

## Challenges for interpreting stable isotope fractionation of carbon and nitrogen in tropical aquatic ecosystems

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### Introduction

Food web studies have greatly benefited from the use of stable isotope analyses, especially of carbon and nitrogen (FRY 2006). Understanding the isotope fractionation between consumers and prey is vital to constructing food webs, but this is not a simple relationship. Earlier field studies on pelagic temperate freshwater systems (VANDERZANDEN & RASMUSSEN 1999, 2001, POST 2002) indicated that fractionation of  $\delta^{13}\text{C}$  was  $\sim 0.5\text{‰}$  and of  $\delta^{15}\text{N}$   $\sim 3.4\text{‰}$  per trophic step. [Notation for trophic fractionation is  $\Delta\delta^{13}\text{C}$  and  $\Delta\delta^{15}\text{N}$ .] In their meta-analysis, VANDERZANDEN & RASMUSSEN (2001) found differences for  $\Delta\delta^{15}\text{N}$  between field and laboratory studies ( $3.4\text{‰}$  vs.  $2.7\text{‰}$   $\delta^{15}\text{N}$ , respectively) and noted large variations for herbivores, although this

was not observed by POST (2002). However, studies in tropical streams in Costa Rica and Puerto Rico (KILHAM & PRINGLE 2000) and in Panama (HUNTE-BROWN 2006, VERBURG et al. 2007) indicated that  $\Delta\delta^{15}\text{N}$  was much lower ( $\sim 1.6\text{‰}$ ) and of  $\Delta\delta^{13}\text{C}$  much higher ( $\sim 1.4\text{‰}$ ) per trophic step (Table 1).

One of the difficulties in comparing isotope fractionation in lakes and streams is that lake food webs tend to have distinct trophic categories, especially in pelagic food webs. It is often difficult to discern clear trophic levels in an examination of a biplot of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for stream food webs, however, possibly due to a greater degree of omnivory in streams, or simply because there is a much broader range of basal resources available that creates a number of simultaneous overlapping food chains. This can also be true for littoral

**Table 1.** Comparisons of average stable isotope fractionation (in ‰) of nitrogen ( $\Delta\delta^{15}\text{N}$  step<sup>-1</sup>) and carbon ( $\Delta\delta^{13}\text{C}$  step<sup>-1</sup>) across food webs in tropical and temperate aquatic ecosystems. Estimates were made based on averages of basal resources and top carnivores divided by the number of trophic steps.

	$\Delta\delta^{15}\text{N}$ step <sup>-1</sup>	$\Delta\delta^{13}\text{C}$ step <sup>-1</sup>	
<b>Tropical Streams:</b>			
Rio Salto, Costa Rica	1.1	0.9	Kilham & Pringle 2000
Quebrada Bisley, Puerto Rico	2.0	1.6	Kilham & Pringle 2000
Quebrada Toronja, Puerto Rico	2.0	2.0	Kilham & Pringle 2000
Rio El Cope, Panama 2000	1.8	1.8	Verburg et al. 2007
Rio El Cope, Panama 2004–5	1.7	0.8	Hunte-Brown 2006
Rio Fortuna, Panama	0.8	1.3	Hunte-Brown 2006
AVERAGE	1.6	1.4	
<b>Temperate Streams:</b>			
Valley Creek, PA	2.9	0.5	Kilham (unpublished)
Lower Lookout Creek, OR	2.2	1.0	Fry 1991
South Fork Eel River, CA (pools)	2.0	0.5	Finlay et al. 2002
South Fork Eel River, CA (riffles)	1.8	2.2	Finlay et al. 2002
AVERAGE	2.2	1.1	
<b>Tropical Lake:</b>			
Lake Tanganyika	2.4	1.5	Verburg 2004
<b>Temperate Lakes:</b>			
Lake Baikal	3.3	0.5	Yoshii et al. 1999
Lakes, Meta-analysis	3.4	0.4	Post 2002
Lakes, Meta-analyses	3.4	0.5	Vanderzanden & Rasmussen 1999, 2001

food webs in lakes (Post 2002). Basal resources in streams are often complex mixtures of materials with varying sources of both carbon and nitrogen. For example, biofilms on stream substrates, which are a dominant benthic food source, are generally mixtures of autotrophs and heterotrophs (Stock & Ward 1989). Furthermore, patchiness of resources is greater in streams than lakes, and flow variation is also greater.

A greater understanding of the important processes leading to this variation in stable isotope fractionation might lead to more information about interactions within food webs. We explored some of these sources of variation in stream food webs in several categories: turnover rates, trophic level, taxon, and environmental conditions.

**Key words:** food webs, isotope fractionation, stable isotopes, tropical streams

## Turnover rate

Stable isotopes in an organism represent an integration of food resources that have accumulated over time. Differences between tissue turnover times (O'Reilly et al. 2002) are sources of variation that must be accounted for in understanding fractionation. Long-lived predatory organisms in aquatic food webs tend to vary less in isotopic composition than those of their prey owing to their generally larger size, lower mass-specific growth, and lower metabolic rates. Olive et al. (2003) showed that the magnitude of trophic enrichment of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was sensitive to feeding rate, excretion rate, and degree of isotopic discrimination during food absorption and excretion. In an exploration of nitrogen turnover rates in tropical primary consumers, McIntyre & Flecker (2006) observed that turnover rates were higher in smaller organisms with higher metabolic rates, and higher in tropical species than in temperate species.

Fractionation can also vary markedly among tissues within an organism because of different rates of metabolic replacement (Gannes et al. 1997, McCutchan et al. 2003). Sweeting et al. (2007) showed that body size was not an important variable in a laboratory study of marine fish, but  $\Delta\delta^{15}\text{N}$  tended to decrease with increased temperature. Barnes et al. (2007) showed that  $\Delta\delta^{15}\text{N}$  decreased with increasing temperature, and  $\delta^{13}\text{C}$  fractionation increased with increasing temperature. This latter effect was attributed to higher lipid contents of tissues in fish reared at colder temperatures. They also suggested an average  $\delta^{13}\text{C}$  fractionation for fish of 2‰ was appropriate in food web studies.

DeNiro & Epstein (1977) showed that lipid synthesis discriminated against  $\delta^{13}\text{C}$ . Within an organism, lipids are depleted in  $^{13}\text{C}$  relative to proteins and carbohydrates by about 6–8‰ (more negative  $\delta^{13}\text{C}$ ). Pinnegar & Polunin (1999) agreed that differences in lipid content were a major determinant of variation in  $\delta^{13}\text{C}$  among tissues. Post et al. (2007) have further explored the issue of lipid content of organisms and showed that this must be taken into consider-

ation in samples from organisms with high lipids. They showed that a sample with high lipid concentration that had not been lipid-extracted would be 3–4‰ more negative than an extracted sample. For aquatic animals, if tissues are <5% lipid or have C:N mass ratio of <3.5, then extraction is not necessary. For higher values, lipid extraction should be considered. For fish tissues, Pinnegar & Polunin (1999) suggest using white muscle tissue because it has the lowest lipid content and is most convenient for comparing both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among species. Sweeting et al. (2007) showed that different tissues had variable fractionation values for  $\delta^{15}\text{N}$ , with muscle tissues ~3.9‰ and liver ~2.0‰ in experiments on fish reared over 2 years on particular diets. They concluded that a  $\Delta\delta^{15}\text{N}$  value of 2.9‰ for whole fish samples is most appropriate.

Trophic fractionation for nitrogen mostly results from the balance between assimilation and nitrogenous excretion (Ponsard & Averbunch 1999). During ingestion, food products are transported across the gut wall, which generally results in depletion of the heavier isotope; therefore, the feces are usually enriched in  $\delta^{15}\text{N}$ . Nitrogen excretory products contain more of the lighter isotope than the organism as they are transported away, so the organism will become enriched in the heavier isotope, creating  $\delta^{15}\text{N}$  increases with trophic steps in a food web. Olive et al. (2003) developed a detailed model to explain the effect of assimilation and excretion on stable isotope trophic fractionation. They concluded that the magnitude of the trophic step enrichment is most sensitive to the degree of isotopic discrimination during food absorption and excretion.

## Trophic level

Organisms at different trophic levels have somewhat different trophic fractionation. A meta-analysis of laboratory studies by McCutchan et al. (2003) showed that variation in trophic enrichment was due to differences in diet and the method of sample preparation. For  $\delta^{13}\text{C}$ , they found an average  $\Delta\delta^{13}\text{C}$  of 0.3‰ for consumers analyzed whole and  $\Delta\delta^{13}\text{C}$  of 1.3‰ for consumers analyzed as muscle tissue. Diet also had an impact. They found that  $\Delta\delta^{15}\text{N}$  values were 1.4‰ for consumers raised on invertebrate diets, 3.3‰ on high protein diets, and 2.2‰ on plant or algal diets. In another meta-analysis of experiments in which diets were known and controlled, Vanderklift & Ponsard (2003) found that  $\Delta\delta^{15}\text{N}$  in both terrestrial and aquatic organisms had an overall average of 2.54‰. They showed that variation was introduced by type of nitrogenous excretion. Most aquatic organisms are ammonolectic, which averaged 2.0‰ per trophic step. Interestingly, marine organisms had much lower  $\Delta\delta^{15}\text{N}$  (1.4‰) than freshwater organisms (2.8‰), a trend also observed by Vanderzanden & Rasmussen (2001). Herbivores tended to have higher  $\Delta\delta^{15}\text{N}$  (3.0‰) than carnivores (2.7‰) and omnivores (2.6‰). Detritivores, however, had much lower  $\Delta\delta^{15}\text{N}$  (0.5‰). Goedkoop et al. (2006) also found  $\Delta\delta^{15}\text{N}$  was very low for detritus-

fed midges. They observed the lowest fractionation rates in food sources with the highest N content and a 4-fold greater  $\Delta\delta^{15}\text{N}$  in food with low N content. Similarly, ADAMS & STERNER (2000) reported that  $\Delta\delta^{15}\text{N}$  between *Scenedesmus* and *Daphnia* was inversely related to the N content of the food.

LANCASTER & WALDRON (2001) investigated within-population variation in stable isotopes in 8 populations of lotic invertebrates. Predatory nymphs were 2‰ higher than their mayfly prey. They observed that carnivores in different food chains within the same food web had  $\delta^{15}\text{N}$  values that indicated they were feeding on different basal resources, which more strongly affected their  $\delta^{15}\text{N}$  values than trophic position. This is one reason that discerning trophic position in streams from an examination of a biplot of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  is difficult. The wide variations in  $\delta^{15}\text{N}$  observed in primary consumers were ascribed at least in part to differential digestion and assimilation of food types such as leaf detritus or periphyton. Variations in  $\delta^{13}\text{C}$  were related to lipid content. They concluded that species from the same functional feeding group and with similar diets can have different isotope values and related some of this variation to relative mobility of species. Individuals with higher mobility exhibited little within-population variation in isotope values because they wander throughout the stream habitat encountering the same range and relative abundance of food, so variation in fractionation produced by faster tissue turnover times is likely to be high relative to variation resulting from food intake.

Taxon-specific effects on trophic fractionation have not been widely researched. VANDERKLIFF & PONSARD (2003) showed that  $\Delta\delta^{15}\text{N}$  was greater for vertebrate consumers (2.9‰) than for invertebrate consumers (2.1‰) at the same trophic level in laboratory studies. However, for biota in temperate lakes, VANDERZANDEN & RASMUSSEN (2001) found  $\Delta\delta^{15}\text{N}$  to be 3.2‰ for carnivores and 2.5‰ for herbivores. They found no significant differences among taxonomic categories for  $\Delta\delta^{13}\text{C}$ .

## Environmental conditions

Watershed area can affect variation in  $\delta^{13}\text{C}$ . FINLAY (2001) found that as watershed size increased, the major source of carbon for organisms came increasingly from autochthonous production. He found a transition from terrestrial to algal carbon sources for lotic food webs as watershed area increased in size to  $>10\text{ km}^2$ . FINLAY (2004) found that the availability of  $[\text{CO}_2\text{ aq}]$  affected fractionation of  $\delta^{13}\text{C}$  during photosynthesis in autotrophs in temperate streams. When  $[\text{CO}_2\text{ aq}]$  was low, discrimination was lower and  $\delta^{13}\text{C}$  in autotrophs was enriched; when  $[\text{CO}_2\text{ aq}]$  was higher, discrimination was greater and  $\delta^{13}\text{C}$  was depleted in autotrophs. He further showed a strong relationship between  $\delta^{13}\text{C}$  in the epilithon and herbivores, with an average fractionation of 0.34‰  $\delta^{13}\text{C}$ . Flow rate has a strong effect on fractionation from the aqueous phase into autotrophs (FINLAY et al. 1999) because increased water velocity increases the supply of  $\text{CO}_2$  to benthic algae, and discrimination against

$^{13}\text{CO}_2$  during photosynthesis increases with  $\text{CO}_2$  availability. The large degree of patchiness in  $\delta^{13}\text{C}$  of epilithic algae, owing to variation in  $[\text{CO}_2\text{ aq}]$  as a result of differential sources of dissolved inorganic carbon and flow rate, provides a wide range of variation in basal food resources. This is likely a major contributor to the large variation in  $\delta^{13}\text{C}$  often observed in herbivores in lotic systems.

Microbial conditioning of food particles can affect dietary isotope signatures. In laboratory studies of deposit-feeding midges, GOEDKOOP et al. (2006) observed a doubling of  $\delta^{15}\text{N}$  (from 6.2 to 11.4‰) after 8 days of microbial colonization on the food particles used in the experiments. In nature, this effect can certainly introduce variation in basal food resources. In studies in Panama, upland streams with tadpoles had higher  $\delta^{15}\text{N}$  values in basal resources than streams in which amphibians had dramatically declined, attributed at least in part to tadpole feeding, egestion, and excretion (HUNTE-BROWN 2006, WHILES et al. 2006).

Note that none of these environmental variables (except temperature) directly affect fractionation rate *per se*. They do affect the stable isotope values of the consumers by changing values in the diets. When detailed diet information is not available, these environmental variables can lead to erroneous estimates of fractionation between diet and consumer.

## Comparisons among food webs

Information on trophic fractionation of stable isotopes helps us understand the often great complexity of food webs, but we need a better knowledge of the factors that introduce variation into this process, some of which were discussed above. How do we make comparisons among food webs using this information?

One approach has been to use mixing models to more quantitatively describe food web interactions (PHILLIPS & KOCH 2002, PHILLIPS & GREGG 2003). However, these models require an isotope fractionation value between consumer and prey to a high degree of accuracy. Small variations (e.g., 0.2‰ of  $\delta^{15}\text{N}$ ) can lead to large changes in interpretation (HUNTE-BROWN 2006); thus, using some mean value, such as  $\Delta\delta^{15}\text{N}$  of 3.4‰, derived from a meta-analysis is usually not appropriate.

Recently, LAYMAN et al. (2007) proposed a method to quantitatively characterize community-wide aspects of trophic structure. They proposed using the convex hull area occupied by species in  $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$  niche space as a representative of the total extent of trophic diversity within a food web. They also used mean nearest-neighbor distance among all species pairs as a measure of species-packing within trophic niche space. They described a total of 6 community-wide metrics that reflect different aspects of trophic structure, which allows for comparisons of different food webs along environmental gradients, or the same food web under changing conditions. However, they acknowledged that caution must be applied when differential fractionation occurs within the food web. The metrics will be most infor-

mative in systems when distinct feeding niches are reflected by different positions of species in  $\delta^{13}\text{C}$ – $\delta^{15}\text{N}$  niche space.

In studying tropical aquatic food webs, we have noted that trophic fractionation of nitrogen ( $\Delta\delta^{15}\text{N}$ ) is generally much less and trophic fractionation of carbon ( $\Delta\delta^{13}\text{C}$ ) much greater than in temperate aquatic food webs. This observation induced us to explore the causes of variation in fractionation. We propose here another metric for comparisons among aquatic food webs: average fractionation across the entire food web. This requires that basal resources (carbon sources) and top carnivores be adequately represented in the food web description. The average value of each of the isotopes is determined for all of the potential basal resources and for the top predators. For each isotope, the values are subtracted from each other and divided by the number of trophic steps in that particular food web. Our estimation of this metric and comparison of tropical and temperate ecosystems (Table 1) show that the general observation holds for lower  $\Delta\delta^{15}\text{N}$  and higher  $\Delta\delta^{13}\text{C}$  for tropical systems compared to temperate systems. In addition, streams tend to have lower average  $\Delta\delta^{15}\text{N}$  and higher  $\Delta\delta^{13}\text{C}$  than lakes within either temperate or tropical systems, and higher  $\Delta\delta^{13}\text{C}$  than lakes within temperate systems.

## Summary

Stable isotopes are useful for elucidating food webs, and one essential aspect of interpretation is accurately determining the enrichment between trophic levels, especially when used in mixing models. The fractionation of the stable isotopes of nitrogen ( $\Delta\delta^{15}\text{N}$ ) and carbon ( $\Delta\delta^{13}\text{C}$ ) between trophic levels in tropical aquatic ecosystems seems to differ compared to typical values found in temperate aquatic ecosystems of about 3.4‰ for  $\delta^{15}\text{N}$  and 0.5‰ for  $\delta^{13}\text{C}$ . In recent studies of upland stream ecosystems in Panama, with and without tadpoles, we found lower fractionation of  $\delta^{15}\text{N}$ , typically 1.0–1.7‰, and much higher fractionation of  $\delta^{13}\text{C}$ , typically 1–1.6‰. Similar values have been observed in other tropical systems, including Puerto Rico and Costa Rica streams and Lake Tanganyika. These large differences in trophic fractionation are enigmatic. We explore sources of variation in fractionation such as tissue turnover rate, stream flow, taxonomic differences, functional group differences, dietary balance, growth rate, and microbial activity to assess possible contributing factors. The ecological information embedded in this variation in trophic fractionation should be embraced and exploited.

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