

Seasonal variation in stable isotope ratios of stream algae

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Introduction

Stable isotopes of carbon and nitrogen are commonly used to trace pathways of organic matter through food webs. Isotope ratios are generally expressed in δ notation. For carbon, $\delta^{13}\text{C}$ signifies the ‰ difference in the $^{13}\text{C}:^{12}\text{C}$ ratio between a sample and a carbonate standard. $\delta^{15}\text{N}$ signifies the ‰ difference in the $^{15}\text{N}:^{14}\text{N}$ ratio between a sample and N_2 in air. Where the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ differs between two sources of organic matter, isotope ratios of a consumer will reflect the relative contributions of the two sources to the growth of the consumer, after correction for the isotopic shift (fractionation) between diet and consumer. Fractionation is small for carbon (i.e. less than 1‰; DENIRO & EPSTEIN 1978, FRY & ARNOLD 1982, PETERSON & HOWARTH 1987) but larger for nitrogen (1–5‰; DENIRO & EPSTEIN 1981, MINAGAWA & WADA 1984). In fact, the step-wise increase in $\delta^{15}\text{N}$ between trophic levels is often used to estimate trophic positions of consumers (WADA et al. 1991).

Estimations of food sources of consumers by use of standard mixing models involve two assumptions: (1) that isotope ratios of food sources have remained constant over recent time; and (2) that the isotope ratio of a consumer reflects recent feeding activity. The degree to which sources and consumers meet these assumptions has seldom been examined critically.

Stable isotope ratios of terrestrial plants can change seasonally, but the degree of seasonal change usually is small compared to variation among individuals in a particular area (LOWDON & DYCK 1974, MCARTHUR & MOORHEAD 1996). Stable isotope ratios of aquatic primary producers can, however, show substantial seasonal change (CIFUENTES et al. 1988, BERNASCONI et al. 1997). Temporal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of algae and aquatic plants has several causes (GOERICKE et al. 1994). Firstly, isotope ratios of inorganic C and N may change seasonally and thus induce changes in the isotope ratios of primary producers. In addition, changes in the availability of inorganic C or N can lead to changes in isotope ratios of aquatic plants and algae, even when

the ratio of the inorganic source is constant. When availability of inorganic nutrients is low, the potential for fractionation between inorganic and organic forms is diminished and isotope ratios of producers may approach the ratios of inorganic sources. Growth rates of producers also can affect isotope ratios because isotope ratios of producers reach equilibrium with inorganic C and N most rapidly when producers are growing rapidly.

When the isotope ratios of a food source are changing, isotope ratios of consumers reflect ratios of sources at the time of sampling (after correction for fractionation by consumers) only if the rate of tissue turnover in consumers is much higher than the rate of change in isotope ratios of sources. Isotope ratios of consumers do track changes in isotope ratios of their food sources, but may lag behind changes in sources even when consumers are growing rapidly (FRY & ARNOLD 1982, HESSEIN et al. 1993). If consumers are growing slowly, their isotope ratios may deviate greatly from ratios of food sources (FRY & ARNOLD 1982, SCRIMGEOUR et al. 1995).

The purposes of this paper are: (1) to report large seasonal changes in stable isotope ratios of stream algae, (2) to show that isotope ratios of consumers can fall outside the range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for food sources if isotope ratios of food sources change over time, and (3) to demonstrate that estimation of food sources is possible when isotope ratios of sources change.

Study site

Data are from a 2-year study of St. Vrain Creek in the Front Range of the Colorado Rockies. The study site is a fifth order reach, located at 40° 13' 05" N latitude and 105° 15' 34" W longitude, at the edge of the Great Plains. Discharge in St. Vrain Creek is dominated by snowmelt from March to September. Vegetation in the watershed is largely pine and spruce–fir forest. Alpine vegetation and talus fields are present above 3,300 m and deciduous trees replace conifers in the riparian zone at low elevation. WARD (1986, 1992) provides additional information on St. Vrain Creek.

Methods

Collection and processing of samples

Macroinvertebrates and their potential food sources were collected on a monthly basis; sampling was less frequent during peak discharge and in mid-winter. Epilithon was scrubbed from rocks and filamentous algae were collected when present in sufficient quantity. Epilithon samples were agitated and sonicated gently to disaggregate cells, passed through 53- μm Nitex mesh, and centrifuged through Ludox AM (DuPont) colloidal silica (specific gravity 1.17; HAMILTON et al. 1992, HAMILTON & LEWIS 1992). Centrifugation typically resulted in separation of bulk epilithon into three fractions. A green band, highly enriched in unicellular algae, was usually found at the top of the silica suspension. The majority of vascular plant detritus was found below the algae, but still near the top of the centrifuge tube. Inorganic material, diatoms, and amorphous detritus were found in a third layer, below the silica. Material from each layer was removed by pipette and collected on Whatman QM/A filters. Additional samples of vascular plant material were collected from live riparian plants. Samples of filamentous algae were rinsed and cleared of non-algal material under a dissecting microscope. Benthic invertebrates were held overnight in filtered stream water to achieve gut clearance, lyophilized, and weighed to the nearest microgram. Samples of algae and terrestrial plant material were lyophilized and all samples were analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with a Fisons Optima mass spectrometer, operated in conjunction with an elemental analyzer. Precision (± 1 SD) for replicate standards was typically better than 0.1‰.

Computer spreadsheet model

A computer spreadsheet model was used to estimate the contribution of algae and terrestrial plant material to the growth of consumers. The spreadsheet model predicts changes over time in isotope ratios of consumers and incorporates temporal changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of food sources, biomass dilution (consumer growth and tissue turnover), and correction for fractionation between diet and consumer. The relative contribution of each food source to the growth of consumers is estimated by varying the estimate of the fraction of algae in assimilated organic matter to achieve the best fit between observed and predicted isotope ratios of consumers.

The isotope ratio of a consumer at time t (c_t) is a function of the initial isotope ratio (c_0) and weight (w_0) of the consumer, growth over the interval ($w_t - w_0$), turnover of tissue, the isotope ratio of assimilated organic matter (r), and the isotopic shift

between diet and consumer (Δ). If tissue turnover for a given interval of time is set equal to the change in weight (FRY & ARNOLD 1982), c_t can be estimated over short intervals of time as follows:

$$c_t = \frac{c_0 w_0 + 2(w_t - w_0)(r + \Delta)}{w_0 + 2(w_t - w_0)} \quad (1)$$

For primary consumers, Δ is set to 0.5‰ for carbon and 1.5‰ for nitrogen. Higher values of Δ for nitrogen (2.5–3.5‰) are applied for predators. Over each interval of time, w_t is estimated from w_0 and the instantaneous growth rate (g):

$$w_t = w_0 e^{gt} \quad (2)$$

For species with identifiable cohorts, g is estimated from the means of measured weights of consumers from each sampling date.

$$g = \frac{\ln(w_t) - \ln(w_0)}{t} \quad (3)$$

Results and discussion

Stable isotope ratios of terrestrial plant material from the riparian zone of St. Vrain Creek remained nearly constant over time (Fig. 1). The $\delta^{13}\text{C}$ of algae varied by about 10‰ across dates and was lowest during runoff when growth rates of algae were low. Variability of $\delta^{13}\text{C}$ of algae was highest during runoff, possibly because rates of turnover in algae varied spatially or taxonomically. $\delta^{15}\text{N}$ of stream algae decreased during seasonal high discharge (Fig. 1), presumably because the $\delta^{15}\text{N}$ of inorganic nitrogen in meltwater is near 0‰ and growth rates of algae are low during runoff. In 1995, the mean $\delta^{15}\text{N}$ of algae decreased from 9.8‰ to 2.2‰ during runoff, and then returned to 9.5‰ by mid October.

The $\delta^{13}\text{C}$ of many stream consumers (e.g. *Pteronarcella badia*; Fig. 2) remained near the annual average $\delta^{13}\text{C}$ of algae over the study period. The mean $\delta^{13}\text{C}$ of *Pteronarcella* and other consumers varied across dates but the magnitude of seasonal change was less for consumers than for algae, and on some dates the $\delta^{13}\text{C}$ of consumers fell outside the range of $\delta^{13}\text{C}$ for sources. The mean $\delta^{15}\text{N}$ of *Pteronarcella* and

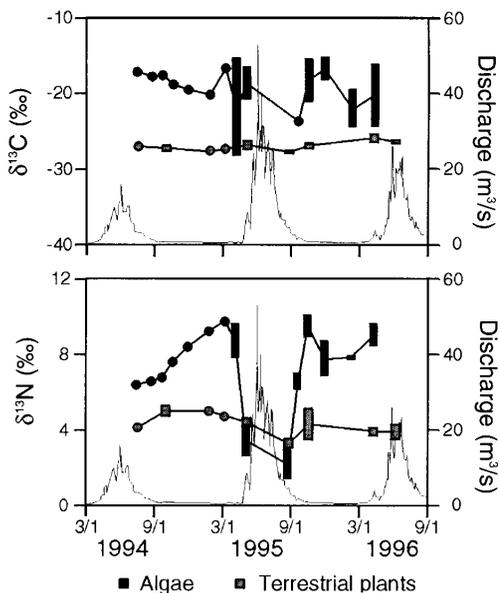


Fig. 1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of algae and terrestrial plant material. Bars indicate means \pm 1 SE. Circles indicate single measurements. Discharge data are from the U.S. Geological Survey.

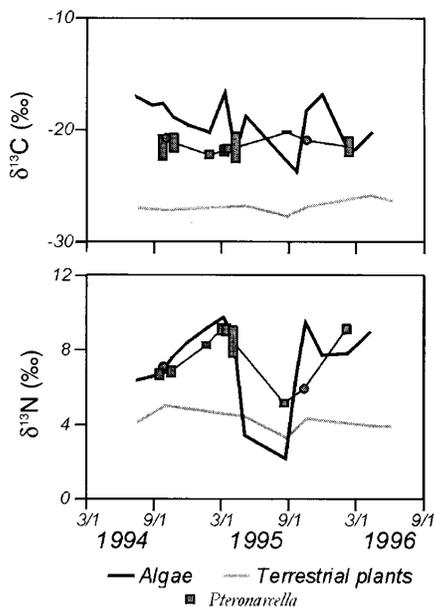


Fig. 2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *Pteronarcella badia*. Bars indicate means \pm 1 SE. Circles indicate single measurements. Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of sources for each date are also shown.

other consumers followed the gradual changes in $\delta^{15}\text{N}$ of algae over much of the study period but fell outside the range of $\delta^{15}\text{N}$ for sources on some dates.

The spreadsheet model made possible estimates of the contribution of algae to consumers over the study period, including periods of time when isotope ratios of consumers fell outside the range of sources, as expected when the isotope ratio of the source is changing rapidly. The time-weighted mean contribution of algal carbon to *Pteronarcella* over the study period, estimated with the biomass-dilution model, was 71% and the time-weighted mean contribution of nitrogen from algae was 70%. The time-weighted mean contributions of C and N cannot be estimated with confidence if a standard two-source mixing model is used to estimate the contribution of algae to *Pteronarcella* on each date (i.e. time-weighted estimates exceed 100% for both C and N if the standard model is used).

Where seasonal oscillations in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ of

algae are large relative to the isotopic separation between algae and terrestrial plant material, special measures must be taken into account for the effects of change in stable isotope ratios of sources. If growth rates of consumers are low relative to rates of turnover in algae, isotope ratios of consumers may fall well outside the range of sources and frequent measurements of isotope ratios of sources are required to explain this apparent paradox. Even where growth rates of consumers are high (e.g. growth rates of chironomids may exceed 0.5 day^{-1} in warm streams of the southeastern United States; STITES & BENKE 1989), frequent measurements of isotope ratios of sources are necessary to capture seasonal shifts in isotope ratios of consumers.

The generality of large temporal changes in isotope ratios of stream algae has not been established. The close ties between $\delta^{15}\text{N}$ of algae and the hydrograph in St. Vrain Creek suggest that similar patterns may be found in other streams where discharge changes over time. In streams with predictable hydrographs (e.g.

snowmelt-dominated streams or most large rivers), monthly sampling of aquatic primary producers for stable isotope analyses should be sufficient. In streams with hydrographs dominated by rainfall (e.g. many small streams receiving low amounts of groundwater), it may be necessary to structure sampling of algae and consumers around large precipitation events to capture changes in isotope ratios.

Conclusions

If isotope ratios of sources change and no account for seasonality is made, grossly erroneous conclusions can be drawn from stable isotope analyses. Proper consideration of changes in isotope ratios of sources and dilution of biomass in consumers can, however, extend the potential of stable isotope methods and make possible estimates of the contribution of sources to consumers at times when isotope ratios of consumers fall outside the range of sources.

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