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USING STABLE ISOTOPES TO ESTIMATE TROPHIC POSITION: MODELS, METHODS, AND ASSUMPTIONS

DAVID M. POST[1,2,3]

[1]*Department of Ecology and Evolutionary Biology, Corson Hall, Cornell University, Ithaca, New York 14853 USA*
[2]*Institute for Ecosystem Studies, Box AB, Millbrook, New York 12545 USA*

Abstract. The stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) provide powerful tools for estimating the trophic positions of and carbon flow to consumers in food webs; however, the isotopic signature of a consumer alone is not generally sufficient to infer trophic position or carbon source without an appropriate isotopic baseline. In this paper, I develop and discuss methods for generating an isotopic baseline and evaluate the assumptions required to estimate the trophic position of consumers using stable isotopes in multiple ecosystem studies. I test the ability of two primary consumers, surface-grazing snails and filter-feeding mussels, to capture the spatial and temporal variation at the base of aquatic food webs. I find that snails reflect the isotopic signature of the base of the littoral food web, mussels reflect the isotopic signature of the pelagic food web, and together they provide a good isotopic baseline for estimating trophic position of secondary or higher trophic level consumers in lake ecosystems. Then, using data from 25 north temperate lakes, I evaluate how $\delta^{15}N$ and $\delta^{13}C$ of the base of aquatic food webs varies both among lakes and between the littoral and pelagic food webs within lakes. Using data from the literature, I show that the mean trophic fractionation of $\delta^{15}N$ is 3.4‰ (1 SD = 1‰) and of $\delta^{13}C$ is 0.4‰ (1 SD = 1.3‰), and that both, even though variable, are widely applicable. A sensitivity analysis reveals that estimates of trophic position are very sensitive to assumptions about the trophic fractionation of $\delta^{15}N$, moderately sensitive to different methods for generating an isotopic baseline, and not sensitive to assumptions about the trophic fractionation of $\delta^{13}C$ when $\delta^{13}C$ is used to estimate the proportion of nitrogen in a consumer derived from two sources. Finally, I compare my recommendations for generating an isotopic baseline to an alternative model proposed by M. J. Vander Zanden and J. B. Rasmussen. With an appropriate isotopic baseline and an appreciation of the underlying assumptions and model sensitivity, stable isotopes can help answer some of the most difficult questions in food web ecology.

Key words: *$\delta^{13}C$; $\delta^{15}N$; isotopic baseline; lake food webs; long-lived consumers; stable isotopes; trophic fractionation; trophic position.*

INTRODUCTION

There is considerable interest in using stable isotopes, particularly those of nitrogen and carbon, to evaluate the structure and dynamics of ecological communities (e.g., Peterson and Fry 1987, Kling et al. 1992, France 1995, Vander Zanden et al. 1999, Post et al. 2000). One advantage of stable isotope techniques is that they combine benefits of both the trophic-level and food web paradigms in food web ecology. Many studies use trophic levels because they are simple to define, characterize the functional role of organisms, and facilitate estimates of energy or mass flow through ecological communities (e.g., Hairston and Hairston 1993). The trophic level concept, however, is limited by the strict use of discrete trophic levels and its limited ability to capture the complex interactions and trophic omnivory that are prevalent in many ecosystems (Paine 1988, Polis and Strong 1996, Persson 1999, Vander Zanden and Rasmussen 1999). In contrast, food webs capture the complexity of trophic interactions in ecological communities, but are time-consuming to construct, often subjective in their resolution and scope (Paine 1988), and typically hold all trophic links to be of equal importance, which makes them ineffectual for tracking energy or mass flow through ecological communities (Paine 1988, Hairston and Hairston 1993, Polis and Strong 1996, Persson 1999, Vander Zanden and Rasmussen 1999). Stable isotope techniques can provide a continuous measure of trophic position that integrates the assimilation of energy or mass flow through all the different trophic pathways leading to an organism. Stable isotopes have the potential to simultaneously capture complex interactions, including trophic omnivory, and to track energy or mass flow through ecological communities (Peterson and Fry 1987, Kling et al. 1992, Cabana and Rasmussen 1996).

The ratio of stable isotopes of nitrogen ($\delta^{15}N$) can be used to estimate trophic position because the $\delta^{15}N$

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[3] Present address: National Center for Ecological Analysis and Synthesis, 735 State Street, Suite 300, Santa Barbara, California 93101-3351 USA. E-mail: post@nceas.ucsb.edu

of a consumer is typically enriched by 3–4‰ relative to its diet (DeNiro and Epstein 1981, Minagawa and Wada 1984, Peterson and Fry 1987). In contrast, the ratio of carbon isotopes ($\delta^{13}C$) changes little as carbon moves through food webs (Rounick and Winterbourn 1986, Peterson and Fry 1987, France and Peters 1997) and, therefore, typically can be used to evaluate the ultimate sources of carbon for an organism when the isotopic signature of the sources are different. In terrestrial ecosystems, $\delta^{13}C$ is often used to differentiate between diets based on plants with different photosynthetic pathways (e.g., C_3 vs. C_4; Rounick and Winterbourn 1986, Peterson and Fry 1987, O'Leary et al. 1992). In lakes, $\delta^{13}C$ is useful for differentiating between two major sources of available energy, littoral (near shore) production from attached algae and detritus, and pelagic (open water) production from phytoplankton, because the $\delta^{13}C$ of the base of the littoral food web tends to be enriched in ^{13}C (less negative $\delta^{13}C$) relative to the base of the pelagic food web (France 1995).

While it is relatively straightforward to use stable isotope ratios to evaluate food web structure and material flow within a single system (e.g., Peterson et al. 1985, Keough et al. 1996, Hansson et al. 1997), many critical questions in ecology are best answered through comparisons across multiple systems (e.g., Kling et al. 1992, Post et al. 2000). When comparing among ecosystems, the $\delta^{15}N$ and $\delta^{13}C$ of an organism alone provides little information about its absolute trophic position or ultimate source of carbon. This is because there is considerable variation among ecosystems in the $\delta^{15}N$ and $\delta^{13}C$ of the base of the food web from which organisms draw their nitrogen and carbon ($\delta^{15}N_{base}$, $\delta^{13}C_{base}$; Rounick and Winterbourn 1986, Zohary et al. 1994, Cabana and Rasmussen 1996, MacLeod and Barton 1998, Kitchell et al. 1999, Vander Zanden and Rasmussen 1999; France, *in press*). Without suitable estimates of $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ in each system, there is no way to determine if variation in the $\delta^{15}N$ and $\delta^{13}C$ of an organism reflects changes in food web structure and carbon flow, or just a variation in the $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$. Obtaining the isotopic baseline required to estimate trophic position is one of the most difficult problems facing the application of stable isotope techniques to multiple-system food web studies.

The simplest model for estimating the trophic position of a secondary consumer is: trophic position $= \lambda + (\delta^{15}N_{\text{secondary consumer}} - \delta^{15}N_{base})/\Delta_n$, where λ is the trophic position of the organism used to estimate $\delta^{15}N_{base}$ (e.g., $\lambda = 1$ for primary producers), $\delta^{15}N_{\text{secondary consumer}}$ ($\delta^{15}N_{sc}$, or any higher consumer) is measured directly, and Δ_n is the enrichment in $\delta^{15}N$ per trophic level. For long-lived consumers, $\delta^{15}N_{base}$ must capture the temporal variation in $\delta^{15}N$ of primary producers and detrital energy sources for those consumers within an ecosystem. Ideally, $\delta^{15}N_{base}$ should also integrate the isotopic signature at a time scale near that of long-lived consumers. It is important to note that there are four unknowns in this equation: trophic position, Δ_n, $\delta^{15}N_{sc}$, and $\delta^{15}N_{base}$. The trophic fractionation of nitrogen (Δ_n) is generally assumed to be between 3‰ and 4‰ (Peterson and Fry 1987), a critical assumption I evaluate in detail in this paper. If a robust estimate of Δ_n is available, solving the above equation still requires the quantification of two of the three remaining unknowns. That is why $\delta^{15}N_{sc}$ is not a sufficient estimate of trophic position without a good estimate of $\delta^{15}N_{base}$.

Where consumers acquire nitrogen from more than one food web, each with a separate set of primary producers or detritus sources (e.g., fish that feed on both littoral and pelagic food webs), the model must further capture any potential spatial heterogeneity in $\delta^{15}N_{base}$. For a two-source food web, trophic position is calculated as: trophic position $= \lambda + (\delta^{15}N_{sc} - [\delta^{15}N_{base1} \times \alpha + \delta^{15}N_{base2} \times (1 - \alpha)])/\Delta_n$, where α is the proportion of nitrogen in the consumer ultimately derived from the base of food web one. When the movement of nitrogen and carbon through the food web is similar, α can be estimated using carbon isotopes such that: $\alpha = (\delta^{13}C_{sc} - \delta^{13}C_{base2})/(\delta^{13}C_{base1} - \delta^{13}C_{base2})$. This two-end-member-mixing model allows for the differentiation between two sources, such as the littoral and pelagic food webs found in lakes, and as written assumes that there is little or no trophic fractionation of carbon and that mixing is linear, assumptions I address further in this paper (see Fry and Sherr [1984] and Schwarcz and Schoeninger [1991] for discussion and expansion of this mixing model). Where the number of important resources (n) is >2, a minimum of $n - 1$ isotope ratios (or other sources of information) are required to resolve the system (Fry and Sherr 1984, Peterson et al. 1985, 1986, Peterson and Fry 1987). For example, Peterson et al. (1986) used two isotope ratios ($\delta^{13}C$ and $\delta^{34}S$) to evaluate the flow of organic material from three dominant producers (upland plants, marine plankton, and the salt marsh plant S*partina*) to macroconsumers.

Variability in $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ derives from differences in the isotopic ratios of carbon and nitrogen available for uptake by organisms at the base of the food web, and through variable expression of fractionation during uptake. In aquatic systems, most primary producers and detrital energy sources have high temporal variation in $\delta^{15}N$ and $\delta^{13}C$, complicating their direct use as indicators of $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ for secondary consumers that integrate $\delta^{15}N$ and $\delta^{13}C$ over much longer time periods (Cabana and Rasmussen 1996). Furthermore, $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ are spatially variable both within a lake (France 1995, Vander Zanden and Rasmussen 1999) and among lakes (Cabana and Rasmussen 1996, del Giorgio and France 1996). Cabana and Rasmussen (1996) and Vander Zanden and Rasmussen (1999) have suggested using long-lived primary consumers to quantify $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ in aquatic food webs because the temporal variance of their isotopic signature is much lower than that of pri-

TABLE 1. Limnological characteristics of the 26 lakes used in this study.

Lake	State	Latitude (°N)	Longitude (°W)	Area (ha)	Max. depth (m)	Total phosphorus (μg/L)	Dissolved organic carbon (mg/L)
Bear	NY	42°20′	79°23′	48	10.8	28.2	8.1
Black	NY	44°28′	75°38′	3 074	5.0	64.9	9.5
Bull Pond	NY	41°20′	74°04′	12	24.1	10.2	3.2
Cayuga	NY	42°56′	76°44′	17 236	132.6	16.9	5.7
Champlain	NY	44°32′	73°20′	113 000	122.0	12.1	3.8
Chautauqua	NY	42°11′	79°25′	5 314	23.0	12.2	7.5
Clear	NY	44°16′	75°49′	64	13.4	6.6	5.4
Cross	NY	43°08′	76°29′	788	19.8	25.4	⋯
Conesus	NY	42°47′	77°43′	1 303	⋯	⋯	⋯
Cuba	NY	42°14′	78°18′	180	6.1	15.1	4.6
Erie	NY	41°40′	81°40′	2 582 000	64.0	5.5	5.8
Honeoye	NY	42°45′	77°30′	714	9.2	36.6	8.3
Hyde	NY	44°14′	75°50′	73	5.5	12.2	3.8
Kegonsa	WI	42°57′	89°15′	1 299	10.2	89.0	⋯
Keuka	NY	42°33′	77°05′	4 718	55.8	2.6	5.1
Mendota	WI	43°06′	89°24′	3 938	25.3	49.0	6.5
Monona	WI	43°03′	89°22′	1 324	22.8	44.0	6.6
Neatahwanta	NY	43°18′	76°26′	277	3.7	230.0	13.1
Oneida	NY	43°12′	75°55′	20 746	16.8	26.8	7.3
Ontario	NY	43°30′	78°00′	1 876 000	225.0	9.0	⋯
Owasco	NY	42°54′	76°32′	2 750	54.0	6.6	5.8
Raquette	NY	43°50′	74°38′	1 994	29.2	5.7	5.3
Round Pond	NY	41°22′	74°01′	7	9.5	18.5	4.1
Silver	NY	42°41′	78°01′	339	11.3	23.5	8.4
Spencer	NY	42°14′	76°30′	26	12.5	7.5	5.6
Upton	NY	41°50′	73°45′	19	18.0	9.4	6.1

Notes: Lakes were located in or bordering New York (NY) and in central Wisconsin (WI). Total phosphorus concentrations are for midsummer (July through early August). Ellipses (⋯) indicate characteristics that were not available or not measured for some lakes used in this study.

mary producers (Cabana and Rasmussen 1996), and because they should reflect the spatial variation within and among lakes (Cabana and Rasmussen 1996, Vander Zanden and Rasmussen 1999). Although these are reasonable expectations, there is no empirical evidence that long-lived primary consumers actually do provide the hypothesized temporal integration of, or reflect the spatial variability in primary producers and detrital energy sources in aquatic food webs.

The goals of this paper are to develop and discuss the methods for obtaining an isotopic baseline, and to evaluate the assumptions required to estimate trophic position in multiple systems studies. While I focus primarily on aquatic examples, the methods, models, and assumptions discussed in this paper are applicable to all ecological systems. Because of their importance to estimating trophic position, I review the trophic fractionation of $\delta^{15}N$ and $\delta^{13}C$. I also discuss the importance of assuming that nitrogen and carbon move through the food web with similar stoichiometry when using $\delta^{13}C$ to estimate the source of nitrogen for consumers. I use time series of primary producers, detritus, and zooplankton collected in three lakes to test the ability of two long-lived primary consumers, filter-feeding mussels, and surface-grazing snails, to estimate $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ for aquatic food webs. I also use mussels and snails collected from 25 north temperate lakes to evaluate patterns of variation in $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ both within and among lakes. I then test the sensitivity of trophic position estimates to assumptions about trophic fractionation and to different methods for estimating the isotopic baseline. Finally, I discuss the general problem of multiple sources, the application of mixing models, and the use of single isotope ratios and nonlinear models to evaluate multiple end members.

METHODS

To test the use of mussels and snails as indicators of the isotopic signature of the base of littoral and pelagic food webs, I collected time series of primary producer and detrital carbon sources in Spencer, Cayuga, and Oneida lakes in central New York State, USA. These lakes were chosen because of their limnological differences (Table 1). Spencer Lake is small, relatively shallow, and with low productivity. In contrast, Cayuga and Oneida lakes are large and moderately productive, but Cayuga is deep and Oneida is shallow. In each lake, I collected a mixture of periphyton (attached algae) and detritus from the littoral zone, and zooplankton and seston (a mixture of phytoplankton and detritus) from the pelagic zone. Each lake was sampled on 5 or 6 dates (every two weeks) from early June to late August. Cayuga and Spencer lakes were sampled in 1997, and Oneida Lake was sampled in 1998. Periphyton and detritus were brushed from rocks, macrophytes, and logs, prefiltered through 75-μm mesh to remove large invertebrates, and then filtered onto precombusted Gellman A/E glass fiber filters (Pall Gelman Laboratory,

Ann Arbor, Michigan). Seston was collected from integrated epilimnetic water samples. Depth for epilimnetic samples was determined from a depth profile of temperature taken in each lake on each sampling date. Seston was prefiltered through 75- or 30-μm mesh and then filtered onto precombusted Gellman A/E glass fiber filters. Seston and periphyton/detritus samples were visually inspected to remove large particulate contaminants. I sampled periphyton and detritus from multiple surfaces and collected different size fractions of seston in order to represent the isotopic signature of the range of material available to long-lived consumers. Bulk zooplankton samples were obtained using a 150-μm mesh net pulled vertically through the epilimnion several times. Again, the depth for epilimnetic samples was determined from a depth profile of temperature taken in each lake on each sampling date. Bulk zooplankton samples contained a mixture of the herbivorous cladocerans, copepods, and rotifers present in the lake on any given sampling date. Each sample was visually inspected using a microscope to remove particulate contaminants and predatory zooplankton such as *Chaoborus*, *Mesocyclops*, *Epischura*, *Leptidora*, and others. I was unable to remove all small predatory zooplankton, such as the predatory rotifer *Asplanchna*; however, because they were a very small proportion of any sample, they would have had very little influence on the isotopic signature. Zooplankton were collected because they are better indicators of $\delta^{13}C_{base}$ for the pelagic food web than are bulk seston samples, which often contain recalcitrant carbon that is not assimilated (Zohary et al. 1994, del Giorgio and France 1996).

Snails and mussels were sampled by SCUBA in late August, at the end of the time series collected in each lake. The dominant snail species were haphazardly collected from the littoral zone of each lake (*Goniobasis* spp. in Cayuga Lake; *Physine* spp., *Planorbella trivoluis* group, and *Fossaria* spp. in Oneida Lake; *Fossaria* spp., and *Physine* spp. in Spencer Lake). In Spencer Lake, I used unionid mussels (Unionidae) and in Cayuga and Oneida lakes I used zebra mussels (*Dreissena polymorpha*). To help eliminate individual variation in isotopic signature, I aggregated the soft tissue of a minimum of 25 snails, 3 unionid mussels, or 25 zebra mussels for each sample. The soft tissue of mussels and snails reflects the isotopic signature of their diets. The shell of snails and mussels is a biologically mediated, carbon-based precipitate that reflects the isotopic signature of the inorganic environment (McConnaughey et al. 1997). Finally, to evaluate the isotopic similarity of unionid and zebra mussels, I also collected unionid and zebra mussels in four other New York lakes where they coexist (Champlain, Conesus, Cross, and Keuka lakes; Table 1).

To evaluate within and among lake patterns of variation in $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$, I collected snails from 25 lakes, mussels from 21 lakes, and time series of seston from 4 lakes without mussels. All of the lakes were located in or bordering New York, or in central Wisconsin (Table 1), and all samples were collected in the summers of 1997–1999. Snail and mussel samples were collected in July and August. Seston samples were collected every other week in 1999 from late May to early August. The methods used follow those outlined above. Among lakes, I looked for relationships among $\delta^{13}C_{base}$ and $\delta^{15}N_{base,}$ and lake area, maximum depth, DOC (dissolved organic carbon) concentrations (mg/L), and total phosphorous concentrations (μg/L).

All samples were dried at 40°C for ≥48 h. Mussel and snail samples were ground into a fine powder and lipids were extracted using methanol-chloroform (2:1 by volume). I performed lipid extraction because lipids are depleted in ^{13}C compared with whole organisms and the lipid content of animal tissue samples is variable (Peterson and Fry 1987, Kling et al. 1992). Stable isotope analysis was performed on a Europa Geo 20/20 continuous flow isotope ratio mass spectrometer (PDZ Europa, Cheshire, UK) at the Cornell University and Boyce Thompson Institute Stable Isotope Laboratory (CoBSIL). The standard deviation for replicate samples I ran throughout this analysis were 0.05‰ for $\delta^{13}C$ and 0.18‰ for $\delta^{15}N$. All stable isotope values are reported in the δ notation where $\delta^{13}C$ or $\delta^{15}N = ([R_{sample}/R_{standard}] - 1)\cdot 1000$, where R is ^{13}C:^{12}C or ^{15}N:^{14}N. Global standard for $\delta^{13}C$ is PeeDee Belemnite and for $\delta^{15}N$ is atmospheric nitrogen. The CoBSIL working standard for animal samples with high nitrogen content was CBT (Cayuga Brown Trout; $\delta^{13}C = -25.06$, $\delta^{15}N = 17.36$; 54.9% C, 12.2% N), and for plant, algal, and detrital samples with low nitrogen content was BCBG (Burnt Cabbage; $\delta^{13}C = -27.03$, $\delta^{15}N = 0.21$; 43.0% C, 3.2% N).

Although the trophic fractionations of nitrogen and carbon have been reviewed previously and are discussed widely (e.g., Fry and Sherr 1984, Minagawa and Wada 1984, Peterson and Fry 1987), a large amount of new data has been collected since those surveys were published, particularly for nitrogen, allowing a more robust statistical evaluation. Using literature data and my results, I collected laboratory and field observations of trophic fractionation for both aquatic and terrestrial organisms ranging in size from copepods to polar bears. I used data from both individual laboratory trials (e.g., DeNiro and Epstein 1978, Adams and Sterner 2000) and whole ecosystem studies (e.g., Hansson et al. 1997). I collected 56 estimates of trophic fractionation for nitrogen and 107 estimates of trophic fractionation for carbon. Fractionation estimates for nitrogen were drawn from Gaebler et al. (1966), Kreitler (1975), Steele and Daniel (1978), DeNiro and Epstein (1981), Macko et al. (1982), Minagawa and Wada (1984), Fry (1988), Sholtodouglas et al. (1991), Hobson and Welch (1992), Kling et al. (1992), Keough et al. (1996), Hansson et al. (1997), Gorokhova and Hansson (1999), Adams and Sterner (2000), and this study. For the analysis of the trophic fractionation of nitrogen, I excluded two

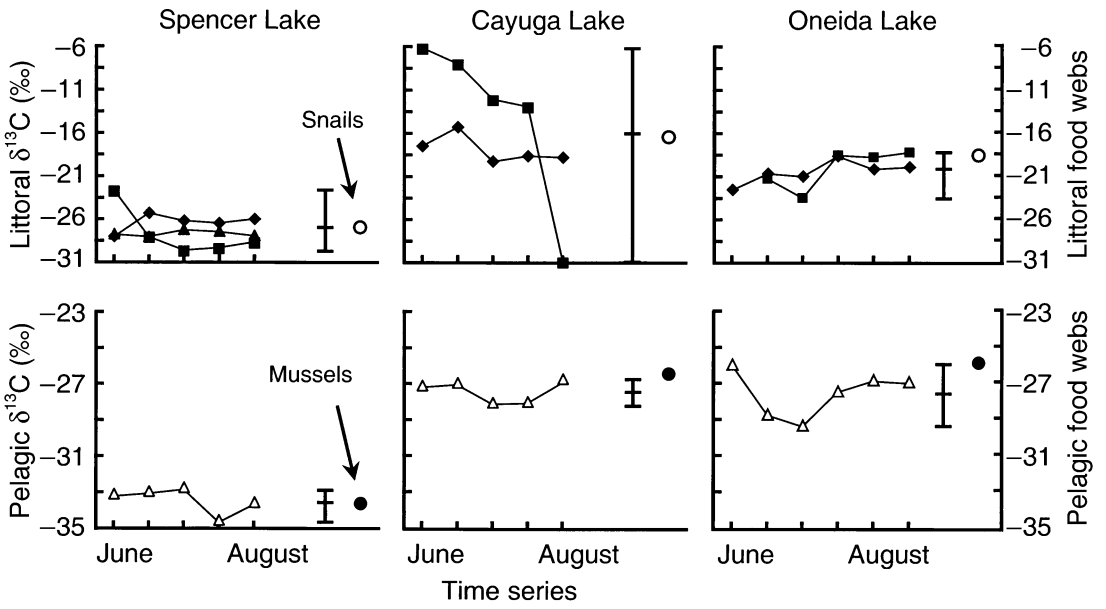

FIG. 1. The plots show $\delta^{13}C$ of time series and primary consumers collected from the littoral and pelagic food webs of Spencer, Cayuga, and Oneida lakes. The littoral time-series data derive from periphyton and detritus removed from logs (filled triangles), rocks (filled diamonds), and macrophytes (filled squares). The pelagic time-series data are herbivorous zooplankton (open triangles). The bars show the range and median of the time series data. The primary consumer data are for snails (open circles) and mussels (closed circles) collected at the end of each time series and are shown by the unconnected symbols near the right of each panel. All of the primary consumers, except the mussels in Cayuga Lake, fall within the range of time-series data, and there is no significant difference between the primary consumers and the median of the time-series data.

data points from DeNiro and Epstein (1981) that were outliers (−0.1 and 9.2; based on visual analysis of normal probability plot). Fractionation estimates for carbon were drawn from DeNiro and Epstein (1978), Fry et al. (1978), Haines and Montague (1979), Petelle et al. (1979), Teeri and Schoeller (1979), Rau and Anderson (1981), Fry and Arnold (1982), Macko et al. (1982), Gu et al. (1996), Focken and Becker (1998).

RESULTS

Long-lived consumers as temporal and spatial integrators

Despite considerable temporal variation, snails and mussels were good temporal integrators of the isotopic variation at the base of pelagic and littoral food webs (Figs. 1 and 2). In all but one case (Cayuga pelagic $\delta^{13}C$), long-lived primary consumers fell within the range of $\delta^{13}C$ and $\delta^{15}N$ values recorded in the time series of observations. Using lake by habitat combinations as replicates ($n = 6$ for both $\delta^{13}C$ and $\delta^{15}N$), there were no significant differences between the median $\delta^{13}C$ and $\delta^{15}N$ of each time series and the $\delta^{13}C$ and $\delta^{15}N$ of snails and mussels (paired t test for means; $t = 2.29$, $P = 0.07$ for $\delta^{13}C$; $t = 2.19$, $P = 0.08$ for $\delta^{15}N$, where I subtracted 3.4‰ from the $\delta^{15}N$ of snails and mussels to remove the expected one trophic level of enrichment). Although not significant at alpha = 0.05, long-lived primary consumers were slightly enriched in $\delta^{13}C$ (1‰) and $\delta^{15}N$ (0.7‰). If this trend is real, it may be due to a combination of isotopic carryover from previous years, coarse temporal sampling, the presence of recalcitrant material in time series samples that was not assimilated, and small physiological differences in isotope fractionation.

Snails and mussels also effectively reflect spatial differences in $\delta^{13}C$ and $\delta^{15}N$ between the littoral and pelagic food webs. Within a single lake, snails had an isotopic signature similar to that of periphyton and detritus that forms the base of the littoral food web, and mussels had an isotopic signature that was similar to that of seston that forms the base of the pelagic food web (Figs. 1 and 2).

There were small isotopic differences between zebra mussels (*Dreissena polymorpha*) and unionid mussels (Unionidae) used to estimate $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ of pelagic food webs (Fig. 3). In three lakes, Champlain, Conesus, and Keuka, I found no differences between $\delta^{13}C$ and $\delta^{15}N$ of unionid and zebra mussels (nested ANOVA with species nested in lake, $F_{3,18} = 1.56$, $P = 0.23$ for $\delta^{13}C$; $F_{3,18} = 2.21$, $P = 0.12$ for $\delta^{15}N$). In all three lakes, the absolute differences between unionid and zebra mussels were <0.4‰ for $\delta^{15}N$ and <0.5‰ for $\delta^{13}C$. In Cross Lake, however, unionid mussels were enriched in $\delta^{15}N$ by 3.3‰, and depleted in $\delta^{13}C$ by 0.9‰ compared with zebra mussels. Unionid mussels in Cross Lake were the largest of any collected and presumably quite old. The isotopic differences were likely due to carryover from previous years, caused by dif-

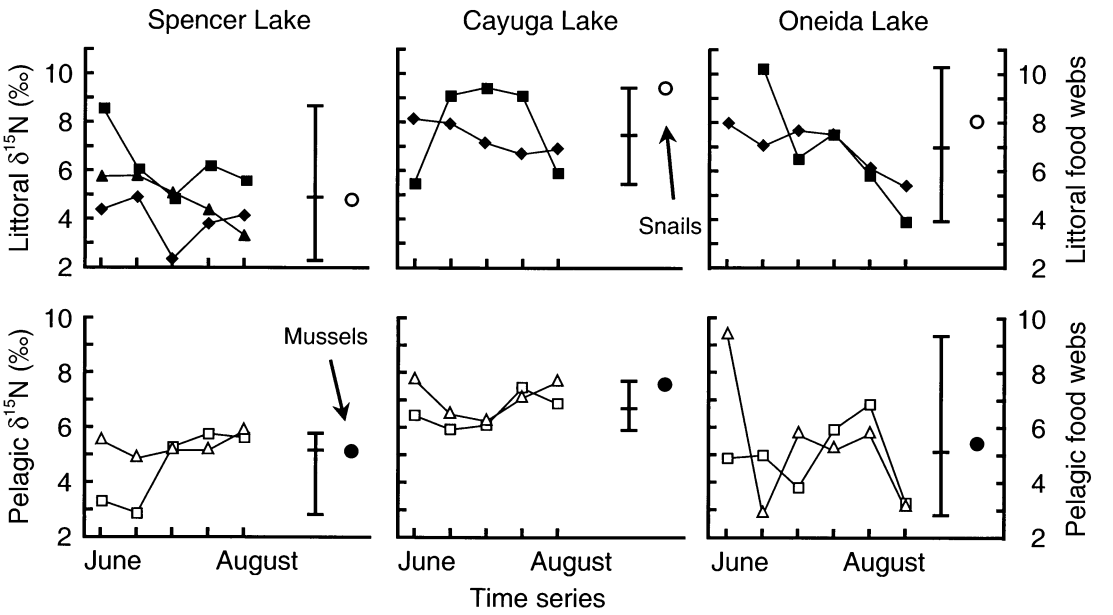

FIG. 2. The plots show $\delta^{15}N$ of time series and primary consumers collected from the littoral and pelagic food webs of Spencer, Cayuga, and Oneida lakes. The littoral time-series data derive from periphyton and detritus removed from logs (filled triangles), rocks (filled diamonds), and macrophytes (filled squares). The pelagic time-series data derive from <30 μm seston (open triangles) and <75 μm seston (open squares). The bars show the range and median of the time-series data. The primary consumer data are for snails (open circles) and mussels (closed circles) collected at the end of each time series and are shown by the unconnected symbols near the right of each panel. All of the primary consumers fall within the range of time-series data, and there is no significant difference between the primary consumers and the median of the time-series data.

ferences in the turnover time of unionid and zebra mussel tissue and temporal changes in the isotopic signature of their food source.

Patterns of variance in $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$

There was considerable variation in $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ both among and within lakes. Among lakes, $\delta^{13}C_{base}$ of littoral food webs varied between −14‰ and −28‰, and $\delta^{13}C_{base}$ of pelagic food webs varied between −20‰ and −34‰. There was a significant positive relationship between $\delta^{13}C_{base}$ and lake area for both littoral and pelagic food webs (Fig. 4; littoral $\delta^{13}C_{base} = -26.12 + 1.57 \times \log(\text{area})$, $n = 25$, $F_{1,23} = 17.5$, $P < 0.001$, $r^2 = 0.43$; pelagic $\delta^{13}C_{base} = -33.3 + 1.74 \times \log(\text{area})$, $n = 25$, $F_{1,23} = 30.6$, $P < 0.001$, $r^2 = 0.57$), but no relationship between $\delta^{13}C_{base}$ and log depth (littoral, $n = 25$, $F_{1,23} = 3.8$, $P = 0.07$; pelagic, $n = 25$, $F_{1,23} = 4.1$, $P = 0.06$), between $\delta^{13}C_{base}$ and log DOC concentrations (mg/L; littoral, $n = 22$, $F_{1,20} = 0.27$, $P = 0.61$; pelagic, $n = 22$, $F_{1,20} = 0.96$, $P = 0.34$), or between $\delta^{13}C_{base}$ and log total phosphorus concentrations (μg/L; littoral, $n = 25$, $F_{1,23} = 1.3$, $P = 0.27$; pelagic, $n = 25$, $F_{1,23} < 0.01$, $P = 0.99$). The slope of the relationship between lake area and littoral $\delta^{13}C_{base}$ was not significantly different from that for lake area and pelagic $\delta^{13}C_{base}$ (ANCOVA, $F_{1,26} = 0.126$, $P = 0.725$), but the intercepts for littoral $\delta^{13}C_{base}$ and pelagic $\delta^{13}C_{base}$ were significantly different with a mean intra-lake difference of 6.7‰ (ANCOVA, $F_{1,26} = 46.67$, $P < 0.001$). The consistent difference between littoral $\delta^{13}C_{base}$ and pelagic $\delta^{13}C_{base}$ further supports the conclusion that mussels and snails effectively reflect the within-lake spatial differences in $\delta^{13}C_{base}$ between the littoral and pelagic food webs.

Among my study lakes, $\delta^{15}N_{base}$ varied between 4.5‰ and 13.6‰ (Fig. 5a), equivalent to nearly three trophic levels of variation. Including data from the literature (Kidd et al. 1998, Vander Zanden and Rasmussen 1999) expands the $\delta^{15}N_{base}$ range to between 0‰ and 13.6‰, equivalent to four trophic levels of variation. In my study lakes, the range of $\delta^{15}N_{base}$ for the pelagic food web was slightly larger (5.1–13.6‰) than that for littoral food webs (4.5–12.9‰). There was no significant relationship between lake area and pelagic $\delta^{15}N_{base}$ ($n = 25$, $F_{1,23} = 0.81$, $P = 0.377$, $r^2 = 0.03$; Fig. 5a), but there was a significant positive relationship between lake area and littoral $\delta^{15}N_{base}$ ($6.1 + 0.71 \times \log(\text{area})$, $n = 25$, $F_{1,23} = 5.98$, $P = 0.023$, $r^2 = 0.21$; Fig. 5a). There was also a significant relationship between littoral $\delta^{15}N_{base}$ and log depth ($n = 25$, $F_{1,23} = 6.06$, $P = 0.022$), but log depth did not explain any additional variance in a model that already included lake area ($F_{1,22} = 1.17$, $P = 0.29$). There was no relationship between pelagic $\delta^{15}N_{base}$ and log depth ($n = 25$, $F_{1,23} = 0.75$, $P = 0.40$), between $\delta^{15}N_{base}$ and log total phosphorus concentrations (littoral, $n = 25$, $F_{1,23} = 0.12$, $P = 0.82$; pelagic, $n = 25$, $F_{1,23} = 0.69$, $P = 0.42$), or between $\delta^{15}N_{base}$ and log DOC concentrations (littoral,

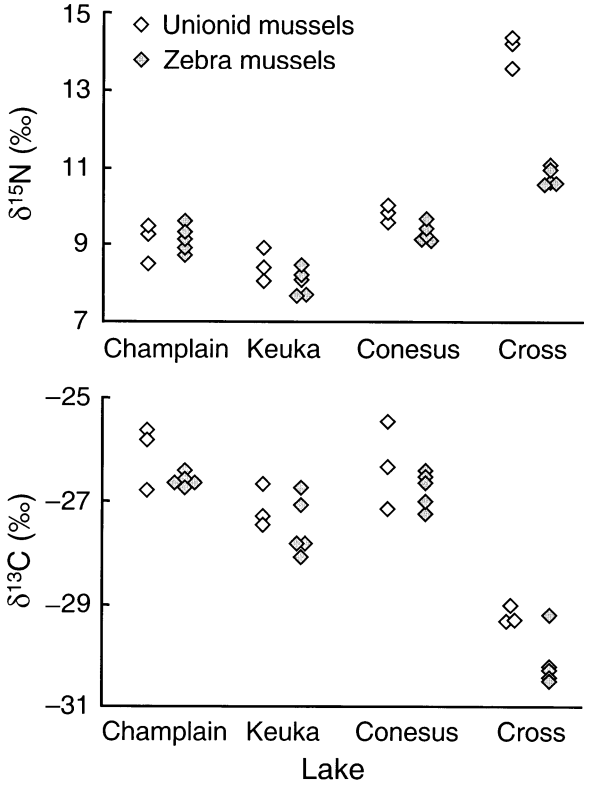

FIG. 3. The plots show $\delta^{15}N$ and $\delta^{13}C$ of unionid and zebra mussels in four lakes in New York State. For each lake, all mussels were collected on a single date. There were no significant differences in $\delta^{15}N$ and $\delta^{13}C$ between unionid and zebra mussels in Champlain, Keuka, and Conesus lakes, but unionid and zebra mussels were significantly different in Cross Lake.

$n = 22$, $F_{1,20} = 0.26$, $P = 0.61$; pelagic, $n = 21$, $F_{1,19} = 0.07$, $P = 0.79$). The mean absolute within-lake difference between littoral and pelagic $\delta^{15}N_{base}$ was small, <1‰. However, because of the positive relationship between littoral $\delta^{15}N_{base}$ and lake area, the difference between littoral and pelagic $\delta^{15}N_{base}$ switched from generally negative (littoral lighter than pelagic) to generally positive (littoral heavier than pelagic) as lake size increased (littoral $\delta^{15}N$ − pelagic $\delta^{15}N = -1.6 + 0.44 \times \log(\text{area})$, $n = 24$, $F_{1,22} = 7.10$, $P = 0.01$, $r^2 = 0.24$; Fig. 5b).

Trophic fractionation of nitrogen and carbon

The mean trophic fractionation of $\delta^{15}N$ was 3.4‰ (1 SD = 0.98, $n = 56$), and the distribution was not significantly different from normal (Shapiro-Wilk W test, $n = 56$, $W = 0.972$, $P = 0.38$; Fig. 6A). There were no significant differences in mean fractionation or in the variability in fractionation between aquatic and terrestrial organisms, between laboratory and field observations, or between carnivores and herbivores/detritivores (Table 2). Likewise, there was no relationship between trophic fractionation of $\delta^{15}N$ and body mass (Table 2).

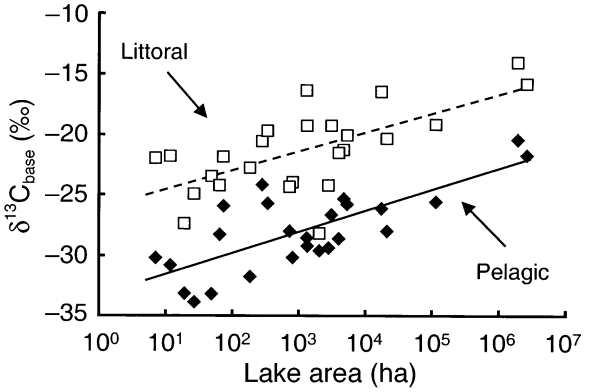

FIG. 4. The relationship between lake area and $\delta^{13}C_{base}$ for the littoral and pelagic food webs of 25 lakes in eastern North America.

The mean trophic fractionation of carbon was 0.39‰ (1 SD = 1.3, $n = 107$) and the distribution was not significantly different from normal (Shapiro-Wilk W test, $n = 107$, $W = 0.985$, $P = 0.78$; Fig. 6B). The mean fractionation was significantly different from zero ($n = 107$, $t = 3.08$, $P < 0.01$), suggesting that organisms generally become enriched in ^{13}C (less negative $\delta^{13}C$) compared with their diet. However, there was relatively large variation around 0.39‰ suggesting

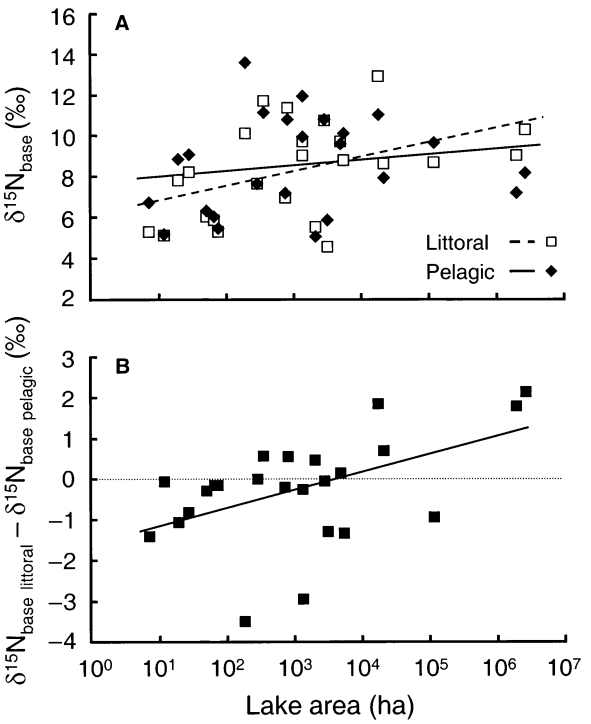

FIG. 5. (A) The relationship between lake area and $\delta^{15}N_{base}$ for the littoral and pelagic food webs, and (B) the relationship between lake area and the difference between the littoral $\delta^{15}N_{base}$ and the pelagic $\delta^{15}N_{base}$ for 24 lakes in eastern North America.

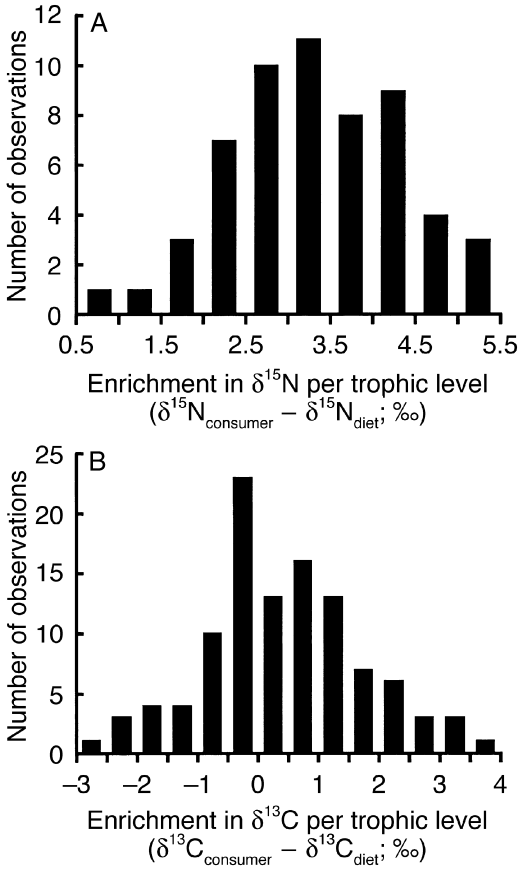

FIG. 6. Frequency distributions of the enrichment in (A) $\delta^{15}N$ and (B) $\delta^{13}C$ per trophic level. The means are 3.4‰ for $\delta^{15}N$ (SD = 0.98, n = 56) and 0.39‰ for $\delta^{13}C$ (SD = 1.3, n = 107), and neither distribution is significantly different from normal. See *Methods* for the list of studies used to produce these figures.

that it will be difficult, ecologically, to distinguish this slight positive fractionation from zero. There were no significant differences in the mean fractionation of $\delta^{13}C$ between aquatic and terrestrial organisms, between laboratory and field observations, or between carnivores and herbivores/deritivores (Table 3). Likewise, there was no relationship between the trophic fractionation of $\delta^{13}C$ and estimated body mass. While the mean fractionation was consistent across all contrasts, trophic fractionation of $\delta^{13}C$ measured in the laboratory was more variable than that measured under field conditions (variance = 2.0 vs. 0.9), and was more variable for herbivores/detritivores than for carnivores (2.0 vs. 0.6; Table 3).

Sensitivity of trophic position to assumptions

Post et al. (2000) used the models and methods outlined in this paper to estimate the trophic position of piscivorous fish from food webs in 25 lakes in order to test theories about the determinants of food-chain length. I used the Post et al. (2000) data to estimate the sensitivity of estimates of trophic position to different assumptions about the trophic fractionation of $\delta^{15}N$ and $\delta^{13}C$, where top predators were getting their carbon, and the use of time series data rather than mussels and snails to estimate $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$. To keep the analysis manageable, I report the effect of different assumptions on estimates of maximum trophic position for each lake (the trophic position of the species with the highest mean trophic position in a food web). All assumptions were compared with a nominal scenario that used snails and mussels as the isotopic baseline, and assumed trophic enrichment of 3.4‰ for $\delta^{15}N$ and 0‰ for $\delta^{13}C$. Trophic position was most sensitive to assumptions about the trophic fractionation of $\delta^{15}N$, and relatively insensitive to assumptions about the trophic fractionation of $\delta^{13}C$ (Table 4). Trophic position was more sensitive to a 1-SD reduction in trophic fraction of $\delta^{15}N$ than to a 1-SD increase, because trophic

TABLE 2. Comparisons for the mean trophic fractionation and variance in the trophic fractionation of $\delta^{15}N$.

Comparison	Fractionation†	df	*F*	*P*
Mean fractionation				
Lab vs. field	3.30 vs. 3.45	1, 53	0.10	0.76
Aquatic vs. terrestrial	3.42 vs. 3.26	1, 53	0.15	0.70
Herbivores‡ vs. carnivores	3.35 vs. 3.45	1, 53	0.04	0.85
Variance in fractionation				
Lab vs. field	1.37 vs. 0.94	26, 31	1.15	0.26
Aquatic vs. terrestrial	0.99 vs. 1.48	43, 14	1.50	0.37
Herbivores‡ vs. carnivores	1.21 vs. 0.91	38, 19	1.33	0.49

Notes: Comparisons are for the setting in which fractionation estimates were made (lab vs. field), the general habitat from which organisms were drawn (aquatic vs. terrestrial), the general trophic habit of organisms (herbivores and detritivores vs. carnivores), and the correlation between the estimated body mass of the consumer and trophic fractionation (n = 52, r = 0.08, P = 0.58; log of estimated body mass used for this analysis).

† Values are the mean fractionation for comparisons of mean, and 1 SD for comparisons of variance.

‡ Herbivores and deritivores were combined for this analysis.

TABLE 3. Comparisons for the mean trophic fractionation and variance in the trophic fractionation of $\delta^{13}C$.

Comparison	Fractionation†	df	*F*	*P*
Mean fractionation				
Lab vs. field	3.35 vs. 0.45	1, 103	0.10	0.75
Aquatic vs. terrestrial	−0.10 vs. 0.51	1, 103	3.03	0.08
Herbivores‡ vs. carnivores	0.50 vs. 0.05	1, 103	1.64	0.20
Variance in fractionation				
Lab vs. field	1.42 vs. 1.06	68, 27	2.17	<0.05
Aquatic vs. terrestrial	0.96 vs. 1.35	85, 20	1.99	0.11
Herbivores‡ vs. carnivores	0.50 vs. 0.05	80, 25	3.51	<0.01

Notes: Comparisons are for the setting in which fractionation estimates were made (lab vs. field), the general habitat from which organisms were drawn (aquatic vs. terrestrial), the general trophic habit of organisms (herbivores and detritivores vs. carnivores), and the correlation between the estimated body mass of the consumer and trophic fractionation ($n = 107$, $r = 0.02$, $P = 0.20$; log of estimated body mass was used for this analysis).

† Values are the mean fractionation for comparisons of the mean, and 1 SD for the comparisons of variance.

‡ Herbivores and deritivores were combined for this analysis.

position = $(\delta^{15}N_{\text{secondary consumer}} - \delta^{15}N_{\text{base}})$/trophic fractionation. As trophic fractionation approaches zero, trophic position increases rapidly and approaches infinity. Estimates based on an incomplete isotopic baseline (e.g., using mussels only) were similar, on average, to the nominal estimates, but an incomplete baseline caused large deviations from the nominal estimates in a few lakes (Table 4). For example, in Cayuga Lake, a partial baseline introduced almost as large a deviation from nominal as assuming a $\delta^{15}N$ trophic enrichment

TABLE 4. The sensitivity of maximum trophic position (MTP) to different methods for estimating the isotopic baseline and assumptions about trophic fractionation.

Assumption or baseline method	Spencer Lake MTP	Spencer Lake Deviation from nominal (%)	Oneida Lake MTP	Oneida Lake Deviation from nominal (%)	Cayuga Lake MTP	Cayuga Lake Deviation from nominal (%)	All lakes, mean deviation (% range)
Nominal estimate†	3.80		4.34		4.74		
Trophic fractionation of $\delta^{15}N = 4.4‰ (+ 1$ SD)‡	3.39	11	3.81	12	4.12	13	12% (10–14%)
Trophic fractionation of $\delta^{15}N = 2.4‰ (- 1$ SD)‡	4.55	20	5.31	20	5.88	24	21% (19–26%)
Trophic fractionation of $\delta^{13}C = 1.3‰ (+ 1$ SD)§	3.89	2	4.26	2	4.39	7	3% (0–9%)
Trophic fractionation of $\delta^{13}C = -1.3‰ (- 1$ SD)§	3.67	4	4.56	5	4.82	2	3% (0–13%)
$\delta^{15}N_{\text{base}}$ − littoral only‖	3.89	2	4.15	4	4.27	10	3% (0–11%)
$\delta^{15}N_{\text{base}}$ − pelagic only‖	3.65	4	4.42	7	4.82	2	7% (0–26%)
$\delta^{15}N_{\text{base}}$ from time series samples¶	3.84	1	4.13	5	5.03	6	⋯#
$\delta^{15}N_{\text{base}}$ from time series (mean + 1 SD)††	3.53	7	3.57	18	4.89	3	⋯#
$\delta^{15}N_{\text{base}}$ from time series (mean − 1 SD)	4.06	7	4.72	9	5.12	8	⋯#

Notes: For Spencer, Oneida, and Cayuga lakes, the table reports MTP and the percentage deviation away from the nominal estimate of MTP for each method and assumption. For all 25 lakes, the table reports the mean percentage deviation and the range of percentage deviations in MTP away from the nominal estimate of MTP. Data are from Post et al. (2000).

† Maximum trophic position (MTP) is the trophic position of the species with the highest average trophic position in a lake's food web. The top predator was largemouth bass in Spencer Lake, walleye in Oneida Lake, and lake trout in Cayuga Lake. The nominal estimate uses long-lived primary consumers for the littoral and pelagic $\delta^{15}N_{\text{base}}$ and assumes a trophic fractionation for $\delta^{15}N$ of 3.4‰ and for $\delta^{13}C$ of 0‰ (see Post et al. 2000 for details).

‡ MTP calculated assuming a trophic fractionation for $\delta^{15}N$ of 3.4 ± 1 SD (1.0‰).

§ MTP calculated assuming a trophic fractionation for $\delta^{13}C$ of 0 ± 1 SD (1.3‰).

‖ MTP calculated without taking into account the spatial variation within a lake, using only $\delta^{15}N_{\text{base}}$ littoral to calculate MTP or using only $\delta^{15}N_{\text{base}}$ pelagic to calculate MTP.

¶ MTP calculated using the mean $\delta^{15}N$ and $\delta^{13}C$ from the time series of pelagic phytoplankton and littoral periphyton/detritus.

There are no all-lake estimates of the mean deviation for time-series assumptions because time-series data were collected only in Spencer, Oneida, and Cayuga lakes.

†† MTP calculated using the mean ± 1 SD of $\delta^{15}N$ from the pelagic and littoral time series.

of 4.4‰. Using a time series of phytoplankton and periphyton/detritus for the isotopic baseline provided estimates of trophic position that were quite similar to the nominal estimates of trophic position; however, because there is large amount of variation around the mean (e.g., Fig. 1), a short or incomplete time series could lead to misleading estimates of trophic position (Table 4; time series mean ± 1 SD).

Discussion

Isotopic baselines and long-lived consumers

Properly applied, stable isotope techniques produce estimates of trophic position that simultaneously capture complex trophic interactions and track energy or mass flow through the reticulate pathways of ecological communities. Thus, they can provide a powerful tool for testing food-chain theory (Post et al. 2000), for evaluating the effects of invasion on food web structure (Vander Zanden et al. 1999), and for estimating the trophic position of and trophic differentiation between species with difficult to quantify diets (Kling et al. 1992; C. L. Holtmeier and D. M. Post, *unpublished manuscript*). All of these applications, however, require estimates of an isotopic baseline appropriate to the question of interest. Obtaining an appropriate baseline is one of the most difficult methodological issues facing the effective application of stable isotopes to trophic food web questions. There are two general baseline methods. One method is to use the $\delta^{15}N$ of a few phylogenetically or ecologically related species within a single ecosystem to estimate shifts in relative trophic position (e.g., Kling et al. 1992, Ponsard and Arditi 2000; C. L. Holtmeier and D. M. Post, *unpublished manuscript*). This approach works well when the question does not require an absolute trophic position, when there are only a few related species, and when the trophic position of one species is well understood and can serve as an isotopic baseline for the specific question. For example, Kling et al. (1992) used a well-studied herbivorous copepod as a baseline to estimate the extent of trophic omnivory in a second copepod with variable feeding behavior. Long-lived primary consumers provide a more general baseline for questions that require quantitative estimates of trophic position and that compare species across multiple ecosystems (e.g., Post et al. 2000).

With the equivalent of nearly two trophic levels' worth of temporal variation in $\delta^{15}N_{base}$ within a single lake, and almost four trophic levels' worth of variation in $\delta^{15}N_{base}$ among lakes, long-lived primary consumers need to accurately reflect the isotopic signature of the base of the food webs they represent. Snails and mussels effectively integrate the temporal variation, and reflect the intra- and inter-lake spatial variation in $\delta^{13}C$ and $\delta^{15}N$ at the base of lake food webs. Surface-grazing snails tend to reflect the isotopic signature of detritus and periphyton that form the base of littoral food webs, while unionids and zebra mussels tend to reflect the isotopic signature of seston that forms the base of pelagic food webs (Figs. 1 and 2).

For any baseline, it is important to avoid large temporal and spatial discontinuities. The turnover rate of tissue for a whole organism, and therefore the turnover rate of isotopes, is correlated with body mass (Peters 1983) and, for a given mass, fast growing organisms have more rapid turnover rates (Fry and Arnold 1982, Hesslein et al. 1993). Large consumers, such as fish, have tissue turnover rates ranging from months to years (Hesslein et al. 1993) and their isotopic signature is representative of their diet over long periods of time. Although small snails and zebra mussels are a considerable improvement over a single or even a few seston, periphyton/detritus, or even zooplankton samples for temporal integration, the turnover time of their tissue is certainly shorter than that of many large secondary consumers. Unionid mussels provide a better temporal match than zebra mussels for large secondary consumers; however, unionids are being replaced by zebra mussels in many places and are not always available. Furthermore, where they are found, unionid species may be endangered or threatened and must be sampled with caution. Small-scale spatial variation in the sources and fractionation of carbon and nitrogen are also likely (MacLeod and Barton 1998). Collecting primary consumers from diverse substrates and at multiple sites should help integrate across small-scale spatial discontinuities.

When appropriate primary consumers are not available or are difficult to obtain, carefully chosen time series of basal resources, such as phytoplankton or periphyton in aquatic ecosystems, can be substituted. A time series of basal resources has two major shortcomings. First, it will not provide the nearly continuous temporal sampling provided by primary consumers and it may therefore miss temporally short-lived but biologically important pulses of productivity. Second, time series samples may contain recalcitrant material that is not normally assimilated by primary consumers and passed up the food web. Whether using a time series of basal resources or primary consumers, it is critical to understand the natural history of the secondary consumer (e.g., where they feed, their tissue turnover time) when choosing an isotopic baseline.

Variation in $\delta^{13}C_{base}$

There were consistent differences between $\delta^{13}C_{base}$ of the littoral and pelagic food webs (Fig. 4), reflecting differential expression of fractionation during the uptake of dissolved inorganic carbon (DIC; see also France 1995). Fractionation associated with carbon fixation is strongly influenced by the availability of DIC. When DIC is strongly limiting, little of the fractionation associated with carbon fixation is expressed (Smith and Walker 1980, Goericke et al. 1994). Local DIC availability is controlled by a combination of

boundary layer thickness at the cell boundary, and uptake rate of primary producers relative to DIC diffusion rate into the unstirred boundary layer. Littoral producers presumably experience less turbulence and have a thicker boundary layer than pelagic producers (France 1995), and therefore are more CO_2 limited, fractionate DIC less during uptake, and are enriched in ^{13}C relative to pelagic producers. Variation around the mean difference of 6.7‰ between pelagic $\delta^{13}C_{base}$ and littoral $\delta^{13}C_{base}$ is probably due to lake specific differences in boundary layer conditions, primary producer growth rates, and isotopic signatures of DIC sources.

The strong relationship between $\delta^{13}C_{base}$ and lake area (Fig. 4) has interesting implications for understanding carbon supply for production in lakes. Changes in $\delta^{13}C_{base}$ are caused by some combination of changes in either the $\delta^{13}C$ of DIC across this lake area gradient or a change in fractionation during DIC uptake and assimilation. The parallel response of the littoral $\delta^{13}C_{base}$ and the pelagic $\delta^{13}C_{base}$ suggests that littoral and pelagic production draw from the same relatively well mixed DIC pool, and that the relationship between lake size and $\delta^{13}C_{base}$ results from a change in the $\delta^{13}C$ of DIC rather than a change in fractionation. Fractionation of carbon during assimilation is generally assumed to be ~ -20‰ for phytoplankton, although there is considerable variation (Peterson and Fry 1987, Goericke et al. 1994). There are, in general, three potential sources for DIC in lakes: respiration (re-mineralization) of organic carbon derived from either internal production or allochthonous inputs (e.g., France, *in press*), atmospheric CO_2, and DIC from weathering of carbonates in the watershed (Wachniew and Różański 1997). Respired carbon is generally isotopically light, with $\delta^{13}C$ from ~ -20 to -35‰ (e.g., France, *in press*), roughly reflecting the isotopic signature of the parent organic material. In contrast, atmospheric CO_2 and weathered carbonates are isotopically heavy, ~ -7 and 0‰, respectively. The $\delta^{13}C_{base}$ data in Fig. 4 suggest that respired carbon (from heterotrophic activity) fuels more of the production in small lakes than in larger lakes. As lake area increases, more in situ production may be derived from atmospheric CO_2 and weathered carbonates. This implies that the ratio of heterotrophy to primary productivity declines (primary productivity becomes more important) as lake size increases. Furthermore, because more respired carbon in small lakes derives from allochthonous carbon inputs, either particulate or dissolved, the positive relationship between lake area and $\delta^{13}C_{base}$ suggests that allochthonous inputs are more important for fueling production in small lakes than in larger lakes.

Assumptions for estimating trophic position

When estimating trophic position using the equation for two nitrogen sources [trophic position $= \lambda + (\delta^{15}N_{sc} - [\delta^{15}N_{base1} \times \alpha + \delta^{15}N_{base2} \times (1 - \alpha)])/3.4$], three assumptions are generally made: the trophic fractionation of $\delta^{15}N$ is 3.4‰, the trophic fractionation of $\delta^{13}C$ is near 0‰, and carbon and nitrogen move through the food web with a similar stoichiometry. Historically, trophic fractionation was thought to result from the excretion of isotopically light nitrogen (Minagawa and Wada 1984). However, it more likely derives from a combination of isotopic fractionation both during assimilation and protein synthesis, and during the excretion of endogenous nitrogen in urine (Ponsard and Averbuch 1999). The mean 3.4‰ trophic enrichment widely observed (Fig. 6) originates from small differences in fractionation during synthesis and excretion, with the ratio of fractionation during assimilation to fractionation during excretion determining the difference between whole body $\delta^{15}N$ and dietary $\delta^{15}N$ (Ponsard and Averbuch 1999).

Although the mean 3.4‰ trophic enrichment was consistent across all comparisons (Table 2), it is important to note that 3.4‰ is a valid approximation of trophic fractionation only when averaged over multiple trophic pathways. Any single trophic transfer is likely to range between ~2‰ and 5‰ (e.g., between algae and *Daphnia* in laboratory experiments; Adams and Sterner 2000) and studies which attempt to quantify trophic differences among just a few feeding links should be cautious in their interpretation of $\delta^{15}N$ differences (e.g., Kling et al. 1992; C. L. Holtmeier and D. M. Post, *unpublished manuscript*) or should explicitly integrate the variance around the mean trophic fractionation of 3.4‰ in estimates of trophic position (e.g., Ponsard and Arditi 2000). When applied to entire food webs, with multiple trophic pathways and many species, a mean trophic fractionation of 3.4‰ is a robust and widely applicable assumption.

Trophic fractionation of carbon

There is continued debate over the trophic fractionation of carbon. It is generally agreed that the trophic fractionation of $\delta^{13}C$ is ~0‰ per trophic level (Rounick and Winterbourn 1996, Peterson and Fry 1987). Like the trophic fractionation of nitrogen, the mean trophic fractionation of $\delta^{13}C$ was consistent across multiple comparisons (Table 3). However, the higher variability of herbivores and detritivores compared with carnivores suggests food quality may influence $\delta^{13}C$ fractionation, but additional work is needed to explore this trend. Regardless of the precise value, small differences in the trophic fractionation of $\delta^{13}C$ have very little effect on calculations of trophic position (Table 4) because differences between littoral $\delta^{15}N_{base}$ and pelagic $\delta^{15}N_{base}$ are generally small (usually <1.5‰, Fig. 4b) and because there are relatively large differences between littoral $\delta^{13}C_{base}$ and pelagic $\delta^{13}C_{base}$. To further elaborate on this point, I calculated the trophic position of 191 fish from 25 lakes (see Post et al. 2000) assuming trophic fractionations for $\delta^{13}C$ of 0‰ and 1‰. The mean difference in trophic position between these two assumptions was 0.01 trophic levels (1 SD = 0.14), and

the mean of the absolute difference was 0.1 trophic levels (1 SD = 0.11). Ninety percent of the fish differed by <0.2 trophic levels and only a few fish differed by >0.25 trophic levels.

Trophic fractionation of $\delta^{13}C$ can be integrated into estimates of α, and therefore into estimates of trophic position, using the equation: $\alpha = [\delta^{13}C_{base2} - (\delta^{13}C_{sc} + \Delta_c t_{sc})]/(\delta^{13}C_{base2} - \delta^{13}C_{base1})$, where Δ_c is the trophic fractionation of $\delta^{13}C$ and t_{sc} is the tropic position of the consumer of interest. Because α is in the equation for trophic position and t is in the equation for α, an iterative routine is required to calculate trophic position and α. Estimates of trophic position generally converge to within 0.01 trophic level after just one or two iterations because trophic position is not particularly sensitive to the trophic fractionation of $\delta^{13}C$ (Table 4).

C:N stoichiometry in a two source model

The two-source trophic-position model calculates the isotopic baseline for an organism using $\delta^{13}C$ to estimate how much nitrogen an organism obtained from each of two sources (e.g., from the littoral and pelagic food webs). The model assumes that carbon and nitrogen move through the food web with a similar stoichiometry. This assumption is acceptable when working with organisms that have similar C:N, such as primary and secondary consumers in lake ecosystems. It is not, however, a good assumption when working with organisms, such as detritivorus fish and crayfish, that feed on prey with very different C:N (Gannes et al. 1997). For example, if an omnivore assimilates an equal mass of detritus (C:N of 100:1) and animal prey (C:N of 6:1), the $\delta^{13}C$ would indicate that the omnivore was acquiring most of its carbon and, assuming similar C:N stochiometry, most of its nitrogen from detritus. In fact, the omnivore might well assimilate nearly equal amounts of nitrogen from each source. If the prey have substantially different $\delta^{15}N$ values, the failure to incorporate differences in C:N stochiometry could lead to large over- or underestimates of trophic position. Even this is a simplistic model because the omnivore, with a C:N similar to the animal prey, may eliminate much of the excess detrital carbon while assimilating most or all of the nitrogen from both sources to maintain nutrient homeostasis (see Gannes et al. 1997 for a discussion of this and related animal physiological issues related to applying stable isotopes to ecological studies).

Multiple sources of N and C in lakes

Tracing multiple sources of nitrogen and carbon is a general problem in the application of stable isotopes to questions of energy flow and trophic position in complex ecosystems (Fry and Sherr 1984, Peterson et al. 1986, Peterson and Fry 1987). Because the model I present here uses a single isotope ratio, $\delta^{13}C$, to estimate α (the proportion of nitrogen in the consumer ultimately derived from different sources), it can only differentiate between two potential sources. Any study using this model must explicitly choose the two most important sources of carbon and nitrogen for the study organisms. In lakes, a logical choice might be the littoral and pelagic food webs. Terrestrial and profundal food webs are certainly potential sources of production for secondary consumers in some lakes, but their importance is not clear (but see France 1997). The $\delta^{13}C$ of terrestrial production ranges between $\sim -15‰$ and $\sim -30‰$ (Peterson and Fry 1987) and therefore can be hard to distinguish from aquatic production. Indirect inputs, such as leaf litter that must first be processed by littoral consumers before becoming available to the rest of the food web, are generally integrated into the isotopic signature of the base of the littoral food web. In contrast, directly available terrestrial inputs, such as insects or pollen, might be important especially in small lakes or for short time periods.

The profundal food web could also be important in deep, well-oxygenated lakes. Vander Zanden and Rasmussen (1999) present evidence that the profundal $\delta^{13}C_{base}$ is more negative than the pelagic $\delta^{13}C_{base}$ by $\sim 2‰$. If profundal resources are important, then they will tend to make the $\delta^{13}C$ of secondary consumers more negative than pelagic $\delta^{13}C_{base}$. There is little evidence of a profundal signal in 191 fish from 25 lakes that I measured (data from Post et al. 2000). Only 10% of the fish had a $\delta^{13}C$ more negative than pelagic $\delta^{13}C_{base}$ and most of those fish were well within 1‰ of $\delta^{13}C_{base}$. Because of sampling and analytical errors, fish within 1‰ are probably not ecologically different from $\delta^{13}C_{base}$. Although this is not an ideal test, it suggests that profundal carbon may contribute to a few fish, but is not generally an important source of carbon to these secondary consumers. Each study should explicitly choose two end members based on the natural history of the secondary consumer of interest. When there are more than two important basal resources, appropriate multiple isotope mixing models should be developed and applied (e.g., Peterson et al. 1985, 1986).

An alternative method for estimating $\delta^{15}N_{base}$

Vander Zanden and Rasmussen (1999) have proposed using many long-lived primary consumers and an empirical curve fitting method to estimate $\delta^{15}N_{base}$. Their approach is an attempt to solve the problem of multiple end members using a single isotope ratio. Their isotopic baseline model fits a non-linear (sigmoid) function to $\delta^{13}C$ (x-axis) and $\delta^{15}N$ data (y-axis) from a variety of primary consumers in 14 lakes in Quebec and Ontario, Canada. To estimate trophic position, they relate the $\delta^{13}C$ of a secondary consumer to the $\delta^{13}C$ of the model equation and derive a lake- and fish-specific $\delta^{15}N$ baseline. Their model assumes a constant negative relationship between the $\delta^{15}N$ and $\delta^{13}C$ of primary consumers, and it uses one isotope, $\delta^{13}C$, to distinguish among multiple potential carbon sources (littoral, pelagic, and profundal).

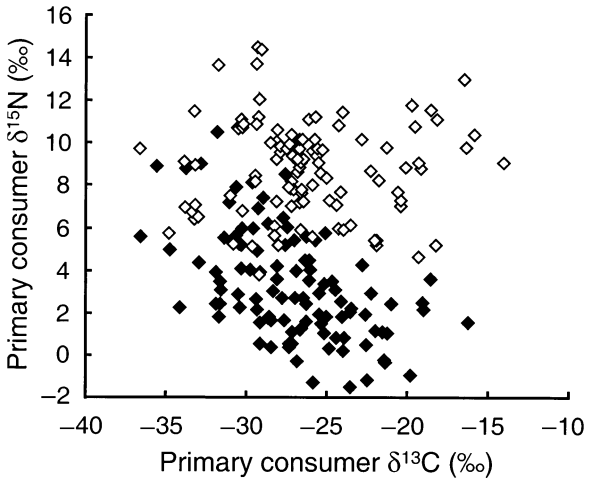

FIG. 7. The plot shows $\delta^{15}N$ and $\delta^{13}C$ values for 203 primary consumers in 40 lakes in eastern North America. The open diamonds are data from this study (99 primary consumers in 26 lakes), and the closed diamonds are data from Vander Zanden and Rasmussen (1999; 104 primary consumers in 14 lakes). There is no significant relationship between $\delta^{15}N$ and $\delta^{13}C$ in these 40 lakes ($n = 203$, $F_{1, 201} = 1.57$, $P = 0.21$).

There are two major limitations to the application of the Vander Zanden and Rasmussen (1999) model. First, they assume that there is a consistent negative relationship between $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$. While this was the case in the limited range of lakes studied by Vander Zanden and Rasmussen (1999), there was no relationship between $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ in a diverse set of lakes studied by Post et al. (2000; Fig. 7). I used data from Vander Zanden and Rasmussen (1999) as well as from this study (40 lakes and 203 primary consumer samples) to test the negative relationship between $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ reported by Vander Zanden and Rasmussen (1999). The 40 north temperate lakes in this analysis span a large range of area, depth, and productivity, and represent much of the trophic and morphological diversity of north temperate lakes (Table 1). In this broad cross section of lakes, there was no general relationship between $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ (Fig. 7, $n = 203$, $F_{1, 201} = 1.57$, $P = 0.21$). The lakes I sampled and the Vander Zanden and Rasmussen lakes had significantly different slopes for the relationship between $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ (ANCOVA, interaction term, $F_{1, 198} = 22.3$, $P < 0.01$); there was no relationship between $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ in my lakes ($n = 97$, $F_{1,95} = 0.01$, $P = 0.91$, $r^2 = 0$), but there was a negative relationship in the Vander Zanden and Rasmussen lakes ($n = 104$, $F_{1, 102} = 40.1$, $P = <0.01$, $r^2 = 0.28$).

It is possible that a negative relationship between $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ exists within each lake, but is obscured in the above analysis by combining data from multiple lakes. I evaluated this possibility by performing an ANCOVA looking for an interaction between lake and $\delta^{13}C_{base}$. A significant interaction indicates that there are significant among-lake differences in the slope of the relationship between $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$. Using all 40 lakes, there was a significant interaction between lake and $\delta^{13}C$ (ANCOVA using raw data, $F_{39, 123} = 1.9$, $P < 0.01$; ANCOVA using linearized data according to Vander Zanden and Rasmussen [1999], $F_{39, 123} = 2.0$, $P < 0.01$). The slope of the relationship between $\delta^{13}C$ and $\delta^{15}N$ for individual lakes ranged between -0.7 and 0.4, and there was no consistent negative slope across these 40 lakes. The lakes used by Vander Zanden and Rasmussen (1999) were all rather similar, deep oligotrophic Canadian Shield lakes, and this may explain why they found a distinct relationship between $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ in their lakes. In my 25 study lakes, the relationship between $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ was variable because the pelagic $\delta^{15}N_{base}$ was not consistently heavier than the littoral $\delta^{15}N_{base}$ (Fig. 5b). As lake area increased, the littoral $\delta^{15}N_{base}$ switched from being lighter than the pelagic $\delta^{15}N_{base}$ in small lakes to being heavier than the pelagic $\delta^{15}N_{base}$ in large lakes (Fig. 5). This caused the relationship between $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ for a single lake to be positively related to lake area in my 25 lakes ($r = 0.45$, $P = 0.02$); larger lakes generally have a more positive slope than smaller lakes.

While there is no reason to doubt the negative correlation found in the Vander Zanden and Rasmussen lakes, the broader analysis of these 40 lakes indicates that there is no consistent relationship between $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$. Without a consistent relationship between $\delta^{13}C$ and $\delta^{15}N$, the Vander Zanden and Rasmussen (1999) method cannot be used generally to estimate trophic position of secondary consumers. Variation in the slope of the relationship between $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ can produce large errors in estimates of trophic position. For example, if the slope in a lake is positive rather than negative (such as in Lake Erie where the slope is 0.36), estimates of trophic position using the Vander Zanden and Rasmussen (1999) model and assuming a negative slope could be off by over two trophic levels.

The second limitation of the Vander Zanden and Rasmussen (1999) model is that it attempts to distinguish among multiple sources using a single isotope ratio. Implicit within the Vander Zanden and Rasmussen (1999) model is a two-end-member-mixing model used to resolve three end members; carbon from littoral, pelagic, and profundal food webs. However, a single isotope ratio cannot provide a unique solution when there are more than two possible sources (Peterson et al. 1986). For example, if the littoral ($\delta^{13}C_{base}$, $\delta^{15}N_{base}$: $-20‰$, 5‰), pelagic ($-25‰$, 5‰), and profundal food webs ($-30‰$, 10‰) are all important sources, then a secondary consumer with a $\delta^{13}C$ of $-25‰$ and a $\delta^{15}N$ of 11.8‰ might derive 100% of its carbon and nitrogen from the pelagic food web or 50% from each the littoral and profundal food webs, producing trophic positions estimates of 4 or 3.3. In fact, there are an infinite number of estimates for the carbon source and trophic po-

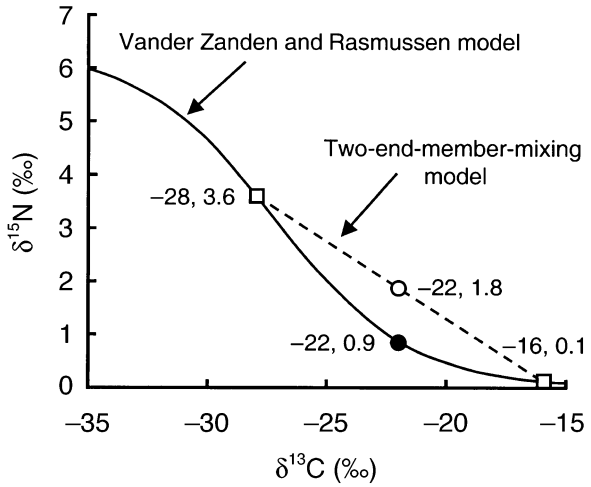

FIG. 8. A comparison of the Vander Zanden and Rasmussen (1999) model and a two-end-member-mixing model for estimating $\delta^{15}N_{base}$. Both models use $\delta^{13}C$ to estimate the source from which a consumer receives its nitrogen, which is then used to estimate $\delta^{15}N_{base}$ for each consumer. In this example, a consumer with a $\delta^{13}C$ of -22 (circles) receives an equal amount of nitrogen from two sources (open squares: $\delta^{13}C = -28$, $\delta^{15}N = 3.6$; and -16, 0.1). The two-end-member-mixing model predicts a $\delta^{15}N_{base}$ of 1.8 (open circle; the appropriate mean for this example) while the nonlinear model developed by Vander Zanden and Rasmussen (1999) predicts a $\delta^{15}N_{base}$ of 0.9 (closed circle).

sition within a range constrained by the range of variation in $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$.

Even assuming the proportion of carbon from two sources is known, the nonlinearity of the Vander Zanden and Rasmussen (1999) model can produce estimates of $\delta^{15}N_{base}$ that differ considerable from those obtained using a two-end-member-mixing model (Fig. 8). If a consumer received an equal amount of carbon and nitrogen from two sources, the consumer's $\delta^{13}C$ and presumed $\delta^{15}N_{base}$would be a mean of the two sources (Fig. 8; Fry and Sherr 1984, Peterson et al. 1986, Peterson and Fry 1987). Depending on the $\delta^{13}C$ of the end members, $\delta^{15}N_{base}$ estimates from the Vander Zanden and Rasmussen model could over- or underestimate $\delta^{15}N_{base}$ by 1‰: the equivalent of almost one-third of a trophic level.

The Vander Zanden and Rasmussen (1999) model could provide a useful $\delta^{15}N_{base}$ for estimating trophic position under two relatively restrictive conditions. First, the relationship between $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ needs to be linear, and $\delta^{13}C_{base}$ needs to explain much of the variation in $\delta^{15}N_{base}$ (i.e., have a high r^2) because variation in $\delta^{15}N_{base}$ translates directly into errors in estimating trophic position. Second, because the slope of the relationship between $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ is not consistent among lakes, the relationship needs to be quantified separately for each lake. Under these conditions, a linear model relating $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ mimics a two-end-member-mixing model and can estimate $\delta^{15}N_{base}$ for secondary consumers. Unfortunately, these conditions may apply in very few natural ecosystems.

Application to food web studies

Stable-isotope-based estimates of trophic position provide a powerful fusion of trophic level and food web paradigms for evaluating realized trophic structure of complex food webs. As Vander Zanden and Rasmussen (1999) point out, stable isotope techniques can provide a time-integrated measure of trophic position that simultaneously captures complex interactions, including trophic omnivory, and tracks energy or mass flow through ecological communities. Stable isotope techniques provide an important tool for answering general questions about trophic structure (e.g., Vander Zanden et al. 1999, Post et al. 2000). However, because there are a limited number of stable isotopes available to ecologists (Peterson and Fry 1987), this approach alone generally does not provide the resolution required to track energy or material flow through a large number of specific food web pathways (Peterson et al. 1986). To obtain the high level of resolution required to discern complex trophic interactions, stable isotopes must be used in conjunction with other information, such as direct diet analyses. Regardless of the research question, the choice of an appropriate baseline is one of the most important decisions in the application of stable isotopes to trophic studies. Although every baseline will suffer from some spatial and temporal variation between the baseline and the secondary consumer of interest, a good baseline will (1) integrate isotopic changes at a time scale near that of the secondary consumer of interest, (2) cover the same time period as the secondary consumer of interest (i.e., be collected in the same year), and (3) capture the spatial variability that contributes to the isotopic signature of the secondary consumer of interest. Long-lived primary consumers, such as snails and mussels, satisfy these criteria in aquatic food webs and can provide an appropriate baseline to quantify the trophic position of secondary consumers.

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