45	Monie Creek	MB 8	0.5	3.0	29.1	4.5	3.09	41.3	29.1	4.6	3.03	40.5
7/1/2008	12:05	Monie Creek	MB 9	0.6	2.3	28.8	6.6	3.31	44.2	28.7	6.7	3.10	41.6
7/1/2008	12:26	Monie Creek	MB 10	0.5	3.0	28.0	9.0	4.26	57.2	28.0	9.1	4.29	57.7
7/31/2008	13:45	Monie Bay	MB 1	0.6	2.6	28.8	11.6	5.65	77.4	28.8	11.6	5.48	76.9
7/31/2008	13:22	Monie Bay	MB 2	0.5	2.0	29.2	10.7	4.87	68.0	29.1	10.7	4.58	63.7
7/31/2008	14:10	Little Creek	MB 3	0.7	3.8	29.1	10.7	3.97	56.1	29.1	10.7	3.78	54.5
7/31/2008	14:25	Little Creek	MB 4	0.8	2.8	29.0	10.9	4.49	66.4	29.1	10.9	4.38	62.9
7/31/2008	14:44	Little Monie Creek	MB 5	0.6	1.9	29.5	10.4	3.51	48.5	29.4	10.4	3.32	46.3
7/31/2008	14:57	Little Monie Creek	MB 6	0.5	0.8	29.1	10.8	4.05	56.6	29.1	10.8	3.98	56.7
7/31/2008	15:03	Little Monie Creek	MB 7	0.6	2.6	29.1	10.9	4.83	67.9	29.1	10.9	4.84	68.1
7/31/2008	12:24	Monie Creek	MB 8	0.5	2.6	29.4	7.5	2.93	40.0	29.3	7.7	2.90	39.1
7/31/2008	15:37	Monie Creek	MB 9	0.5	2.0	29.3	9.3	3.79	50.4	29.3	9.3	3.63	49.7
7/31/2008	13:04	Monie Creek	MB 10	0.4	3.0	29.1	10.5	4.02	55.1	29.0	10.5	4.08	55.9
9/8/2008	12:54	Monie Bay	MB 1	0.9	2.3	27.4	13.4	6.77	91.9	25.5	13.5	5.77	74.0
9/8/2008	15:07	Monie Bay	MB 2	0.8	1.3	27.3	12.8	5.53	77.5	26.7	12.9	5.19	71.5

Date	Time	Regions	Site	Chlorophyll a	Phaeophytin	TN	NH4	NO23	NO2	TP	PO4	TSS	TVS	WQI
				(µg L-1)	(µg L-1)	(mg L-1)	(mg L-1)	(mg L-1)	(mg L-1)	(mg L-1)	(mg L-1)	(mg L-1)		
4/15/2008	15:25	Monie Bay	MB 1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/15/2008	nd	Monie Bay	MB 2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/15/2008	13:15	Little Creek	MB 3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/15/2008	13:33	Little Creek	MB 4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/15/2008	14:20	Little Monie Creek	MB 5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/15/2008	14:41	Little Monie Creek	MB 6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/15/2008	14:59	Little Monie Creek	MB 7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/15/2008	11:53	Monie Creek	MB 8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4/15/2008	12:24	Monie Creek	MB 9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.00
4/15/2008	12:44	Monie Creek	MB 10	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.00
4/29/2008	nd	Monie Bay	MB 1	12.5	-4.6	0.79	0.012	0.113	0.0047	0.0283	0.0057	48.3	9.7	nd
4/29/2008	nd	Monie Bay	MB 2	6.2	2.5	0.83	0.005	0.031	0.0025	0.0351	0.0047	41.3	7.0	nd
4/29/2008	nd	Little Creek	MB 3	1.3	1.4	0.78	0.037	0.022	0.0016	0.0286	0.0030	61.0	9.7	nd
4/29/2008	nd	Little Creek	MB 4	-1.3	8.2	0.60	0.054	0.028	0.0044	0.0237	0.0047	43.7	7.3	nd
4/29/2008	nd	Little Monie Creek	MB 5	13.7	0.3	0.82	0.007	0.027	0.0021	0.0585	0.0061	33.3	9.0	nd
4/29/2008	nd	Little Monie Creek	MB 6	-3.7	16.0	0.83	0.025	0.049	0.0041	0.0412	0.0042	41.0	8.3	nd
4/29/2008	nd	Little Monie Creek	MB 7	0.0	4.4	0.74	0.099	0.025	0.0094	0.0288	0.0042	38.7	8.0	nd
4/29/2008	nd	Monie Creek	MB 8	51.1	11.7	1.09	0.014	0.016	0.0037	0.0723	0.0057	24.3	10.0	0.00
4/29/2008	nd	Monie Creek	MB 9	37.4	4.5	0.96	0.024	0.005	0.0028	0.0626	0.0055	37.3	10.7	0.00
4/29/2008	nd	Monie Creek	MB 10	nd	nd	0.90	0.012	0.011	0.0015	0.0426	0.0043	51.7	10.3	0.00
5/6/2008	2:12	Monie Bay	MB 1	7.5	-2.2	0.72	0.031	0.127	0.0058	0.0438	0.0054	50.7	7.3	0.25
5/6/2008	1:45	Monie Bay	MB 2	7.5	-3.1	0.77	0.015	0.006	0.0028	0.0360	0.0052	52.0	11.0	0.50
5/6/2008	2:38	Little Creek	MB 3	5.0	-1.5	0.76	0.010	0.017	0.0021	0.0362	0.0027	52.3	8.0	0.50
5/6/2008	2:47	Little Creek	MB 4	6.2	-3.6	0.80	0.006	0.029	0.0022	0.0340	0.0029	41.0	7.0	0.50
5/6/2008	3:10	Little Monie Creek	MB 5	7.5	-2.2	1.16	0.003	0.008	0.0012	0.0469	0.0029	44.7	7.7	0.25
5/6/2008	3:25	Little Monie Creek	MB 6	3.7	-2.9	1.11	0.015	0.038	0.0020	0.0491	0.0037	20.0	4.3	0.25
5/6/2008	3:40	Little Monie Creek	MB 7	6.2	-3.6	0.70	0.003	0.024	0.0017	0.0348	0.0029	47.0	8.0	0.75
5/6/2008	12:37	Monie Creek	MB 8	15.0	0.8	1.07	0.004	0.009	0.0014	0.0807	0.0041	30.7	9.0	0.00
5/6/2008	1:02	Monie Creek	MB 9	10.0	-7.4	1.26	0.059	0.005	0.0082	0.0644	0.0074	32.7	8.0	0.25
5/6/2008	1:20	Monie Creek	MB 10	6.2	8.6	0.91	0.039	0.009	0.0045	0.0617	0.0058	71.3	12.7	0.25
5/22/2008	13:40	Monie Bay	MB 1	7.5	-3.1	1.63	0.024	0.060	nd	0.0454	0.0037	62.3	10.7	0.50
5/22/2008	15:25	Monie Bay	MB 2	8.7	0.0	2.91	0.023	0.027	nd	0.1097	0.0031	104.5	16.5	0.50
5/22/2008	14:07	Little Creek	MB 3	2.5	-4.2	0.80	0.019	0.014	nd	0.0255	0.0024	33.7	6.7	0.75
5/22/2008	14:25	Little Creek	MB 4	3.7	-2.9	0.76	0.231	0.013	nd	0.0238	0.0199	40.7	7.3	0.75
5/22/2008	14:45	Little Monie Creek	MB 5	2.5	-1.6	1.27	0.083	0.096	nd	0.0884	0.0174	45.7	9.3	0.25
5/22/2008	15:00	Little Monie Creek	MB 6	6.2	-5.4	0.72	0.030	0.027	nd	0.0458	0.0084	18.3	5.0	0.25
5/22/2008	15:10	Little Monie Creek	MB 7	3.7	1.5	1.58	0.077	0.007	nd	0.0446	0.0122	44.3	8.7	0.50
5/22/2008	12:21	Monie Creek	MB 8	10.0	1.4	6.29	0.040	0.138	nd	0.3240	0.0211	24.0	8.0	0.25
5/22/2008	12:45	Monie Creek	MB 9	16.2	2.1	0.96	0.028	0.094	nd	0.0690	0.0121	23.0	6.7	0.00
5/22/2008	13:03	Monie Creek	MB 10	13.7	2.0	1.11	0.025	0.013	nd	0.0627	0.0036	37.0	8.3	0.50
6/17/2008	13:40	Monie Bay	MB 1	13.7	-0.6	1.07	0.010	0.007	0.0022	0.0547	0.0041	54.3	10.0	0.50
6/17/2008	15:25	Monie Bay	MB 2	10.0	1.4	1.02	0.046	0.003	0.0150	0.0599	0.0041	49.3	8.0	0.50
6/17/2008	14:10	Little Creek	MB 3	8.7	0.0	1.09	0.003	0.004	0.0006	0.0472	0.0029	36.7	5.7	0.25
6/17/2008	14:25	Little Creek	MB 4	8.7	-0.9	0.81	0.031	0.017	0.0006	0.0487	0.0041	43.7	7.7	0.50
6/17/2008	14:45	Little Monie Creek	MB 5	16.2	1.3	1.26	0.003	0.011	0.0006	0.0946	0.0072	35.3	8.0	0.00
6/17/2008	15:00	Little Monie Creek	MB 6	nd	nd	1.10	0.024	0.003	0.0052	0.0630	0.0033	40.3	6.7	nd
6/17/2008	15:05	Little Monie Creek	MB 7	7.5	-0.5	1.01	0.003	0.004	0.0006	0.0571	0.0024	47.3	10.3	0.50
6/17/2008	12:20	Monie Creek	MB 8	11.2	0.1	1.24	0.101	0.034	0.0016	0.0973	0.0215	29.7	6.3	0.25
6/17/2008	12:50	Monie Creek	MB 9	16.2	3.0	1.22	0.087	0.015	0.0235	0.0809	0.0096	49.7	8.3	0.00
6/17/2008	13:05	Monie Creek	MB 10	13.7	2.0	1.07	0.005	0.009	0.0010	0.0651	0.0025	18.3	4.3	0.25
7/1/2008	14:18	Monie Bay	MB 1	7.5	0.4	0.73	0.009	0.003	0.0007	0.0560	0.0034	45.0	12.3	0.50
7/1/2008	14:38	Monie Bay	MB 2	10.0	-3.0	0.84	0.006	0.003	0.0006	0.0554	0.0030	37.7	10.0	0.50
7/1/2008	13:35	Little Creek	MB 3	8.7	-7.9	0.84	0.041	0.008	0.0007	0.0511	0.0053	33.0	7.3	0.25
7/1/2008	13:50	Little Creek	MB 4	7.5	-2.2	0.79	0.023	0.002	0.0014	0.0505	0.0049	28.3	8.3	0.50
7/1/2008	12:56	Little Monie Creek	MB 5	18.7	-3.0	1.03	0.053	0.007	0.0066	0.0910	0.0112	30.3	9.0	0.00
7/1/2008	13:05	Little Monie Creek	MB 6	7.5	0.4	0.81	0.006	0.003	0.0006	0.0560	0.0038	44.7	11.7	0.25
7/1/2008	13:19	Little Monie Creek	MB 7	11.2	-5.1	0.78	0.003	0.003	0.0006	0.0524	0.0030	32.3	10.3	0.50
7/1/2008	11:45	Monie Creek	MB 8	18.7	2.2	1.17	0.004	0.005	0.0010	0.0891	0.0073	28.7	9.0	0.00
7/1/2008	12:05	Monie Creek	MB 9	12.5	2.4	1.02	0.003	0.003	0.0011	0.0729	0.0065	35.7	9.7	0.25
7/1/2008	12:26	Monie Creek	MB 10	12.5	-2.9	0.98	0.008	0.003	0.0006	0.0641	0.0034	52.7	12.7	0.25
7/31/2008	13:45	Monie Bay	MB 1	13.7	-1.5	0.67	0.011	0.003	0.0006	0.0374	0.0028	48.0	8.7	0.50
7/31/2008	13:22	Monie Bay	MB 2	10.0	3.1	0.90	0.022	0.003	0.0009	0.0507	0.0032	48.0	9.3	0.25
7/31/2008	14:10	Little Creek	MB 3	11.2	0.1	0.87	0.021	0.003	0.0006	0.0512	0.0030	48.3	9.3	0.25
7/31/2008	14:25	Little Creek	MB 4	10.0	-2.1	0.95	0.015	0.002	0.0020	0.0405	0.0030	39.7	8.7	0.25
7/31/2008	14:44	Little Monie Creek	MB 5	15.0	2.5	0.99	0.031	0.006	0.0015	0.0816	0.0125	41.7	7.7	0.00
7/31/2008	14:57	Little Monie Creek	MB 6	7.5	1.3	1.21	0.017	0.004	0.0018	0.0624	0.0039	50.3	8.0	0.25
7/31/2008	15:03	Little Monie Creek	MB 7	10.0	0.5	0.88	0.022	0.002	0.0085	0.0572	0.0031	49.0	7.7	0.25
7/31/2008	12:24	Monie Creek	MB 8	15.0	8.6	1.30	0.048	0.009	0.0065	0.0897	0.0102	43.7	11.3	0.00
7/31/2008	15:37	Monie Creek	MB 9	15.0	2.5	1.46	0.020	0.001	0.0006	0.0651	0.0052	42.7	8.3	0.00
7/31/2008	13:04	Monie Creek	MB 10	11.2	3.6	0.97	0.017	0.003	0.0006	0.0537	0.0044	62.3	9.0	0.25

Appendix 2: Oyster Muscle Nitrogen and Carbon

Region	Site	Replicate	Oyster $\delta^{15}\text{N}$	Oyster $\delta^{13}\text{C}$	%N	%C	C/N ratio
Monie Bay	1	1	13.10	-22.96	13.18	45.73	4.05
Monie Bay	1	2	12.56	-22.76	13.34	45.26	3.96
Monie Bay	1	3	13.46	-22.74	12.59	43.87	4.07
Monie Bay	1	4	12.27	-22.80	13.19	46.22	4.09
Monie Bay	1	5	12.44	-22.79	13.08	45.27	4.04
Monie Bay	2	1	11.22	-24.49	13.08	45.22	4.03
Monie Bay	2	2	11.20	-25.14	12.23	45.19	4.31
Monie Bay	2	3	11.13	-24.63	12.09	41.61	4.01
Monie Bay	2	4	11.18	-25.83	11.30	46.75	4.83
Monie Bay	2	5	11.73	-24.84	6.12	22.39	4.27
Little Creek	3	1	11.06	-26.03	12.91	44.63	4.03
Little Creek	3	2	12.56	-26.50	10.99	42.31	4.49
Little Creek	3	3	13.18	-25.87	10.78	38.54	4.17
Little Creek	3	4	12.04	-25.49	13.35	45.04	3.94
Little Creek	3	5	11.79	-26.10	10.91	39.54	4.23
Little Creek	4	1	11.62	-24.82	12.45	43.42	4.07
Little Creek	4	2	11.83	-24.93	12.19	42.31	4.05
Little Creek	4	3	11.73	-24.43	13.47	44.85	3.88
Little Creek	4	4	10.66	-25.29	13.44	45.61	3.96
Little Creek	4	5	14.32	-24.78	11.13	40.81	4.28
Little Monie Creek	5	1	12.07	-25.68	13.00	44.62	4.00
Little Monie Creek	5	2	11.53	-27.10	12.40	44.22	4.16
Little Monie Creek	5	3	12.58	-26.81	12.38	43.67	4.12
Little Monie Creek	5	4	12.87	-26.41	12.74	44.37	4.06
Little Monie Creek	6	1	11.23	-25.67	12.67	46.47	4.28
Little Monie Creek	6	2	11.78	-24.91	13.33	46.67	4.08
Little Monie Creek	6	3	12.28	-24.23	12.83	44.81	4.08
Little Monie Creek	6	4	11.67	-24.49	13.31	45.37	3.98
Little Monie Creek	6	5	11.12	-25.32	13.43	46.29	4.02
Little Monie Creek	7	1	11.23	-24.74	13.49	46.52	4.02
Little Monie Creek	7	2	9.79	-25.71	14.68	50.55	4.02
Little Monie Creek	7	3	10.63	-25.15	13.69	46.76	3.98
Little Monie Creek	7	4	10.84	-24.90	13.56	46.55	4.01
Monie Creek	10	1	13.42	-25.27	10.82	40.89	4.41
Monie Creek	10	2	13.49	-24.94	9.92	37.00	4.35
Monie Creek	10	3	13.46	-24.95	11.07	41.65	4.39
Monie Creek	10	4	14.96	-25.59	10.21	38.98	4.45
Monie Creek	10	5	13.33	-24.65	12.76	44.22	4.04

Appendix 3: Photos



Monitoring water quality. Removing fouling organisms from oyster cages and ropes (left) and using a YSI probe to measure temperature, salinity, dissolved oxygen (right). Cages became fouled regularly, particularly those in Monie Bay (with bryozoans, barnacles, sea squirts), and station MB6 (with wideongrass). Occasionally, blue crabs, mud crabs and other organisms were also found and removed from oyster cages.



Filtering water samples in the field using a portable filtering apparatus. This apparatus attaches to tubing and a hand pump. Filter papers were prepared for the field by placing them into pre-labeled tin foil packets for each metric of interest (chlorophyll a, TSS/TVS, seston $\delta^{15}\text{N}$) which were sorted by sites in plastic zippered bags labeled by site. These bags also contained the test-tube for TN/TP and the three plastic vials for DIN/DIP, all of which were pre-labeled with site number, date, and analysis to be conducted. Filtering in the field saves time that would otherwise later be spent in the laboratory, provided enough hands are available in the field.



This pile driver was observed in 2008 in Monie Creek. A new dock has been built near station MB 8, in the station furthest upstream in this creek. Rural residential development further upstream has been observed along much of Monie Creek's banks.



Heading upstream in Monie Creek towards the boat ramp at the end of Drawbridge Rd. The unique blue-roofed house can be seen in the background and used as a landmark.