

## Interpreting stable isotopes in food webs: Recognizing the role of time averaging at different trophic levels

**Abstract**—Lake Tanganyika, East Africa, has a simple pelagic food chain, and trophic relationships have been established previously from gut-content analysis. Instead of expected isotopic enrichment from phytoplankton to upper level consumers, there was a depletion of  $^{15}\text{N}$  in August 1999. The isotope signatures of the lower trophic levels were an indicator of a recent upwelling event, identified by wind speed and nitrate concentration data, that occurred over a 4-d period several days prior to sampling. The isotope structure of the food web suggests that upwelled nitrate is a nutrient source rapidly consumed by phytoplankton, but the distinctive signature of this nitrate is diluted by time averaging in the upper trophic levels. This time averaging is a consequence of the fact that the isotopic signature of an organism is related to variable nitrogen sources used throughout the life of the organism. This study illustrates the importance of recognizing differences in time averaging among trophic levels.

Stable isotopes are becoming a standard analytical tool in food web ecology. Differences in carbon and nitrogen isotope ratios between consumers and their diet provide information on energy flows, nutrient sources, and trophic relationships. Typically, carbon provides information on the primary energy source (e.g. benthic vs. pelagic photosynthesis), while nitrogen allows discrimination among trophic levels. Relative enrichment with increasing trophic level often allows a better interpretation of dietary relationships than gut-content analysis alone because isotope ratios record material that is actually assimilated (Michener and Schell 1994). Recent field studies have shown an average enrichment of  $0.05\text{‰} \pm 0.63 \delta^{13}\text{C}$  and  $3.49\text{‰} \pm 0.23 \delta^{15}\text{N}$  (Vander Zanden and Rasmussen in press) and are similar to the commonly used trophic fractionation values of  $1\text{‰} \delta^{13}\text{C}$  and  $3.4\text{‰} \delta^{15}\text{N}$  (Michener and Schell 1994). The ease of stable isotope analyses makes them an appealing tool in ecology, but care must be taken in interpretation of the results (Gannes et al. 1997). An isotopic ratio of an organism is usually understood to represent its diet, but it should be remembered that this isotopic ratio is also time specific, representing an average ratio related to tissue turnover rate and the life of the organism.

The pelagic food web in Lake Tanganyika, East Africa, provided an excellent example of how stable isotopic analyses may not be straightforward. Lake Tanganyika is a deep (mean 570 m; max 1470 m), large (mean width 50 km; length 650 km) lake located a few degrees south of the equator. The lake is permanently stratified and is anoxic below approximately 150 m. Lake Tanganyika has a simple pelagic food web, and trophic relationships have been established previously from gut-content analysis (Fig. 1) (Coulter 1991). The zooplankton are dominated by the copepod *Tropodiatomus simplex*, a major dietary component of the two clupeid fish species, *Stolothrissa tanganyicae* and *Limnothrissa mio-*

*don*. The upper trophic level is represented by four *Lates* species. Of these, *Lates angustifrons* and *Lates mariae* rely on nearshore fishes as a food source throughout their lives. *Lates microlepis* spend their larval and juvenile stages near shore and recruit to the pelagic as adults, whereas *Lates stappersi* has a fully pelagic life cycle. Thus, the most abundant species of the pelagic food web form a linear food 'chain' from phytoplankton to the copepod *T. simplex* to the zooplanktivorous *Stolothrissa* to the predatory *L. stappersi*. This linear food chain should be apparent as a distinct sequential enrichment in the carbon and nitrogen isotopes with increasing trophic level (Michener and Schell 1994).

**Methods**—Our study took place near Kigoma, Tanzania, during the dry season. Food web samples were collected approximately 15 km offshore ( $4^{\circ}50'S$ ,  $29^{\circ}29'E$ ) during the night of 1 August 1999. Phytoplankton were collected using vertical tows with a  $50\text{-}\mu\text{m}$  mesh net ( $n = 6$ ). Samples were filtered through  $100\text{-}\mu\text{m}$  mesh to remove zooplankton, then collected on  $0.45\text{-}\mu\text{m}$  glass-fiber filters and rinsed with 0.01 N HCl and distilled water. Zooplankton were caught using vertical tows with a  $100\text{-}\mu\text{m}$  mesh net ( $n = 4$ ). They were placed in filtered lake water for 2 h to clear gut contents then collected on  $0.45\text{-}\mu\text{m}$  glass-fiber filters and rinsed with 0.01 N HCl and distilled water. Fish specimens of *Stolothrissa* ( $n = 6$ ), *Limnothrissa* ( $n = 5$ ), *Lates stappersi* juveniles ( $n = 4$ ), and *Lates stappersi* ( $n = 4$ ) were obtained from local fishermen who were fishing adjacent to the site when the plankton samples were collected. For all species except *Stolothrissa*, we collected a section of white muscle tissue from behind the dorsal fin. As *Stolothrissa* were too small to obtain a large muscle sample, we used the entire body after removing the head, tail, and viscera. Fish samples were washed with distilled water, dried, and homogenized before analysis. All samples were dried at  $50^{\circ}\text{C}$  and stored wrapped in aluminum foil.

Samples were analyzed at the University of Waterloo Environmental Isotope Lab on an isochrom continuous flow stable isotope mass spectrometer (Micromass) coupled to a Carla Erba Elemental Analyzer (CHNS-O EA1108). The isotope ratios are expressed in delta notation with respect to deviations from standard reference material (Pee Dee belemnite carbon and atmospheric nitrogen). Standard error is  $0.2\text{‰}$  for carbon and  $0.3\text{‰}$  for nitrogen. Statistical analyses were done using JMP IN (SAS Institute).

**Isotope structure of the food web**—Overall, the isotope structure of the pelagic food web was not that of a linear food chain (Fig. 2). There was a general trend of carbon enrichment but a depletion of nitrogen with trophic level increase. The enrichment in  $\delta^{13}\text{C}$  across the lower food web from phytoplankton to *Stolothrissa* was larger than would

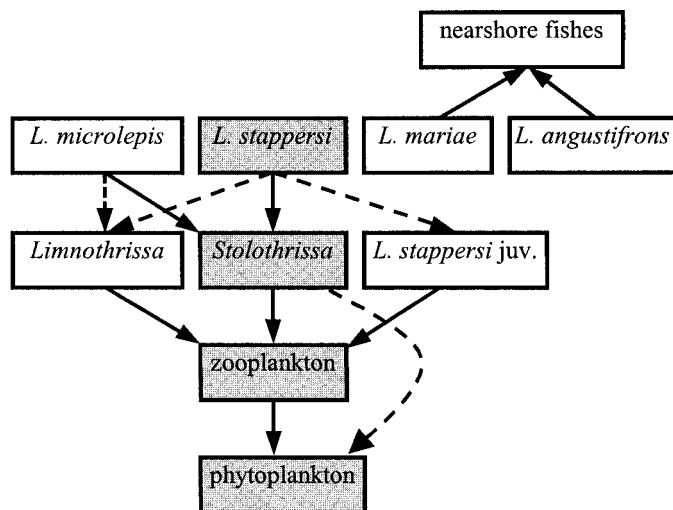


Fig. 1. The pelagic food web of Lake Tanganyika. The solid lines indicate major food preferences; the dashed lines indicate other prey relationships. *Lates mariae* and *Lates angustifrons* also include a minor proportion of *Stolothrissa tanganyicae* and *Limnothrissa miodon* among their prey. The shaded boxes represent the dominant species of the pelagic zone and illustrate the linear food chain relationship. Modified from Coulter (1991).

be predicted. We expected a total  $\delta^{13}\text{C}$  enrichment of 2‰ across two trophic levels (Michener and Schell 1994), while the enrichment here was 4‰. Furthermore, the  $\delta^{15}\text{N}$  ratios did not show the expected 3.4‰ enrichment with increasing trophic level.

*The upper food web*—For the upper pelagic food web, isotopic analyses were consistent with the expected diet specificity in these fish species. The primary prey item for adult *L. stappersi* is *Stolothrissa*, and this relationship was clearly seen in the isotope data, where *L. stappersi* was approximately 1‰ enriched in  $\delta^{13}\text{C}$  and 2‰ enriched in  $\delta^{15}\text{N}$  relative to *Stolothrissa*. Juvenile *L. stappersi* were significantly depleted in  $\delta^{15}\text{N}$  compared to the adults (one-sided *t*-test,  $p < 0.02$ ), which reflects the fact that their diet includes relatively more copepods (Mannini et al. 1999). The other clupeid species, *Limnothrissa*, includes smaller *Stolothrissa* in its diet, which is likely the reason for its isotopic enrichment relative to *Stolothrissa*.

*A recent change in the nutrient source*—We used the isotopic ratios of the fish species and established isotopic relationships between trophic levels to predict isotope values for the primary energy source (Fig. 2). Both zooplankton and phytoplankton are depleted in  $\delta^{13}\text{C}$  compared to predicted ratios. Given the  $\delta^{15}\text{N}$  values of the upper food web and likely importance of atmospheric N fixation in Lake Tanganyika (Hecky 1991), we predicted phytoplankton would have a  $\delta^{15}\text{N}$  value near 0‰. Instead, phytoplankton had a  $\delta^{15}\text{N}$  of between 6 and 8‰, similar to that of zooplankton and slightly enriched compared to the upper consumer levels. A discrepancy of this magnitude cannot be explained easily by either a change in phytoplankton productivity rates

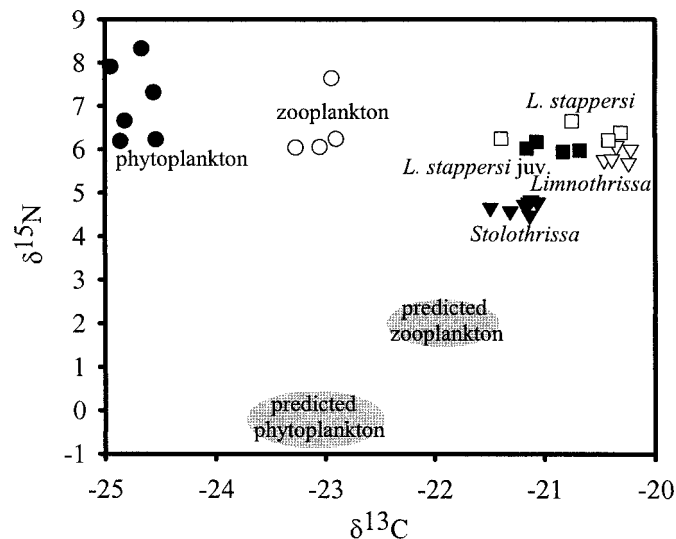


Fig. 2. The isotope structure of the pelagic food web. Each data point represents an individual organism. Predicted values for zooplankton and phytoplankton are based on isotopic values of the upper food web using standard trophic enrichments (1‰  $\delta^{13}\text{C}$  and 3.4‰  $\delta^{15}\text{N}$ ) and the enrichments seen in the upper food web (1‰  $\delta^{13}\text{C}$  and 2‰  $\delta^{15}\text{N}$ ). Closed circles are phytoplankton, open circles are zooplankton, solid triangles are *Stolothrissa*, open triangles are *Limnothrissa*, solid squares are *Lates stappersi* juveniles, and open squares are *L. stappersi* adults.

or species composition and thus implies a change in nitrogen source.

Although the primary sources of new nitrogen are atmospheric deposition and biological nitrogen fixation, internal loading of deep-water nutrients is also an important nutrient source for the pelagic zone (Hecky et al. 1991). Lake Tanganyika is permanently stratified, but strong winds during the dry season cause seiche activity with a 28- to 36-d period, leading to episodic vertical metalimnion entrainment (Plisnier et al. 1999). Wind speeds vary diurnally, but daily mean speeds were significantly higher in the week preceding sampling ( $1.48 \pm 0.09 \text{ m s}^{-1}$ ) than in the week following sampling ( $0.81 \pm 0.08 \text{ m s}^{-1}$ ), with four consecutive days where speeds were higher than the long-term seasonal mean (Johannes et al. 1999). Concurrently, nitrate concentration profiles showed upwelling of nitrate from 100-m deep around 28 July (Fig. 3) (Johannes et al. 1999). These data provide strong evidence that an upwelling event occurred 4 to 6 d prior to sampling the food web.

This deep-water nitrogen is likely enriched in  $^{15}\text{N}$  (Francois et al. 1996). The metalimnion of Lake Tanganyika has low oxygen levels and elevated nitrate concentrations (Hecky et al. 1991). As denitrification occurs in the suboxic section of the water column, the lighter isotope is selectively removed and the remaining nitrate becomes increasingly enriched in  $^{15}\text{N}$ . Field studies in temperate lakes have shown that fractionation during nitrogen assimilation by phytoplankton can be  $-4$  to  $-5\%$  if nitrogen is in excess (Fogel and Cifuentes 1993), which implies that the nitrogen source for these phytoplankton would require a  $\delta^{15}\text{N}$  of at least 10 to 14‰. Denitrification has fractionations in the range of 10

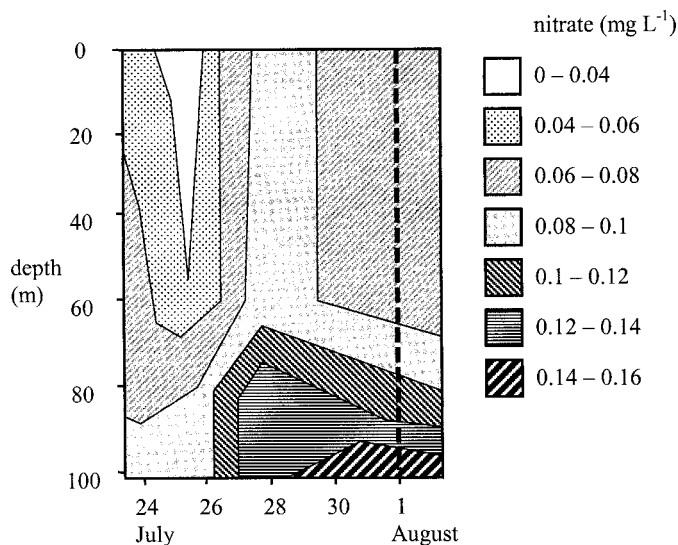


Fig. 3. Nitrate concentration profiles. Nitrate concentrations from surface waters to the upper metalimnion off Kigoma Bay throughout the week prior to sampling. The dashed line indicates the sampling date. Upwelling of water from 100-m deep is indicated by the increase in nitrate concentrations around 28 July. Data from Johannes et al. (1999).

to 30‰ (Wada and Hattori 1991), which would lead to an enriched nitrate pool in the suboxic metalimnion of Lake Tanganyika. For other lakes, investigators have measured deep-water nitrate values of 15.1‰ (Yoshioka et al. 1988) or have calculated values between 10 to 30‰ (Teranes and Bernasconi 2000).

A short-term, episodic nutrient input from deeper water to the epilimnion would alter phytoplankton isotopic signals, eventually causing them to have  $\delta^{15}\text{N}$  signatures in the range observed in this study. With high nitrate concentrations, discrimination against the heavier nitrogen isotope occurs and initially phytoplankton are depleted relative to dissolved nitrogen. Following Rayleigh fractionation kinetics, the remaining nitrate becomes relatively enriched. As the algal bloom continues, however, demand for nitrogen remains high and discrimination against the heavier isotope decreases as nitrogen concentrations decline. The pelagic zone of Lake Tanganyika is usually nitrogen limited (Hecky et al. 1991), implying that Rayleigh fractionation continues to near completion. Thus, phytoplankton eventually attain an isotope value similar to that of their nitrogen source.

Supporting evidence that phytoplankton were recording upwelling in their nitrogen isotopic ratios existed in their carbon isotope signal. Under conditions of high nutrient availability, phytoplankton productivity increases and discrimination against the heavier carbon isotope eventually decreases, giving them an enriched carbon signal (Farquhar et al. 1989). The fact that the phytoplankton signal in Lake Tanganyika was relatively depleted after upwelling indicates a source of depleted dissolved inorganic carbon (DIC). In general, deep-water DIC is depleted relative to surface DIC through dissolution and oxidation of carbonate and organic carbon. Craig (1974) found that metalimnetic DIC in Lake Tanganyika was depleted by 1.5‰ relative to surface water.

Uptake of this upwelled DIC by phytoplankton would cause them to have a  $\delta^{13}\text{C}$  value that was depleted compared to average conditions.

Time averaging explains the lack of trophic enrichment with respect to  $\delta^{15}\text{N}$  and the large  $\delta^{13}\text{C}$  enrichment (2‰) between the lower trophic levels. This straightforward food chain has different turnover times associated with each trophic level. Phytoplankton population growth rates are  $1.2\text{ d}^{-1}$  or higher (Hecky 1991), and thus their stable isotope signal represents carbon and nitrogen uptake and sources over the last few days. Preliminary evidence suggests that the time to full development for copepods in Lake Tanganyika is 31 to 45 d (Hyvonen 1997); thus, their isotopic ratios represent an average of phytoplankton consumed both preupwelling and postupwelling. The clupeid *Stolothrissa integrata* integrates diet over a period of several months to 1 year, and the predatory *L. stappersi* has a life span of several years (Coulter 1991). The effect of this greater temporal integration is seen in the upper food web in Lake Tanganyika, where the short-term fluctuation in nutrient source is not apparent. Time averaging of this type has been invoked in Lake Ontario, where the isotopic signature of late summer zooplankton may be reflecting phytoplankton consumed earlier in the summer (Leggett et al. 1999).

In summary, this study illustrates the importance of understanding the temporal resolution of different trophic levels and the effect of time averaging in stable isotope ecology. Isotopic signals of primary producers are subject to greater variation than other trophic levels in this system because of constantly changing nutrient sources and concentrations. It is precisely because of this variation that food web modeling using isotopes is usually based on the isotope signal of the primary consumer, whose longer term integration is assumed to reduce the short-term variability found in primary producers (i.e., Post et al. 2000). However, this work suggests that temporal variation may be significant at the primary consumer level and could affect assessment of relative trophic level. Temporal integration must be considered when comparing organisms whose isotopic compositions average over different time scales.

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## Photoreceptors in the cnidarian hosts allow symbiotic corals to sense blue moonlight

**Abstract**—In many species of symbiotic corals, spawning occurs synchronously several nights after the full moon. This process is correlated with the level of lunar irradiance, but the mechanism by which these cnidarian/zooxanthellate symbioses can detect such low levels of light remains unknown. Here we report the first biophysical evidence that the host animal exhibits extraordinarily sensitive photoreception in the blue region of the spectrum. Using a high-resolution laser-induced signal to detect tentacle scattering, we measured the effect of low irradiance on the contraction of polyps in the corals that normally have their tentacles extended in darkness. Similar to

most deep-sea invertebrates, the action spectra of coral photoreception reveal a maximum sensitivity in the blue, at 480 nm, with a spectral band width (at full-width half-maximum) of ca. 110 nm. The spectra closely overlap the maximal transparency of oligotrophic tropical waters, thus optimizing the perception of low light at depth. The detected threshold of photoreception sensitivity is  $\sim 1.2 \times 10^{15}$  quanta  $\text{m}^{-2} \text{s}^{-1}$  in the blue region. This makes corals capable of sensing the blue portion of lunar irradiance, as evidenced from the recorded slight contractions of polyp tentacles under variations in moonlight intensity.