

Long-lived organisms provide an integrative footprint of agricultural land use

CARLA L. ATKINSON,^{1,5} ALAN D. CHRISTIAN,² DANIEL E. SPOONER,^{3,4} AND CARYN C. VAUGHN¹

¹Oklahoma Biological Survey, Department of Biology and Ecology and Evolutionary Biology Graduate Program, University of Oklahoma, Norman, Oklahoma 73019 USA

²University of Massachusetts Boston, Biology Department, Boston, Massachusetts 02125 USA

³Trent University, Department of Biology, Peterborough, Ontario K9J 7B8 Canada

⁴United States Geological Survey, Leetown Science Center, Northern Appalachian Research Laboratory, Wellsboro, Pennsylvania 16901 USA

Abstract. Nitrogen (N) fertilizer runoff into rivers is linked to nutrient enrichment, hydrologic alteration, habitat degradation and loss, and declines in biotic integrity in streams. Nitrogen runoff from agriculture is expected to increase with population growth, so tracking these sources is vital to enhancing biomonitoring and management actions. Unionid mussels are large, long-lived, sedentary, primary consumers that transfer particulate material and nutrients from the water column to the sediments through their filter feeding. Because of these traits, mussels may provide a temporal integration of nitrogen inputs into watersheds. Our goals were to (1) establish a baseline $\delta^{15}\text{N}$ signature for unionid mussels in watersheds not heavily influenced by agriculture for use in comparative analyses and (2) determine if mussels provide an integrative measure of N sources in watersheds with varying percentages of agriculture across large spatial scales. We compiled tissue $\delta^{15}\text{N}$ data for 20 species of mussels from seven geographic areas, including 23 watersheds and 42 sample sites that spanned varying degrees of agricultural intensification across the eastern United States and Canada. We used GIS to determine land cover within the study basins, and we estimated net anthropogenic nitrogen inputs (NANI) entering these systems. We then determined the relationship between mussel tissue $\delta^{15}\text{N}$ and percentage of land in agriculture (%AG) and net anthropogenic N loading. The $\delta^{15}\text{N}$ of mussel tissue could be predicted from both %AG and net anthropogenic N loading, and one component of NANI, the amount of N fertilizer applied, was strongly related to the $\delta^{15}\text{N}$ of mussel tissue. Based on our results, mussels occupying a system not affected by agricultural land use would have a baseline $\delta^{15}\text{N}$ signature of approximately 2.0‰, whereas mussels in basins with heavy agriculture had $\delta^{15}\text{N}$ signatures of 13.6‰. Our results demonstrate that mussels integrate anthropogenic N input into rivers at a watershed scale and could be a good bioassessment tool for tracking agriculture N sources.

Key words: baseline; bioindicator; biomonitoring; $\delta^{15}\text{N}$; geographic information systems; NANI; nitrogen management tools; stable isotopes; unionid mussels.

INTRODUCTION

Nitrogen is an important limiting resource for primary production, but has become increasingly prevalent due to anthropogenic inputs (Vitousek 1997, Elser 2011). Reactive N is responsible for biodiversity losses, eutrophication, hypoxia, habitat degradation, and acidification (with sulfur) of both marine and freshwater habitats (Vitousek 1997, Howard 2000). Agriculture has a large effect on stream nutrient concentrations, sediment load, water flow, and stream channel placement (Gordon et al. 2008) and is the largest single contributor to anthropogenic nitrogen in many rivers (Boyer et al. 2002, Howarth et al. 2012). Even though rivers have the capacity to process much of

the N entering a catchment (Boyer et al. 2002, Galloway et al. 2003), N entering coastal areas from rivers is a large pollution problem because it stimulates algal blooms and subsequent oxygen depletion (Dodds 2006, Elser 2011). Although management practices to decrease the amount of N entering waterways have been instigated (e.g., riparian buffers, wetland protection), the success of these practices in mitigating N pollution varies regionally or is largely unknown (Riseng et al. 2011). The ability to biologically track N loading to watersheds in a way that complements predictive modeling has become of great interest in recent years (e.g., Lefebvre et al. 2007, Hong et al. 2011), and further tools are needed to determine watershed N loading across large spatial scales and multiple time scales.

Nitrogen stable isotopes reflect both the nitrogen source and the outcomes of processes that transform N (Robinson 2001, Vander Zanden et al. 2005, Diebel and Vander Zanden 2009). Consequently, N isotope ratios

Manuscript received 1 April 2013; revised 10 July 2013; accepted 11 July 2013. Corresponding Editor: C. C. Cleveland.

⁵ E-mail: carlalatkinson@gmail.com

have been suggested as surrogate measures of nutrient loading and processing in stream watersheds (Lefebvre et al. 2007). Differences in land use among watersheds is correlated with the source $\delta^{15}\text{N}$ signal of macrophytes (Cole et al. 2004), marine plants (Costanzo et al. 2001), invertebrates, and fish (Fry and Allen 2003, Anderson and Cabana 2005, Fertig et al. 2009). Nitrogen from both animal manure and synthetic fertilizers can be transformed by processes such as volatilization and denitrification (Groffman et al. 2006), leading to gaseous losses of N that fractionate N isotopes and result in elevated $\delta^{15}\text{N}$ values of the remaining N. For this reason, manure and synthetic fertilizer both often have enriched $\delta^{15}\text{N}$ values relative to background values (Hogberg 1990, Kendall 1998). Consequently, food web components become more enriched in $\delta^{15}\text{N}$ in areas receiving high fertilizer inputs. The $\delta^{15}\text{N}$ value of primary consumer tissue reflects an integration of the N assimilated by the consumer over a particular time period, which varies as a function of the life span and tissue turnover of the consumer (Peterson and Fry 1987). Thus, $\delta^{15}\text{N}$ provides an integrated temporal and spatial measure of N sources and land use rather than a one-time snapshot of N concentrations. The information gained by isotope analyses may allow managers to collect fewer samples while still obtaining information on the N entering a stream reach over a time relevant to an animal's tissue turnover.

Freshwater mussels (Mollusca: Bivalvia: Unionidae) are large, long-lived (typically 10–25 yr, but up to 190 yr [Haag and Rypel 2011]) primary consumers that may provide a temporal integration of nitrogen inputs into watersheds. Freshwater mussels play an important ecological role through their filter feeding. This feeding activity transfers organic materials and nutrients from the water column to the surrounding benthic area and stimulates increased primary and secondary production (Howard and Cuffey 2006, Vaughn et al. 2007). Adult mussels can ingest and assimilate a wide range of suspended fine particulate organic matter (FPOM), ranging in size from 1 μm up to at least 40 μm (Brönmark and Malmqvist 1982, Atkinson et al. 2011). Thus, mussels are able to assimilate a wide variety of particulates originating from both aquatic and terrestrial sources. Despite some differences in diet, mostly due to particle size preferences (Leff et al. 1990, Galbraith et al. 2009, Atkinson et al. 2011), different taxa of unionids tend to have similar isotopic signatures within a site allowing cross-species comparisons (Christian et al. 2004, Atkinson et al. 2010). Additionally, adult mussels are sedentary and rarely move farther than a few meters laterally per year (Kappes and Haase 2012) and less than 0.5 m in a week (Allen and Vaughn 2009), so the isotopic signatures of their tissues should provide a good representation of N inputs into a specific stream reach.

Our goals were to (1) establish a baseline $\delta^{15}\text{N}$ signature for unionid mussels in watersheds not heavily influenced by agriculture for use in comparative analyses

and (2) determine if freshwater mussels provide an integrative measure of N sources in watersheds with varying percentages of agriculture across large spatial scales. We compiled $\delta^{15}\text{N}$ data for a total of 20 species of freshwater mussels from seven geographic areas, including 23 watersheds and 42 sample sites that spanned varying degrees of agricultural intensification across the eastern United States and Canada. We then determined the relationship between mussel tissue $\delta^{15}\text{N}$ with the percentage of land in agriculture (%AG) and net anthropogenic N loading across this broad geographic scale, and used these data to estimate the $\delta^{15}\text{N}$ of mussels occupying a watershed with little to no agricultural land use.

METHODS

Study areas and sample collection.—We compiled $\delta^{15}\text{N}$ tissue data for 20 species of freshwater mussels from seven geographic areas, including 23 watersheds and 42 sample sites that spanned varying degrees of agricultural intensification across the eastern United States and Ontario, Canada (Fig. 1, Table 1, Appendix A). These data included samples that we collected ourselves (Red River, Buffalo, Darby, Ouachita, Ichawaynochaway, and Ontario) and published studies (Neuse Basin).

Isotope sample processing.—Foot muscle or mantle tissue samples were collected (Naimo et al. 1998) from each individual mussel, dried (45°C), and ground. Isotope ratios are expressed in the delta (δ) notation: $\delta^{15}\text{N}$ (units of ‰) = $(R_{\text{sample}} - R_{\text{standard}}/R_{\text{standard}}) \times 1000$, where R is the $^{15}\text{N}:^{14}\text{N}$ ratio. A bovine protein (peptone) lab standard was referenced against an international standard and precision averaged to 0.1‰ or less. Stable isotope analyses were performed as follows: Red River drainage and Ichawaynochaway Creek samples were analyzed at the University of Georgia Stable Isotope Facility using a Finnigan Delta Plus mass spectrometer (Thermo-Finnigan, Bremen, Germany); Buffalo River, Darby Creek basin, and Ouachita River samples were analyzed at the University of Alaska Fairbanks Stable Isotope Facility using a Europa 20–20 continuous flow-isotope ratio mass spectrometer (Sercon, Cheshire, UK); Ontario study sites samples were analyzed at Trent University Water Quality Center using a EuroEA3028-HT EuroVector Elemental Analyzer (EuroVector SpA, Milan, Italy) coupled with a Micromass IsoPrime Continuous Flow Isotope Ratio Mass Spectrometer (Micromass, Manchester, UK).

GIS analysis.—We derived watershed areas for each sampling point using the Spatial Analyst Toolkit in ArcMap 10.0 (Environmental System Research Institute, Redlands, California, USA) with a 30-m digital elevation model (DEM) from the National Elevation Dataset. We obtained land cover (30-m resolution) for the United States from the 2006 National Land Cover Database (Homer et al. 2004). Land cover for the Ontario sites was obtained from the Ontario Land

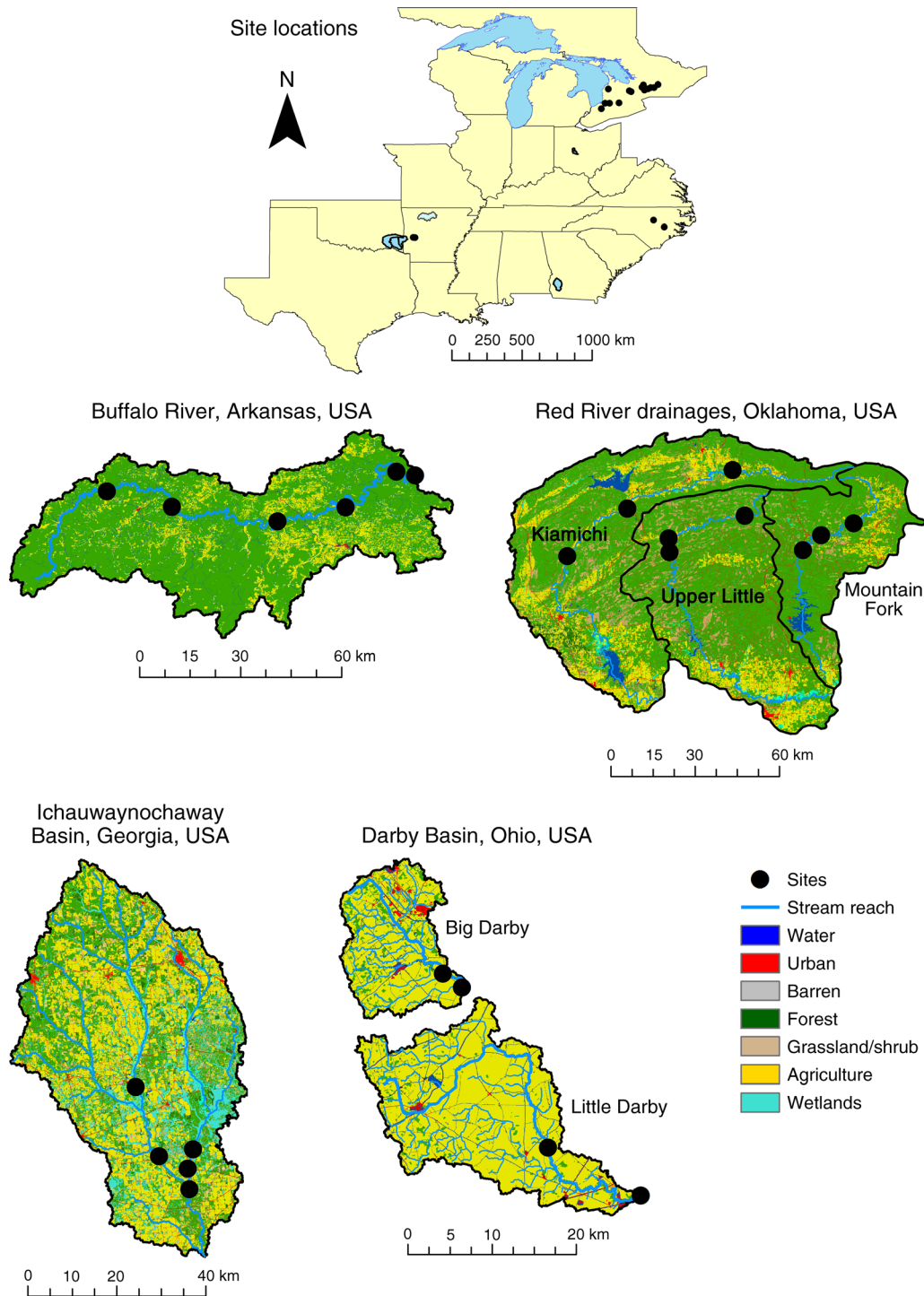


FIG. 1. Maps of study areas. Black dots indicate individual sampling sites. Watersheds with more than two sampling sites within them are shown with the land use in that basin. All the sites sampled within Ontario, Canada, are within individual watersheds.

Cover (OLC) data base. Land cover was delineated for each individual sample site.

Net anthropogenic nitrogen inputs.—We assessed net anthropogenic nitrogen inputs (NANI) to the most

downstream sampling point of each individual U.S. watershed ($n = 10$) in our database using the NANI Calculator Toolbox (Hong et al. 2011). This model has been used to estimate total riverine N flux from

TABLE 1. Description of data sets used in the analyses.

Study area and data sources†	Major drainage basin	Dominant land use	Watershed points used in analyses‡	Range of site watershed areas (km ²)	Species sampled§
Buffalo River (6); A. D. Christian, unpublished data	White River, Arkansas, USA	forest and pasture	Buffalo River (1)	482–3037	<i>Actinonaias ligamentina</i> (5), <i>Cyclonaias turbiculata</i> (5), <i>Lampsilis reeviana</i> (5), <i>Ptychobranchus occidentalis</i> (5)
Darby Creek Basin (4); Christian et al. (2004); A. D. Christian, unpublished data	Scioto River, Ohio, USA	agriculture (corn and soybeans) (Ohio EPA 1987)	Big Darby, Little Darby (2 total)	179–399	<i>Elliptio dilatata</i> (3–10), <i>Ptychobranchus fasciolaris</i> (3–10)
Ichawaynochaway Basin (5); Atkinson et al. (2010)	Flint River, Georgia, USA	agriculture (cotton, corn peanuts), forest	Ichawaynochaway, Chickasawhatchee (2 total)	773–2557	<i>Elliptio crassidens</i> (5–10)
Neuse Basin (2); Bucci et al. (2011)	Neuse River, North Carolina, USA	agriculture	Neuse (1)	74–240	<i>Elliptio complanata</i>
Ouachita River (2); A. D. Christian, unpublished data	Ouachita River, Arkansas, USA	forest, some cattle and chickens	Ouachita (1)	982–1040	<i>Actinonaias ligamentina</i> (3–5), <i>Elliptio dilatata</i> (3–5), <i>Ptychobranchus occidentalis</i> (3–5)
Ontario, Canada (14); Spooner et al. (2013)	Lakes Erie and Huron	agriculture (row crop corn and soy)	Beaverton, Uxbridge, Nottawasaga, Fleetwood, East Cross, Nonquon, Fish Creek, Sydenham, Thames, Cavanville, Indian, Humber, Ausable, Teeswater (14 total)	128–2123	<i>Lasmigona costata/compressa</i> (5–10), <i>Elliptio dilatata/complanata</i> (5–10)
Kiamichi, Little and Mountain Fork Rivers (9); C. L. Atkinson, unpublished data	Red River, Oklahoma, USA	forest, some cattle and chickens (Matthews et al.) 2005)	Kiamichi, Little, Mt. Fork (3)	74–2044	<i>Amblema plicata</i> (5), <i>Actinonaias ligamentina</i> (5), <i>Fusconaia flava</i> (5), <i>Lampsilis</i> spB (2–5),¶ <i>Quadrula pustulosa</i> (4–5), <i>Quadrula verrucosa</i> (4–5)

† Number of sites in parentheses.

‡ Number of points in parentheses.

§ Number of individuals per site in parentheses.

¶ This species is currently being described.

landscapes to coastal ecosystems (Galloway et al. 2004, Howarth et al. 2012). Databases included in the NANI Toolbox are county-level Agricultural Census data for the Agricultural Census years 1987, 1992, 1997, 2002, and 2007 (data available online),⁶ county-level Census data for the population in Census years 1990 and 2000 (data available online),⁷ county-level USGS nutrient input estimates for annual fertilizer application in years 1987 to 2001 (data available online),⁸ and 36-km² grid scale of the U.S. Environmental Protection Agency's community multi-scale air quality (CMAQ) data for nitrogen deposition annually available from 2002 to 2006. We were unable to obtain net anthropogenic nitrogen input data for Ontario, thus those watersheds

were not used to determine the relationship between NANI and mussel tissue $\delta^{15}\text{N}$.

Statistical analyses.—We investigated spatial autocorrelation among sites in the data set using Moran's *I*. We used spatial analysis in macroecology (SAM 4.0; Rangel et al. 2010) to assess spatial autocorrelation between the percentage of agriculture in the watershed and $\delta^{15}\text{N}$ of mussel tissue. To determine if the percentage of land in agriculture (%AG) and $\delta^{15}\text{N}$ of mussel tissue followed the same spatial patterns, we used an ordinary least-squares regression (OLS) to determine the relationship of their Moran's *I* values. To avoid problems of spatial autocorrelation in further analyses, we grouped data by watershed ($n = 24$ watersheds) and used average %AG and $\delta^{15}\text{N}$ values of the watersheds. The watersheds used in the following analyses included one point each for the Buffalo, Kiamichi, Little, Mountain Fork, Big Darby, Little Darby, Ouachita, and Neuse rivers, and 14 separate watersheds in Ontario. Prior to analysis,

⁶ <http://www.agcensus.usda.gov/>⁷ <http://www.census.gov/>⁸ <http://pubs.usgs.gov/sir/2006/5012>

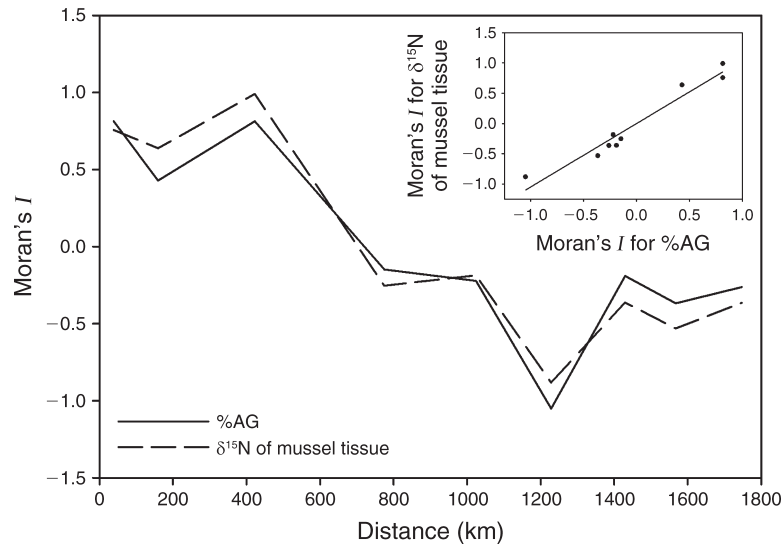


FIG. 2. Correlogram of Moran's I with the data used in the analyses. Moran's I for the percentage of land in agriculture (%AG) and $\delta^{15}\text{N}$ of mussels overlapped significantly. The distance on the x axis was determined by SAM 4.0 and represents the straight-line distance between sites. The insert shows the ordinary least-squares regression for the Moran's I values ($R^2 = 0.94$, $P < 0.0001$).

%AG was arcsine-square-root transformed to meet assumptions of normality (Gotelli and Ellison 2004). Because error in predictors was high in relation to the response, we used reduced major axis regression (RMA) to evaluate the relationship between the arcsine square-root of %AG and the $\delta^{15}\text{N}$ of mussel tissue. Because most streams are impacted by agriculture, the y intercept was used to investigate what the $\delta^{15}\text{N}$ of mussel tissue may be in a watershed without agriculture. RMA was performed in the lmodel2 package in R 2.14.1 (R Core Development Team). Ordinary least-squares (OLS) regression was used to determine the relationship between agriculture and both NANI and total N in fertilizer applied to the landscape. OLS regression also was used to determine the relationship between NANI and $\delta^{15}\text{N}$ of mussel tissue. To determine what components of NANI (non-food crop N (e.g., cotton and tobacco), N fertilizer applied, N deposition, agricultural N fixation, and/or food crop N (e.g., wheat, corn) were important in influencing the $\delta^{15}\text{N}$ of mussel tissue, a backward stepwise multiple linear regression with AIC selection was used to examine significant predictors using the MASS package using R 2.14.1 (R Development Core Team 2011). Prior to multiple linear regressions, we checked for multicollinearity using Pearson correlations in the Hmisc package using R 2.14.1. Significant predictors of $\delta^{15}\text{N}$ found with Akaike information criterion (AIC) selection were then used in an OLS regression to determine their univariate influence on $\delta^{15}\text{N}$.

RESULTS

Spatial patterns

For both %AG and $\delta^{15}\text{N}$ values, our data exhibited positive spatial autocorrelation for sites geographically

closer to each other (positive Moran's I values) and negative correlation for sites further apart (negative Moran's I values; Fig. 2). The patterns of spatial autocorrelation of these two variables were similar and our regression results suggested that the Moran's I for $\delta^{15}\text{N}$ increased as Moran's I for %AG increased ($R^2 = 0.94$, $P < 0.0001$; Fig. 2).

Mussel tissue $\delta^{15}\text{N}$ and %AG and NANI.—Our results show that mussel tissue $\delta^{15}\text{N}$ was positively related to percent agriculture in watersheds (Fig. 3; $R^2 = 0.74$, $y = 11.79x + 1.05$, $P < 0.0001$). The y -intercept suggests that mussels occupying a watershed without agriculture would have a $\delta^{15}\text{N}$ value around $1.05\text{‰} \pm 0.92\text{‰}$ (mean \pm SD). Additionally, NANI ($R^2 = 0.58$, $y = 33.4x + 2171$, $P < 0.001$) and the amount of N applied as fertilizer ($R^2 = 0.90$, $y = 84.4x - 573$, $P < 0.001$) in a watershed were both significantly positively predicted by %AG in a watershed (Fig. 4). Furthermore, net NANI was a significant predictor of $\delta^{15}\text{N}$ in watersheds (Fig. 5; $R^2 = 0.60$, $y = 0.002x + 2.09$, $P < 0.01$), demonstrating a signal of human application of nitrogen to the landscape. The y intercept of the net NANI relationship suggests that mussels in a watershed not affected by anthropogenic nitrogen would have a $\delta^{15}\text{N}$ value around $2.09\text{‰} \pm 1.6\text{‰}$. Collectively, the relationship of $\delta^{15}\text{N}$ to the %AG in a watershed and NANI indicate that a $\delta^{15}\text{N}$ value ranging from 0.13‰ to 3.69‰ would represent a mussel in a system not influenced by agriculture. Among the components of NANI, only the amount of nitrogen fertilizer applied to a watershed appeared as a strong predictor of $\delta^{15}\text{N}$ ($t_5 = 3.87$, $P = 0.01$, backward stepwise regression). Accordingly, it was closely and positively related to mussel $\delta^{15}\text{N}$ ($R^2 = 0.83$, $y = 0.0009x + 5.4$, $P < 0.0001$).

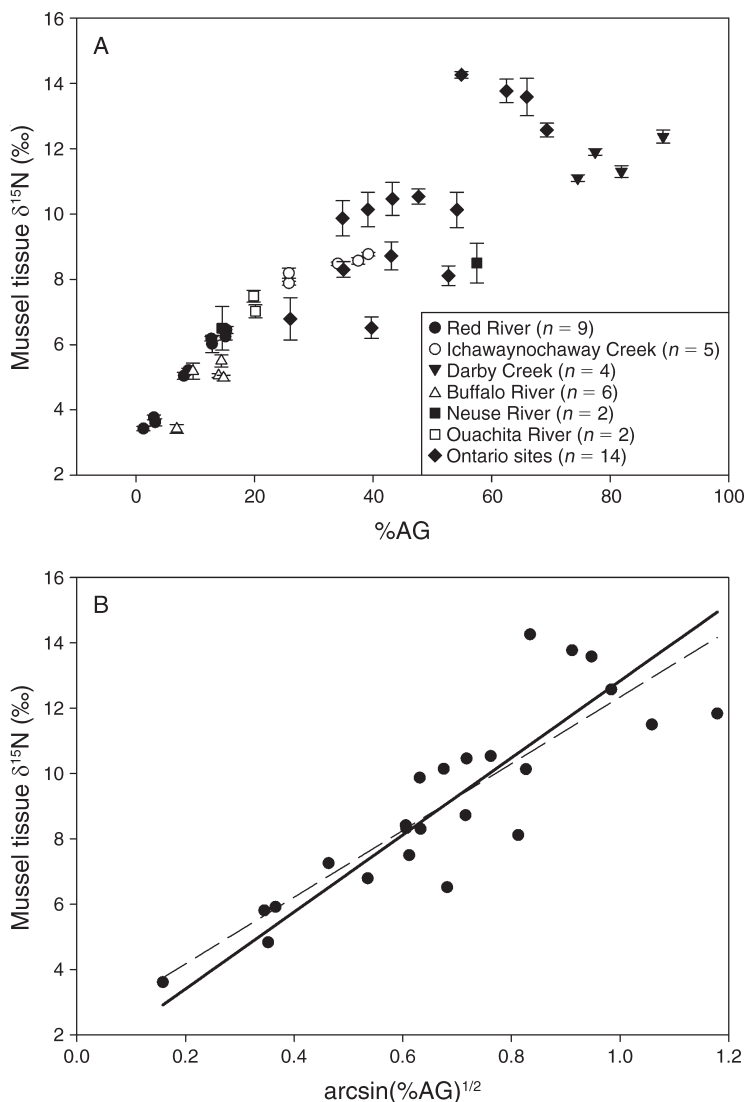


FIG. 3. (A) Plot of %AG vs. mussel tissue $\delta^{15}\text{N}$ values (mean \pm SE) for all of the sites in the seven watersheds used in this study. (B) Watershed-level grouped data (arcsine-square-root-transformed %AG and mean mussel tissue $\delta^{15}\text{N}$) and regression lines for both reduced major axis (RMA) regression (solid line; $y = 11.79x + 1.05$; $P < 0.0001$) and ordinary least-squares (OLS) regression (dashed line; $y = 10.21x + 2.13$; $P < 0.0001$).

DISCUSSION

Our results show that primary consumers, especially freshwater mussels, are a good integrator of land use influences and should be a focal component of stream biomonitoring. Continuous monitoring of water quality can be time consuming and expensive and results are often difficult to summarize in an ecologically meaningful way (Olden and Poff 2003). We found that the nitrogen isotope ratio of freshwater mussel tissue could be predicted from both the percent agriculture in the watershed and NANI. Our results indicate that mussels biogeochemically integrate nitrogen loading on the landscape and are good indicators of anthropogenic N inputs. We suggest that biomonitoring isotope ratios of

mussels would be an efficient way to assess agricultural runoff into streams.

Our results combined with other studies demonstrate that the $\delta^{15}\text{N}$ signature of primary consumers nicely reflects variation in anthropogenic N loading. For example, a study of 82 streams by Anderson and Cabana (2006) demonstrated a significant curvilinear relationship between stream N concentration and primary consumer $\delta^{15}\text{N}$. Other studies confirm a large range in primary consumer $\delta^{15}\text{N}$ across gradients of land use (Fry and Allen 2003, Anderson and Cabana 2005) and nutrient enrichment (Bergfur et al. 2009, Diebel and Vander Zanden 2009). While the relationship between agricultural land use and stream water nitrogen loads has been well established (Vitousek 1997, Lefebvre et al.

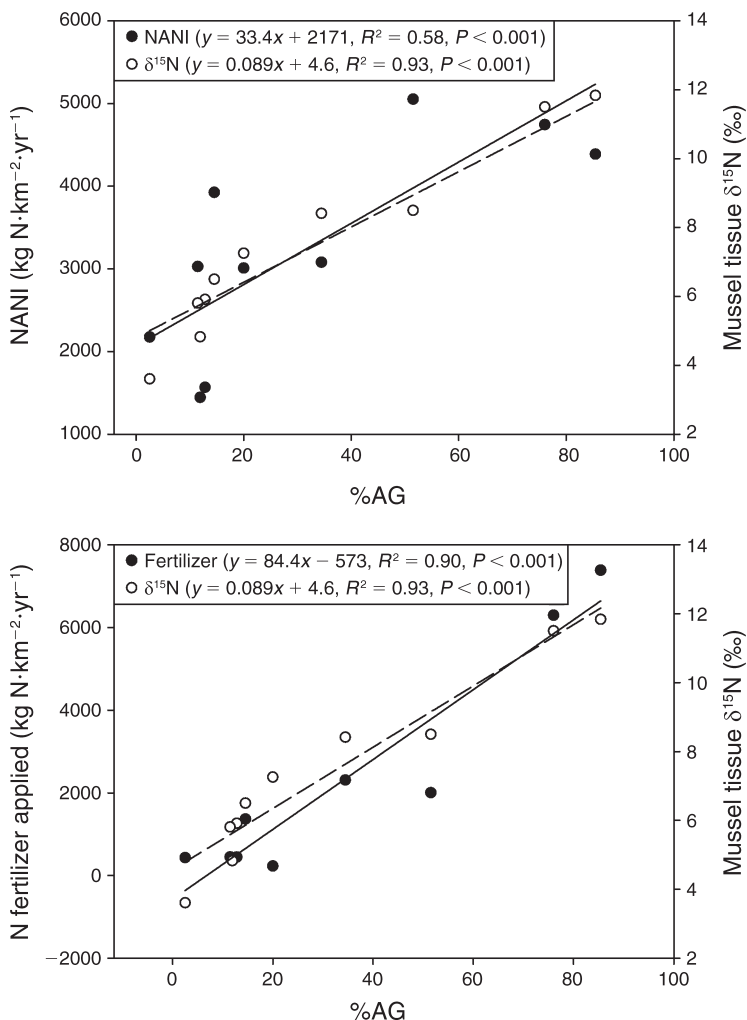


FIG. 4. Relationship between %AG and (A) the amount of net anthropogenic nitrogen inputs (NANI) and mussel δ¹⁵N and (B) N fertilizer applied within the watershed and mussel δ¹⁵N. The slope of both NANI and N fertilizer are similar to the slope of δ¹⁵N.

2007), the relationship between agricultural land use and δ¹⁵N is not as clear (Diebel and Vander Zanden 2009). However, based on our results, we would expect riverine mussels to have a δ¹⁵N of approximately 2.0‰ without agriculture, allowing us to establish a theoretical baseline δ¹⁵N signature for future bioassessments.

Better predictions about how much N is entering watersheds at a variety of spatial and temporal scales and the effects of these N subsidies on ecosystem processes would be valuable. Models to calculate NANI can be used, however real-time measurements are necessary to understand amounts of N actually reaching streams. While the NANI toolbox allows prediction of the amount of N entering a watershed from various sources, it may not be very sensitive to how much N actually enters a stream over a variety of temporal and spatial scales (e.g., stream buffers may mitigate some N). Also, some of the data layers used by NANI are several years old, so the analyses may not reflect which N

sources are currently influencing the river. In contrast, the δ¹⁵N of various tissue compartments within mussels, assuming tissue-specific nitrogen turnover times, are a better representation of N that is entering a given area over a specific time period (dependent on the turnover time of the tissue). For example, Howarth et al. (2012) suggested a NANI value of 1070 kg N·km⁻²·yr⁻¹ or lower as a threshold of N that rivers can process without exporting excess amounts to coastal waterways. Our regression analysis suggests that this would be a δ¹⁵N value of 3.8‰ ± 1.6‰ (mean ± SD) in freshwater mussels. Collectively, the use of NANI as a predictive tool (Appendix C) in conjunction with field monitoring tools, such as δ¹⁵N in consumer tissues, will be useful for future N management.

In this study, we only considered the influence of agriculture on freshwater mussel tissue N isotope signatures. There was some scatter in our data and other anthropogenic land uses, such as higher urban

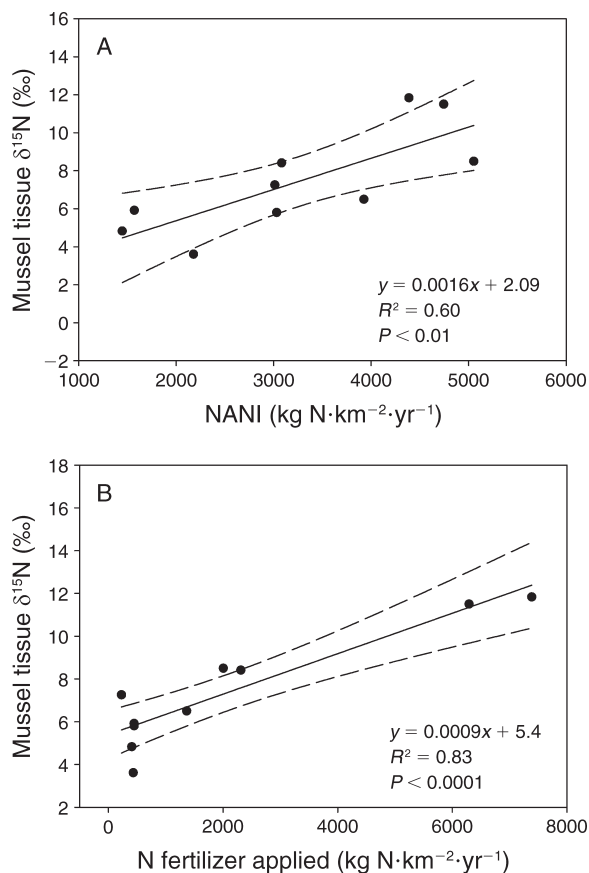


FIG. 5. Relationship between (A) NANI and (B) average N fertilizer applied vs. $\delta^{15}\text{N}$ of mussel tissue with 95% confidence intervals (dashed lines).

cover, are also likely to drive the $\delta^{15}\text{N}$ signatures of aquatic organisms. For example, previous studies have shown that high $\delta^{15}\text{N}$ values of inorganic N derived from sewage (Kendall 1998) can be traced in aquatic food webs influenced by urban development (e.g., Cabana and Rasmussen 1996, Steffy and Kilham 2004, Vander Zanden et al. 2005). Additionally, land cover such as forest and wetlands may mitigate the influence of N loading (Zedler 2003). While human- and animal-derived wastes have $\delta^{15}\text{N}$ values that are elevated (Tucker et al. 1999, Vander Zanden et al. 2005) and inorganic fertilizers typically have lower values (0‰) of $\delta^{15}\text{N}$ (Kendall 1998), N from both synthetic fertilizers and animal manure can be transformed in watersheds by processes (e.g., assimilation, nitrification, denitrification) leading to fractionation of N through gaseous loss of ^{14}N and disproportionate retention of ^{15}N within the watershed (Robinson 2001, Groffman et al. 2006). These differing initial signatures of fertilizer and the varying usage of fertilizer across different types of agriculture could have led to the variability in $\delta^{15}\text{N}$ values across locations, yet the $\delta^{15}\text{N}$ signature of freshwater mussels was correlated strongly with the percentage of agriculture in watersheds, suggesting that

mussel tissue signatures are good indicators of human disturbance.

The linkage between land use, anthropogenic nitrogen, and the assimilation of this nitrogen into food webs shows a direct connection between the influence of humans on watersheds and the biochemical makeup of organisms. These relationships can be complex because of system-specific differences in background $\delta^{15}\text{N}$, the type of N inputs, and hydrology (Fry et al. 2003, Hoffman et al. 2012, Howarth et al. 2012). Although this study does not completely agree with existing N isotope cycling models (Fry 2006, Diebel and Vander Zanden 2009), our results are similar to other recent studies that show that $\delta^{15}\text{N}$ content of organisms track anthropogenic N inputs (Lefebvre et al. 2007, Hoffman et al. 2012, Spooner et al. 2013). We found that freshwater mussels reflect watershed-scale changes in N entering rivers as indicated by the biochemical makeup of nitrogen ($\delta^{15}\text{N}$) in their tissue. Thus, mussels should be a useful future monitoring tool for riverine N because they integrate N entering the river across both time and space. For example, threshold nitrogen concentrations of 0.3–1.0 mg N/L alter species composition of algae (specifically diatoms; Black et al. 2011), chlorophyll concentrations in streams (Dodds et al. 2002b), and N uptake capacity (Dodds et al. 2002a). However, such thresholds can be hard to identify because N concentrations can vary widely over time such that typical water quality samples of N in stream water may not represent the total N loading to the stream (as suggested by Appendix B). There is often a time lag between N loading to the watershed and when the N enters the stream, usually linked to discharge (Golladay and Battle 2002). Freshwater mussels are long lived and sedentary; thus, individual populations could be sampled over time as part of long-term monitoring across multiple watersheds. Plus, different tissue types from freshwater mussels, such as mantle tissue (Berg et al. 1995) and hemolymph (Gustafson et al. 2007), can be sampled nonlethally, allowing for continual monitoring without compromising mussel populations. Additionally, different tissue types have varying turnover times (Raikow and Hamilton 2001, Gustafson et al. 2007); thus, there is a potential to examine N loading expressed in mussel tissue across different seasons and time scales.

Excessive nitrogen loading to water bodies is responsible for loss of biodiversity, eutrophication, hypoxia, and habitat degradation in coastal ecosystems globally (Turner and Rabalais 1994, Howard 2000, Dodds 2006, Riseng et al. 2011). Thus, adequate monitoring and mitigation of N loading is essential. Our results show that the N signature in primary consumer tissue can be used as a bioassessment tool that integrates watershed-level land use change with incoming stream nitrogen fluxes. We suggest setting baseline N signatures (for example $\delta^{15}\text{N}$ of 3.8‰) and resampling populations over time in sensitive rivers to assess management outcomes. Freshwater mussels and other long-lived primary

consumers may be an ideal tool to achieve these biomonitoring objectives.

ACKNOWLEDGMENTS

We are especially appreciative of Tom Maddox for his assistance in the stable isotope laboratory at the University of Georgia. Jeff Kelly and Michael Patten provided statistical advice. Kenneth Forshay, Stephen Golladay, Jason Julian, Jeff Kelly, and Michael Patten were exceptionally helpful throughout the writing process. C. Atkinson was supported in part by the United States Environmental Protection Agency (EPA) under the Science to Achieve Results (STAR) Graduate Fellowship Program. EPA has not officially endorsed this publication, and the views expressed herein may not reflect the views of the EPA. Partial support for this project was provided to A. Christian by the Ohio Chapter of the Nature Conservancy, the USDA Ouachita National Forest, the U.S. Department of Interior Buffalo National River, and National Science Foundation Award #09-42371 (DBI:MRI-R12; Hannigan and Christian). D. Spooner was supported in part by Canada's Natural Sciences and Engineering Council (NSERC) Discovery and Strategic grants (to M. A. Xenopoulos).

LITERATURE CITED

- Allen, D. C., and C. C. Vaughn. 2009. Burrowing behavior of freshwater mussels in experimentally manipulated communities. *Journal of the North American Benthological Society* 28:93–100.
- Anderson, C., and G. Cabana. 2005. Delta N-15 in riverine food webs: effects of N inputs from agricultural watersheds. *Canadian Journal of Fisheries and Aquatic Sciences* 62:333–340.
- Anderson, C., and G. Cabana. 2006. Does delta N-15 in river food webs reflect the intensity and origin of N loads from the watershed? *Science of the Total Environment* 367:968–978.
- Atkinson, C. L., M. R. First, A. P. Covich, S. P. Opsahl, and S. W. Golladay. 2011. Suspended material availability and filtration–biodeposition processes performed by a native and invasive bivalve species in streams. *Hydrobiologia* 667:191–204.
- Atkinson, C. L., S. P. Opsahl, A. P. Covich, S. W. Golladay, and L. M. Conner. 2010. Stable isotope signatures, tissue stoichiometry, and nutrient cycling of a native and invasive freshwater bivalve. *Journal of the North American Benthological Society* 29:496–505.
- Berg, D. J., W. R. Haag, S. I. Guttman, and J. B. Sickel. 1995. Mantle biopsy: a technique for nondestructive tissue-sampling of freshwater mussels. *Journal of the North American Benthological Society* 14:577–581.
- Bergfur, J., R. K. Johnson, L. Sandin, and W. Goedkoop. 2009. Effects of nutrient enrichment on C and N stable isotope ratios of invertebrates, fish and their food resources in boreal streams. *Hydrobiologia* 628:67–79.
- Black, R. W., P. W. Moran, and J. D. Frankforter. 2011. Response of algal metrics to nutrients and physical factors and identification of nutrient thresholds in agricultural streams. *Environmental Monitoring and Assessment* 175:397–417.
- Boyer, E. W., C. L. Goodale, N. A. Jaworski, and R. W. Howarth. 2002. Anthropogenic nitrogen sources and relationships to riverine nitrogen export in the northeastern USA. *Biogeochemistry* 57:137–169.
- Brönmark, C., and B. Malmqvist. 1982. Resource partitioning between unionid mussels in a Swedish lake outlet. *Holarctic Ecology* 389–395.
- Bucci, J. P., J. F. Levine, and W. J. Showers. 2011. Spatial variability of the stable isotope (delta N-15) composition in two freshwater bivalves (*Corbicula fluminea* and *Elliptio complanata*). *Journal of Freshwater Ecology* 26:19–24.
- Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences USA* 93:10844–10847.
- Christian, A. D., B. N. Smith, D. J. Berg, J. C. Smoot, and R. H. Findlay. 2004. Trophic position and potential food sources of 2 species of unionid bivalves (Mollusca: Unionidae) in 2 small Ohio streams. *Journal of the North American Benthological Society* 23:101–113.
- Cole, M. L., I. Valiela, K. D. Kroeger, G. L. Tomasky, J. Cebrian, C. Wigand, R. A. McKinney, S. P. Grady, and M. H. C. da Silva. 2004. Assessment of a delta N-15 isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. *Journal of Environmental Quality* 33:124–132.
- Costanzo, S. D., M. J. O'Donohue, W. C. Dennison, N. R. Loneragan, and M. Thomas. 2001. A new approach for detecting and mapping sewage impacts. *Marine Pollution Bulletin* 42:149–156.
- Diebel, M. W., and M. J. Vander Zanden. 2009. Nitrogen stable isotopes in streams: effects of agricultural sources and transformations. *Ecological Applications* 19:1127–1134.
- Dodds, W. K. 2006. Nutrients and the “dead zone”: the link between nutrient ratios and dissolved oxygen in the northern Gulf of Mexico. *Frontiers in Ecology and the Environment* 4:211–217.
- Dodds, W. K., et al. 2002a. N uptake as a function of concentration in streams. *Journal of the North American Benthological Society* 21:206–220.
- Dodds, W. K., V. H. Smith, and K. Lohman. 2002b. Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. *Canadian Journal of Fisheries and Aquatic Sciences* 59:865–874.
- Elser, J. J. 2011. A world awash with nitrogen. *Science* 334:1504–1505.
- Fertig, B., T. J. B. Carruthers, W. C. Dennison, A. B. Jones, F. Pantus, and B. Longstaff. 2009. Oyster and macroalgae bioindicators detect elevated delta(15)N in Maryland's coastal bays. *Estuaries and Coasts* 32:773–786.
- Fry, B. 2006. *Stable isotope ecology*. Springer, New York, New York, USA.
- Fry, B., and Y. C. Allen. 2003. Stable isotopes in zebra mussels as bioindicators of river-watershed linkages. *River Research and Applications* 19:683–696.
- Fry, B., A. Gace, and J. W. McClelland. 2003. Chemical indicators of anthropogenic nitrogen loading in four Pacific estuaries. *Pacific Science* 57:77–101.
- Galbraith, H. S., S. E. Frazier, B. Allison, and C. C. Vaughn. 2009. Comparison of gill surface morphology across a guild of suspension-feeding unionid bivalves. *Journal of Molluscan Studies* 75:103–107.
- Galloway, J. N., J. D. Aber, J. W. Erisman, S. P. Seitzinger, R. W. Howarth, E. B. Cowling, and B. J. Cosby. 2003. The nitrogen cascade. *BioScience* 53:341–356.
- Galloway, J. N., et al. 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry* 70:153–226.
- Golladay, S. W., and J. Battle. 2002. Effects of flooding and drought on water quality in gulf coastal plain streams in Georgia. *Journal of Environmental Quality* 31:1266–1272.
- Gordon, L. J., G. D. Peterson, and E. M. Bennett. 2008. Agricultural modifications of hydrological flows create ecological surprises. *Trends in Ecology and Evolution* 23:211–219.
- Gotelli, N. J., and A. M. Ellison. 2004. *A primer of ecological statistics*. Sinauer Associates, Sunderland, Massachusetts, USA.
- Groffman, P. M., M. A. Altabet, J. K. Bohlke, K. Butterbach-Bahl, M. B. David, M. K. Firestone, A. E. Giblin, T. M. Kana, L. P. Nielsen, and M. A. Voytek. 2006. Methods for measuring denitrification: diverse approaches to a difficult problem. *Ecological Applications* 16:2091–2122.
- Gustafson, L., W. Showers, T. Kwak, J. Levine, and M. Stoskopf. 2007. Temporal and spatial variability in stable

- isotope compositions of a freshwater mussel: implications for biomonitoring and ecological studies. *Oecologia* 152:140–150.
- Haag, W. R., and A. L. Rypel. 2011. Growth and longevity in freshwater mussels: evolutionary and conservation implications. *Biological Reviews* 86:225–247.
- Hoffman, J., J. Kelly, G. Peterson, A. Cotter, M. Starry, and M. Sierszen. 2012. Using $\delta^{15}\text{N}$ in fish larvae as an indicator of watershed sources of anthropogenic nitrogen: response at multiple spatial scales. *Estuaries and Coasts* 35:1453–1467.
- Hogberg, P. 1990. Forests losing large quantities of nitrogen have elevated N15-N14 ratios. *Oecologia* 84:229–231.
- Homer, C., C. Huang, L. Yang, B. Wylie, and M. Coan. 2004. Development of a 2001 National Land Cover Database for the United States. *Photogrammetric Engineering and Remote Sensing* 70:829–840.
- Hong, B., D. P. Swaney, and R. W. Howarth. 2011. A toolbox for calculating net anthropogenic nitrogen inputs (NANI). *Environmental Modelling and Software* 26:623–633.
- Howard, E. A. 2000. Nutrient pollution of coastal rivers, bays, and seas. *Issues in Ecology* 7:1–15.
- Howard, J. K., and K. M. Cuffey. 2006. The functional role of native freshwater mussels in the fluvial benthic environment. *Freshwater Biology* 51:460–474.
- Howarth, R., D. Swaney, G. Billen, J. Garnier, B. Hong, C. Humborg, P. Johnes, C.-M. Mörth, and R. Marino. 2012. Nitrogen fluxes from the landscape are controlled by net anthropogenic nitrogen inputs and by climate. *Frontiers in Ecology and the Environment* 10:37–43.
- Kappes, H., and P. Haase. 2012. Slow, but steady: dispersal of freshwater molluscs. *Aquatic Sciences* 74:1–14.
- Kendall, C. 1998. Tracing nitrogen sources and cycling in catchments. *Isotope Tracers in Catchment Hydrology* 519–576.
- Lefebvre, S., J. C. Clement, G. Pinay, C. Thenail, P. Durand, and P. Marmonier. 2007. N-15-nitrate signature in low-order streams: effects of land cover and agricultural practices. *Ecological Applications* 17:2333–2346.
- Leff, L. G., J. L. Burch, and J. V. McArthur. 1990. Spatial distribution, seston removal, and potential competitive interactions of the bivalves *Corbicula fluminea* and *Elliptio complanata*, in a coastal plain stream. *Freshwater Biology* 24:409–416.
- Matthews, W. J., C. C. Vaughn, K. B. Gido, and E. Marsh-Matthews. 2005. Southern Plains rivers. Pages 282–325 in C. B. Arthur and E. C. Colbert, editors. *Rivers of North America*. Academic Press, Burlington, Maryland, USA.
- Naimo, T. J., E. D. Damschen, R. G. Rada, and E. M. Monroe. 1998. Nonlethal evaluation of the physiological health of unionid mussels: methods for biopsy and glycogen analysis. *Journal of the North American Benthological Society* 17:121–128.
- Olden, J. D., and N. L. Poff. 2003. Redundancy and the choice of hydrologic indices for characterizing streamflow regimes. *River Research and Applications* 19:101–121.
- Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18:293–320.
- R Development Core Team. 2011. R 2.14.1. R Project for Statistical Computing, Vienna, Austria. www.r-project.org
- Raikow, D. F., and S. K. Hamilton. 2001. Bivalve diets in a midwestern US stream: a stable isotope enrichment study. *Limnology and Oceanography* 514–522.
- Rangel, T. F., J. A. F. Diniz, and L. M. Bini. 2010. SAM: a comprehensive application for spatial analysis in macroecology. *Ecography* 33:46–50.
- Riseng, C. M., M. J. Wiley, R. W. Black, and M. D. Munn. 2011. Impacts of agricultural land use on biological integrity: a causal analysis. *Ecological Applications* 21:3128–3146.
- Robinson, D. 2001. Delta N-15 as an integrator of the nitrogen cycle. *Trends in Ecology and Evolution* 16:153–162.
- Spooner, D. E., P. C. Frost, H. Hillebrand, M. T. P. O., Arts, and M. A. Xenopoulos. 2013. Nutrient loading associated with agriculture land use dampens the importance of consumer-mediated niche construction. *Ecology Letters* 16:1115–1125.
- Steffy, L. Y., and S. S. Kilham. 2004. Elevated delta N-15 in stream biota in areas with septic tank systems in an urban watershed. *Ecological Applications* 14:637–641.
- Tucker, J., N. Sheats, A. E. Giblin, C. S. Hopkinson, and J. P. Montoya. 1999. Using stable isotopes to trace sewage-derived material through Boston Harbor and Massachusetts Bay. *Marine Environmental Research* 48:353–375.
- Turner, R. E., and N. N. Rabalais. 1994. Coastal eutrophication near the Mississippi river delta. *Nature* 368:619–621.
- Vander Zanden, M. J., Y. Vadeboncoeur, M. W. Diebel, and E. Jeppesen. 2005. Primary consumer stable nitrogen isotones as indicators of nutrient source. *Environmental Science and Technology* 39:7509–7515.
- Vaughn, C. C., D. E. Spooner, and H. S. Galbraith. 2007. Context-dependent species identity effects within a functional group of filter-feeding bivalves. *Ecology* 88:1664–1662.
- Vitousek, P. M. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* 7:737–750.
- Zedler, J. B. 2003. Wetlands at your service: reducing impacts of agriculture at the watershed scale. *Frontiers in Ecology and the Environment* 1:65–72.

SUPPLEMENTAL MATERIAL

Appendix A

Mussel tissue $\delta^{15}\text{N}$ data used in the study ([Ecological Archives A024-022-A1](#)).

Appendix B

A figure showing that water chemistry of the Oklahoma, USA, sites was not significantly related to the percentage of agriculture in the watershed, while the $\delta^{15}\text{N}$ of mussels was significantly positively related ([Ecological Archives A024-022-A2](#)).

Appendix C

Relationship between net anthropogenic nitrogen inputs (NANI) and the percentage of N flux that is NANI ([Ecological Archives A024-022-A3](#)).