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Cultural eutrophication is reflected in the stable isotopic composition of the eastern mudsnail, *Nassarius obsoletus*

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Abstract

In aquatic ecosystems, biological indicators are used in concert with nutrient concentration data to identify habitat impairments related to cultural eutrophication. This approach has been less commonly implemented in coastal areas due to the dominance of physical conditions in structuring biological assemblage data. Here, we describe the use of the stable isotopic composition of *Nassarius obsoletus*, the eastern mudsnail, as an indicator of cultural eutrophication for 40 locations in coastal estuaries in New York, USA. We found ¹⁵N enrichment in mudsnail tissue where watersheds had high population densities, land use patterns were more urbanized, and when sampling sites were adjacent to wastewater treatment plant discharges. Stable carbon isotopes were responsive to salinity and watershed forest cover, with more saline sites reflecting a predominantly C4 or algal carbon isotopic signature and more forested sites a lighter isotopic signature reflecting greater inputs of C3 terrestrial detrital carbon. Mudsnail nitrogen isotopic composition had a high level of separation between more impacted and pristine watersheds (6.6–14.1‰), highlighting its utility as an indicator. We thus propose that stable isotope values of estuarine biota, such as the eastern mudsnail, can be used in concert with water quality data to identify areas where improvements in water quality are needed and can also be used to identify sources of detrital carbon to estuarine environments.

Introduction

Although dinitrogen gas is the dominant constituent of the earth's atmosphere, nitrogen often is the primary limiting nutrient for biological productivity as it is not readily available in chemical forms that organisms can assimilate (Galloway et al., 2003). Consequently, enhanced delivery of fixed nitrogen leads to alterations in the growth, function, and structure

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of ecological communities (Fox et al., 2012). Nitrogen enrichment can support greater productivity, both in terms of primary production (Valiela 1983) and at higher trophic levels (Oczkowski et al. 2009). However, nitrogen over-enrichment has been associated with declines in ecological integrity through increased weed abundance (Bidwell et al., 2006), suppression of rare taxa (Clark and Tillman, 2008; Bobbink et al., 2010), biodiversity declines at the landscape level (Howarth et al., 2000), loss of seagrass and wetland habitat (Orth et al., 2006; Deegan et al., 2012) and hypoxia or anoxia in aquatic ecosystems (Diaz, 2001).

Human activities have more than doubled the supply of biologically active nitrogen to terrestrial and aquatic systems globally since 1700 (Vitousek et al., 1997). Fossil fuel combustion releases biologically active nitrogen to the atmosphere, which is then deposited at the earth's surface via wet and dry atmospheric deposition (Galloway et al., 2004). Fertilizers used in agriculture and waste resulting from animal husbandry enhance nitrogen supplies to rivers and other aquatic ecosystems (Robertson and Vitousek, 2009). Furthermore, anthropogenically mediated land use change including urbanization, biomass burning, and wetland drainage mobilize nitrogen from long-term biological storage pools, exposing downstream and downwind communities to nitrogen enrichment (Lobert et al., 1990; Vitousek and Matson, 1993). Similarly, human wastewater discharges increase nitrogen supply to downstream ecosystems, especially when the population density is high, or the waste is untreated or minimally treated (Boynton and Nixon, 2012; DiMilla et al., 2011; Monti and Scorca, 2003).

The remediation of poor water quality in coastal areas necessitates first recognizing the impairment. Although water quality sampling programs usually include the measurement of dissolved nitrogen species (nitrate, nitrite, ammonium), several factors can obfuscate the detection of nitrogen over-enrichment. First, assimilation of nitrogen by phytoplankton, macroalgae or other opportunistic species (Fong et al. 2004) may mask chemical detection. Also, nutrient concentrations often vary by an order of magnitude or more on relatively short spatial and temporal scales as a function of flow conditions, precipitation, season (Ward et al. 1986), and other factors that are challenging to adjust for rigorously unless many samples are collected and analyzed. Consequently, water quality data is most often considered in concert with bioindicator data for recognition of ecosystem impairment. Typical bioindicators include macroinvertebrates, fish, algal, or amphibian communities (Whitton and Kelly, 1995; Marco et al., 1999; Azrina et al., 2006; Kuklina et al., 2013). Rather than reflecting a singular point in time, species assemblages integrate conditions over the lifespan of the individuals and reflect species-tolerances to environmental gradients (Metcalf 1999).

Here, we evaluate the stable isotope composition of the eastern mudsnail, *Nassarius obsoletus*¹, as a potential eutrophication indicator. *Nassarius obsoletus* is an abundant gastropod found on the Atlantic coast of Canada and the United States (Scheltema, 1964), which inhabits the sub- and inter-tidal zone of coastal areas (salinity >15‰). The snails are deposit feeders, ingesting diatoms that grow on the sediment surface (Connor and Edgar, 1982; Crisp, 1969), and feed opportunistically on macroalgae and carrion (Crisp, 1969;

¹We follow Turgeon et al., 1998 and refer to the snail as *Nassarius obsoletus*; previously, it has been referred to as *Ilyanassa obsoleta*.

Giannotti and McGlathery, 2001). The snails may live to up to five years (Scheltema, 1964), can be found in dense aggregations ($>1500\text{ m}^{-2}$) (Kelaheer et al., 2003; Nichols and Roberston, 1979), and have well developed sensory systems which allow them to quickly detect and scavenge food sources (Rahman et al., 2000; Trott and Dimock, 1978).

Previously, the abundance of *N. obsoletus* has been reported to increase in response to nutrient enrichment in a long-term landscape-scale fertilization experiment, exploiting the increased benthic algae availability (Johnson and Short, 2013). The snail also has been reported to exploit plot-scale enrichment experiments, aggregating around fertilized plots and consuming diatoms (M. Potpova, personal communication).

Previous studies have reported on the stable isotope ratios of coastal organisms, such as the wetland macrophyte *Spartina alterniflora* (Wigand et al., 1999; Cole et al., 2004), the ribbed mussel *Geukensia demissa* (McKinney et al. 2001), and the common mummichog *Fundulus heteroclitus* (Bannon and Roman, 2008; Wigand et al., 2015) as indicators of nitrogen source and load. Nitrogen isotopic composition has been found to be enriched in coastal organisms exposed to increasing concentrations of human wastewater, reflecting trophic enrichment of ^{15}N . Heavier nitrogen isotopic composition has also been associated with high nitrogen inputs, regardless of source, as biologically mediated denitrification removes ^{14}N preferentially, enriching the ^{15}N pool (Fry and Allen, 2003; Oczkowski et al., 2008).

This study tests the extent to which the stable isotopic composition of *N. obsoletus* reflects watershed land use and population patterns as surrogate for nitrogen load and source for Long Island and Staten Island, New York, where water quality issues are a priority for coastal management. We hypothesized that high human population density and/or nearby treated wastewater inputs would be reflected in the stable isotope ratio of the organisms. *N. obsoletus* is ever-present and easy to collect, as well as having an omnivorous feeding strategy, which enhances its selection as an indicator in comparison with other coastal wetland organisms. We were especially interested in stable isotope composition of snails collected from estuaries whose waters are dominated by agricultural nitrogen sources to determine whether $\delta^{15}\text{N}$ ratios are primarily related to nitrogen source or level of ecosystem enrichment.

Materials and Methods

Collections of the eastern mudsnail, *Nassarius obsoletus*, were made from 40 estuarine wetlands spanning the east to west population density gradient found on Long Island (New York, USA), including from the boroughs of Brooklyn, Queens, and Staten Island (Fig. 1; Supplemental Information). At each estuarine marsh, fifty mud snails were collected by hand, frozen, separated into snail tissue and shell, dried, and homogenized by using a Spex Shatterbox. Each snail did not comprise sufficient sample, so samples were analyzed as composites. For a sub-set of the sites (21), snail lengths of ten random individuals were measured prior to analysis, and shells and tissue were analyzed separately. Size measurements were conducted to constrain the impacts, if any, of snail size on isotopic composition, as organisms often feed at higher trophic levels at larger body size (Griffen and Valiela, 2001). Snail shell and tissue were analyzed separately to identify the relative

importance of fractionation that might be relative for analysis of fossil material, which would by necessity only include shell (Oczkowski et al. 2016).

Stable nitrogen and carbon isotope ratios ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$) were measured using a Vario Cube elemental analyzer interfaced to an Isoprime 100 isotope ratio mass spectrometer (IRMS). Isotope ratios for carbon and nitrogen are reported in permille notation as:

$$\delta^a X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \text{ ‰} \text{ where } R \text{ is the abundance ratio of the less common (a) to}$$

more common isotope (Dawson et al., 2002). The standard for nitrogen is atmospheric nitrogen gas; the standard for carbon is PeeDee Belemnite; by definition standards have $\delta=0$. All samples were analyzed in triplicate for stable isotopic and elemental composition with a mean difference between triplicates of 0.06‰ for $\delta^{13}\text{C}$, and 0.09‰ for $\delta^{15}\text{N}$.

Short soil cores (0–5cm) were also collected from five sites at each study location for analysis of salinity. Soil salinity was measured by reconstituting dried homogenized soils with a 5:1 ratio of water to soil, allowing the mixture to equilibrate, centrifuging, measuring salinity on the supernatant, then back-calculating soil salinity based on the original measured soil moisture (Watson and Byrne, 2009). Salinity was measured using a YSI 63 handheld conductivity meter.

For each collection site, catchment areas were delineated using the National Hydrography Watershed Boundary Dataset (WBD) at the subwatershed hydrologic level (USGS, 2012). Land use patterns for each estuary were constrained by clipping the 2011 National Land Cover Dataset to each hydrologic unit, and were recalculated to exclude herbaceous wetlands and open water. Population density was measured for each watershed by joining the WBD dataset to a population geodatabase (Minnesota Population Center, 2011). To identify the impacts of nearby wastewater treatment plants, if any, outfalls within 10km of each sampling location were mapped using the New York State Pollutant Discharge Elimination System (SPDES) in concert with permitted discharges (NYSDEC, 2004). An index of WWTP influence was calculated for each sampling site as:

$$WWTP \text{ Index} = \sum_{i=1}^n \frac{D_1}{d_1} + \frac{D_2}{d_2} + \frac{D_3}{d_3} + \dots + \frac{D_n}{d_n}$$

where D is the permitted discharge for each outfall (in millions of gallons per day) within 10-km and d is the proximity of the outfall in km to the collection site.

Biplots were used to examine individual relationships between land use land cover variables factors, including WWTP discharges and population density, with $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of mudsnails. Step-wise multiple regression models were built using partial least squares (PLS) regression (which is unaffected by multicollinearity; Wold et al., 2002). Population density and the WWTP index were log-transformed prior to analysis. Relationships between snail length and width and stable isotope ratios were examined using regression, and relationships between snail shell and tissue stable isotope ratios were examined using a paired t-test.

Results

Mudsnail tissue $\delta^{15}\text{N}$ was found to vary from 6.6‰ to 14.1‰, and $\delta^{13}\text{C}$ was found to vary from -9.9 to -17.3‰ across coastal New York. The site with the highest snail tissue $\delta^{15}\text{N}$ mean value was located in Jamaica Bay, New York City, while the site with the lowest snail tissue $\delta^{15}\text{N}$ mean value was at Pipes Cove, on far eastern Long Island (Fig. 1). Stable nitrogen isotopic signatures of mudsnails were higher with higher population density ($r^2=0.28$, $p<0.01$), in western Long Island ($r^2=0.36$, $p<0.01$), with increasing wastewater discharges ($r^2=0.21$, $p<0.01$), and based on watershed land use, with more urban watersheds associated with enrichment in ^{15}N ($r^2=0.55$, $p<0.01$). Stable carbon isotopic composition of mudsnails varied significantly with salinity and land cover such that higher salinities were associated with more enriched $\delta^{13}\text{C}$ ratios ($r^2=0.11$, $p=0.02$), and more depleted signatures were associated with forested watersheds ($r^2=0.37$, $p=0.01$) (Fig. 2).

Full PLS regression models expressing isotopic composition as a function of watershed land use, population density, sewage inputs, and longitude collectively explained 62% of the variability in the $\delta^{15}\text{N}$ values and 36% of the variability in the $\delta^{13}\text{C}$ values. Strong predictors of nitrogen isotope composition were watershed forest cover and other land cover variables, longitude, population density, and WWTP discharges (Table 1). Small declines in model skill were observed upon removing longitude, population density, and salinity from the multiple regression model (decline in r -squared value of 0.02, 0.01, and 0.01, respectively). Larger drops in skill were noted for removal of land use values and nearby sewage discharges. Removal of land cover data was associated with a decline from 63% to 41% of variance explained, while removal of data on treated sewage discharge data was associated with a decline from 63% to 54% of variance explained.

Examination of stable isotopes of snail shells vs. tissue revealed that snail shell contained significantly enriched $\delta^{15}\text{N}$ ratios ($t=-1.89$, $p=0.03$), and more depleted $\delta^{13}\text{C}$ signatures ($t=52.8$, $p<0.001$) than snail tissue (Fig. 3). There was also a negative relationship between snail size and $\delta^{15}\text{N}$ tissue ratio, with larger snails associated with lighter ^{15}N signatures (Fig. 3). A trend was also identified between snail size and longitude, such that western Long Island snails were found to be of smaller size than eastern Long Island ($r^2=0.375$, $p=0.03$). Regression of snail tissue vs. shell against indicators suggested a tighter correlation between indicators and tissue $\delta^{15}\text{N}$ ($r^2=0.80$) vs. compared with shell $\delta^{15}\text{N}$ ($r^2=0.71$) for the 20 sites where we compared the two.

Discussion

While previous research has validated the use of $\delta^{15}\text{N}$ ratios in vertebrates (Lake et al., 2001; Pruell et al., in press), invertebrates (McKinney et al., 2001), and macrophytes (Wigand et al., 1999; Cole et al. 2004) as indicators of wastewater inputs and development in aquatic environments, our focus on mudsnails advances their use as a potential indicator of water quality. In concert with earlier work (Pruell et al., 2006; Johnson and Short, 2013), this study suggests that the eastern mudsnail, *Nassarius obsoletus*, may act as an indicator of nutrient enrichment both in terms of its stable isotopic composition and abundance. The abundance of the mud snail is known to increase in response to ecosystem level fertilization

of tidal creeks (Johnson and Short, 2013; Pascal et al., 2013). As a detritivore, *N. obsoletus* feeds on diatoms as well as decaying organisms and macroalgae, and as such it appears to opportunistically expand its population in response to the rapid biomass production and turnover associated with eutrophication (Valiela et al., 1997). The mud snail may be especially valuable as an indicator as its relatively long lifespan means it is integrating the stable isotope regime of the water column and sediment over several years. Previous studies of *N. obsoletus* growth have reported a lifespan of five years (Scheltema, 1964), significantly longer than other common coastal organisms (e.g. mummichogs, fiddler crabs) which typically live for three years (Fritz and Garside, 1975; Valiela et al., 1977; Burns, 1976; Montague, 1980), or macrophytes whose biomass turns over seasonally (Hopkinson et al. 1978). Previously, *N. obsoletus* was found to vary little in stable nitrogen isotope composition over seasonal and yearly time-scales and was thus thought to act as a stable indicator of long-term condition (Pruell et al., 2006).

Because previous studies have found that estuarine biota increase in ^{15}N as a function of size (Bannon and Roman, 2008), we measured mean snail size of composite samples. A pattern of increasing $\delta^{15}\text{N}$ with increasing size is often seen with fish and other organisms as trophic position increases with age (Griffin and Valiela, 2001). In this study, we found – rather paradoxically – that larger snails tended to have lower $\delta^{15}\text{N}$ ratios. Because smaller snail size was also found to significantly covary with longitude (smaller snails were found in western Long Island), we hypothesized that more eutrophic conditions may be associated with a larger number of smaller snails, which are commensurately more enriched in ^{15}N than snails found under less eutrophic conditions (Fig. 4).

The large range of nitrogen stable isotopic values found for *N. obsoletus* across Long Island also suggests that this gastropod is an effective indicator. Because nitrogen stable isotope signatures vary between organisms within sites – usually by several permille – the large amplitude of variability makes it easier to detect between-site differences. Here, we found that mudsnail tissue nitrogen isotopic composition varied across Long Island by 7.5‰. In comparison, values in ribbed mussel tissue (*Geukensia demissa*) were found to vary by 2.7‰ in Narragansett Bay and by 3.5‰ across Massachusetts and New York. Similarly, macroalgae has been reported to vary by 2–4‰ over strong wastewater input and population density gradients (Pruell et al., 2006) and hard clams (*Mercenaria mercenaria*) not at all (Oczkowski et al., 2008). Our results suggest that in comparison with other organisms, *N. obsoletus* is a valuable organism to analyze when differences between sites are subtle.

We also noted strong fractionation in $\delta^{13}\text{C}$ between mud snail shell and tissue (11‰) (Fig. 3). This pattern is common for land snails where respiratory gas exchange retains the isotopically heavier HCO_3^- but is less common for marine snails where shell tends to reflect a fractionation induced isotopic signature of dissolved inorganic carbon (McConnaughey and Gillikin, 2008). The comparison of shell and tissue thus suggests that *N. obsoletus* – as an pulmonate intertidal organism – is physiologically a land snail, even though it overwinters subtidally (Sindermann, 1960). Analysis of extant material might help constrain analysis of the abundant fossil marine gastropod material found in shell middens and other coastal sedimentary deposits (Stoner 1997). Comparisons between indicators and the $\delta^{15}\text{N}$ signature

of *N. obsoletus* tissue vs. shell suggested that both tissue and shell were highly correlated with indicators of watershed nitrogen impairment, with tissue displaying the tightest correlation for the 20 sites tested.

In streams and rivers, assessments of water quality impairment and appropriate remediation strategies are often arrived at using a combination of water chemistry measurements in concert with assessment of biological assemblage data (e.g., macroinvertebrates, fish, amphibians, algae). The use of complementary data helps strengthen interpretations based on chemistry using organismal data that reflects environmental conditions on a much longer time-frame. Similarly, use of water chemistry data can help verify expert opinion regarding the habitat requirements of species assemblages. However, in coastal environments, physical processes such as wave exposure, inundation, salinity, and tidal exchange are often the dominant control on biological assemblages. Also, biological assemblages in coastal areas are often dominated by a small number of abundant species that vary geographically and include, but are not limited to, cordgrass (*Spartina* spp.), pickleweed (*Salicornia* spp.), mummichogs, killifish, gobies, mussels, and crabs. While some sensitive species can be used as indicators of ecosystem health, such as stable eelgrass beds (Romero et al. 2007) or benthic invertebrates (Dauer et al. 2000), the analysis of species assemblages using a biological condition gradient approach is not well supported for most coastal ecosystems. Here we propose that the stable isotopic ratios of coastal organisms can provide an important perspective complementary to water chemistry measurements, and we advocate that gastropods can play an important role in such work.

Conclusions

This study demonstrates that the stable isotopic composition of the gastropod, *Nassarius obsoletus*, reflects land use and wastewater inputs to coastal areas for Long Island, NY. Specifically, $\delta^{15}\text{N}_{\text{tissue}}$ values ranged from 6.6‰ to 14.1‰ across Long Island, which spans the full range of values that are found in estuarine organisms across the Atlantic coast of North America (Kwak and Zedler 1977). The isotopic composition of *N. obsoletus* was found to be much more variable than analyses of fish and mussels in the Northeast, for which the range of values has been found to be more compressed (10–15‰). In comparison with terrestrial ecosystems (Niemi and McDonald 2004), biologically-based indicators of ecosystem health are not well developed in coastal areas. We postulate that that stable isotopic analyses of coastal organisms, such as we conducted here, can play an important role in detection of poor water quality. We conclude that marine gastropods such as the eastern mudsnail, *Nassarius obsoletus*, are good candidates for analysis due to their high densities, relatively lengthy lifespans, and integrated feeding strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Core Ideas

- We analyzed *Nassarius obsoletus* tissue and shell for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$
- $\delta^{15}\text{N}$ values strongly reflected anthropogenic trophic enrichment from wastewater
- $\delta^{13}\text{C}$ values reflected C4 vs. C3 plant signatures based on salinity and forest cover
- Stable isotope signatures of estuarine biota are useful indicators of water quality

Figure 1.

Map of Long Island showing spatial patterns in stable nitrogen and carbon isotopic ratios across Long Island, including the NYC boroughs of Brooklyn and Queens in *N. obsoletus* tissue at the 40 locations sampled.

Figure 2.

Relationship between stable isotopic composition of *N. obsoletus* tissue and watershed population density, longitude, wastewater inputs, salinity, and land use. For watershed land use, individual land cover types typically had small correlations with stable isotopic ratios. To show the composite influence of land cover on isotopic ratios, we show predicted vs. actual isotopic ratios based on a multiple regression model considering all land cover types.

Stable isotopic composition of snail shell vs. tissue

Stable isotopic composition vs. snail size

Figure 3.

Stable isotopic composition of snail shells vs. tissues and relationship between snail size and stable nitrogen isotope ratio.

Hypothesized relationship between snail demography and stable isotopic composition

Figure 4.

A conceptual model of the hypothesized relationship between the stable nitrogen isotopic ratios of snails, snail demographic patterns, and watershed characteristics.

Table 1.

Variable Importance in Projection Scores for full PLS regression models. Values above 0.75 are considered significant.

Predictor	Snail $\delta^{15}\text{N}_{\text{tissue}}$	Snail $\delta^{13}\text{C}_{\text{tissue}}$
Population	1.55	0.85
WWTP index	1.31	0.80
Salinity	1.27	1.64
Longitude	1.75	0.98
Developed open space	1.04	1.35
Low density development	1.01	1.15
Medium density development	1.16	1.06
High density development	0.77	0.75
Barren land	1.30	0.86
Deciduous forest	1.02	0.77
Evergreen forest	0.83	0.66
Mixed forest	1.97	2.02
Shrub/Scrub	1.01	1.84
Grassland	0.70	0.91
Pasture/Hay	0.71	1.43
Cultivated crops	0.87	1.52
Woody wetlands	0.97	0.96