Primary Consumer Stable Nitrogen Isotopes as Indicators of Nutrient Source

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Non-point source loading of nitrogen and phosphorus is a primary cause of eutrophication of inland waters, although the diffuse and variable nature of nutrient inputs makes it difficult to trace and identify nutrient pathways. Stable nitrogen isotope values (δ^{15}N) in aquatic biota are thought to reflect anthropogenic nutrient inputs, and they may be a promising tool for tracing nutrient sources in watersheds. We measured δ^{15}N of aquatic consumers from a suite of 27 Danish lakes spanning a range of trophic states (oligotrophic to eutrophic) and land uses (forest, urban, agriculture). Primary consumer δ^{15}N values (PC$_{\delta^{15}N}$) varied more than 14‰ among lakes. Models of PC$_{\delta^{15}N}$ were developed from limnological, nitrogen loading, and nitrogen source variables using an information-theoretic approach (Akaike's Information Criteria, AIC). Models based on land use/ land cover performed best, indicating that elevated δ^{15}N is not only associated with high nitrogen loading, but is also reflective of nitrogen source. Urban and agricultural land use in the watershed, and particularly within the riparian buffer areas, was quantitatively linked to elevated biotic δ^{15}N.

Introduction

Urban and agricultural landscapes are dominant sources of phosphorus and nitrogen loading to aquatic ecosystems. Excess nutrient loading has led to widespread eutrophication of aquatic systems, with consequent algal blooms, fish kills, habitat and biodiversity loss, and impairment of aquatic ecosystems (*1*, *2*). Traditionally, phosphorus has been considered the major threat to lakes, but recent studies indicate that also nitrogen, most often derived from non-point sources, plays a role in shallow lakes (*3*). While progress has been made controlling nutrient point sources to aquatic systems in the developed world, non-point source loading persists and is a growing threat to aquatic systems (*1*, *2*, *4*, *5*). Curbing non-point source pollution has proven to be a

difficult challenge; it occurs at many sites across vast landscapes, and inputs are highly variable in space and time (*5−7*). Identifying nutrient sources and pathways to aquatic ecosystems is critical for achieving nutrient reduction, and there is a need for reliable indicators of anthropogenic nutrient source.

Current indicators of lake eutrophication focus on the impacts of nutrient loading such as nuisance algal blooms, loss of submerged macrophytes, hypolimnetic oxygen depletion, and reduced water clarity (*3*, *4*, *8*). This approach of a posteriori detection is far from ideal, as solving the problem in already-impacted ecosystems requires costly remediation (*3*, *4*). A preferable approach would be to identify nutrient loading early, preferably prior to ecosystem-level impacts. Control of non-point source nutrient loading requires an understanding of nutrient sources and the flow pathways that link aquatic ecosystems and their catchments. Measuring nutrient levels in runoff waters and streams is one approach, although nutrient inputs are highly variable in space and time, thus requiring repeated measuring and intensive monitoring (*5*). Nutrient loading can also be modeled for watersheds, although processes governing nutrient movement are poorly known; such models require validation and are generally site-specific (*9*).

As an alternative, recent studies indicate that stable nitrogen isotope ratios (^{15}N/^{14}N) can be a useful tool for tracing nitrogen sources in watersheds (*10−14*). Dissolved inorganic nitrogen (DIN) sources to aquatic ecosystems tend to have distinct isotopic values. Human wastewater and livestock waste nitrogen are enriched in δ^{15}N (10−20‰), while synthetic fertilizers and atmospheric nitrogen typically have low δ^{15}N values (atmospheric, 2−8‰; inorganic fertilizer, −3 to 3‰) (*15*, *16*). In addition, biogeochemical transformations of nitrogen (denitrification, ammonia volatilization) can result in substantial isotopic fractionation (*17*). Ultimately, DIN (nitrate, ammonia) in aquatic ecosystems is taken up by aquatic plants, whose tissues are isotopically reflective of their DIN source. Isotopic signatures of aquatic plants are subsequently passed on to herbivores and other aquatic consumers, which serve as integrators of temporal isotopic variation at a lower level in the system (DIN and aquatic plants). This provides the foundation for how nutrient loading and source pathways to aquatic ecosystems may be inferred from biota δ^{15}N, which is easy to measure (*10−14*).

The goal of this paper is to assess the utility of macroinvertebrate primary consumer δ^{15}N as an indicator of lake trophic status, nutrient loading, and watershed land use for a series of intensively studied Danish lakes. We develop models based on Akaike's information criteria (AIC) that compare physical/watershed, limnological (water quality), nutrient loading, and land use/land cover information as potential drivers of primary consumer δ^{15}N. In addition, we use land use data and macroinvertebrate isotopic signatures to empirically estimate isotopic value associated with specific watershed and riparian land uses.

Methods

Stable Isotope Analysis. In October and November of 1998, we collected benthic macroinvertebrates from 27 lakes in central and northern Jutland, Denmark (Figure 1).

Study lakes were selected to span a wide range of land uses, loading sources, and limnological attributes (mean annual total phosphorus concentrations ranged from 11 to 270 mg/m³). We used sweep nets to collect macroinvertebrates from littoral (shallow water) habitats (depth < 1 m) that included reed beds, macrophytes, cobble, sand, and

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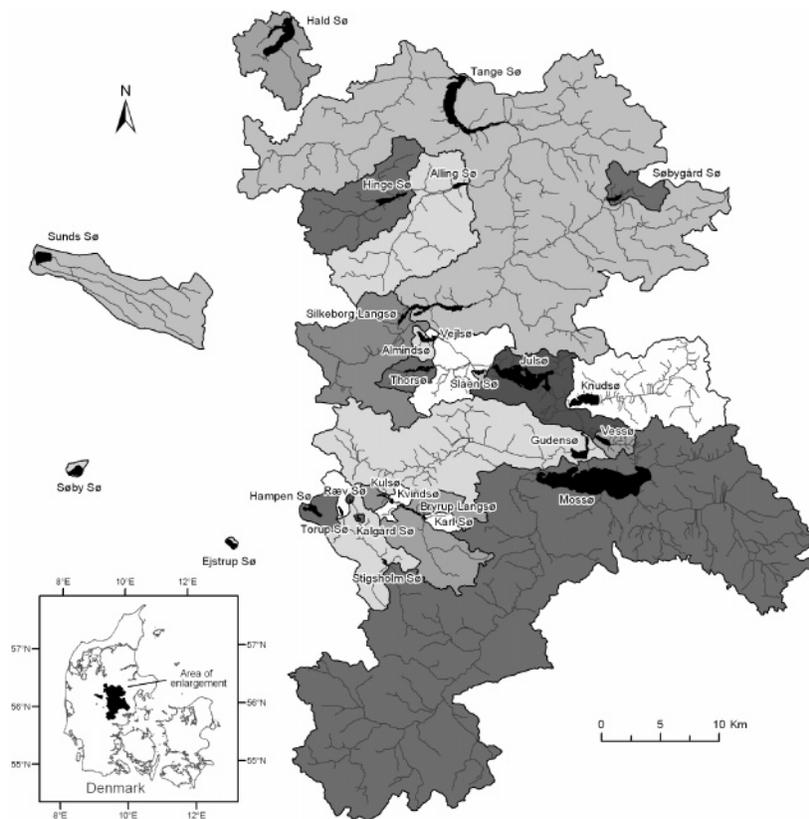
FIGURE 1. Map showing the location of the 27 Danish study lakes and their contributing watersheds.

open sediments. Invertebrates were sorted and separated to the family or genus level within 24 h and allowed to purge their guts for another 24 h. The foot muscle of unionid mussels was sampled, and gastropods were removed from their shells. Whole body was used for all other macroinvertebrate taxa. Individuals of the same taxa from a lake were pooled for isotopic analysis to achieve an adequate sample weight, with the exception of unionid mussels and crayfish, for which individuals from a lake were analyzed separately and individual values were averaged. Taxa were classified according to general dietary preference (*Herbivore-detritivores*: Bivalvia, Gastropoda, Amphipoda, Isopoda, Ephemeroptera, Trichoptera, Chironomidae (minus Tanypodinae), Corixidae, Tipulidae, Oligochaeta. *Predator-omnivores*: Decapoda, Dytiscidae, Odonata, Tanypodinae, Hirudinae, Megaloptera) using published dietary descriptions, particularly Merritt and Cummins (*18*) and Thorp and Covich (*19*).

Samples were dried at 60 °C for 24 h, ground with mortar and pestle, and approximately 0.5 mg was placed into a 5 × 8 mm tin capsule. N stable isotope ratios were analyzed on a FinneganMAT Delta Plus mass spectrophotometer at G.G. Hatch Isotope Laboratories, Ottawa, Ontario, Canada. Stable isotope ratios are expressed in delta (δ) notation, defined as the parts per thousand (‰ or "per mil") deviation from a standard material (atmospheric nitrogen, set at 0‰; $\delta^{15}N = ([R_{sample}/R_{standard}] - 1) \times 1000$, where $R = {}^{15}N/{}^{14}N$. 20% of the samples were analyzed in duplicate; the standard error of the mean for replicates was <0.15‰.

For each lake, we calculated the mean $\delta^{15}N$ of macroinvertebrate primary consumer taxa, and for all macroinvertebrate taxa (primary consumers, omnivores, and predators). Mean primary consumer $\delta^{15}N$ was closely correlated with the mean $\delta^{15}N$ for all macroinvertebrate taxa (all macroinvertebrates = 1.03*primary consumer − 0.132; $r^2 = 0.99$), indicating strong lake-specific differences in $\delta^{15}N$, independent of which taxa are considered. Hereafter, we

used primary consumer $\delta^{15}N$ ($PC_{\delta^{15}N}$) as the dependent variable in our analysis. Because $\delta^{15}N$ increases with trophic level (*20*), use of primary consumers reduces variance associated with animal trophic position and is consistent with the approach used in previous studies (*10, 21*).

Lake Data. Limnological sampling was conducted as part of the lake monitoring program coordinated by the National Environmental Research Institute (NERI). Variables used in the analysis are presented in Table 1. Chlorophyll-*a*, Secchi depth transparency, and total phosphorus and total nitrogen represent averages for April to October were measured using standard limnological methods reported elsewhere (*22, 23*). Total nitrogen loading was estimated for each lake by measuring stream input nitrogen concentrations throughout the year and calculating total loads (t y^{-1}) from flow data (*23, 24*). Land cover data (1:25 000, National Environmental Research Institute, Silkeborg, Denmark) were analyzed in a GIS to estimate whole watershed land use/land cover and land use within a 100 m buffer of the stream network contributing to each study lake. Farm animal numbers (total animal units/watershed) and densities (animals/km^2 in watershed) were derived for each lake watershed from point coverage of farms that indicated the number of animals on each farm (Central Livestock Register of the Danish Ministry of Food, Agriculture and Fisheries).

Statistics and Data Transformations. To meet assumptions of normality and constant variance for statistical analysis, some explanatory variables were transformed. We tested several different data transformations [log(x), x^2, sqrt-(x), exp(x), exp($-x$), $1/x$, $1/-x$, arcsin(sqrt(x)] and examined for normality of residuals in plots of the transformed variable (explanatory variables) versus primary consumer $\delta^{15}N$. For most variables, log-transformation was the best, although several variables required no transformation (Table 1).

Our goal in model building was to compare best-performing models derived from different classes of variables, each class representing a different set of potential drivers of

TABLE 1. Variable Name, Variable Class, Variable Description (Including Units), and Data Transformation for the Predictor Variables Used in This Study

variable	category	description	transformation
catarea	physical/watershed	catchment area (km^2)	$\log(x)$
lakearea	physical/watershed	lake area (ha)	$\log(x)$
catlake	physical/watershed	catchment area/lake area	$\log(x)$
rettime	physical/watershed	water retention time in lake (days)	$\log(x)$
TN	limnological	mean total nitrogen (mg/L) − April−Oct.	$\log(x)$
chla	limnological	mean summer season chla (μg/L)	$\log(x)$
sec	limnological	mean summer season secchi transparency (m)	
TP	limnological	mean total phosphorus (mg/L) − April−Oct.	$\log(x)$
Nload	loading	total nitrogen loading to lake (tonnes/year)	$\log(x)$
Nloadv	loading	total nitrogen loading/lake volume (10^6 m^3)	$\log(x)$
aniunit	source	no. of animal livestock units in watershed	$\log(x)$
aniden	source	density of animal livestock units in watershed (animals/km^2)	$\log(x)$
lcnat	source	watershed landcover − % natural vegetation	
bufnat	source	% natural vegetation within riparian buffer (100 m of stream network)	

$\delta^{15}N_{PC}$. Our overall approach for model selection was based on the information-theoretic approach described by Burnham and Anderson (25). Candidate models were constructed and ranked using Akaike Information Criteria corrected for small sample size (AIC_c). AIC_c quantifies predictive power of candidate models (model performance) based on evaluation of the Kullback−Leibler distance between fitted models and the underlying data-generating mechanism. Candidate models were defined to represent the following classes of variables, reflective of potential drivers of nitrogen isotopic signatures: (1) physical and watershed variables, (2) limnological (lake water quality) variables, (3) nutrient loading variables, and (4) watershed and riparian land use variables. Raw AIC_c values were estimated for each model: lower values correspond to better model performance. AIC_c values for individual models were expressed relative to the lowest AIC_c value generated from the four variable classes (Δ_i; minimum AIC_c value = 0). Within each of the four variable classes, all possible candidate models were examined, and Δ_i values were calculated and ranked in order of ascending AIC_c. The predictor variables included in the best-performing model from each model class (finalist variables) were included for consideration in a global model of $\delta^{15}N_{PC}$. In addition, stepwise multiple regression was used to produce a separate model from among the finalist predictor variables.

Because riparian and watershed land use/land cover were strong predictors of $\delta^{15}N_{PC}$, we used a model fitting approach to estimate $\delta^{15}N$ values associated with each of the four general land use/land cover types (urban, agricultural, natural, aquatic). Primary consumer $\delta^{15}N$ from a system can be modeled as the weighted average of "source" isotopic signatures associated with the four land use categories:

$$\delta^{15}N_{PC} = (a*\%\text{urb}) + (b*\%\text{ag}) + (c*\%\text{nat}) + (d*\%\text{aq}) \quad (1)$$

where $a = \delta^{15}N_{urb}$, $b = \delta^{15}N_{ag}$, $c = \delta^{15}N_{nat}$, $d = \delta^{15}N_{aq}$. Whole-watershed and riparian buffer land use data are available for each of our 27 lakes, thus allowing us to estimate the $\delta^{15}N$ value associated with each of the land use categories using eq 1. Because these land use variables sum to 100, this produces multicollinearity. Data were reduced to three variables from four (%urb, %ag, %nat, %aq, $\Rightarrow$ %ag, %nat, %aq), and eq 1 was rearranged to:

$$\delta^{15}N_{PC} = 100a + (b - a)*\%\text{ag} + (c - a)*\%\text{nat} + (d - a)*\%\text{aq} \quad (2)$$

Using the regression coefficients from eq 2 (three variables),

we simply estimated "a", "b", "c", and "d", which correspond with isotopic signatures associated with these different land uses.

Results

General Patterns in Macroinvertebrate $\delta^{15}N$. Analysis of Variance (ANOVA) indicated that 78% of the total variation in individual primary consumer $\delta^{15}N$ values was explained by a "lake" variable ($p < 0.0001$). Additional variation was explained by "taxa" ($p < 0.0001$); 93% of the total variation in macroinvertebrate $\delta^{15}N$ was explained by lake and taxa. There was no significant interaction between taxa and lake. Mean primary consumer $\delta^{15}N$ values varied widely (spanning from 1.0‰ to 15.2‰) among lakes (Table 2).

Modeling $\delta^{15}N_{PC}$. Models of $\delta^{15}N_{PC}$ were developed separately from physical/hydrological, limnological, nitrogen loading, and land use/land cover variables. Overall, variables indicative of eutrophication and human land use and activities were positively correlated with $\delta^{15}N_{PC}$. Physical/hydrological variables had relatively low explanatory power (Table 3, model a, AIC = 136.2). Limnological (water quality) variables performed better, with the best model including total nitrogen and total phosphorus concentrations (Table 3, model b, AIC = 131.8). There was a slight improvement in model performance by considering nitrogen loading variables (Table 3, model c, 130.5). The best-performing model was derived from land use variables (Table 3, model d, AIC = 119.6) and included the following variables: number of animal units, animal density, and natural buffer (percentage of the 100 m riparian buffer within the watershed stream network classified as "natural" land use). The variables from best-performing models a−c were added to the variables included in model d: a very small number of candidate models slightly outperformed the best model based on land use variables from above. The best-performing model added two additional variables to those in model d: lake area and N loading/lake volume (Table 3, model d, AIC = 118.7).

Stepwise multiple regression (SAS, $p < 0.15$ significance cutoff) using all finalist variables from models a−d produced a model that included just two significant predictor variables: "Nload" and "bufnat". The AIC score for this model was lower than that for any other model examined (Table 3, model e, AIC = 115.1), which is consistent with our finding that land use and nutrient loading variables are the best predictors of biota $\delta^{15}N$.

Estimating $\delta^{15}N_{PC}$ Associations with Land Use Categories. We estimated $\delta^{15}N$ signatures associated with each of four major land use/land cover categories (urban, farm,

TABLE 2. Primary Consumer δ^{15}N and Predictor Variables (Those Variables Included in Best Model for Each Variable Class) for the 27 Danish Lakes

| lake	δ^{15}N	lakearea	rettime	TP	TN	Nload	Nloadv	aniunit	aniden	bufnat
Alling Sø	11.0	40	5.3	0.115	3.04	308	496.8	10 414	77	3.9
Almind Sø	4.9	53	876	0.014	0.32	3.8	0.7	0	0	91.6
Bryrup Langsø E.	11.2	38	94	0.06	4.56	69.9	40.6	5104	106	8.3
Ejstrup Sø	9.6	42	91	0.103	2.13	8.9	15.1	0	0	65.8
Gudensø	12.6	133	4.3	0.102	1.20	944	290.5	49 643	62	24.9
Hald Sø	10.3	340	545	0.051	0.97	160	3.6	1514	44	20.8
Hampen Sø	5.2	76	550	0.023	0.64	1.6	0.5	96	12	94.1
Hinge Sø	11.0	91	18	0.128	3.83	142	125.7	5211	97	1.1
Julsø	12.8	565	44	0.099	2.02	1052	24.0	57 402	62	27.1
Kalgård Sø	5.1	10.5	730	0.019	0.46	0.71	2.6	0	0	93.6
Karlsø	7.2	7.6	150	0.119	1.79	0.55	3.2	830	223	14.2
Knudsø	12.5	191	365	0.021	1.96	68.6	2.7	5398	69	24.5
Kul Sø	15.0	16	11	0.093	2.23	83.4	270.8	5620	99	11.2
Kvind Sø	14.8	15	10	0.094	2.9	79.3	289.4	5581	105	8.9
Mossø	15.2	1690	262	0.084	1.51	324	2.1	41 713	68	11.6
Rævsø	4.8	4.6	730	0.02	0.60	0.6	3.7	0	0	82.0
Silkeborg Langsø W.	8.1	50	9.3	0.091	1.21	50.6	56.2	1638	24	61.1
Slåensø	1.0	20	300	0.014	0.26	2.2	1.5	0	0	100.0
Søby Sø	7.6	73	137	0.02	0.35	4.03	2.0	546	45	50.6
Søbygård Sø	14.5	40	26	0.273	1.87	34.4	312.7	0	0	19.7
Stigsholm Sø	12.1	21	6	0.08	2.06	75.5	444.1	1733	46	40.3
Sunds Sø	11.4	127	53	0.034	2.14	62.9	28.6	3395	62	1.2
Tange Sø	12.6	420	11	0.093	2.3	2244	134.4	106 912	58	19.7
Thorsø	7.1	69	156	0.034	0.65	9	3.1	12	1	89.3
Torup sø	6.4	18	375	0.043	2.37	2.7	3.4	20	4	41.2
Vejlsø	6.6	16.5	100	0.06	0.79	0.3	0.5	0	0	61.8
Vessø	11.2	53	730	0.039	1.37	2.3	1.0	235	36	26.7

TABLE 3. Best-Performing Models from Each Variable Group for Predicting δ^{15}N$_{PC}$[a]

model and variable group	AIC$_C$ value	Δ_i	r^2
(a) physical/watershed (**lakearea**, **rettime**, catarea, catlake) model: δ^{15}N = 10.236 + 2.691*log(lakearea) − 2.712*log(rettime)	136.2	17.5	0.52
(b) limnological (**TN**, **TP**, sec, chla) model: δ^{15}N = 13.587 + 5.488*log(TN) + 3.586*log(TP)	131.8	13.1	0.59
(c) nutrient loading (**Nload**, **Nloadv**) model: δ^{15}N = 5.986 + 1.987*log(Nload) − 0.925*log(Nloadv)	130.5	11.8	0.61
(d) land use (**aniunit**, **aniden**, lcnat, **bufnat**) model: δ^{15}N = 13.797 + 1.585*log(aniunit) − 3.340*log(aniden) − 0.098*bufnat	119.6	0.9	0.76
(e) global model 1 (**aniunit**, **aniden**, **bufnat**, Nload, **Nloadv**, TN, TP, **lakearea**, rettime) model: δ^{15}N = 8.51 − 0.060*log(aniunit) − 0.484*log(aniden) −0.071*(bufnat) + 1.285*log(Nloadv) + 1.935*log(lakearea)	118.7	0[b]	0.80
(f) global model 2 (derived from stepwise multiple regression) model: δ^{15}N = 10.530 + 1.330*log(Nload) − 0.065*(bufnat)	115.1		0.77

[a] Candidate variables for each variable group are shown in parentheses; variables included in the best model for each group are indicated in bold and were considered in the global model (model e, variables from best previous model, model d, were retained in the global model). Δ_i is model performance relative to global model 1. [b] Best-performing model from among the variable groups: Δ_i value is set to 0 to serve as a benchmark for assessing other models.

natural, and aquatic) from GIS data for both whole watershed and riparian buffer land use (eq 2). Whole-watershed land use data gave the following δ^{15}N estimates (±1 SE): urban = 21‰ (±8.58), agricultural = 13‰ (±1.56), natural = 3‰ (±1.79), aquatic = 9‰ (±3.4; model r^2 = 0.47), indicating an association between human activities and elevated biota δ^{15}N. Riparian buffer (100 m) land use data generally gave similar δ^{15}N estimates: urban = 31‰ (±12.66), agricultural = 13‰ (±0.94), natural = 4‰ (±1.06), aquatic = 14‰ (±6.65; model r^2 = 0.71). Comparison of r^2 values indicates that riparian land use performs much better than watershed-level land use. Also note that urban land use within the riparian area shows a stronger δ^{15}N response relative to the watershed level land use. Predicted and observed δ^{15}N$_{PC}$ values from these two models were plotted (Figure 2).
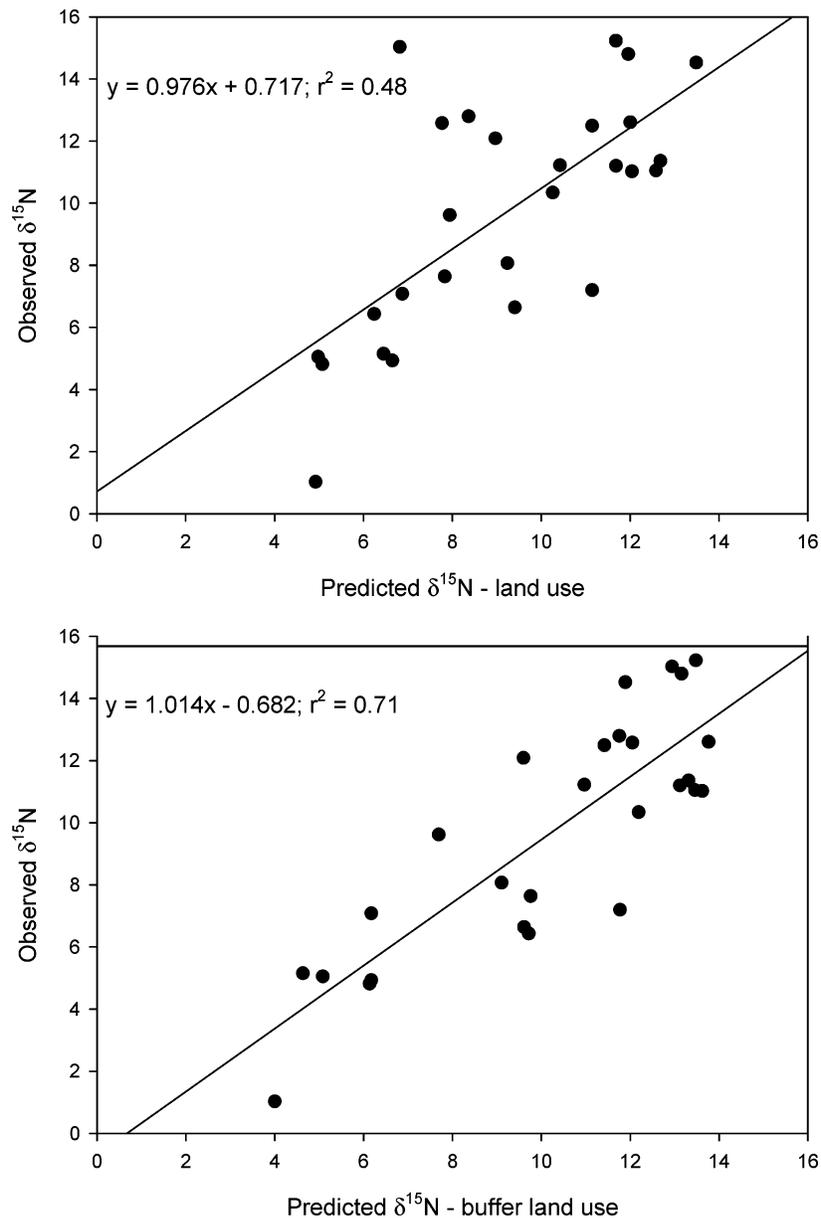
FIGURE 2. Predicted vesus observed $\delta^{15}N_{PC}$ from eq 2 using (a) whole-watershed land use data, and (b) land use within the 100 m buffer of the watershed stream network.

Discussion

A major finding of this study is that biota isotopic signatures in lakes appear to reflect riparian zone and watershed land use, both of which are indicative of nutrient sources to lakes. Limnological attributes and total nitrogen loading were also correlated with $\delta^{15}N_{PC}$, but had lower overall explanatory power. This finding suggests that $\delta^{15}N$ signatures associated with particular nitrogen sources such as barnyards outweigh effects of gross nitrogen loading to lakes, although both total loading and nutrient source are positively correlated with $\delta^{15}N$. Further, the importance of the number and density of farm animals in the watershed points to manure as an important driver of biotic $\delta^{15}N$ enrichment.

To more specifically examine whether land use adds significant explanatory power to a model predicting $\delta^{15}N$ based on total nitrogen loads, we used stepwise regression to test whether residuals of the $\delta^{15}N$−total N loading (t/y) relationship were explained by land use/land cover variables. As expected, nitrogen loading by itself was a significant ($p < 0.001$) predictor of primary consumer $\delta^{15}N$ values ($r^2 = 0.43$). Two variables, agricultural and urban land use within

a 100 m riparian zone, both explained a significant amount of variation of the residuals of the loading model, and a regression model with these three variables provided for high explanatory power ($r^2 = 0.73$), although nitrogen loading became only marginally significant ($p = 0.07$) upon inclusion of agricultural and urban riparian land use variables. Thus, while biota $\delta^{15}N$ does reflect lake trophic state and total nutrient loading, inclusion of land use data explains residual variance from models that consider nutrient loading alone.

Because land use emerged as an important driver of biotic $\delta^{15}N$ from our AIC modeling approach, we used watershed and riparian buffer land use data to estimate a $\delta^{15}N$ value corresponding with each major land use type. Our analysis does not imply that these land uses/nutrient sources have a single or fixed isotopic signature or that they will always have these signatures. Rather, what we present is simply an estimation approach that provides best fit estimates of $\delta^{15}N$ values associated with different watershed and riparian buffer land uses. The strong $\delta^{15}N$ signal for both urban (21‰− 31‰) and agricultural (13‰) land use is consistent with the results of our AIC models. It is notable that land use in the

riparian zone of the contributing stream network was a much better predictor of biota δ^{15}N than watershed-scale land use. This finding is corroborated by previous research (26) showing that aquatic systems reflect and respond to land use at a local scale (i.e., within the riparian buffer zone). It also highlights the important role of stream riparian corridors as a critical interface between land and water, whose influence on nutrient dynamics is disproportionate relative to their overall area.

Previous Studies of Biotic δ^{15}N. Previous studies have linked anthropogenic nutrient loading to elevated biotic δ^{15}N. For example, Cabana and Rasmussen (10) found that primary consumer δ^{15}N in lakes increased with human population density in the watershed, although linkages between biota δ^{15}N and specific nutrient sources or land uses were not assessed. An important finding in the present study is that both agricultural and urban nitrogen inputs were linked to elevated δ^{15}N in the biota. Several recent studies have correlated elevated δ^{15}N to urban and wastewater nutrient inputs (13, 14, 27). McKinney et al. (27) reported mussel δ^{15}N to be positively correlated with residential development in coastal marshes of eastern North America, and negatively correlated with agricultural and recreation land use. Similarly, groundwater nitrate δ^{15}N was elevated in the presence of wastewater nitrogen, but did not reflect total nitrogen loading, atmospheric sources, or fertilizer inputs (11, 12). Steffy and Kilham (28) also found that groundwater DIN (dissolved inorganic nitrogen) and biotic δ^{15}N were elevated in suburban watersheds with septic systems relative to comparable watershed on a sewer system, again demonstrating the utility of this approach for detecting wastewater inputs to aquatic ecosystems.

In contrast, other recent studies report that elevated biotic δ^{15}N correlates with agricultural land use. Harrington et al. (29) reported positive relationships between percent agricultural land use, stream nitrate levels, and stream nitrate δ^{15}N. The elevated δ^{15}N of nitrate was passed on to the biota (fish), such that biotic δ^{15}N reflected agricultural land use, although it should be noted that urban nitrogen sources in this watershed were presumably minor. In western Canada, Hebert and Wassenaar (30) found that duckling feather δ^{15}N was positively correlated with the proportion of land use in agriculture. Similarly, this study was conducted in a region with a minimal degree of urbanization. In these studies, the specific origin of agricultural nitrogen inputs to these aquatic systems was not known. This is important because agricultural nitrogen sources may vary widely in isotopic signatures and will be subject to fractionation associated with biogeochemical transformations. Inorganic fertilizer nitrogen has low δ^{15}N values (15, 16), while nitrogen derived from barnyard manure tends to be elevated in δ^{15}N as a result of volatilization of ammonia (15, 16). Inorganic fertilizer inputs with initially low δ^{15}N can yield elevated δ^{15}N values if denitrification rates are high (31). Because denitrification rates tend to increase as a function of nitrogen concentration, high loadings of inorganic nitrogen fertilizer could still result in elevated δ^{15}N in DIN and biota.

The above discussion highlights the fact that elevated δ^{15}N in DIN and the biota may reflect a nitrogen source that is elevated in δ^{15}N or, alternatively, nitrogen transformations (denitrification, ammonia volatilization) that result in substantial δ^{15}N enrichment. Lake landscape position could also play a role, with the effect of nitrogen source being most evident high in the watershed, and the role of biogeochemical transformations increasing downstream through the drainage network (32). In the present study, multiple processes are likely responsible for the among-lake variation in δ^{15}N, although the relatively poor performance of limnological variables indicates that in-lake nitrogen processing appears to be less important than nitrogen loading, nutrient source, and perhaps broader-scale catchment processes.

Application to Monitoring and Management. Stable nitrogen isotope values of both biota and DIN sources have been used to assess nutrient loading (29, 33, 34). An ideal indicator of nutrient loading and source pathways should be temporally stable (10). δ^{15}N values of DIN often exhibit a high degree of intra-annual variability. For example, using data from McClelland and Valiela (12), the coefficient of variation (CV) for groundwater DIN δ^{15}N was 140%. Using data from Lindau et al. (35), the CV of DIN δ^{15}N surface water runoff was 53%. In contrast, mussels from different sites within a lake differed little in $\delta^{15}N_{PC}$ (among site CV = 2%) (27), and the δ^{15}N of relatively long-lived consumers such as benthic macroinvertebrates showed relatively little temporal variation (10). Clearly, long-lived components of the biota such as primary consumers serve as integrators of underlying isotopic variability in DIN and are thus useful as a monitoring tool.

Anthropogenic nutrient loading is an important driver of eutrophication in aquatic systems. Traditionally, studies of lake eutrophication have focused on the consequences of gross increases in nutrient loading on biotic communities and have put less emphasis on tracking nutrient source pathways (8). Reduction of anthropogenic nutrient loading through best management practices in agriculture is an important management goal for improving water quality and reflects a shift toward watershed-based approaches for addressing water quality issues (5). Reducing nutrient loads requires an understanding of nutrient sources, and how they are transported from catchments to aquatic ecosystems. Biota δ^{15}N appears to be reflective anthropogenic nutrient loading to aquatic systems under a wide range of conditions (10, 36), suggesting value as a monitoring tool for anthropogenic nutrient loading (36). Finally, the fact that biotic δ^{15}N is more reflective of riparian zone land use than whole-watershed land use indicates that nutrient management/reduction efforts are likely to show greatest benefits to aquatic systems when concentrated in riparian zones.

Literature Cited

(1) Smith, V. H. Cultural eutrophication of inland, estuarine, and coastal waters. In *Successes, limitations, and frontiers in ecosystem science*; Pace, M. L., Groffman, P. M., Eds.; Springer-Verlag: New York, 1998.

(2) Carpenter, S. R.; Caraco, N. F.; Correll, D. L.; Howarth, R. W.; Sharpley, A. N.; Smith, V. H. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.* **1998**, *8*, 559−568.

(3) Jeppesen, E.; Sammalkorpi, I. Lakes. In *Handbook of Ecological Restoration, Volume 2: Restoration practice*; Perrow, M., Davy, T., Eds.; Cambridge University Press: Cambridge, 2002; pp 297−324.

(4) Cooke, G. D.; Welch, E. B.; Peterson, S. A.; Newroth, P. R. *Restoration and management of lakes and reservoirs*; Lewis Publishers: Boca Raton, FL, 1993.

(5) Kronvang, B.; Jeppesen, E.; Conley, D. J.; Søndergaard, M.; Larsen, S. E.; Ovesen, N. B. An analysis of pressures, state and ecological impacts of nutrients in Danish streams, lakes and coastal waters and ecosystem responses to nutrient pollution reductions. *J. Hydrol.* **2005**, *304*, 274−288.

(6) Carpenter, S. R.; Lathrop, R. C. Lake restoration: capabilities and needs. *Hydrobiologia* **1999**, *395/396*, 19−28.

(7) Portielje, R.; Van der Moelen, D. T. Relationships between eutrophication variables: from nutrient loading to transparency. *Hydrobiologia* **1999**, *408/409*, 375−387.

(8) Johnes, P. J. Understanding lake and catchment history as a tool for integrated lake management. *Hydrobiologia* **1999**, *395/396*, 41−60.

(9) Soranno, P. A.; Hubler, S. L.; Carpenter, S. R.; Lathrop, R. C. Phosphorus loads to surface waters: A simple model to account for spatial pattern of land use. *Ecol. Appl.* **1996**, *6*, 865−878.

(10) Cabana, G.; Rasmussen, J. B. Comparison of aquatic food chains using nitrogen isotopes. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10844−10847.

(11) McClelland, J. W.; Valiela, I.; Michener, R. H. Nitrogen-stable isotope signatures in estuarine food webs: a record of increasing urbanization in coastal watersheds. *Limnol. Oceanogr.* **1997**, *42*, 930−937.

(12) McClelland, J. W.; Valiela, I. Linking nitrogen in estuarine producers to land-derived sources. *Limnol. Oceanogr.* **1998**, *43*, 577−585.

(13) Lake, J. L.; McKinney, R. A.; Osterman, F. A.; Pruell, R. J.; Kiddon, J.; Ryba, S. A.; Libby, A. D. Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems. *Can. J. Fish. Aquat. Sci.* **2001**, *58*, 870−878.

(14) McKinney, R. A.; Lake, J. L.; Charpentier, M. A.; Ryba, S. Using mussel isotope ratios to assess anthropogenic nitrogen inputs to freshwater ecosystems. *Environ. Monit. Assess.* **2002**, *74*, 167−192.

(15) Heaton, T. H. E. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. *Chem. Geol.* **1986**, *59*, 87−102.

(16) Valiela, I.; Geist, M.; McClelland, J.; Tomasky, G. Nitrogen loading from watersheds to estuaries: verification of the Waquoit Bay Nitrogen Loading Model. *Biogeochemistry* **2000**, *49*, 277−293.

(17) Owens, N. J. P. Natural variation in ^{15}N in the marine environment. *Adv. Mar. Biol.* **1987**, *24*, 390−451.

(18) Merritt, R. W.; Cummins, K. W. *An introduction to the aquatic insects of North America*; Kendall/Hunt Publishing: Dubuque, IA, 1978.

(19) Thorp, J. H.; Covich, A. P. *Ecology and classification of North American freshwater invertebrates*; Academic Press: San Diego, CA, 1991.

(20) Vander Zanden, M. J.; Rasmussen, J. B. Variation in δ^{15}N and δ^{13}C trophic fractionation: Implications for aquatic food web studies. *Limnol. Oceanogr.* **2001**, *46*, 2061−2066.

(21) Vander Zanden, M. J.; Rasmussen, J. B. Primary consumer δ^{15}N and δ^{13}C and the trophic position of aquatic consumers. *Ecology* **1999**, *80*, 1395−1404.

(22) Jeppesen, E.; Søndergaard, M.; Jensen, J. P.; Mortensen, E.; Hansen, A.-M.; Jørgensen, T. Cascading trophic interactions from fish to bacteria and nutrients after reduced sewage loading: an 18-year study of a shallow eutrophic lake. *Ecosystems* **1998**, *1*, 250−267.

(23) Søndergaard, M.; Jensen, J. P.; Jeppesen, E.; Møller, P. H. Seasonal dynamics in the concentrations and retention of phosphorus in shallow Danish lakes after reduced loading. *Aquat. Ecosyst. Health Manage.* **2002**, *5*, 19−29.

(24) Kronvang, B.; Grant, R.; Larsen, S. E.; Svendsen, L. M.; Kristensen, P. Non-point-source nutrient losses to the aquatic environment in Denmark: impact of agriculture. *Mar. Freshwater Res.* **1995**, *46*, 167−177.

(25) Burnham, K. P.; Anderson, D. R. *Model selection and inference: a practical information-theoretic approach*; Springer-Verlag: New York, 1998.

(26) Gergel, S. E.; Turner, M. G.; Kratz, T. K. Dissolved organic carbon as an indicator of the scale of watershed influence on lakes and rivers. *Ecol. Appl.* **1999**, *9*, 1377−1390.

(27) McKinney, R. A.; Lake, J. L.; Allen, M.; Ryba, S. Spatial variability in mussels used to assess base level nitrogen isotope ratio in freshwater ecosystems. *Hydrobiologia* **1999**, 17−24.

(28) Steffy, L. Y.; Kilham, S. S. Elevated δ^{15}N in stream biota in areas with septic tank systems in an urban watershed. *Ecol. Appl.* **2004**, *14*, 637−641.

(29) Harrington, R. R.; Kennedy, B. P.; Chamberlain, C. P.; Blum, J. D.; Folt, C. L. ^{15}N enrichment in agricultural catchments: Field patterns and applications to tracking Atlantic salmon (*Salmo salar*). *Chem. Geol.* **1998**, *147*, 281−294.

(30) Hebert, C. G.; Wassenaar, L. I. Stable nitrogen isotopes in waterfowl feathers reflect agricultural land use in western Canada. *Environ. Sci. Technol.* **2001**, *35*, 3482−3487.

(31) Kellman, L.; Hillaire-Marcel, C. Nitrate cycling in streams: Using natural abundances of NO_3-δ^{15}N to measure in-situ denitrification. *Biogeochemistry* **1998**, *43*, 273−292.

(32) Toda, H.; Uemura, Y.; Okino, T.; Kawanishi, T.; Kawashima, H. Use of nitrogen stable isotope ratio of periphyton for monitoring nitrogen sources in a river system. *Water Sci. Technol.* **2002**, *46*, 431−435.

(33) Komor, S. C.; Anderson, H. W. Nitrogen isotopes as indicators of nitrate sources in Minnesota sand-plain aquifers. *Ground Water* **1993**, *31*, 260−270.

(34) Douglass, T. A.; Chamberlain, C. P.; Blum, J. D. Land use and geologic controls on the major elemental and isotopic (δ^{15}N and ^{87}Sr/^{86}Sr) geochemistry of the Connecticut River watershed, USA. *Chem. Geol.* **2002**, *189*, 19−34.

(35) Lindau, C. W.; Delaune, R. D.; Alford, D. P. Monitoring nitrogen pollution from sugarcane runoff using N^{15} analysis. *Water, Air, Soil Pollut.* **1997**, *89*, 389−399.

(36) Savage, C.; Elmgren, R. Macroalgal (*Fucus vesiculosus*) δ^{15}N values trace decrease in sewage influence. *Ecol. Appl.* **2004**, *14*, 517−526.