# Comparison of aquatic food chains using nitrogen isotopes

(food web/trophic level/sewage/eutrophication/nutrient cycling)

GILBERT CABANA*† AND JOSEPH B. RASMUSSEN

Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, QC Canada H3A 1B1

ABSTRACT      Recent studies have shown the utility of $\delta^{15}N$ to model trophic structure and contaminant bioaccumulation in aquatic food webs. However, cross-system comparisons in $\delta^{15}N$ can be complicated by differences in $\delta^{15}N$ at the base of the food chain. Such baseline variation in $\delta^{15}N$ is difficult to resolve using plankton because of the large temporal variability in the $\delta^{15}N$ of small organisms that have fast nitrogen turnover. Comparisons using large primary consumers, which have stable tissue isotopic signatures because of their slower nitrogen turnover, show that $\delta^{15}N$ increases markedly with the human population density in the lake watershed. This shift in $\delta^{15}N$ likely reflects the high $\delta^{15}N$ of human sewage. Correcting for this baseline variation in $\delta^{15}N$, we report that, contrary to expectations based on previous food-web analysis, the food chains leading up to fish varied by about only one trophic level among the 40 lakes studied. Our results also suggest that the $\delta^{15}N$ signatures of nitrogen at the base of the food chain will provide a useful tool in the assessment of anthropogenic nutrient inputs.

The nitrogen pools of animals are enriched in $^{15}N$ relative to their food with top predators having the highest concentrations of this stable isotope (1–4). In laboratory experiments, the enrichment in $\delta^{15}N$ {$\delta^{15}N = [(^{15}N/^{14}N_{sample})/(^{15}N/^{14}N_{standard}) - 1] \times 1000$}, where atmospheric nitrogen is the reference material, in animals relative to their diet is on average $+3.4\%o$ for a wide variety of animal taxa (5). This has led to a general approach to the measurement of food-web processes in the field (6, 7) that has been extended to the modeling of the biomagnification of persistent contaminants such as mercury (8, 9) and organochlorines (10, 11). Recently, between-lake variation in the $\delta^{15}N$ of piscivores has been proposed to distinguish lakes where high concentration of persistent contaminants in fish is attributable to longer than usual food chains from those where point-source contamination should be suspected (12). However, variation in the $\delta^{15}N$ signature of primary producers at the base of the food chain can produce variation in $\delta^{15}N$ within the same species of predator (13, 14), independently of the length of food chain supporting the production of such top consumers. Thus, the general applicability of $\delta^{15}N$ as a time-integrated measure of variation of trophic level among populations of the same consumer species depends on our ability to first identify and measure between-habitat variation in the $\delta^{15}N$ signature of primary producers or that of other organisms with fixed low trophic level and then adjust the $\delta^{15}N$ values of consumers to these reference values so that their $\delta^{15}N$ truly reflect variation in trophic level and not variation in $\delta^{15}N$ at the base of the food chains.

A survey of the literature showed that small marine and freshwater organisms tend to show greater temporal variability in their $\delta^{15}N$ signature than larger organisms (Fig. 1). For example, the $\delta^{15}N$ of phytoplankton or small size fraction of organic matter as well as dissolved nitrogen in the form of

nitrate and ammonium can vary by up to 6–10‰ during the year (15,16, 25–29). This high temporal variation in $\delta^{15}N$, which has been linked to variation in the $\delta^{15}N$ of nitrogen source (15), as well as to factors influencing the fractionation in nitrogen uptake by primary producers such as temporal variation in nitrogen concentration (25, 26), and the species succession of primary producers from nonnitrogen fixers to nitrogen fixers (29), renders the identification and measurement of $\delta^{15}N$ at the base of the food chain potentially problematic. Thus differences in baseline $\delta^{15}N$ are difficult to resolve using lake plankton signatures because of the large temporal variability in the $\delta^{15}N$ signatures of small organisms that have faster nitrogen turnover.

Unionid mussels are microsestonic filter feeders of inorganic nitrogen users such as phytoplankton and bacteria but are orders of magnitude larger (about 10 cm long) than planktonic organisms and live for many years, thus making their tissue pools less sensitive to the short-term seasonal fluctuations that make the average $\delta^{15}N$ signature of planktonic organisms difficult to assess with precision. By collecting such mussels from a wide range of lakes, we have been able to show that considerable but consistent variation across lakes in baseline $\delta^{15}N$ does exist (mussel $\delta^{15}N$ ranges from $+1.2\%o$ to $+9.0\%o$). A survey (23) of the $\delta^{15}N$ signature of the major sources of nitrogen (atmospheric, fertilizers, soil, and sewage) showed that nitrogen derived from sewage stands out as having very high $\delta^{15}N$ (modal value of $+15\%o$), compared with the other sources (modal values between $-5$ and $+5\%o$). This shift in $\delta^{15}N$ can be attributed not only to the relatively high trophic level of humans (and, therefore, of their excreted nitrogen), but also to a fractionation occurring during ammonification and subsequent volatilization of nitrogenous waste products, which results in much greater proportionate losses of the light isotope (23). We hypothesized that the highly variable $\delta^{15}N$ observed in primary consumers is related to anthropogenic influences on the lake and its watershed. Fig. 2 shows that the $\delta^{15}N$ signature of mussels and other smaller shorter-lived primary consumers for which statistically sound seasonal averages are available is strongly correlated to the human population density of the lake watershed. The empirically derived asymptotic $\delta^{15}N$ values of primary consumers from aquatic systems dominated by anthropogenic nitrogen input and that of those from pristine habitats were, respectively, 11.0‰ and 3.3‰. This result suggests that the $\delta^{15}N$ signature of primary producers would average [after accounting for the fractionation factor of $+3.4\%o$ (5) between primary consumers and primary producers] around 7.5‰ and 0‰ for the two extreme conditions. While anthropogenic impacts measured as human density account for most (68%) of the among-lake variation in the $\delta^{15}N$ of primary consumers, other sources of variability in the $\delta^{15}N$ of primary producers, subsequently passed on to primary consumers, could be related to the relative importance of denitrification (28), nitrogen fixation

*Present address: Department of Integrative Biology, University of California at Berkeley, Berkeley, CA 94720.
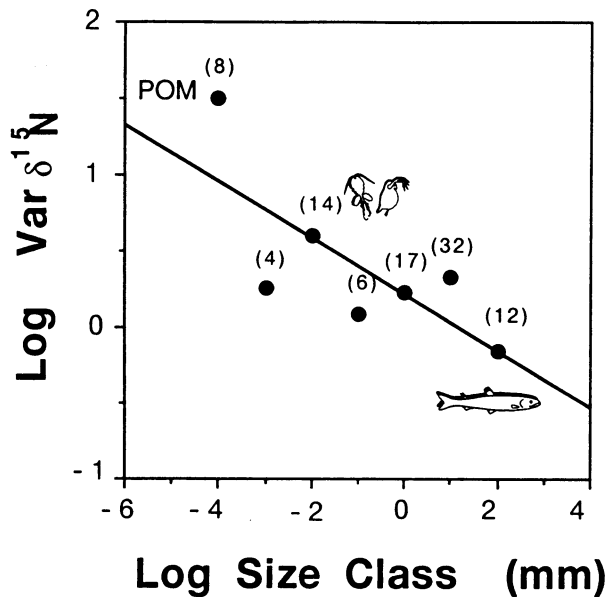†To whom reprint requests should be addressed.

Ecology: Cabana and Rasmussen

*Proc. Natl. Acad. Sci. USA 93 (1996)* 10845



FIG. 1. Arithmetic average of temporal variance in $\delta^{15}N$ plotted as a function of organism or particle logarithmic size class. Temporal variability in $\delta^{15}N$ is much greater in plankton-sized organisms than in fish and macroinvertebrates. For example, the temporal variance of phytoplankton-size ($10^{-4}$ mm) organisms or particulate organic matter (POM) is expected to be about 10 times as large as that of a large invertebrate or fish ($10^2$ mm). To achieve the same precision in $\delta^{15}N$ as measured by the standard error, phytoplankton would have to be sampled 10 times more frequently than a large invertebrate or a fish The number of time series of $\delta^{15}N$ for each size class is shown in parenthesis for each average. Temporal series for each kind of organism/particle ranged from 1 to 12 months and the number of $\delta^{15}N$ values per time series varied between 2 and 10. The least-square regression line is shown ($P < 0.001$). Data are from refs. 14–22, and 29.

(16), and input of low $\delta^{15}N$ nitrogen from fertilizers reaching the lake.

In addition to being related to watershed development, this between-lake variability in the signature of base consumers can also be very useful in correcting the signatures of higher level consumers to provide a more accurate reflection of their trophic position. To demonstrate this, we compared the $\delta^{15}N$ signatures of walleye (*Stizostedion vitreum*) and yellow perch (*Perca flavescens*), respectively, a top predator and an intermediate consumer (33, 34), with unionid mussels collected from the same lake and found them to be significantly correlated (Fig. 3), with perch tending to be on average nearly 6‰ (almost two full trophic levels) and walleye 8‰ above the primary consumer signal provided by the unionid mussels. Since walleye and yellow perch do not consume unionid mussels (34, 35), the covariation reflects the common influence of factors that affect the baseline variation of the $\delta^{15}N$ signature in these lakes. Although the $\delta^{15}N$ of walleye and yellow perch are highly variable across lakes, respectively, 70% and 30% of the variability seen in their $\delta^{15}N$ signatures reflect the variation in $\delta^{15}N$ at the base of the food chain, and therefore, only the residual variation should be considered to reflect between-lake variation in the length of the food chain leading up to these fish. We computed estimates of food-chain length for each lake by subtracting mean mussel $\delta^{15}N$ from fish $\delta^{15}N$ and then comparing these differences to the value of 3.4‰ expected for a single trophic level increment. Assigning the first trophic level to primary producers, fish feeding exclusively on herbivores, such as zooplanktivorous fish, would have a trophic position of 3 (as shown by the first dashed line at the left in Fig. 4); consumers feeding entirely on secondary consumers, such as a predator of zooplanktivorous fish, would
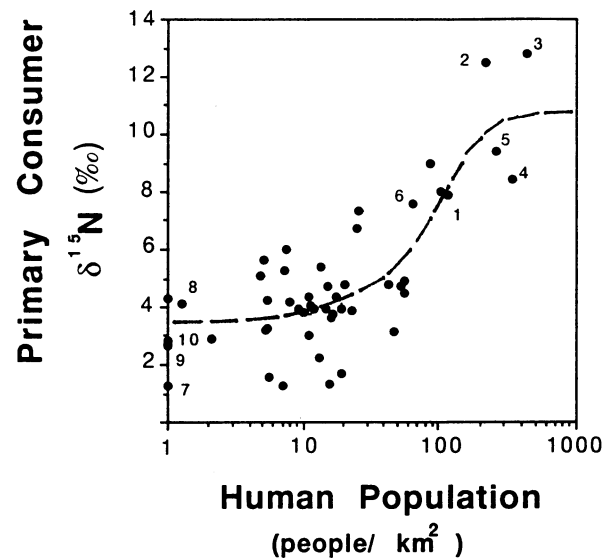


**Human Population**
**(people/ km²)**

FIG. 2. Variation in the $\delta^{15}N$ signature of primary consumers reflects the human population density of the watershed, likely a reflection of the high $^{15}N$ content of domestic sewage. Unionid mussel data (*Lampsilis* sp., *Anodonta* sp., and *Elliptio* sp.) from Quebec and Ontario lakes are show as unlabeled solid circles (present study). $\delta^{15}N$ data sources: 1, $\delta^{15}N$ data for zebra mussel (*Dreissena polymorpha*) from Lake Oneida, United States, were kindly provided by E. L. Mills (Cornell University); 2, gizzard shad (*Dorosoma cepedianum*), a herbivorous fish (24) from Lake Erie, Canada (present study); 3, average for four species of herbivorous cladocerans and copepods (*Eodiaptomus japonicus, Bosmina* sp., *Diaphanosoma brachyurum*, and *Cyclops vicinus*) from lake Kasumigaura, Japan, over 14 months (29); 4, average for six species of herbivorous zooplankton (*Eodiaptomus japonicus, Bosmina fatalis, Bosmina longirostris, Bosminopsis dietersi, Diaphanosoma brachyurum*, and *Cyclops vicinus*) over 7 months from lake Suwa, Japan (16); 5, average for four species of mollusks (*Assiminea lutea, Nuttalia olivacea, Corbicula japonica*, and *Laternula limicola*) from Gamo lagoon, Japan (30); 6, average for "benthic animals" from the Otsuchi river, Japan (30); 7 and 8, caddis fly larvae from Iliamna Lake and Sashin Creek, AL (13, 19); 9, average for four species of herbivorous chironomids (*Phaenospectra* sp., *Trichotanypus* sp., *Pseudodiamesa* sp., and *Orthocladius* sp.) over 2 months from an alpine lake in Colorado (31); 10, average $\delta^{15}N$ for the isopod *Asellus aquaticus* and the pelecypod *Pisidium* sp. from lake Blacksastjarn, Sweden (32). Watershed areas and human population were calculated from topographic and population maps, and governmental census data. The dashed curve fitted by the iterative nonlinear fitting method PROC NLIN, Marquardt method, SAS (33), $\delta^{15}N = 10.96/[1 + \exp(-0.84 - 0.0171_{pop})]$, accounted for 68% ($P < 0.001$) of the observed variation in $\delta^{15}N$ and the estimated asymptotes were 10.96‰ for aquatic systems completely dominated by anthropogenic nitrogen inputs and 3.30‰ for prisitine lakes and rivers. Sites from remote areas for which no detailed population census were available were considered to have a population density of 1 person per km² (these four data points were not included in the statistical analysis).

have a trophic position of 4. Finally, quaternary consumers feeding exclusively on tertiary consumers, such as a predator of piscivorous fish, would be calculated as having a trophic position of 5. The results indicate that the variation in trophic position of walleye and yellow perch relative to that of primary consumers spanned about one trophic level among lakes. For example, the lake-specific trophic position of yellow perch ranged between that of a secondary consumer (e.g., an invertebrate-feeding perch) and that of a tertiary consumer (e.g., a piscivorous perch), a range of variation that is consistent with the feeding ecology of this species (34). Among-lake variation in trophic position of about one trophic level was similarly observed in four other predatory fish species, largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieui*), rock bass (*Ambloplites rupestris*), and pike (*Esox lucius*)
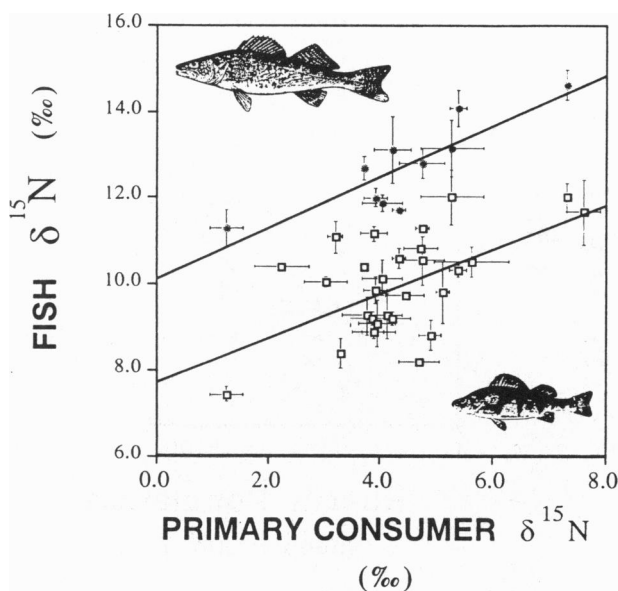
FIG. 3.   Mean (±SEM) $\delta^{15}N$ for walleye (solid circles, 9 lakes) and yellow perch (open squares, 29 lakes) plotted against mean (±SEM) $\delta^{15}N$ of primary consumers (unionid mussels). The $\delta^{15}N$ signature of each fish species reflects variation in the $\delta^{15}N$ of lower consumers of the same system. The least-squares regression line is shown for each species of fish ($r^2 = 0.70$ and $0.31$ for walleye and perch, respectively; $P < 0.01$ for both). Since walleye and yellow perch do not consume unionid mussels, the covariation reflects the common influence of factors that determine the baseline $\delta^{15}N$ in these lakes.

Table 1.   Among lake variation in the trophic position of six species of fish

| Species | Lakes | $r^{2*}$ | Trophic position,[†] min. to max |
|---|---|---|---|
| Walleye | 9 | 0.70[‡] | 4.0–4.8 |
| L. bass | 7 | 0.52[§] | 3.9–4.4 |
| Rock bass | 16 | 0.50[‡] | 3.5–4.4 |
| Pike | 10 | 0.41[§] | 3.6–4.4 |
| Perch | 29 | 0.34[§] | 3.0–3.8 |
| S. bass | 15 | 0.34[§] | 3.6–4.7 |

*% of total variance in fish $\delta^{15}N$ explained by mussel $\delta^{15}N$.
[†]Trophic position calculated as [(mean fish $\delta^{15}N$ − mean mussel $\delta^{15}N$)/3.4] + 2. Fish and mussel $\delta^{15}N$ values were based on 1–12 adult-size individuals per lake. All samples are from Quebec and Ontario, Canada (longitude = 72° to 80°; latitude = 44° to 47°). A total of 40 lakes were studied. Average SE of duplicate measurements made on a Europa Tracermass mass spectrometer was 0.3‰.
[‡]$P < 0.05$.
[§]$P < 0.01$.

(Table 1). Altogether, these $\delta^{15}N$ data from 40 lakes and 6 species of fish strongly suggest that the length of food chains leading up to these carnivorous fish varies only by about one trophic level among lakes. This pattern greatly contrasts with the results of food-web studies on similar lakes that were based on the presence or absence of species and their hypothesized feeding relationships (36, 37) rather than on a method reflecting patterns of mass transfer, like the nitrogen isotope method presented here. For example, a survey of zooplankton food webs (35) from the same region as the present study showed that the modal food chain length within lake as determined by the simple presence or absence of species varied between one and eight trophic levels among lakes. Clearly, this wide variation in food chain length within this lower trophic level community does not propagate itself in terms of mass transfer

to higher trophic level species, such as predatory fish, a likely result of variable omnivory (8) and the negligeable importance of many trophic links in terms of mass transfer.

Thus, isotopic signatures of higher trophic level consumers, if corrected for baseline $\delta^{15}N$ variation as indicated by long-lived primary consumers, will provide a measure of food-chain length related to bottom-up mass transfer that can be compared between lakes. This method will further strengthen the usefulness of this modeling approach, not only for the problem of spatial variation in contaminant biomagnification (8–12, 37–39) but also for the testing of hypotheses relating food-chain length to environmental and demographic variables (40–42). Unlike commonly reported estimates of food-chain length that are based on the enumeration of species and and their feeding relationships, the nitrogen isotope presented here does not require detailed taxonomic information on all the species present in the food web and is, therefore, free of the biases related to variable taxonomic resolution (43, 44). Our results also suggests that $\delta^{15}N$ signatures at the base of the food chain will provide a useful tool in the assessment of anthropogenic nutrient inputs such as human sewage, which has been identified as an important contributor to the nitrogen budget of aquatic systems on a global scale (45).
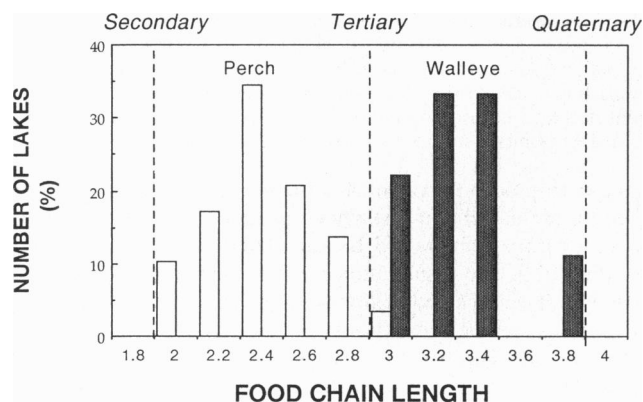
FIG. 4.   Frequency distribution of the lake-specific length of the food chain leading to walleye (solid bars) and to yellow perch (open bars) based on the $\delta^{15}N$ data presented in Fig. 3. For each lake, the length of the food chain is calculated as [($\delta^{15}N_{fish}$ − $\delta^{15}N_{mussel}$)/3.4] + 2. Thus a fish having a $\delta^{15}N$ signature exactly one trophic level (3.4‰) above that of primary consumers (mussel) would be considered to be at trophic level 3.

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