

Stable Nitrogen Isotopes of Plankton and Benthos Reflect a Landscape-level Influence on Great Lakes Coastal Ecosystems

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ABSTRACT. As populations and human activities increase in coastal watersheds, an understanding of the connections of aquatic ecosystems to the adjacent terrestrial landscape is necessary to identify, monitor, and protect vulnerable coastal habitats. This study investigates the relationships between land-use patterns and $\delta^{15}\text{N}$ values of aquatic organisms in coastal ecosystems, across a defined watershed gradient for the U.S. portion of the Great Lakes shoreline. $\delta^{15}\text{N}$ measured in plankton and benthic invertebrates reflects a range of basin wide land-use gradients and demonstrates a strong connection between watershed-based anthropogenic activities and exposure in aquatic biota. For example, benthos $\delta^{15}\text{N}$ values range over 12‰ across sites in our study, but regression analyses suggest that over 50% of the variability is explained by the regional landscape. Further, multiple taxa at comparable trophic position showed similar patterns in relation to watershed-scale land use. Our results suggest that within the coastal environment, the expression of landscape in aquatic biota is stronger in habitats such as embayments and wetlands than open nearshore. These results support the use of $\delta^{15}\text{N}$ in Great Lakes coastal biota as an exposure indicator of watershed-scale N loading.

INDEX WORDS: Great Lakes, coastal habitat, stable nitrogen isotope, landscape disturbance gradient, plankton, benthos.

INTRODUCTION

As populations and human activities increase in coastal watersheds around the Great Lakes, there is a need to develop an understanding of the connections of aquatic ecosystems to the adjacent terrestrial landscape (Krieger *et al.* 1992, Edsall and Charlton 1997, Goforth and Carman 2005). This understanding is critical to the development of stressor-response relationships, which ultimately form the basis for management strategies protecting vulnerable coastal habitats. In addition, there is a renewed emphasis to include coastal components in Great Lakes-wide monitoring programs (Neilson *et al.* 2003, Niemi *et al.* 2004, Mackey and Goforth 2005). Ideally, these monitoring approaches would not only report on aquatic conditions, but also link those conditions to factors in watersheds that may

contribute to the conditions. In freshwater ecology, there is a strong history of studies connecting streams with their watershed character, and small lakes with their local and regional landscape setting. To date, local- and regional-scale studies (McClelland *et al.* 1997, Valiela and Bowen 2002, Wigand *et al.* 2003) have defined how the character of coastal watersheds influences nutrient loading to ecosystems of marine coastal estuaries. However, this relationship has yet to be defined for Great Lakes coastal ecosystems. Further, the linkage between coastal ecosystems, including different coastal habitats and their adjacent contributing landscape has not been previously established at the scale of the Great Lakes basin.

Among the potential impacts of increased anthropogenic activity in coastal watersheds is the alteration of nutrient dynamics. Conversion of natural vegetation to agricultural and urban land can lead to increased nutrient input to coastal waters (Vitousek

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et al. 1997, Howarth *et al.* 2002). Increased nutrient loads can lead to eutrophication, degradation of habitat, and alteration of food webs and biological community structure (Smith *et al.* 1999, Valiela and Bowen 2002, Sierszen *et al.* 2006a). Methods to detect anthropogenic loading are needed, to identify susceptible habitats and provide early warning of incipient effects to Great Lakes coastal aquatic ecosystems.

Stable isotope analyses are commonly used tools in studies of aquatic ecosystems to examine food-web relationships and energy pathways (Vander Zanden and Rasmussen 1999, Post 2002). Stable isotopes of nitrogen ($\delta^{15}\text{N}$) have also been used to identify anthropogenic contributions in nitrogen loads to aquatic systems, thereby establishing linkages between landscape and aquatic ecosystems (McClelland and Valiela 1998). In addition, $\delta^{15}\text{N}$ has been proposed as a tool to indicate incipient effects of increased nutrient loading (Carmichael *et al.* 2004).

In this study we used an analysis of U.S. watersheds across the Great Lakes basin (Danz *et al.* 2005) to establish a population of sites across a gradient of potential anthropogenic stressors. This approach (detailed in Methods) provides a simple framework to evaluate whether $\delta^{15}\text{N}$ measured in organisms from coastal waters could confirm the connection to the adjacent landscape character. Isotopic signatures are likely to be more meaningful than water quality measures (which can have high temporal variability in dynamic coastal waters), because they integrate exposure over time. Further, while water quality parameters indicate the potential for exposure, isotopic signatures can verify assimilation of nitrogen into biological tissues. Previous studies have demonstrated that $\delta^{15}\text{N}$ measured in aquatic organisms reflects some of the major human activities (agriculture and development, population growth, and wastewater), that occur in contributing watersheds (McClelland *et al.* 1997, Cabana and Rasmussen 1996, McKinney *et al.* 2002, Cole *et al.* 2004). Since $\delta^{15}\text{N}$ can demonstrate food web incorporation of watershed N, we anticipate that $\delta^{15}\text{N}$ would serve as an “exposure indicator” that establishes relationships between coastal receiving systems and watersheds across the Great Lakes.

The objective of this paper is to investigate relationships between land use patterns and $\delta^{15}\text{N}$ isotope values in coastal ecosystems, across a defined watershed gradient for U.S. Great Lakes shorelines. We explore several aspects of that relationship:

1. Stable nitrogen isotope signatures ($\delta^{15}\text{N}$) in selected organisms, relative to four different landscape metrics;
2. Variation in $\delta^{15}\text{N}$ among several different biological components (planktonic and benthic macroinvertebrates), again in relation to the landscape character; and
3. Variation across the landscape gradient in $\delta^{15}\text{N}$ among different ecosystems that represent differing degrees of hydrologic connection to the landscape (coastal wetlands, embayments, and open nearshore waters).

METHODS

Study Design and Site Selection

This study examines stable nitrogen isotope data from samples collected from 2002 to 2004 during related studies of two different coastal habitats. These habitats consisted of 1) open or “high energy” shorelines, and 2) closed or semi-enclosed shorelines, hereafter referred to as open nearshore and embayments, respectively. In addition, data from a third class of coastal habitat, wetlands, are evaluated for comparison. Sites within each of these habitats (Fig. 1) were randomly chosen from among a Great Lakes basin-wide population of possible sites, representing a gradient of natural conditions and human impacts in the adjacent terrestrial watershed (see Land-use PC Gradients below). Selection of sites along this gradient was further refined to include sites from within each of the Great Lakes.

For both open nearshore and embayment sites, sampling locations corresponded to specific points along a grid path developed to describe each system comprehensively. In 2002 and 2003, we collected samples from 30 open nearshore sites. Samples were collected from late Jul to early Sept in 2002, and mid-Jun to early Aug in 2003. At each site, samples were collected along a transect perpendicular to shore at stations corresponding to depths of approximately 5, 10, and 20 m. In 2004, samples were collected from 15 embayment sites from mid-July to late Aug. Embayments were defined as shoreline indentations where the distance from the bay mouth to the inner shoreline was greater than the distance across the mouth. Samples were collected at ≤ 15 m depths within each embayment, as well as from corresponding depths along the shoreline outside the bay mouth. Within embayments, benthic samples were collected at each of up to three unique substrate locations as identified by

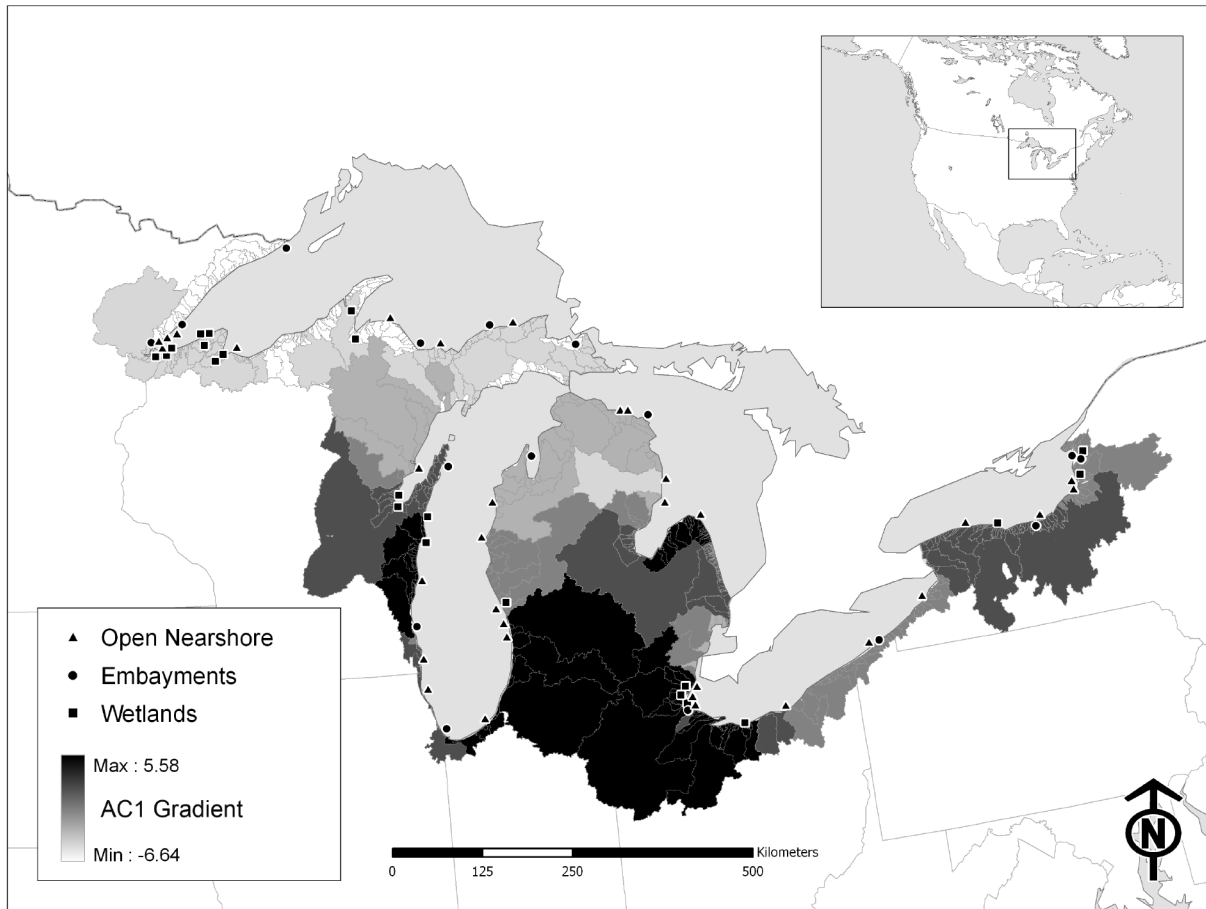


FIG. 1. Map showing sampling locations for each coastal habitat relative to AC1 gradient. The AC1 gradient represents a relative measure of agricultural activity in segment-sheds (Danz et al. 2005, 2007). Higher levels of activity are shown as darker shades (Black = highest activity).

acoustic seabed classification (QTC IMPACT; www.questertangent.com). Coastal wetlands were sampled for stable isotopes from mid-Jul to late Aug in 2002 to 2004 at 22 sites. For each wetland, sampling stations consisted of three to five transects which were chosen to spatially represent the entire wetland and its vegetated habitats.

Land-use PC Gradients

We compared our $\delta^{15}\text{N}$ data to four gradients in watershed character across the U.S. Great Lakes basin using integrated measures of landscape-level anthropogenic stress defined by Danz et al. (2005). Briefly, GIS data on over 200 environmental variables compiled into seven stressor categories were summarized for 762 shoreline segments and associated drainage-shed units (segment-sheds) that com-

prise the U.S. portion of the basin. Principal components (PC) analysis was then used to develop an aggregate measure within each stress category. The resulting PC scores reflect levels of anthropogenic stress, which are described below for each of the stress categories used in our study. All sampled sites were assigned the PC score of their corresponding segment-shed for landscape metric-isotope regression analyses.

Our exploratory study was to establish a linkage to landscape at the coarse scales of the available GIS data and we focused on composite PC scores of major stressor categories as the landscape metrics, rather than search for an underlying specific variable or mechanism by which one might, for example, begin to develop a watershed “loading” model. We followed the general site selection approach of Danz et al. (2005), but added sites to ensure a con-

tinuous representation across the range of values in the agriculture stressor category (see below). Danz *et al.* (2005) originally defined seven categories of stressors and later examined five of these categories in more detail (Danz *et al.* 2007). We restricted our analyses to the first PC (representing the highest percent of overall variance explained in each category) of four landscape categories. Our study used the PC scores and range originally generated by Danz *et al.* (2005), not the subsequent transformed, standardized scaling of Danz *et al.* (2007).

A brief description of each of the four land-use PC gradients used in our study is listed below. Details on individual data sources are in Appendix 1 of Danz *et al.* (2007).

1. Agriculture (labeled AC1)—21 variables characteristic of the major types of stresses associated with agricultural activities, including nutrient runoff, fertilizers, pesticide application, and erosion. Agricultural land cover *per se* was included in the land cover category described below. The AC1 gradient was used as the primary gradient across which the sites were distributed. Analyses of Danz *et al.* (2007) show that this PC is structured by a set of related variables including N, P, K fertilizer use and/or export as well as erosion potential, and it thus roughly represents nutrient loading potential, from low to high.
2. Land cover (labeled LC1)—23 land use/land cover variables derived primarily from the National Land Cover Dataset and also from the Natural Resources Inventory. This PC ranges from primarily forested (high scores) to primarily agricultural and urbanized landscapes (low scores).
3. Human population (labeled PD1)—14 variables representing human population density, road density, developed land, and wetlands converted to developed areas. This PC correlated with a set of factors: population density, amount of roads, and the relative area of developed land.
4. Point source pollution (labeled PS1)—79 variables representing point sources of pollution, including mines, power plant emissions, and facilities with permitted wastewater discharges from the National Pollutant Elimination Discharge System. This PC reflected overall discharge of a wide range of chemical pollutants from point sources in wastewater.

With respect to our analyses, note that while PCs within each of the four gradient categories listed above are independent of each other, there are some strong correlations among gradients. Among the four PC gradients we used in this study the highest correlation (across 762 sites) was between AC1 and LC1 ($r^2 = 0.84$) (Danz *et al.* 2007).

Macroinvertebrate Sample Collection

At each open nearshore and embayment sampling station, multiple benthic samples were collected to ensure adequate numbers of different types of organisms. Amphipods, chironomids, Hexagenia mayflies, mussels, and other invertebrates were removed from sediments, rinsed with 1N HCl and deionized (DI) water, sorted by organism type, and frozen. For open nearshore sites, paired t-tests revealed that, within taxa, $\delta^{15}\text{N}$ from 5 and 10 m depth stations were not different. Therefore $\delta^{15}\text{N}$ values for these stations were combined for each taxon and are reported as a mean for the site. Likewise, within embayments, $\delta^{15}\text{N}$ values within taxa from different sampling locations (bottom types) were very similar (s.e. $\leq 0.8\text{‰}$). Therefore, $\delta^{15}\text{N}$ values were combined and reported as means, by taxa for; 1) all stations within an embayment, and 2) all stations outside each embayment.

Macroinvertebrates were collected at multiple wetland sampling stations using sweep nets. Material obtained from a 3–5 m area was collected on a 253 μm sieve, transferred to storage containers, and frozen. In the laboratory, amphipods were removed from thawed samples, rinsed with 1N HCl and DI water, and dried at 60°C. $\delta^{15}\text{N}$ data from individual wetland stations were combined and reported as a mean for each wetland.

Plankton Sample Collection

Plankton samples were collected at open nearshore and embayment sites (< 20 m depth) by horizontal net tow using an 80 μm mesh, 0.2 m diameter net. A cable depressor was used to facilitate depth control. The net was towed at approximately 5 knots for 2–4 minutes, and undulated between near bottom and near surface to encompass the majority of the water column. In the open nearshore, plankton tows were conducted along the depth contour corresponding to each of the fixed station depths, passing through the station at the midpoint of the tow. In embayments, plankton tows were conducted along a transect roughly centered in the

embayment. Two size fractions of plankton were extracted from each plankton sample using a 253 μm sieve; a $> 253 \mu\text{m}$ (large) and 80–253 μm (small) fraction. All visible debris was removed from samples. Samples were rinsed with 1N HCl followed by DI water, and frozen.

Wetland plankton samples were collected by horizontal net tows as well, but using a larger 0.5 m diameter (80 μm mesh) net, and towed at much slower speeds. Tows of approximately 100 m were conducted in areas adjacent to each transect which were free of vegetation. Samples were passed through a coarse (1 mm) sieve to remove any debris, collected on 50 μm Nitex filters, rinsed with 1N HCl followed by DI water, and frozen. In the laboratory, plankton samples were picked under a dissecting scope to remove detritus prior to analysis.

Stable Isotope Sample Processing and Analysis

All samples were oven-dried at 60°C and ground to a homogeneous powder. Samples were analyzed for nitrogen isotopes using a Carlo-Erba NC2500 elemental analyzer interfaced with a Micromass Isochrome isotope ratio mass spectrometer. Isotope ratios are expressed as δ values, with units of parts-per-thousand (‰) deviations from the ratios found in atmospheric N. Isotopic standards were used to correct for instrument drift. Samples enriched with the heavier isotope (^{15}N) have δ values that are relatively more positive. Replicate analysis over the course of this study typically differed by less than 0.2‰.

Data Constraints and Manipulation

Comparisons are limited to data collected in $\leq 15\text{m}$ depth, unless specified otherwise. This restriction is because $\delta^{15}\text{N}$ of Lake Superior benthos is relatively constant in shallow zones ($< 20\text{m}$ depth) but increases significantly beyond that depth (Sierszen *et al.* 2006b). As a result, our 2004 embayment sampling was restricted to $\leq 15\text{m}$ to control depth-related variability from our data. This depth effect was not observed in plankton data for Lake Superior or in plankton or benthos data from the other Great Lakes. However, as a precaution, and for general compatibility among data sets, we chose to use $\leq 15\text{m}$ depth data for our analyses.

For statistical comparisons and to simplify figures we combined data sets where appropriate. For example, to examine relationships between land use

and $\delta^{15}\text{N}$ in biota in the general nearshore environment, we combined data from the 2002–2003 open nearshore studies with the 2004 embayment data. This combined nearshore population, hereafter referred to as “nearshore combined,” provides greater statistical power to examine differences among groups of organisms and between different indicators of land use.

Statistical Analysis

Linear regression (Systat, v. 11.0) was used to evaluate strength and significance of relationships (r^2 ; p value; s.e.) between $\delta^{15}\text{N}$ of biota and various PC gradients reflected in our sites. To determine whether $\delta^{15}\text{N}$ values of 1) different benthic invertebrates or plankton size fractions, 2) biota from different coastal systems, or 3) biota collected in different years, were different in their relationship to individual PC gradients, we used analysis of covariance (ANCOVA; Systat v.11.0) to test for significant differences between slopes and intercepts of their respective regression lines (high F ratio, low P value). Paired t-tests were used ($\alpha = 0.05$) to compare population means between $\delta^{15}\text{N}$ of biota within and outside embayments, as well as between means of biota collected at open nearshore sites adjacent to tributary inputs and those without nearby tributary input.

RESULTS

Patterns Among Taxa

$\delta^{15}\text{N}$ of biota from the nearshore combined data increased linearly with increasing AC1 landscape gradient (Fig. 2a–e). We used AC1 as a representative land-use indicator for these comparisons, but similar comparisons were made with each of the four land-use indicators. All biota exhibited similar patterns; however, some subtle differences were observed among taxa. Both size fractions of plankton (Fig. 2a) showed a similar and highly significant ($p < 0.0001$) relationship to AC1, with the larger fraction consistently about 1‰ more enriched in ^{15}N than the smaller fraction (intercepts significantly different; $p = 0.02$). Amphipods, chironomids, and mayflies (*Hexagenia* spp.) also exhibited highly significant relationships and relatively strong correlations with AC1 (Fig. 2b–d). Zebra mussels (*Dreissena* spp.) were found only at sites in the upper (i.e., more disturbed) portion of the AC1 gradient (Fig. 2e), and as a result, show a weaker relationship to AC1 ($r^2 = 0.19$; $p = 0.03$). *Dreissena*

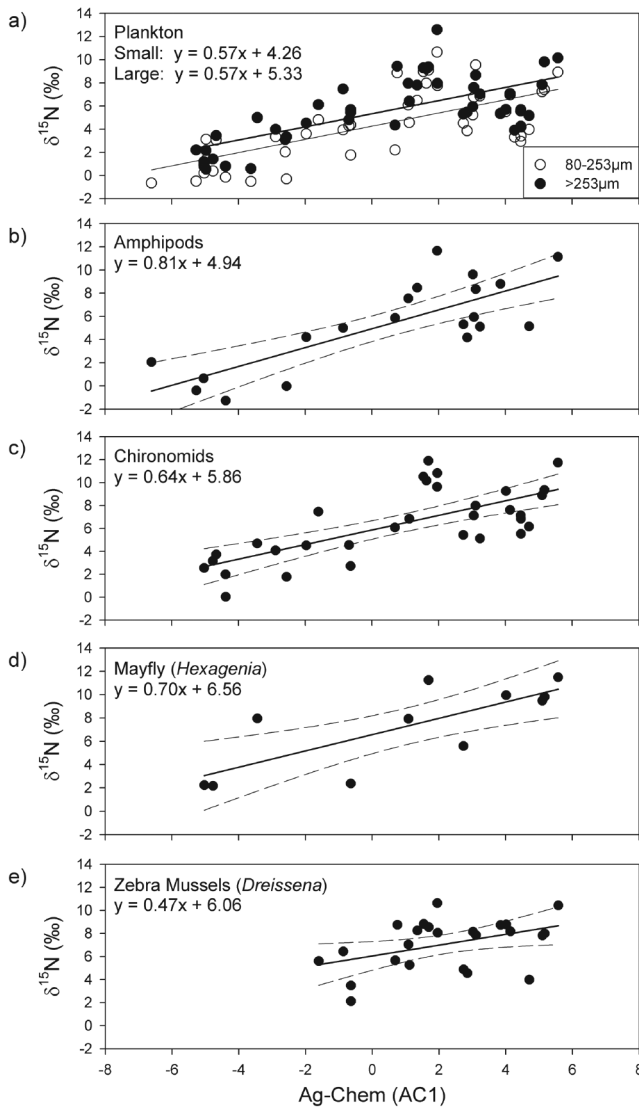


FIG. 2. Relationship of mean $\delta^{15}\text{N}$ with AC1 gradient for a) small (thin line, $r^2 = 0.46$; $p < 0.0001$; $S.E. = 2.23$) and large (thick line, $r^2 = 0.47$; $p < 0.0001$; $S.E. = 2.10$) plankton fractions, b) amphipods ($r^2 = 0.62$; $p < 0.0001$; $S.E. = 2.38$), c) chironomids ($r^2 = 0.51$; $p < 0.0001$; $S.E. = 2.20$), d) *Hexagenia* mayflies ($r^2 = 0.60$; $p = 0.0054$; $S.E. = 2.42$), and e) *Dreissenid* mussels ($r^2 = 0.19$; $p = 0.0332$; $S.E. = 2.02$). Dashed lines indicate 95% confidence intervals.

did, however, exhibit a stronger relationship with LC1 ($p = 0.0007$), possibly because *Dreissena* spanned a greater range of LC1 than it did for AC1 (data not shown).

Amphipods and chironomids were the most common and abundant invertebrates captured among

sites; however, no single benthic taxon was found at all sites. This limited the comparisons we could make among benthic taxa because of reduced sample size or land-use indicator range. To facilitate statistical comparisons among land-use gradients and habitats using benthic invertebrate $\delta^{15}\text{N}$ data, we combined data for amphipods and chironomids to calculate a “mean” benthos $\delta^{15}\text{N}$. To justify this action, analysis of covariance (ANCOVA) was performed on benthic invertebrate regression data in embayments and open nearshore. These analyses showed that for $\delta^{15}\text{N}$ relationship to AC1 (as well as the other landscape indicators), intercept was not significantly different between amphipods and chironomids, nor was there a significant interaction term for the slopes. Therefore, these two data sets were combined for comparisons among land-use gradients as well as between embayments and open nearshore systems. *Hexagenia* was excluded from the mean benthos calculations due to small sample size.

The slopes for small and large size fraction plankton were also statistically indistinguishable across all of the land-use indicators. Consequently, to simplify figures and discussion, we used only one fraction for further comparisons. We chose the small fraction because particle size was more consistently defined at the upper and lower end by our size-fractionation procedures.

Patterns Among Land-use Indicators

We used benthos and small plankton nearshore combined data to evaluate the relationships of $\delta^{15}\text{N}$ to the ag-chem (AC1), land cover (LC1), population density (PD1), and point source (PS1) land-use indicators (Fig. 3a–d). Due to the independent and somewhat arbitrary scaling of these four land-use gradients, comparisons of slope and intercept among these gradients are not meaningful. However, comparisons of the strength of the relationship can be made. Both plankton and benthos $\delta^{15}\text{N}$ exhibit highly significant relationships to AC1 and LC1 with relatively strong correlations ($r^2 > 0.45$). For the PD1 gradient, both plankton and benthos again exhibited highly significant relationships; however, the correlation was considerably stronger for plankton ($r^2 = 0.45$) than for benthos ($r^2 = 0.233$). Relationship with PS1 was significant for plankton, but not for benthos, although both plankton and benthos showed poor correlations ($r^2 = 0.18$ and 0.07 respectively).

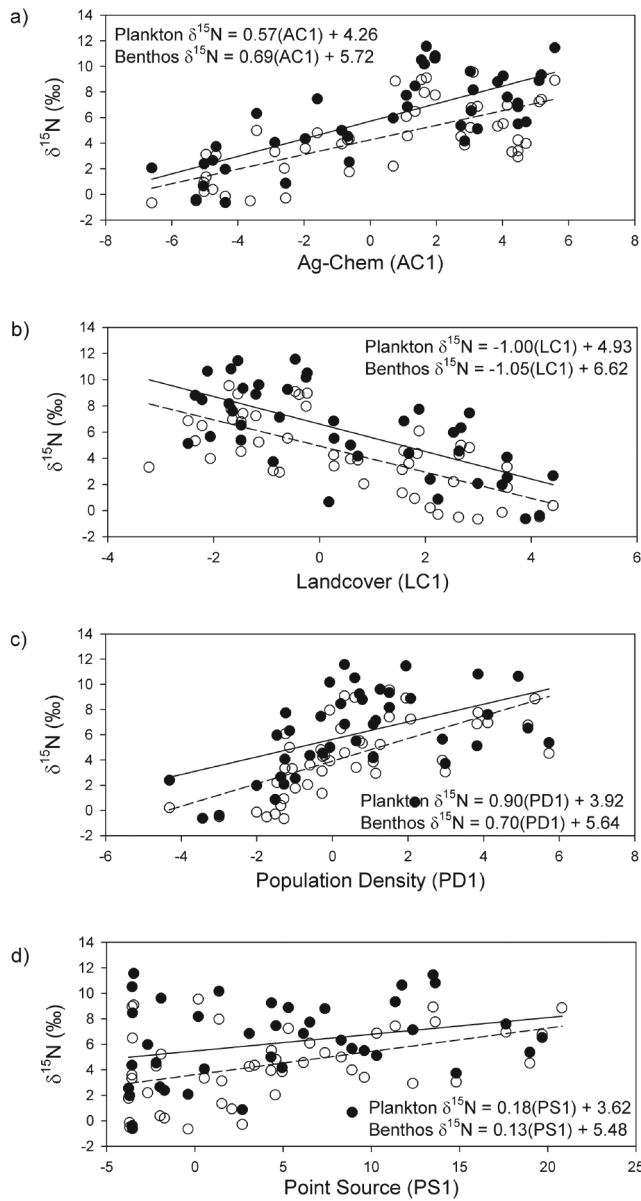


FIG. 3. Relationship of 80–253 μm plankton (open circles) and benthos (filled circles) mean $\delta^{15}\text{N}$ for different land-use indicator gradients; a) AC1, b) LC1, c) PD1, and d) PS1. Regression statistics are as follows for a) AC1: plankton $r^2 = 0.46$, $p < 0.0001$, S.E. = 2.23; benthos $r^2 = 0.55$, $p < 0.0001$, S.E. = 2.25, b) LC1: plankton $r^2 = 0.47$, $p < 0.0001$, S.E. = 2.22; benthos $r^2 = 0.46$, $p < 0.0001$, S.E. = 2.48, c) PD1: plankton $r^2 = 0.45$, $p < 0.0001$, S.E. = 2.27; benthos $r^2 = 0.23$, $p < 0.0014$, S.E. = 2.95, and d) PS1: plankton $r^2 = 0.19$, $p < 0.0025$, S.E. = 2.75; benthos $r^2 = 0.08$, $p < 0.080$, S.E. = 3.24.

Importance of Habitat Type

To examine whether embayments are unique nearshore systems, we analyzed embayments and open nearshore systems separately. Further, for comparison to another coastal system (with potentially even greater terrestrial connectivity), we also included data from Great Lakes coastal wetlands. Highly significant relationships with the AC1 gradient were observed for both plankton (80–253 μm) and benthos $\delta^{15}\text{N}$ for all three habitat types (Figs. 4a,b). However, correlations (r^2) for both plankton and benthos relationships were higher in wetlands and embayments than in open nearshore sites, even though the open nearshore sample size was larger. In addition, slopes of the plankton regressions were steeper, suggesting greater sensitivity to changes in land-use for embayments ($p = 0.05$) and (to a lesser extent) for wetlands than for open nearshore sites. Slope for benthos $\delta^{15}\text{N}$ in embayments versus AC1 was not significantly different from that of open nearshore; however, the wetlands regression was somewhat steeper than either embayment or open nearshore regressions.

For a more direct evaluation of the influence of terrestrial systems on embayments relative to other nearshore zones, we also compared plankton and benthos samples from within embayments with those collected along adjacent shorelines outside the embayment mouth. $\delta^{15}\text{N}$ data inside relative to outside embayments, paired by site (Fig. 5), show a significant trend of enrichment of ^{15}N within embayments for both plankton ($p = 0.03$) and benthos ($p < 0.01$). The most enriched sites were enclosed harbors with relatively narrow connections to the open lake. However, biota from other embayments also were enriched in ^{15}N , though to a lesser degree. This pattern of ^{15}N enrichment of plankton and benthos within embayments relative to outside, is also reflected in higher intercept values versus AC1 (data not shown) within embayments (plankton $\beta_0 = 4.79$; benthos $\beta_0 = 5.85$) relative to outside (plankton $\beta_0 = 3.54$; benthos $\beta_0 = 4.81$). Since the slopes are similar, the net result is an approximate 1‰ upward shift of the $\delta^{15}\text{N}$ regression line (i.e., ^{15}N enrichment) for both plankton ($p = 0.09$) and benthos ($p = 0.24$) within embayments relative to outside.

To test whether the influence of landscape could be detected in deeper nearshore regions, we compared the strength of relationships between land-use indicators and $\delta^{15}\text{N}$ of biota from different depth strata. We examined the relationship of plankton

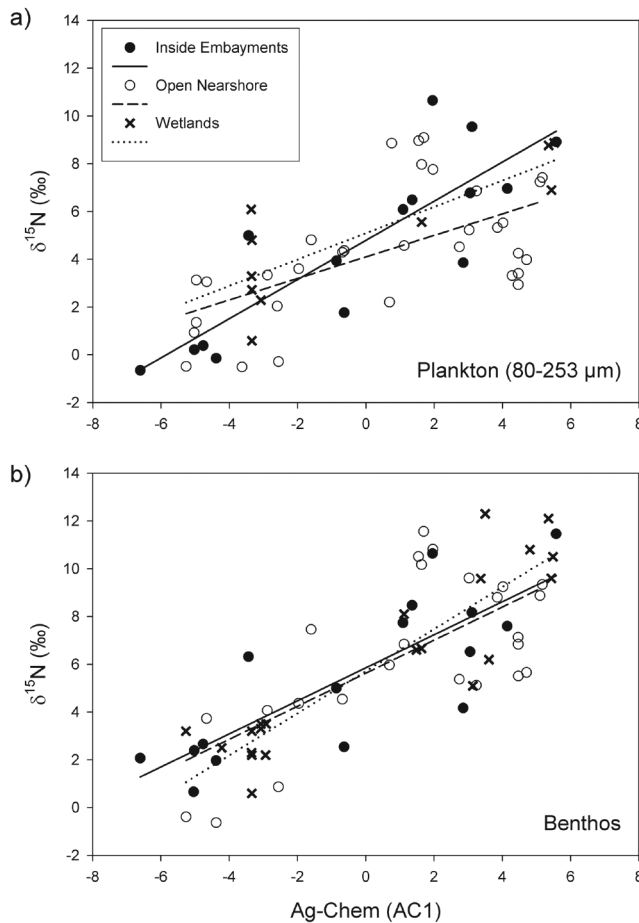


FIG. 4. Comparison of relationship with AC1 gradient between embayments, open nearshore, and coastal wetland habitats for a) plankton (80–253 μm) and b) benthos mean $\delta^{15}\text{N}$. Legend symbols apply to both figures. Regression equations for the relationships are: open nearshore plankton $\delta^{15}\text{N} = 0.45(\text{AC1}) + 4.10$ ($r^2 = 0.35$; $p = 0.0004$; $S.E. = 2.21$), embayment plankton $\delta^{15}\text{N} = 0.82(\text{AC1}) + 4.79$ ($r^2 = 0.72$; $p < 0.0001$; $S.E. = 2.04$), and wetlands plankton $\delta^{15}\text{N} = 0.55(\text{AC1}) + 5.09$ ($r^2 = 0.69$; $p = 0.0031$; $S.E. = 1.65$). Regression equations for the benthos relationships are: open nearshore benthos $\delta^{15}\text{N} = 0.69(\text{AC1}) + 5.63$ ($r^2 = 0.47$; $p = 0.0002$; $S.E. = 2.50$), embayment benthos $\delta^{15}\text{N} = 0.69(\text{AC1}) + 5.63$ ($r^2 = 0.66$; $p < 0.0001$; $S.E. = 2.01$), and wetlands benthos $\delta^{15}\text{N} = 0.88(\text{AC1}) + 5.71$ ($r^2 = 0.82$; $p < 0.0001$; $S.E. = 1.59$).

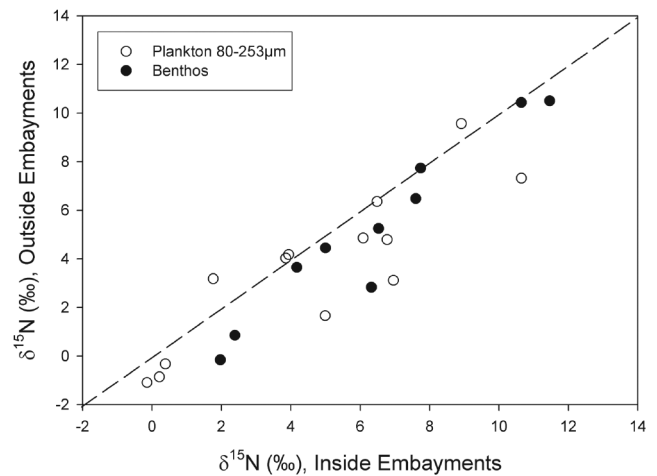


FIG. 5. Comparison of $\delta^{15}\text{N}$ for plankton and benthos from paired samples inside and outside embayment sites at similar depths. The dashed line represents a 1:1 relationship.

and benthos $\delta^{15}\text{N}$ to AC1, for 0–15 m, as well as 15–30 m depth stations outside embayments and at open nearshore sites. There were no samples taken at depths greater than 15 m inside embayments for comparison. As with the 0–15 m data, we obtained highly significant relationships for 15–30 m open nearshore plankton and benthos. However, subtle differences were observed. The p-values for benthos and plankton relationships outside embayments were nearly two and three orders of magnitude larger, respectively, at 15–30 m depths than at 0–15 m depths (Fig. 6) indicating somewhat weaker relationships with landscape at greater depths. Correlations for plankton relationships outside embayments reflect this trend as well, with 55% of the variation in $\delta^{15}\text{N}$ explained by AC1 at 15–30 m, compared with 73% at 0–15 m. Proportion of variance in $\delta^{15}\text{N}$ explained by AC1 did not decrease with depth for benthos outside embayments or for benthos or plankton from open nearshore sites. The fit of regressions for plankton and benthos was lower in the open nearshore than at embayment sites for both depth zones (Fig. 6), further supporting the differences between nearshore systems described above.

Importance of tributary input in the open nearshore was also evaluated using data from the 2002/2003 nearshore study. As part of the study design, plankton and benthic invertebrates were collected from similar depths offshore of tributaries

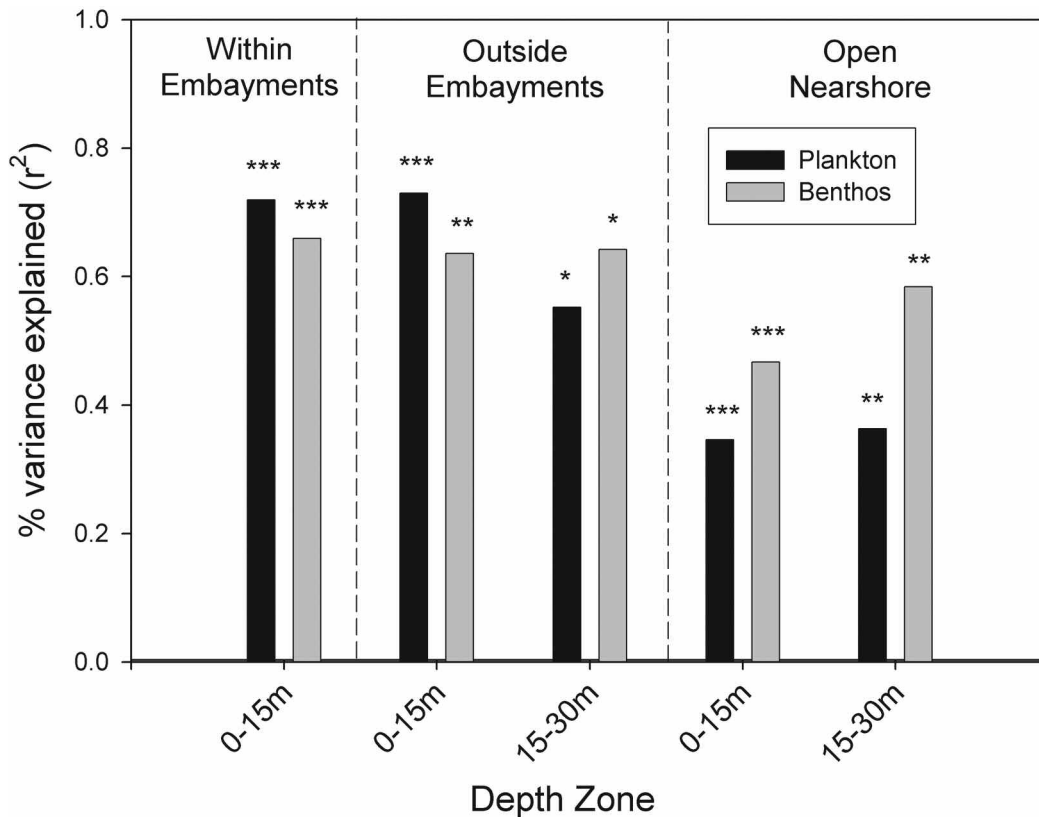


FIG. 6. Plankton and benthos $\delta^{15}\text{N}$ regression statistics (r^2 and p -value) for relationships with the AC1 land-use gradient for embayment (within and outside) and open nearshore habitats at 0–15 m and 15–30 m depths. * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.

and from an adjacent shoreline, 4–6 km from the tributary. Paired site comparison revealed no significant difference in nitrogen isotope signatures between the two zones (Fig. 7).

DISCUSSION

Our data indicate that $\delta^{15}\text{N}$ of biota in Great Lakes coastal habitats strongly reflect landscape-scale attributes, and that $\delta^{15}\text{N}$ of biota may serve as an exposure indicator of landscape-scale disturbance. However, inferring specific N sources that contribute to elevated $\delta^{15}\text{N}$ in coastal biota is difficult. Individual watersheds may contain a variety of different N sources which contribute to the N load to coastal systems. Wastewater, for example, is typically enriched in $\delta^{15}\text{N}$ (Bedard-Haughn *et al.* 2003), and should be detectable through coastal ecosystems. A number of studies have related increased wastewater or sewage loading to increased $\delta^{15}\text{N}$ of groundwater and biota in marine, estuarine,

and freshwater systems (Cole *et al.* 2004, McClelland *et al.* 1997, McClelland and Valiela 1998, Cabana and Rasmussen 1996). In contrast, inorganic fertilizer has low $\delta^{15}\text{N}$ due to the fixation of atmospheric nitrogen during manufacture (Kendall 1998). Yet, studies have reported both poor correlation (Chang *et al.* 2002) and increases in $\delta^{15}\text{N}$ in aquatic systems with increasing percent of watershed agriculture (Harrington *et al.* 1998, Mayer *et al.* 2002, Fry *et al.* 2003). These contrasting results suggest that the isotopic ratio of land-derived N entering aquatic coastal systems is influenced not only by the relative contributions from different sources, but also by biogeochemical transformations occurring in situ and in landscapes as N moves from terrestrial to aquatic systems (Bedard-Haughn *et al.* 2003, Kendall 1998). Denitrification in particular can have strong isotopic effects, potentially increasing $\delta^{15}\text{N}$ from values typically measured for inorganic fertilizer (–3 to +3‰) to values resembling those of manure or septic tank effluent (+10 to

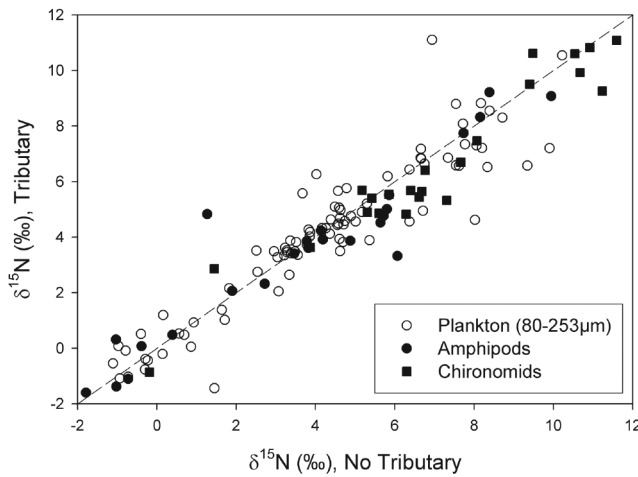


FIG. 7. Comparison of $\delta^{15}\text{N}$ values at open nearshore sites with and without tributary input for plankton, amphipods, and chironomids. Dashed line indicates 1:1 relationship.

+20‰) (Krietler and Browning 1983, Kendall 1998). Therefore, elevated $\delta^{15}\text{N}$ measured in coastal biota may reflect one or more ^{15}N -enriched sources, or result from enrichment of N via transformations occurring in the watershed. Watersheds, in turn, do not process N uniformly, and denitrification can vary considerably. Fry *et al.* (2003) suggested that watershed $\delta^{15}\text{N}$ increased with increasing anthropogenic inputs, but varied with denitrification rates. Thus, watersheds may act as integrators of multiple N sources and alter $\delta^{15}\text{N}$ through biogeochemical transformation processes, making it difficult to detect $\delta^{15}\text{N}$ patterns associated with specific N sources or land uses.

Given the difficulty in relating $\delta^{15}\text{N}$ in coastal biota to specific watershed sources, other metrics may be useful in establishing the link between landscape and coastal ecosystems. Our study used several different aggregate land-use indices developed for the Great Lakes basin (Danz *et al.* 2005, 2007) that have been shown to predict nutrient concentrations (e.g., total nitrogen) in Great Lakes coastal wetlands (Trebitz *et al.* 2007, Morrice *et al.* 2007). While not all of the individual variables in these indices directly relate to N sources (Appendix 1, Danz *et al.* 2007), the overall indices represent classes of anthropogenic activities that can influence N loading to coastal systems. Using these basin-wide indices, our results suggest that $\delta^{15}\text{N}$ measured in benthic and planktonic organisms reflects land-use patterns that incorporate a variety of

anthropogenic stressor variables which directly or indirectly relate to N loading. We found that of the four land-use indices we examined, plankton and benthic invertebrate $\delta^{15}\text{N}$ related most strongly to land-use patterns reflected in the AC1 index, which incorporates variables primarily related to agriculture, such as fertilizer and livestock waste application or export. $\delta^{15}\text{N}$ also showed strong relationships to the LC1 index which is comprised of variables describing the proportion of surface area occupied by different anthropogenic land-uses or vegetative classes, and to a lesser extent, to the PD1 index which includes human population variables such as wetland loss, amount of urbanization, and population density. Interestingly, much weaker relationships were observed with the PS1 index, which is dominated by variables related to wastewater discharges. This may be due to differences in the types (e.g., sewage treatment versus industrial wastewater discharges) and relative locations in the watershed of point-sources. Alternatively, the weak correlation with this metric may reflect a small contribution of wastewater relative to the volume of non-point source influenced input to these systems. As indicated previously, some correlations occur among the different land-use indicators (Danz *et al.* 2005), and further refinement (related to N loading) may improve correlations; however, these results suggest that $\delta^{15}\text{N}$ in Great Lakes biota reflects several broad classes of watershed-scale land use. Similarly, Vander Zanden *et al.* (2005) found that elevated $\delta^{15}\text{N}$ in primary producers was linked to both agricultural and urban landscapes surrounding lakes; however, they also reported that riparian buffer zones were better predictors of biota $\delta^{15}\text{N}$ than watershed-scale land use in those smaller systems. Thus, gradients in $\delta^{15}\text{N}$ may be present at different scales in different systems, and vary in importance depending upon the spatial scale of analysis. Analysis at finer scales of watershed characterization (e.g., Vander Zanden *et al.* 2005, Lake *et al.* 2001) and specific mechanisms within Great Lakes landscapes is a logical step for further evaluations of individual watersheds and their coastal receiving water systems.

This linkage of aquatic biota with their adjacent watershed characteristics must be accounted for to evaluate $\delta^{15}\text{N}$ relationships among biota that inhabit different geographical regions. For example, nitrogen stable isotope ratios are often used to indicate trophic position within food-webs because the $\delta^{15}\text{N}$ of the consumer is typically enriched 3–4‰ relative to its diet (Minagawa and Wada 1984,

Peterson and Fry 1987). Absolute measure of trophic position, however, requires that $\delta^{15}\text{N}$ of consumers be interpreted relative to that of an appropriate baseline (Vander Zanden and Rasmussen 1999, Post 2002). Our data indicate that food-web base $\delta^{15}\text{N}$ values throughout the Great Lakes basin exhibit considerable spatial variability, which is influenced by regional land-use patterns. For example, benthos $\delta^{15}\text{N}$ values range over 12‰ across sites in our study, but regression analyses suggest that over 50% of the variability is explained by the regional landscape.

To evaluate the importance of taxonomy in these relationships of biota and land use, we compared several species of benthic invertebrate and two size classes of plankton. Taxonomic groups at comparable trophic positions were similar in their $\delta^{15}\text{N}$ responses to land-use indicators. While the four groups of benthic invertebrates analyzed were not an exhaustive list of all the species captured in our samples, they represent the only larger-bodied invertebrates common enough to compare across sites. Oligochaetes were also quite common, but difficulty in obtaining a debris-free sample with enough mass for isotopic analysis precluded their use in this study. Benthic community analysis done on replicate ponar samples (G.S. Peterson, U.S. Environmental Protection Agency, Mid-Continent Ecology Division, 2005) indicate that amphipods collected from embayments, open nearshore sites (≤ 15 m depth), and wetlands were primarily *Gammarus* spp. and *Hyalella* spp. *Diporeia* spp. was occasionally found at embayment and open nearshore sites in Lakes Superior and Michigan. Taxonomic information on chironomids collected from embayments and open nearshore habitats was not available. The similarities in functional group and trophic position among these taxa suggest that their $\delta^{15}\text{N}$ patterns should not be significantly different within a given habitat, and our data indicate that amphipods, chironomids, and potentially other detritivores such as *Hexagenia* should provide similar $\delta^{15}\text{N}$ information.

The responses of small and large plankton were also similar for all four land-use indicators; however, an enrichment of approximately 1‰ was consistently observed in the larger size fraction. This appears to reflect the relative trophic position of the two fractions. Analysis of voucher samples shows that the large fraction was dominated by cladocerans and adult copepods, while the small fraction was generally a mixture of phytoplankton and copepod nauplii. The slight $\delta^{15}\text{N}$ enrichment (less than a

full trophic level) reflected differences in the taxonomic composition of the two size fractions. This same pattern of enrichment is also seen in the benthos, which was ^{15}N -enriched relative to plankton, reflecting the dietary importance of sedimented plankton to detritivorous benthos.

In addition to taxonomic differences we explored relationships between land-use patterns and $\delta^{15}\text{N}$ in biota from different coastal habitats. Coastal habitats may differ from each other in their connectivity to the adjacent landscape, and may vary in their vulnerability to, and expression of, watershed disturbance. For example, embayments (including harbors) may inhibit mixing of land-derived nutrient inputs with offshore waters, effectively concentrating and potentially magnifying nutrient impacts, relative to other nearshore habitats. Coastal wetlands may represent an even greater level of isolation from the open lake. We hypothesize that these three coastal habitats, from open nearshore to embayments to wetlands, represent a continuum in degree of connection to terrestrial landscape. In our study, $\delta^{15}\text{N}$ in biota from all coastal systems examined (open nearshore, embayments, and wetlands) had significant relationships to watershed scale land-use gradients. However, some had stronger correlations than others. Plankton and benthos in embayments and wetlands had stronger relationships (higher r^2 and lower S.E. values) with AC1 than did biota from open nearshore. Plankton were also more sensitive to changes in land use in embayments than in open nearshore, based on the slope of the regressions. These results suggest that coastal habitat can influence exposure to land-derived N, and consequently, the expression in aquatic biota.

While differences in the landscape linkage were observed *across* habitats, distinctions or patterns *within* habitats were inconsistent and more ambiguous, or not detectable in our study. In the open nearshore there were apparent differences in the strength of relationship to landscape between plankton and benthos; however these differences were not observed in the embayment data set (Fig. 6). Perhaps open nearshore is a unique environment in this respect. Likewise, relationships with landscape at different depth strata showed a somewhat stronger relationship at shallower depths (plankton only) outside embayments. This depth pattern was not observed in the open nearshore. Similarly, while biota inside embayments were often enriched in ^{15}N , attempts to distinguish specific characteristics which related to differences in enrichment were

largely unsuccessful. Some of the more enriched sites were identified as harbors with comparatively little connection to the open lake. These sites represent an extreme in embayment morphology. Embayment morphology is not easily quantified and the wide range of morphological characteristics encompassed by our sites made it difficult to further refine or identify relationships among embayments. Measures such as the ratio of embayment size to watershed area, and degree of enclosure, were explored to try to explain the variability among sites, but none were significant. Therefore, we were unable to define embayment characteristics based on patterns of $\delta^{15}\text{N}$. Further, it is not clear whether similarity in $\delta^{15}\text{N}$ of organisms within and immediately outside an embayment is due to the embayment being similar to the open nearshore zone, or due to influence of the embayment outside its mouth. Declining strength of landscape- $\delta^{15}\text{N}$ relationships with depth outside embayments, but not in the open nearshore habitats, suggests the latter.

Likewise, our study detected no significant differences between $\delta^{15}\text{N}$ in biota from open nearshore zones with and without tributary input. These data suggest that tributary influence on $\delta^{15}\text{N}$ of biota is not distinguishable within open nearshore habitats. Related studies at these sites using high-resolution towed sensors (CTD, fluorescence, transmissometer) suggest that tributary signals are widely dispersed and distributed along open shorelines (Yurista and Kelly 2007). These results indicate that isotope ratios of Great Lakes open nearshore biota may reflect larger regional processes with greater influence than that of individual watersheds. We were not able to make a comparable analysis among embayments, as all embayment sites had some tributary input. Efforts to quantify tributary flow relative to embayment volume did not yield significant relationships with $\delta^{15}\text{N}$ of biota.

Temporal dynamics can also influence the $\delta^{15}\text{N}$ of aquatic biota and contribute to variability in the $\delta^{15}\text{N}$ relationship with landscape. Nitrogen isotope ratios in organisms that have rapid growth and isotope turnover rates, such as plankton, may vary seasonally (Wainright and Fry 1994, O'Reilly and Hecky 2002). Seston $\delta^{15}\text{N}$ values at two offshore sites in Lake Michigan varied approximately 3‰ and 6‰ respectively, over a temporal period from Apr through Sept (McCusker *et al.* 1999). $\delta^{15}\text{N}$ was elevated in spring, lower through the summer months, and elevated again in mid to late Sept. Leggett *et al.* (2000) reported similar patterns of

^{15}N -enrichment during spring and autumn in plankton from Lake Ontario. Seasonal variability, in turn, may vary among species and trophic levels (Kidd *et al.* 1999, Pruell *et al.* 2006). Yoshioka *et al.* (1994) found considerable seasonal variation in the $\delta^{15}\text{N}$ of plankton, but little temporal variation in benthos. While the potential for a seasonal effect exists in our data, we attempted to minimize this by sampling within a relatively narrow seasonal window. Our sample collection from multiple habitat types across the U.S. Great Lakes shoreline required sampling over 3 years. Samples across all years were collected between mid-Jun and early Sept; however, the majority of sites (over 75%) were sampled within less than a 5 week period from 24 Jul to 29 Aug. Furthermore, we sampled sites within years from south to north to follow the latitudinal progression of seasonal change. It is difficult to directly address temporal variability in our data because of the complications with land use and geography; however, given our sampling design, variability in our $\delta^{15}\text{N}$ relationships due to seasonal effects is likely minimized. We also attempted to analyze the interannual variation in our plankton and benthos $\delta^{15}\text{N}$ relationships with AC1 using ANCOVA and found no significant differences among years. We caution against sampling over more broad temporal periods, and against sampling during spring or fall when $\delta^{15}\text{N}$ of Great Lakes lower food-web biota appears to be more dynamic (McCusker *et al.* 1999, Leggett *et al.* 2000). While temporal effects may contribute to some scatter in these data, the observed strength of $\delta^{15}\text{N}$ relationships to Great Lakes basin land-use indicators is perhaps even more compelling given the potential inclusion of temporal variation. Analysis at reduced geographic scales or across smaller gradients, however, may be more limited by these and other sources of error.

SUMMARY AND CONCLUSIONS

Our results show a strong correlation between $\delta^{15}\text{N}$ in biological tissue from Great Lakes coastal waters and several landscape metrics which broadly characterize the adjacent watershed. In particular, $\delta^{15}\text{N}$ was shown to strongly reflect a land-use metric related to agricultural fertilizer and livestock waste application and export (AC1), as well as a metric describing watershed land cover (LC1). We also demonstrate that $\delta^{15}\text{N}$ measured in different biological components within a given trophic position have consistent values and show similar patterns

and sensitivity relative to watershed-scale land use. Our results suggest that coastal habitats such as embayments and wetlands may express a stronger connection to land use in aquatic biota than the open nearshore environment. Therefore, these habitats may be at higher risk of pollution, and may represent effective monitoring locations for detection of incipient effects. Overall, our results suggest that $\delta^{15}\text{N}$ of coastal biota reflects anthropogenic activity in Great Lakes watersheds and functions as an exposure indicator of watershed-scale N loading. As such, $\delta^{15}\text{N}$ may serve as a useful local measure by which to gauge and confirm land use influence on receiving waters.

ACKNOWLEDGMENTS

Assistance in the field and laboratory was provided by Captain Sam Miller of the R/V *Lake Explorer*, Mario Picinich, Lee Anderson, Tim Corry, Steve Skolasinski, Charlie Butterworth, Evan Slocum, Jon Van Alstine, Matthew Able, and Anne Cotter. We thank Ted Angradi and Cathleen Wigand and the journal reviewers for their highly constructive and critical manuscript reviews. This experimental design was made possible with help from collaborators with the Great Lakes Environmental Indicator project, funded by the U.S. EPA Agreement EPA/R-8286750. The information in this document was wholly funded by U.S. EPA and has been approved for publication after review by agency's National Health and Environmental Effects Research Laboratory. Approval does not signify that the contents reflect the views of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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- Submitted: 28 September 2006*
Accepted: 14 September 2007
Editorial handling: Gerald J. Niemi