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Oyster $\delta^{15}N$ as a Bioindicator of Potential Wastewater and Poultry Farming Impacts and Degraded Water Quality in a Subestuary of Chesapeake Bay

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ABSTRACT

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Anthropogenic nitrogen contributes to water quality degradation, but it is difficult to distinguish sources once they are mixed in coastal ecosystems. Natural abundances of stable nitrogen isotopes ($\delta^{15}N$) were measured in oyster (*Crassostrea virginica*) tissues (muscle, gills, and mantle) during summer 2006 to summer 2008 to identify nitrogen sources in Monie Bay (a subestuary of Chesapeake Bay) receiving freshwater inputs from three tributary creeks. The creeks (estimated flushing times: 3.5, 5.7, and 37.2 d) vary in size and potential nitrogen sources: septic systems and poultry operations (Monie Creek), crop fertilizer (Little Monie Creek), and wetlands, forest, or both (Little Creek). Grand mean oyster tissue $\delta^{15}N$ values (11.8 ± 0.4‰ in muscle, 10.4 ± 0.4‰ in gills, and 10.5 ± 0.3‰ in mantle) indicated a mixture of human and animal sources. Potential nitrogen loss from denitrification (15.1–24.5%) likely did not substantially modify isotopic values, and $\delta^{15}N$ values were greater than would be expected from atmospheric sources, refuting these alternative explanations. Though dilute, spatial patterns supported the inference that human waste, poultry waste, or both entered Monie Bay from its watershed and the adjacent Wicomico River watershed (*via* mixing). Calculated nitrogen generation from poultry manure in the watershed (containing 2.5×10^3 people) was 2.9×10^4 to 1.0×10^6 kg of total nitrogen (TN) *per* year (equivalent to 6.8×10^3–2.3×10^5 people), whereas throughout Delmarva Peninsula (containing 1.2×10^6 people) it was 3.9×10^6 to 1.3×10^8 kg TN y^{-1} (equivalent to 9.0×10^5–3.1×10^8 people). Conservatively estimated (based on 0.038 kg chicken^{-1} y^{-1}), poultry in the Monie Bay watershed generated an amount of nitrogen equivalent to that generated by 263% of the human population. Throughout Delmarva Peninsula, poultry generated an amount of nitrogen equivalent to that generated by 76% of the human population. Estuaries commonly receive nutrients from both inside and outside their watersheds, and oyster $\delta^{15}N$ values elucidated this process locally.

ADDITIONAL INDEX WORDS: *Bioindicators, nitrogen sources, water quality, stable nitrogen isotopes, oysters, poultry manures, land use, land cover*.

INTRODUCTION

Nitrogen from human and animal waste sources (*e.g.* wastewater treatment plants, rural on-site septic treatment systems, and manures) and abiotic sources (*e.g.* chemically synthesized agricultural fertilizers) continue to result in degradation of water quality in Chesapeake Bay (Fisher *et al.,* 2006; Kemp *et al.,* 2005) and other U.S. estuaries (Bricker *et al.,* 2007). Sources are difficult to distinguish with conventional water quality monitoring data (*e.g.* total nitrogen, or TN) due to biogeochemical alteration in the watershed, stream, and estuary. The burgeoning field of biological indicators (*e.g.* Costanzo *et al.,* 2001; McClelland and Valiela, 1998) reports that stable nitrogen isotopes ($\delta^{15}N$) in a range of organisms (Vanderklift and Ponsard, 2003) such as macrophytes (*e.g.* Cole *et al.,* 2004), finfish (*e.g.* Lake *et al.,* 2001), and mollusks (*e.g.* Piola *et al.,* 2006) can be used to distinguish between chemically synthesized agricultural fertilizers and human or animal wastes. Oysters are filter feeders that integrate nitrogen derived from microorganisms, phytoplankton, detritus, and inorganic particles (Langdon and Newell, 1996). Furthermore, oyster muscle $\delta^{15}N$ integrates these sources over roughly 4 months, and oyster mantle and gill $\delta^{15}N$ integrate over roughly 2 to 3 months (Fertig *et al.,* 2010), providing a benefit over direct measurements on groundwater (Aravena, Evans, and Cherry, 1993) or the water column (Lefebvre *et al.,* 2007), which only provide an instantaneous measurement.

Determination of nitrogen source by measuring $\delta^{15}N$ is possible, because natural sources and synthetic fertilizers are "fixed" from atmospheric N_2 (0‰) and have correspondingly

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low $\delta^{15}N$ values: generally –4 to +4‰ (Bateman and Kelly, 2007; Hübner, 1986; Macko and Ostrom, 1994; Vitoria *et al.,* 2004). More positive $\delta^{15}N$ values are indicative of human waste, animal waste, or both types of nitrogen sources, but these cannot be distinguished from one another because they are due to the same process of fractionation. Human sewage wastes are fractionated to +5 to +8‰ (Fry, 2006). Poultry manure has been measured to have $\delta^{15}N$ values of 11.0 ± 4.4‰ in Spain (Curt *et al.,* 2004), while poultry manure from the Delmarva Peninsula has $\delta^{15}N$ values of 10.4 ± 2.2‰ and $\delta^{13}C$ values of –16.9 ± 0.7‰ (n = 13). Atmospheric deposition of ammonia originating from animal feeding operations has been observed to be negative (–10 to –15‰ or lower) but would likely not vary greatly over small geographic areas. Fractionation is due to a combination of ammonia volatilization and denitrification at the source of the signal or due to microbial processing employed by wastewater treatment facilities (Fry, 2006; Kendall, 1998; McClelland and Valiela, 1998; Sweeny and Kaplan, 1980; Tucker *et al.,* 1999). Denitrification can, however, result in large variability of nitrate $\delta^{15}N$ values—to within or well above the range observed for human, animal, or both waste signals (Heaton, 1986; Herbel and Spalding, 1993; ; Kendall, Elliott, and Wankel, 2007). Interpretation of source must therefore be balanced against alternative hypotheses, because terrestrial nitrogen sources have a complicated pathway to aquatic biological indicators and isotopic signatures can be modified or multiple sources can be mixed (Fry, 2006; Kendall, 1998). Nitrogen from manures can be further fractionated by volatilization (Cline and Kaplan, 1975; Fry, 2006; Kendall, 1998) or can dissolve and be denitrified, both of which elevate $\delta^{15}N$ in the remaining nitrate (Mariotti *et al.,* 1982; Shearer and Kohl, 1988). Phytoplankton assimilates dissolved inorganic nitrogen and is consumed by oysters, with an enrichment of 3 to 4‰ at each trophic step due to digestion and waste elimination (Adams and Sterner, 2000; Minagawa and Wada, 1984). Oyster tissue $\delta^{15}N$ values in Monie Bay have been found to match expected values generated from a 50:50 mixture of ammonium and nitrate $\delta^{15}N$ modified by two trophic shifts (Fertig *et al.,* 2010), suggesting minimal fractionation by phytoplankton not associated with trophic shifts.

Oyster $\delta^{15}N$ presents an opportunity to link land use, nitrogen sources, and aquatic living resources (*e.g*. Dennison *et al.,* 1993; Wazniak *et al.,* 2007) to better understand water quality in a rural subestuary of Chesapeake Bay. Nitrogen source identification can direct nutrient reduction priorities in Monie Bay, part of the Chesapeake Bay National Estuarine Research Reserve System (NERRS), designated for monitoring, education, and conservation (Kennish, 2004). The current study addressed the following questions: (1) Can oyster $\delta^{15}N$ link water quality, nitrogen source, and living resources? (2) What role does denitrification play in the nitrogen isotope ratios observed in Monie Bay and its creeks? (3) What are the nitrogen sources available to Monie Bay and its creeks?

METHODS

Study Location and Experimental Design

The Chesapeake Bay, Maryland, NERRS site includes Monie Bay (38°13′30″ N, 75°50′00″ W), a subestuary of Chesapeake

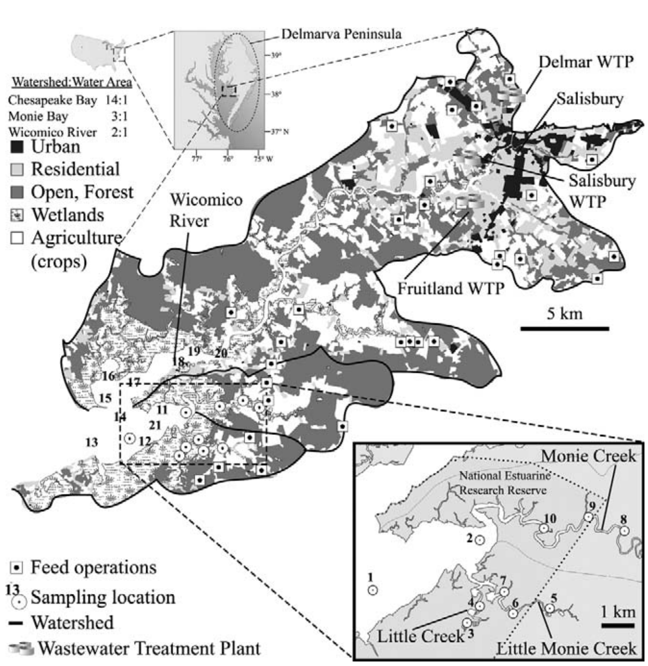

Figure 1. Map locating Monie Bay within Delmarva Peninsula. Land use for the watersheds of Little Creek/Little Monie Creek, Monie Creek, and Wicomico River are shown by color. Locations of wastewater treatment plants and poultry feed operations are noted. Locations of oyster deployment are noted and numbered.

Bay that served as a "natural laboratory" (Figure 1) for linking terrestrial nitrogen sources with aquatic living resources. Monie Bay is a small (1–2 km wide and 4 km long), shallow (1.9 ± 0.1 m), tidally influenced embayment that receives freshwater inputs from three creeks, varying in watershed size and flushing time. Water at the mouth of Monie Bay mixes with that at the mouth of the adjacent Wicomico River. Flushing, springtime flows, intermittent precipitation, and tidal exchange with Monie Bay control salinities in Little Monie Creek and Little Creek, while a first-order stream provides freshwater to Monie Creek year-round (Jones, Murray, and Cornwell, 1997). Tidal scouring, rather than fluvial input, formed these creeks (Ward, Kearney, and Stevenson, 1998), but freshwater input, associated with land use, over spatial and seasonal patterns is a key driver of nutrient delivery (Apple, Del Giorgio, and Newell, 2004).

Forests and wetlands generally dominate Monie Bay's rural and remote watershed (located in Somerset County, Maryland), but comparisons between creeks were made to identify anthropogenic sources of nitrogen, which include agricultural fertilizers, residential septic systems, and poultry feeding operations. While land use in the watersheds of Monie Creek (45.0 km^2) and Little Monie Creek (17.9 km^2) are similar, with more than 50% forest cover, only 3% developed, and the remainder roughly split between wetlands and agriculture (Figure 1), comparisons of nitrogen sources can be made between septic and poultry (Monie Creek) and crop agriculture (Little Monie Creek) due to minimal residential development or poultry production in the Little Monie Creek watershed. Little Creek, with the smallest subwatershed (9.4 km^2), is dominated by wetlands (63%) and forests (35%) and was used as a reference creek because virtually no agriculture (1%) or development (1%) was present (Figure 1). Most forests in Monie Bay's watershed are actually tree farms that are left unfertilized (due to economic constraints; L. Fykes, *personal communication*). Poultry production is also located in the watershed, and poultry houses were counted from tiled digital orthoimagery (1-m ground sample distance) collected during the agricultural growing season (USDA, 2005) and more broadly from Google Earth imagery. While no wastewater treatment plants are located in the watershed of Monie Bay, the watershed of the adjacent Wicomico River contains three (2002 nitrogen loads: Salisbury $\approx 1.8 \times 10^5$ kg N y^{-1}, Fruitland $\approx 9.1 \times 10^3$ kg N y^{-1}, and Delmar $\approx 5.9 \times 10^3$ kg N y^{-1}). To link land use, nitrogen source, and water quality, conventional water quality monitoring and oyster $\delta^{15}N$ values were measured at 10 stations selected to compare inputs from septic and poultry feeding operations (Monie Creek), crop fertilizers (Little Monie Creek), and reference wetlands and forests (Little Creek), as well as downstream effects in Monie Bay (Figure 1). Station 1 was located at the mouth of Monie Bay, at its intersection with the mouth of Wicomico River.

Water Quality Monitoring

Water quality (temperature, salinity, Secchi depth, TN, total phosphorus) was measured at all 10 stations according to NERRS System-Wide Monitoring Program protocols (Mills, Kennish, and Moore, 2008). Total nutrients were considered to include organic fractions, which are at least moderately bioavailable (Seitzinger, Sanders, and Styles, 2002; Wiegner *et al.,* 2006). Water quality data were collected six times in 2006 (June 22, July 11, July 25, August 14, September 7, and October 10), four times in 2007 (June 19, July 27, September 17, and October 8), and nine times in 2008 (April 15 for YSI measurements, April 29 for chlorophyll and nutrient samples, May 6, May 22, June 17, July 1, July 31, September 8, and October 15). Water quality at the additional six stations in 2007 was collected concurrent with oyster deployment and collection (June 19 and October 8). The Maryland Department of Health and Mental Hygiene's Division of Environmental Chemistry analyzed water samples for chlorophyll-*a* with standard methods 10200H. The Chesapeake Biological Laboratory Nutrient Analytical Services Laboratory measured nutrient concentrations (D'Elia, Steudler, and Corwin, 1977; Technicon, 1977, 1986). Water quality data were tested for normality (Proc Univariate, SAS, Inc.) and were nonnormally distributed (Shapiro-Wilk, $p < 0.05$); thus, the nonparametric Kruskal-Wallis test was used for analysis of variance and Spearman's rank was used for correlation coefficients, including that of correlation with oyster data.

Deploying, Sampling, and Analyzing Oyster Biological Indicators

Oysters were deployed according to methods described previously (Fertig *et al.,* 2009, 2010). Spatial variability and nitrogen source identification were sampled by deploying oysters at three sites in Monie Creek, three sites in Little Monie Creek, two sites in Little Creek, and two sites in Monie Bay (Figure 1). Oysters were deployed in summer 2006 (22 June to 10 October), 2007 (19 June to 8 October), and 2008 (15 April to 15 October). Oysters were deployed at an additional six sites (including Wicomico River) during 2007 (Figure 1). Fouling organisms, predators, and trapped sediments were removed regularly to maintain water flow through the bags. Upon collection, oysters were kept on ice in the field and frozen (–20°C) at the laboratory until processing. In 2006, 100 muscle, 100 gill, and 100 mantle samples were collected; in 2007, 63 muscle, 28 gill, and 27 mantle samples were collected; and in 2008, 38 muscle samples were collected. Tissues were thawed, rinsed, thoroughly dried (60°C for 48 hours minimum), and ground (with mortar and pestle wiped free of particles between samples). Subsamples of tissue from individual oysters (1.0 ± 0.2 mg dry weight) were placed in tin capsules (Elemental Microanalysis, pressed, standard weight, 8 × 5 mm) for the elemental content (percent nitrogen [%N] and percent carbon [%C]) and isotopic ratio. The molar C:N ratio was calculated. University of California at Davis's Stable Isotope Facility measured $\delta^{15}N$ and $\delta^{13}C$, where $\delta^{15}N$ or $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 10^3$ and $R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$ with a PDZ Europa automated nitrogen carbon analyzer for gas, solids, and liquids interfaced to a PDZ Europa 20:20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, U.K.). During analysis, samples were interspersed with several replicates of at least two laboratory standards that were selected to be compositionally similar to the samples being analyzed and were previously calibrated against National Institute of Standards and Technology Standard Reference Materials (IAEA-N1, IAEA-N2, IAEA-N3, USGS-40, and USGS-41). A sample's preliminary isotope ratio was measured relative to reference gases analyzed with each sample and were finalized by correcting the values for the entire batch based on the known values of the included laboratory standards. The long-term standard deviation is 0.3‰ for ${}^{15}N$ and 0.2‰ for ${}^{13}C$.

Estimating Flushing Times and Nitrogen Removal

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

$$Q_f = Q_{out} = 1/3 \times \text{precipitation} \quad (1)$$

$$V\, ds_{in}/d_t = 0 = -Q_{out}s_{in} + E(s_{out} - s_{in}) \quad (2)$$

where

Q_f = freshwater input (in cubic meters *per* day)
Q_{out} = advective transport out of the creek (in cubic meters *per* day)
E = nonadvective exchange between Monie Bay and each of the creeks (in cubic meters *per* day)
s_{in} = salinity inside the creek (in parts *per* thousand)
s_{out} = salinity in Monie Bay (in parts *per* thousand)
V = volume of each creek (in cubic meters)

Assumptions for utilizing these equations were (1) that each creek's water volume remained constant and not stratified, (2) that differences in groundwater inputs were negligible because topography and soils were similar, and (3) that therefore Q_f was one-third of the precipitation (assuming the other two-thirds of the precipitation underwent soil infiltration or evapotranspiration). Precipitation volumes were obtained from average daily precipitation from 1971 to 2000 across the area of each creek's watershed (Maryland State Climatologist Office, 2008), while mean measured salinity values for each creek in 2006 were utilized as a constant to solve the equation for E (nonadvective exchange). Flushing times were calculated by dividing creek volume by nonadvective exchange. Nitrogen removal (%) was calculated as $r = 23.4\ m^{0.204}$, where m is months and r is nitrogen removal due to denitrification across lakes, river reaches, estuaries, and the continental shelf (Seitzinger *et al.,* 2006). Estimates of both flushing times and nitrogen source inputs were calculated with the assumption of no net nitrogen import or export across watershed boundaries (manure shipping is not long distance due to economics; L. Fykes, *personal communication*), leaving future studies to quantify nitrogen budgets, manure contributions, and terrestrial–aquatic coupling coefficients.

RESULTS

Water Quality

Monie Bay and its tributaries had degraded overall water quality, largely attributed to concentrations of TN (grand mean 64.4 ± 15.0 μM), total phosphorus (grand mean 1.74 ± 0.41 μM), and associated chlorophyll-*a* (grand mean 10.7 ± 3.0 μg L^{-1}) and dissolved oxygen (4.93 ± 1.15 mg L^{-1}) over summer 2006, 2007, and 2008 (Table 1). Total nitrogen and total phosphorus were positively correlated (Spearman $r = 0.75$, $p < 0.01$). Dissolved oxygen concentrations were negatively correlated with TN (Spearman $r = -0.49$, $p < 0.01$) and total phosphorus ($r = -0.36$, $p < 0.05$) but not with chlorophyll-*a*.

Concentrations of TN increased with distance from the mouth of Monie Bay (Spearman $r = 0.66$, $p < 0.01$), as did those of total phosphorus (Spearman $r = 0.72$, $p < 0.01$) and chlorophyll-*a* (Spearman $r = 0.36$, $p < 0.05$), while dissolved oxygen concentrations decreased with distance from the mouth of Monie Bay (Spearman $r = -0.64$, $p < 0.01$; Figures 2a–d). Total nitrogen was significantly higher in Monie Creek than in Monie Bay (Kruskal-Wallis multiple comparison test, $p < 0.05$). The highest concentrations of TN were observed at site 8 (128.8 ± 142.7 μM in 2008 and 85.5 ± 15.3 μM in 2006) and Site 9 (80.7 ± 10.7 μM in 2007). Total phosphorus was significantly higher in Monie Creek than in Little Creek, Wicomico River, and Monie Bay (Kruskal-Wallis multiple comparison test, $p < 0.05$). The highest concentrations of total phosphorus were observed at site 8 (3.67 ± 1.22 μM in 2006 and 3.50 ± 3.00 μM in 2008) and Site 5 (3.10 ± 0.86 μM in 2006; Figures 2a and b and Table 1). Monie Creek had significantly higher chlorophyll-*a* concentrations than did Little Creek (Kruskal-Wallis multiple comparison test, $p < 0.05$). The highest concentrations of chlorophyll-*a* were observed at site 8 (19.2 ± 8.0 μg L^{-1} in 2006 and 19.2 ± 14.3 μg L^{-1} in 2008) and site 15 (16.2 ± 0.0 μg L^{-1} in 2007).

Oyster Biological Indicators

Grand mean oyster tissue $\delta^{15}N$ values were enriched: 11.8 ± 0.4‰ in muscle, 10.4 ± 0.4‰ in gills, and 10.5 ± 0.3‰ in mantle (Figures 3a–c), and $\delta^{13}C$ values were –25.8 ± 0.8‰ in muscle, –26.6 ± 2.0‰ in gills, and –27.0 ± 0.8‰ in mantle (Figures 3d–f). Grand mean tissue %N content was 12.8 ± 0.7% in muscle, 9.5 ± 0.7% in gills, and 9.3 ± 0.7% in mantle (Figures 3g–i), and tissue %C content was 43.1 ± 1.8% in muscle, 41.7 ± 1.2% in gills, and 41.0 ± 2.1% in mantle (Figures 3j–l).

The observations of the most enriched oyster muscle $\delta^{15}N$ values were in Monie Creek (13.7 ± 0.7‰ at site 10 in 2008) and Monie Bay (13.7 ± 0.3‰ at site 1 in 2006), as well as the Wicomico River (13.4 ± 0.3‰ at site 19 in 2007). Gill and mantle tissues at these sites were also enriched in $\delta^{15}N$.

Oyster $\delta^{13}C$ decreased with distance from the mouth of Monie Bay in the muscle (Spearman $r = -0.43$, $p < 0.05$), the gills (Spearman $r = -0.53$, $p < 0.05$), and the mantle (Spearman $r = -0.73$, $p < 0.01$).

Percent nitrogen did not significantly vary with distance from the mouth of Monie Bay for any tissue. Percent carbon significantly increased with distance in the gills (Spearman $r = 0.54$, $p < 0.05$) and mantle (Spearman $r = 0.54$, $p < 0.05$) but not in the muscle tissue.

Oyster $\delta^{15}N$ in all three tissues exhibited a significant (Spearman, $p < 0.05$) inverse relationship with TN concentrations in the water column, though oyster muscle had the weakest correlation and fit (Spearman, $p = 0.04$, $R^2 = 0.13$) compared to mantle and gills (Spearman, $p < 0.01$, $R^2 > 0.4$; Figures 4a–c).

Nitrogen Removal

Potential nitrogen removal from the tributaries of Monie Bay by denitrification was low (15.1–24.5%) due in part to quick flushing times (3.5–37.2 d) and high nonadvective exchange (maximum of $\sim 1.3 \times 10^5\ m^3\ d^{-1}$ in Little Monie Creek; Table 2), though it may be marginally higher when considering storm events, which may be important biogeochemically. Monie Creek had both the highest total nutrient load and the highest estimated nitrogen removal, both of which were influenced by watershed area ($4.5 \times 10^7\ m^2$), water volume ($\sim 2.5 \times 10^6\ m^3$), mean daily precipitation ($\sim 1.4 \times 10^5\ m^3\ d^{-1}$), slow flushing time (37.2 d; Table 2), slow exchange with Monie Bay (about half that of Little Monie Creek), and land use (including rural residences and poultry feeding operations; Figure 1). In comparison to its creeks, Monie Bay was larger than any of its tributaries ($\sim 1.3 \times 10^7\ m^3$) and was more saline (11.7 ppt) even though it received the highest mean daily precipitation ($\sim 2.2 \times 10^5\ m^3\ d^{-1}$), because its watershed area ($7.2 \times 10^7\ m^2$) was the sum of that of its three tributaries. Nitrogen removal could not be calculated for Monie Bay, because it would be a circular reference for the calculation.

DISCUSSION

Elevated allochthonous nutrient concentrations from multiple nitrogen sources were found in upper reaches of Monie Bay's tributaries (Figures 2a and b; Jones, Murray, and Cornwell, 1997), which led to degraded water quality (Table 1) consistent with land uses (Figure 1; Cornwell, Stribling, and

Table 1. *Surface water quality data observed* N *times in 2006, 2007, and 2008.*

Distance (km)	Site	Year	N	Temperature (°C)	Salinity (ppt)	Secchi Depth (m)	TN (μM)	Total Phosphorus (μM)	Dissolved Oxygen (mg L^{-1})	Chlorophyll-a (μg L^{-1})
Monie Bay										
0.00	1	2006	6	25.1 (3.3)	12.2 (1.3)	0.6 (0.2)	57.3 (3.1)	1.74 (0.31)	7.75 (0.83)	14.0 (3.9)
0.00	1	2007	3	26.8 (1.2)	13.9 (2.8)	0.9 (0.3)	63.9 (3.5)	1.41 (0.22)	5.88 (0.23)	9.1 (1.9)
0.00	1	2008	8	23.1 (5.4)	12.1 (2.1)	0.8 (0.4)	64.1 (25.1)	1.40 (0.30)	5.47 (1.51)	10.4 (3.2)
1.2	12	2007	2	26.7 (1.6)	13.9 (3.7)	1.0 (0.4)	40.0 (3.0)	0.94 (0.00)	6.29 (0.42)	10.0 (0.0)
2.06	13	2007	2	26.1 (1.2)	14.3 (3.7)	0.8 (0.5)	41.8 (2.5)	1.09 (0.22)	6.66 (0.78)	8.7 (0.0)
2.06	21	2007	2	26.0 (0.9)	13.8 (3.7)	0.7 (0.4)	38.6 (0.0)	1.25 (0.00)	3.03 (3.99)	7.5 (0.0)
2.56	11	2007	2	26.3 (1.4)	13.6 (3.8)	0.8 (0.4)	44.3 (10.1)	1.09 (0.22)	5.78 (0.25)	11.2 (0.0)
3.64	2	2006	6	25.7 (3.3)	11.2 (1.7)	0.5 (0.3)	59.8 (10.0)	1.78 (0.43)	7.15 (0.81)	13.0 (4.2)
3.64	2	2007	4	25.8 (3.0)	13.7 (2.3)	0.6 (0.3)	67.1 (6.8)	1.56 (0.00)	4.18 (2.78)	7.5 (2.7)
3.64	2	2008	7	24.7 (4.2)	11.3 (2.2)	0.7 (0.3)	79.7 (57.5)	1.71 (0.82)	5.12 (1.32)	8.4 (1.7)
Little Creek (wetlands)										
4.96	4	2006	6	25.4 (3.6)	11.2 (1.7)	0.7 (0.3)	57.6 (6.5)	1.53 (0.28)	6.53 (1.21)	10.7 (4.7)
4.96	4	2007	4	25.4 (3.3)	13.8 (2.3)	0.7 (0.2)	66.9 (10.1)	1.35 (0.18)	5.15 (0.67)	7.0 (0.9)
4.96	4	2008	8	23.3 (5.3)	11.5 (2.0)	0.7 (0.3)	53.7 (9.7)	1.17 (0.34)	4.38 (1.44)	5.5 (3.8)
6.49	3	2006	6	25.1 (3.7)	10.4 (2.0)	0.7 (0.3)	61.2 (8.4)	1.50 (0.33)	5.66 (0.73)	8.0 (2.8)
6.49	3	2007	4	25.3 (3.5)	13.8 (2.4)	0.7 (0.1)	76.9 (12.7)	1.46 (0.18)	4.44 (0.34)	6.4 (2.0)
6.49	3	2008	8	23.3 (5.3)	11.1 (2.0)	0.7 (0.2)	58.4 (10.9)	1.28 (0.34)	4.09 (1.36)	5.9 (3.7)
Little Monie Creek (crop agriculture)										
5.02	7	2006	6	25.4 (3.5)	11.2 (1.7)	0.5 (0.1)	59.4 (6.4)	1.78 (0.27)	6.43 (1.29)	11.0 (4.3)
5.02	7	2007	4	25.7 (3.2)	13.8 (2.3)	0.7 (0.2)	63.3 (7.6)	1.46 (0.18)	5.04 (0.52)	8.3 (0.7)
5.02	7	2008	8	23.4 (5.1)	10.4 (3.7)	0.8 (0.3)	66.9 (21.6)	1.45 (0.34)	5.95 (3.02)	6.0 (3.9)
6.22	6	2006	6	25.3 (3.5)	10.5 (2.2)	0.6 (0.2)	64.0 (4.8)	2.14 (0.37)	5.62 (1.11)	12.2 (2.2)
6.22	6	2007	4	25.7 (3.3)	13.8 (2.4)	0.7 (0.2)	67.1 (9.9)	1.77 (0.36)	4.37 (0.06)	10.6 (3.6)
6.22	6	2008	8	23.4 (4.9)	11.0 (2.2)	0.7 (0.2)	66.4 (14.5)	1.69 (0.27)	3.89 (1.15)	4.8 (4.4)
7.77	5	2006	6	25.7 (3.7)	9.1 (3.0)	0.6 (0.2)	71.8 (8.7)	3.10 (0.86)	4.54 (1.04)	13.2 (3.6)
7.77	5	2007	4	25.8 (3.9)	13.7 (2.5)	0.7 (0.1)	80.0 (18.2)	2.19 (0.94)	4.25 (0.48)	12.2 (4.0)
7.77	5	2008	8	23.8 (5.1)	9.9 (3.1)	0.6 (0.2)	75.6 (12.7)	2.42 (0.56)	3.19 (0.87)	11.6 (5.8)
Monie Creek (septic/manures)										
8.05	10	2006	6	25.1 (3.5)	9.5 (1.8)	0.7 (0.1)	62.9 (6.3)	1.95 (0.21)	5.83 (1.16)	11.5 (3.2)
8.05	10	2007	4	25.9 (2.9)	13.2 (2.6)	0.7 (0.1)	74.0 (13.3)	1.67 (0.18)	3.85 (2.28)	8.4 (2.8)
8.05	10	2008	8	23.0 (5.1)	10.1 (2.6)	0.5 (0.1)	66.6 (12.1)	1.73 (0.34)	4.10 (1.09)	10.6 (3.5)
11.00	9	2006	6	25.1 (3.7)	6.8 (2.5)	0.6 (0.1)	74.3 (11.0)	2.62 (0.45)	4.77 (0.79)	15.5 (4.9)
11.00	9	2007	4	25.7 (2.8)	12.5 (2.8)	0.7 (0.2)	80.7 (10.7)	1.98 (0.36)	4.14 (0.28)	9.7 (2.6)
11.00	9	2008	8	23.5 (5.2)	8.0 (3.3)	0.6 (0.1)	75.8 (20.8)	2.04 (0.39)	3.47 (0.94)	15.9 (10.5)
12.90	8	2006	6	25.6 (3.2)	4.5 (3.2)	0.5 (0.1)	85.5 (15.3)	3.67 (1.22)	3.88 (1.50)	19.2 (8.0)
12.90	8	2007	4	26.0 (2.9)	11.7 (3.1)	0.6 (0.2)	79.5 (4.6)	2.50 (0.31)	3.75 (0.41)	12.1 (0.8)
12.90	8	2008	8	23.5 (5.3)	6.1 (3.6)	0.5 (0.0)	128.8 (142.7)	3.50 (3.00)	2.96 (1.06)	19.2 (14.3)
Wicomico River (development/manures)										
1.62	14	2007	2	26.4 (1.6)	13.8 (4.4)	0.9 (0.5)	37.9 (1.0)	0.94 (0.00)	5.58 (0.30)	7.5 (0.0)
2.66	15	2007	2	26.6 (1.3)	13.9 (4.6)	0.8 (0.6)	47.5 (14.6)	1.41 (0.22)	5.50 (1.43)	16.2 (0.0)
3.59	17	2007	2	26.1 (1.0)	13.2 (4.9)	0.8 (0.6)	57.5 (24.7)	1.41 (0.66)	5.21 (0.65)	10.0 (0.0)
3.86	16	2007	2	26.6 (1.2)	13.4 (3.8)	0.8 (0.1)	50.4 (19.7)	1.25 (0.44)	5.07 (1.38)	
6.4	18	2007	2	25.9 (0.6)	12.0 (5.2)	0.6 (0.4)	57.9 (14.1)	1.41 (0.22)	5.01 (1.00)	12.5 (0.0)
7.66	19	2007	2	26.1 (0.3)	12.0 (4.9)	0.8 (0.4)	59.6 (17.7)	1.41 (0.22)	5.39 (1.05)	12.5 (0.0)
8.91	20	2007	2	25.6 (0.6)	11.5 (5.4)	0.6 (0.4)	55.4 (1.5)	1.72 (0.22)	2.91 (3.63)	12.5 (0.0)

Oxygen measurements are made at bottom. Distance from station 1 at the mouth of Monie Bay is noted. The mean (standard deviation) of each quality component is reported.

Stevenson, 1994). Spatial patterns of water quality and oyster $\delta^{15}N$ data showed that nutrients were transported to Monie Bay *via* its creeks and were diluted upon reaching larger volume water bodies (Figures 2a–d). Water quality in the NERRS site (Figures 2a–d) was similar to observations made by long-term monitoring stations in the Lower Eastern Shore region. The inverse correlations between the stable nitrogen isotope values and TN concentrations for oyster muscle, gill, and mantle $\delta^{15}N$ (Spearman $R^2 > 0.4$ and $p < 0.01$ for mantle and gill and $R^2 = 0.13$ and $p < 0.05$ for muscle) (Figures 4a–c) linked water quality with septic and poultry nitrogen sources. The watershed's small scale and the tight isotopic variations compared to those of other ecosystems demonstrate the high sensitivity of oyster $\delta^{15}N$ to relatively small inputs in areas with highly contrasting watershed characteristics.

Due to patterns of water circulation and flushing, Monie Bay acted as a nutrient sink for both its watershed and that of the Wicomico River. Nutrient concentrations decreasing toward the mouth of Monie Bay (Figures 2a and b) indicate dilution from terrestrial sources, yet the sources of these diluted nutrients were likely septic, wastewater, and animal manures, because $\delta^{15}N$ values at the mouth of Monie Bay were enriched

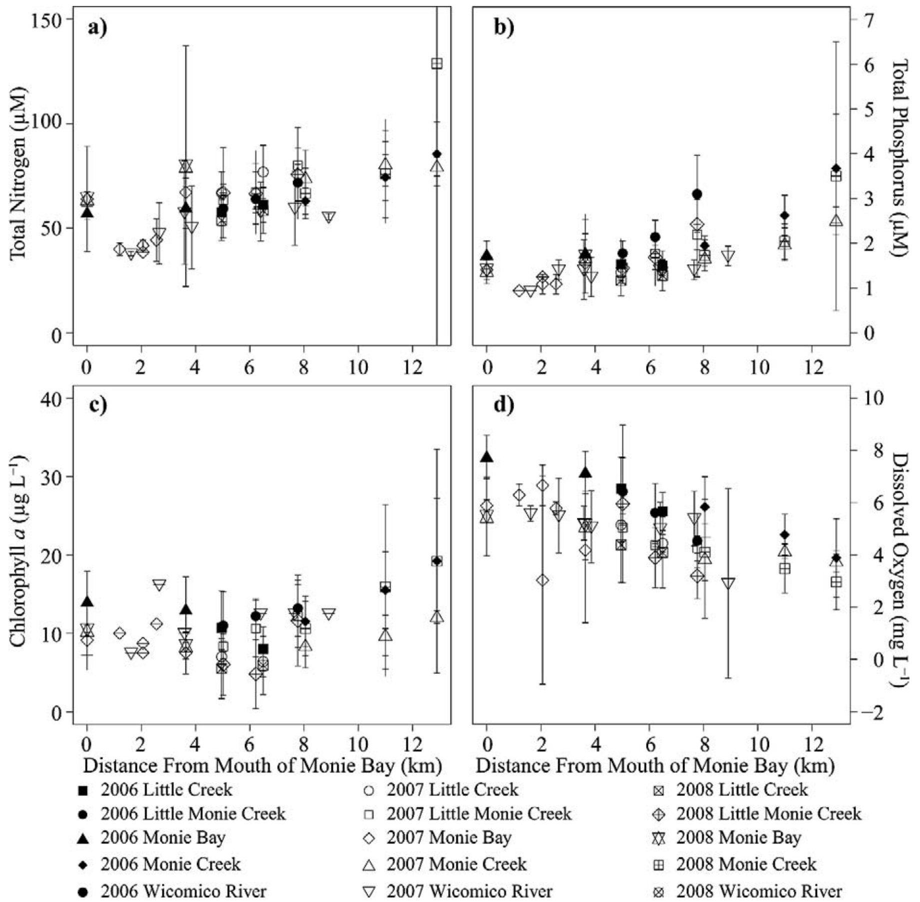

Figure 2. (a) Total nitrogen, (b) total phosphorus, (c) chlorophyll-*a*, and (d) dissolved oxygen concentrations in Little Creek, Little Monie Creek, Monie Creek, Monie Bay, and Wicomico River during summer 2006, 2007, and 2008 plotted against distance (in kilometers) from the mouth of Monie Bay (site 1).

compared to those elsewhere in this system (Figures 3a–c). Fertig *et al*. (2009) reported that nutrient concentration and bioindicator δ^{15}N values may be inversely related as dilution, mixing, and other factors distinguish different types of information. Though Monie Creek likely contributed nutrients, the Wicomico River was likely a main source, because δ^{15}N values at the mouth of Monie Bay matched those farthest upstream in Wicomico River and both were more enriched than values observed in Monie Creek (Figures 3a–c). The Wicomico River watershed includes nitrogen sources such as septic (6543 systems; MD DNR, 1999), wastewater effluents (2002 nitrogen loads in Salisbury $\approx 1.8 \times 10^5$ kg TN y^{-1}, Fruitland $\approx 9.1 \times 10^3$ kg TN y^{-1}, and Delmar $\approx 5.9 \times 10^3$ kg TN y^{-1}), and poultry ($\sim 3.7 \times 10^6$ kg TN y^{-1}; Table 3).

Animal, human, or both wastes were inferred to be important nitrogen sources to Monie Bay and its tributary creeks. While only 19 poultry houses in the Monie Creek watershed were counted by digital orthoimagery (USDA, 2005), these contained an estimated effective year-round population of 23,661 chickens house^{-1}. Poultry population in Monie Bay's watershed was assumed to be proportional to the number of chickens sold (USDA, 2002) and chicken houses (USDA, 2005) in Somerset County (Figure 5a), accounting for an average of 4.7 flocks y^{-1}. Poultry production in the Monie Bay watershed produced about 8.1×10^5 kg TN (untreated) y^{-1} (Table 3), based on an estimated 58 kg manure chicken^{-1} y^{-1} (containing 1.9 kg TN y^{-1}; Naber and Bermudez, 1990), roughly equivalent to that defecated by 1.9×10^5 people (assuming 4.3 kg TN generated *per* person *per* year; Crites and Tchobanoglous, 1998), which is an order of magnitude greater than the human population in Somerset County, Maryland (Figure 5b). In addition, residential septic systems along Monie Creek (699 throughout Monie Bay's watershed; MD DNR, 1999) likely enriched the oyster δ^{15}N signal. Nitrate from agricultural fields likely dominated terrestrial inputs to the outlier in Little Monie Creek, as indicated by oyster δ^{15}N values (11.6 ± 0.6‰ in muscle; Figures 3a–c), which were near the expected value (12.0‰; Bateman and Kelly, 2007; Harrington *et al.,* 1998) for this source (Choi *et al.,* 2007) based upon combining nitrate δ^{15}N values (6.0‰) in nearby watersheds with similar hydric soils and flat topography (T. Fisher and T. Jordan, *personal communication*) with two trophic level shifts (plankton

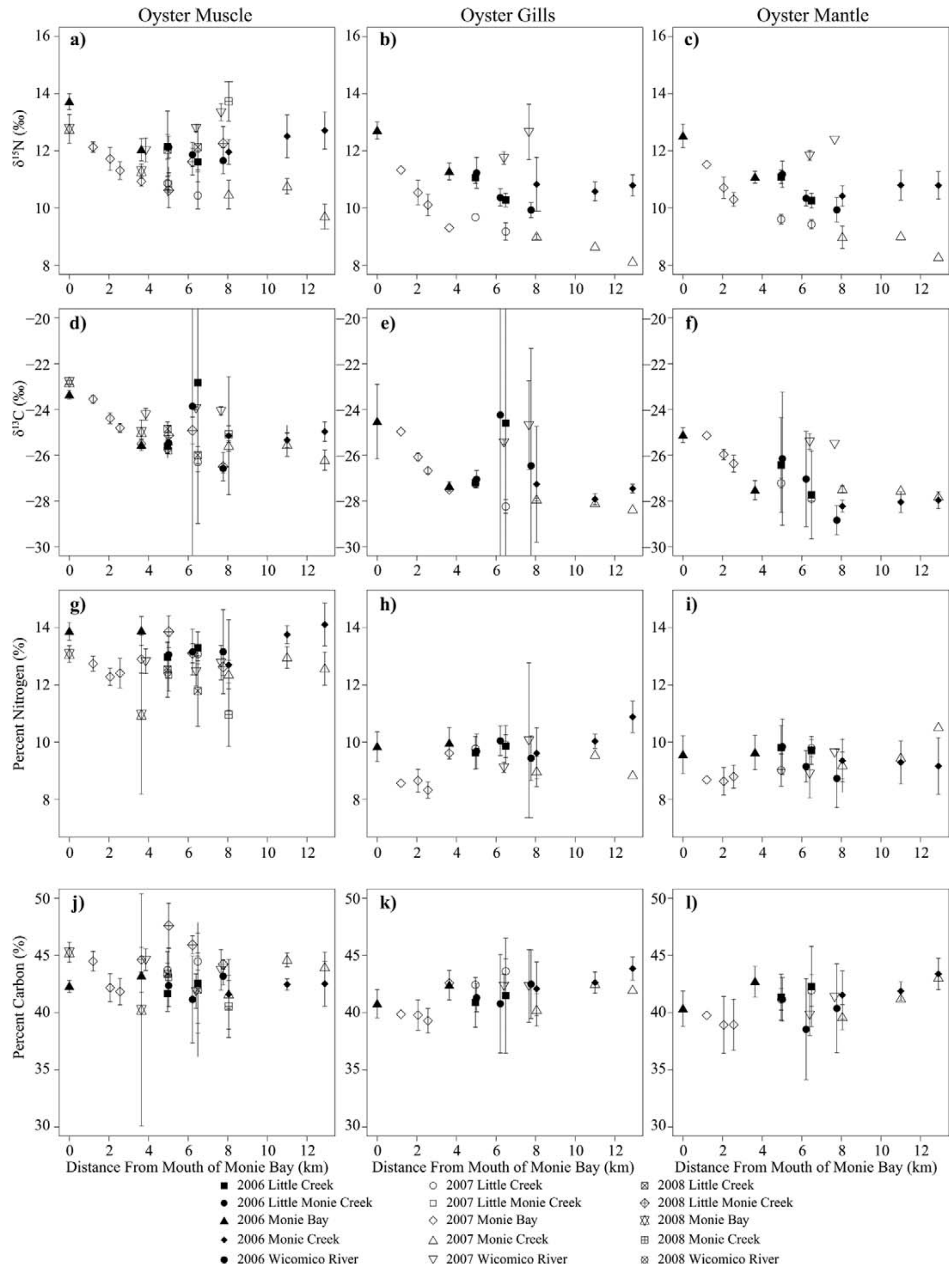

Figure 3. Oyster muscle, gills, and mantle (a–c) $\delta^{15}N$, (d–f) $\delta^{13}C$, (g–i) %N, and (j–l) %C in Little Creek, Little Monie Creek, Monie Creek, Monie Bay, and Wicomico River during summer 2006, 2007, and 2008 plotted against distance (in kilometers) from the mouth of Monie Bay (site 1).

assimilation and oyster consumption) of 3.0‰ each (Adams and Sterner, 2000; Fry, 2006; Minagawa and Wada, 1984). Fertig *et al*. (2010) identified that oysters in Monie Bay ultimately derive nitrogen from a 50:50 mix of nitrate and ammonium. Therefore, reduced nutrient concentrations for this creek could likely be achieved through limiting nutrient inputs from local poultry manure generation (Table 3) in Delmarva Peninsula because Monie Bay, a subembayment of Chesapeake Bay, received a

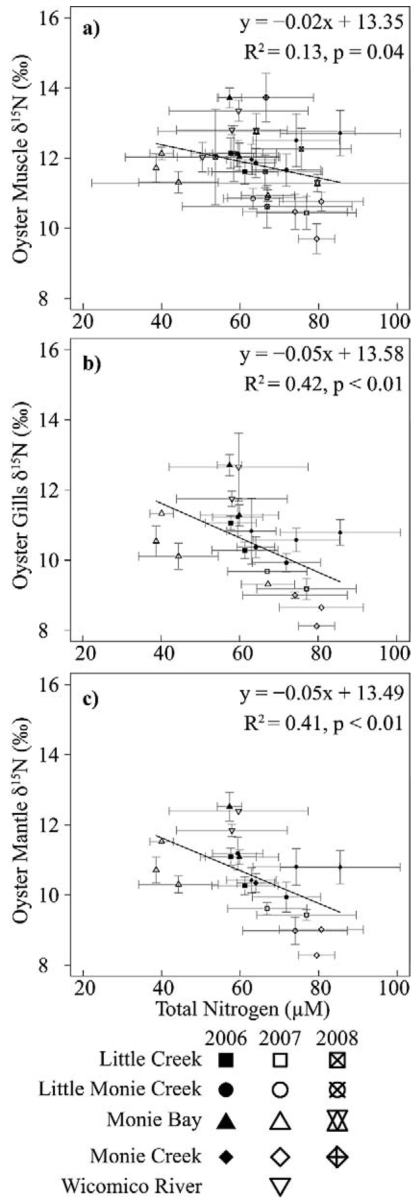

Figure 4. (a) Oyster muscle, (b) gills, and (c) mantle $\delta^{15}N$ vs. water column TN concentration in Little Creek, Little Monie Creek, Monie Creek, Monie Bay, and Wicomico River during summer 2006, 2007, and 2008.

portion of its anthropogenic inputs from the approximately 166,500-km^2 Chesapeake Bay watershed (Figures 1 and 6).

Poultry sources generate large nutrient loads not only to the NERRS site but also to the adjacent Wicomico River watershed and throughout Delmarva Peninsula (Table 3 and Figure 6). Poultry manure in Somerset County is spread locally as fertilizer during spring (L. Fykes, *personal communication*; Figures 1 and 6), which likely contributed to the elevated $\delta^{15}N$ signal along upstream portions of Monie Creek in 2006 (Figures 3a–c). Poultry litter applications increase soil TN content (Kingery *et al.,* 1994) and phosphorus content (Griffin, Honeycutt, and He, 2003) and adversely impacted water quality (Woli, Nagumo, and Hatano, 2002). Countywide, nearly 63.9×10^6 broilers and other meat-type chickens from Somerset County (24th in the nation) were sold in 2002 (Figure 5a; USDA, 2002), produced from approximately 300 poultry houses (counted from orthoimagery; Figure 6), while only about 2.8×10^4 people resided in this county in 2002 (Table 3 and Figure 5b; MDP, 2000). Within the Wicomico River watershed ($\sim$28,000 people), poultry generated an amount of nitrogen equivalent to that generated by about 16,000 people (a conservative comparison of poultry manure to septic systems based on 0.038 kg $chicken^{-1}$ y^{-1}; Lichtenberg, Parker, and Lynch, 2002) to about 5.5×10^5 people (comparing wet poultry manure to septic systems; Table 3). Throughout Delmarva Peninsula ($\sim 1.2 \times 10^6$ people; Table 3 and Figure 6), the average annual poultry population ($\sim 1.0 \times 10^8$ birds; Figure 5a) generated 3.9×10^6 kg TN y^{-1} (conservatively) to 1.3×10^8 kg TN y^{-1} (wet manure), which was the equivalent of that generated by 9.0×10^5 people (conservative estimate of poultry manure to septic systems) to 3.1×10^8 people (wet manure compared to septic systems; Table 3). Therefore, conservatively estimated, the poultry population in Delmarva Peninsula (Figure 6) generated an amount of nitrogen equivalent to 76.4% of the amount generated by the actual human population.

Consistent with the quantities of nitrogen generated by animal and human wastes, the alternative hypothesis that isotopic signatures of nitrogen source were modified by denitrification (Fry, 2006; Kendall, 1998) was considered unlikely due to the magnitude of estimations of potential nitrogen loss *via* denitrification (15–25%) compared to 40 to 50% loss through denitrification in other estuaries (Seitzinger *et al.,* 2006). However, the equation that this estimation relies upon (Seitzinger *et al.,* 2006) is generalized across many systems and assumes that residence time is the only variable controlling denitrification, when at smaller scales other drivers

Table 2. *The simple conservative box model for calculations of flushing time, nonadvective exchange, and potential nitrogen removal via denitrification in Monie Bay and its three tributary creeks.*

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

Salinity was measured in 2006, while daily precipitation was averaged over 1971–2000 (Maryland State Climatologist Office, 2008).

Table 3. Relative inputs to Monie, Wicomico, and Delmarva Peninsula watersheds from sewage, septic, and poultry manure sources.

		Monie Bay	Wicomico River	Delmarva Peninsula	References
Human population (2002)		2576	28,028	1,172,776	MDP, 2000 U.S. Census Bureau, 2000 MD DNR, 2009
Average annual chicken population (2002)		763,560	1,788,912	101,008,080	Naber and Bermudez, 1990 USDA, 2002 USDA, 2005
Chicken manure "people equivalents"	Wet: septic	233,542	547,156	30,894,305	Naber and Bermudez, 1990
	Dry: sewage	83,378	195,343	11,029,751	U.S. EPA, 2002
	Dry: septic	61,398	143,848	8,122,131	
	Conservative: sewage	9207	21,570	1,217,916	
	Conservative: septic	6780	15,884	896,654	
Sewage systems (2002)		0	3	27	A. Brockenbrough, VDEQ, *personal communication* P. Hansen, DNREC, *personal communication* MDE, 2009
Sewage inputs (kg TN y^{-1})		0	196,212	556,090	Crites and Tchobanoglous, 1998 MD DNR, 2009 U.S. EPA, 2002 Tchobanoglous, Burton, and Stensel, 2003
Septic systems		699	7233	181,953	A. Butler, MDP, *personal communication* J. Davis, VDHES, *personal communication* J. Volk, DNREC, *personal communication*
Septic inputs (kg TN y^{-1})		10,304	112,112	3,133,864	U.S. EPA, 2002 Tchobanoglous, Burton, and Stensel, 2003
Manure inputs (kg TN y^{-1})	Wet	1,008,478	2,362,720	133,407,228	Lichtenberg, Parker, and Lynch, 2002
	Dry	265,129	621,160	35,072,840	Parker and Li, 2006
	Conservative	29,276	68,589	3,872,777	USDA, 2005

Poultry manure "people equivalents" are estimated based on the assumed generation 1.9 kg TN $chicken^{-1}$ y^{-1} and 4.3 kg TN $person^{-1}$ y^{-1} (Crites and Tchobanoglous, 1998; Naber and Bermudez, 1990; Tchobanoglous, Burton, and Stensel, 2003) and a conservative estimate of 0.038 kg TN $chicken^{-1}$ y^{-1} (Lichtenberg, Parker, and Lynch, 2002).
DNREC = Delaware Department of Natural Resources and Environmental Control, VDEQ = Virginia Department of Environmental Quality, VDHES = Virginia Department of Health, Eastern Shore District.

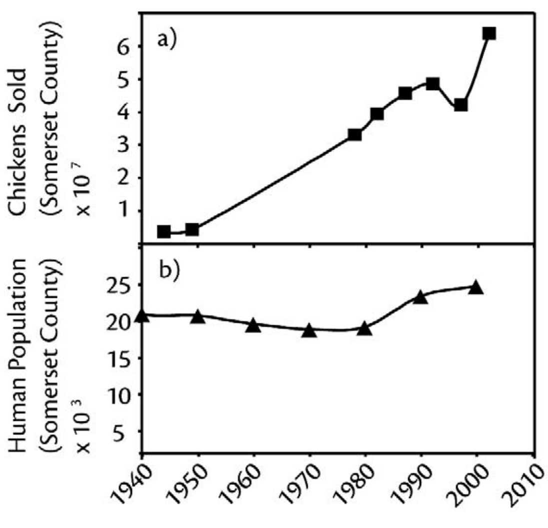

Figure 5. Historical records of (a) chickens sold in Somerset County (USDA, 2002) and (b) human population in Somerset County (MDP, 2000).

(vertical mixing rates, amount of fringing wetlands, *etc*.) may also contribute to denitrification. While larger water bodies like Tomales Bay, California, also receive multiple sources of nutrients, these ecosystems often exhibit slower flushing and more extensive nutrient recycling, resulting in nitrogen bioavailability largely controlled by denitrification (Smith *et al.,* 1989), which influenced spatial patterns of seagrass $\delta^{15}N$ values (Fourqurean *et al.,* 1997).

Atmospheric deposition of ammonia originated from animal feeding operations is another potential alternative source of nitrogen. This source has negative $\delta^{15}N$ values (–10 to –15‰) and is thus concluded to unlikely be a major contributor in this system, because oyster tissues reflecting this nitrogen source would be expected to have $\delta^{15}N$ values of –4 to –7‰ yet the minimum oyster $\delta^{15}N$ values observed were 8.1‰ (oyster gills in Monie Creek during 2007).

Tidal advection likely transported nitrogen from Monie Bay into its tributary creeks, with more impact on Little Monie Creek and Little Creek than on Monie Creek. This pattern is consistent with localized physical processes including tidal scouring, which formed these creeks (Ward, Kearney, and Stevenson, 1998). It took more than six times as long to flush Monie Creek (37.2 d) as to flush Little Creek or Little Monie Creek (3.5 or 5.7 d, respectively; Table 2), so nitrogen sources from Monie Creek's watershed (poultry and septic; Figure 1)

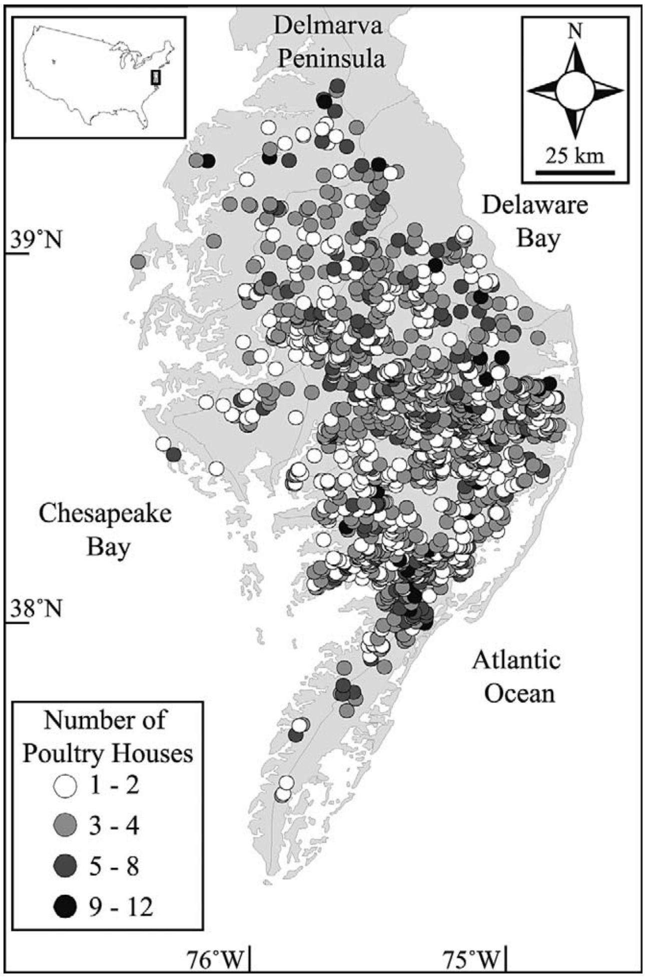

Figure 6. Location and enumeration of individual poultry houses (in grayscale) throughout Delmarva Peninsula (data from Google Earth).

had a larger impact on water quality there than did the respective watersheds of Little Monie Creek and Little Creek because terrestrially derived nitrogen remained in the tributary for a longer period. Likewise, poultry, septic, and wastewater nitrogen sources that entered the mouth of Monie Bay (Figures 1, 2a–d, and 3a–c) encroached more upon Little Monie Creek and Little Creek than upon Monie Creek. As the spatial end member, geographically situated at the mouth of the Wicomico River, Monie Bay itself was likely influenced by allochthonous nitrogen sources (*i.e*. the Wicomico River) through bottom layer circulation patterns typical of Chesapeake Bay tributaries (Fisher *et al.,* 2006; Testa *et al.,* 2008).

Due to the small watershed area of Monie Bay (72.3 km^2), anthropogenic activities with associated nitrogen inputs generally occurred within 6 km of its creeks, in contrast to larger ecosystems (*e.g*. Jordan, Correll, and Weller, 1997). Atmospheric deposition may be important to ecosystems with a high surface area/volume ratio (Giblin and Gaines, 1990; Paerl, 1995). Estuaries often receive nutrient inputs from both inside and outside their watersheds, *e.g*. Californian estuaries (inflowing river and midestuary sources; Cohen and Fong, 2006), or along the Delmarva Peninsula (a groundwatershed not aligned with the topographically defined watershed; Kasper, 2006; Winter, Rosenberry, and LaBaugh, 2003). Oyster $\delta^{15}N$ values in this NERRS site provided a powerful tool to elucidate interactions among the watersheds of Monie Bay and Wicomico River. Nutrient concentration reductions in the NERRS site require holistic management and input reductions from poultry, septic, and wastewater sources of nitrogen derived from inside and outside its topographically defined watershed.

CONCLUSIONS

Elevated allochthonous nutrient concentrations from multiple nitrogen sources were found in upper reaches of Monie Bay's tributaries, which led to degraded water quality consistent with land uses. The 19 poultry houses located in the Monie Creek watershed contained an estimated effective year-round population of 23,661 chickens house^{-1} and produced about 8.1×10^5 kg TN (untreated) y^{-1}, roughly equivalent to that defecated by 1.9×10^5 people. Throughout Delmarva Peninsula ($\sim 1.2 \times 10^6$ people), the average annual poultry population ($\sim 1.0 \times 10^8$ birds) generated 3.9×10^6 kg TN y^{-1} (conservatively) to 1.3×10^8 kg TN y^{-1} (wet manure), which was the equivalent of that generated by 9.0×10^5 people (conservative estimate of poultry manure to septic systems) to 3.1×10^8 people (wet manure compared to septic systems). Conservatively estimated, the poultry population in Delmarva Peninsula generated an amount of nitrogen equivalent to 76.4% of the amount generated by the actual human population. Effects of nitrogen generation from poultry production can be seen in fluctuations of local long-term water quality, because water quality declined during periods of increased county chicken sales. Consistent with the quantities of nitrogen generation from animal, human, or both wastes, the alternative hypothesis that elevated isotopic signatures were due to denitrification prior to assimilation by phytoplankton and ultimately oysters was rejected, because estimated nitrogen loss associated with denitrification was not dominant. Because of the small watershed area of Monie Bay, anthropogenic activities with associated nitrogen inputs generally occurred within 6 km of its creeks. However, atmospheric deposition may be important to ecosystems with a high surface area/volume ratio. Tidal advection flushed nitrogen in Monie Bay into its tributary creeks, with more impact on Little Monie Creek and Little Creek than on Monie Creek. As a result, nutrients enter the aquatic component of this ecosystem from both its watershed and other watersheds (*i.e*. that of the adjacent Wicomico River). Ecosystems around the world receive nutrient inputs from both inside and outside their watersheds, and oyster $\delta^{15}N$ values provided a powerful tool to elucidate this process. Nutrient concentration reductions in the NERRS site require holistic management and input reductions from poultry, septic, and wastewater sources of nitrogen derived from inside and outside its topographically defined watershed.

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