CHAPTER 10

Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems

JACQUES C. FINLAY AND CAROL KENDALL

Introduction

The recent expansion of natural abundance stable isotope methods has rapidly increased our understanding of freshwater food web ecology. Investigators now routinely use stable isotopes to answer questions related to plant and animal ecophysiology, trophic structure, and energy pathways within freshwater ecosystems and at their interfaces with marine and terrestrial ecosystems. Stable isotope studies are particularly useful in aquatic settings because of limited opportunities for direct observations, high degree of spatial complexity and diverse potential sources of nutrients, organic matter, and prey.

All applications of natural abundance methods to trace nutrient and energy sources and trophic interactions depend on variation in isotope ratios in organisms and their environment. While natural abundance stable isotope techniques have been applied to food web research for over 25 years (i.e., Fry et al. 1978; Rounick et al. 1982), the increased availability of automated preparation systems for analyses of carbon (C), nitrogen (N), and sulfur (S) stable isotopes has led to a recent, rapid increase in data. These data have allowed greater exploration of isotopic variation in the environment, leading to many new applications in ecological studies. Moreover, increased knowledge of isotopic variability at the base of food webs, especially in aquatic ecosystems, provides an opportunity to re-evaluate poorly tested assumptions and improve older, established methods.

Isotopic variation of basal resources (detritus and primary producers at the base of food webs) and prey determine if, how, and when stable isotope techniques may be applied. Overlapping or variable isotopic compositions may make natural abundance methods ineffective (e.g., Phillips & Gregg 2003). However, if recognized, such isotopic variability may sometimes prove to be a useful natural signal that may actually enhance the power of isotope methods. Because isotope methods are still rapidly evolving, however, there is currently limited ability to predict when and where natural abundance tracers will be effective.

Excellent introductions to stable isotope approaches in freshwaters are available elsewhere (see Peterson & Fry 1987; Hershey & Peterson 1996). In this chapter, we explore use of natural abundance stable isotopes (primarily $\delta^{13}C$ and $\delta^{15}N$, and to a lesser extent, $\delta^{34}S$) in ecosystems, with an emphasis on nutrient and energy flow at the base of aquatic food webs. Our focus is on freshwaters, especially rivers, and their interface with surrounding watersheds, although the underlying principles apply to many other ecosystems. Below, we review the environmental basis for variation in $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of organic matter at the base of river food webs, and explore broad patterns, biogeochemical predictors, and applications of natural abundance measurements in river environments.

Overview of river food webs and stable isotope approaches

Food webs in rivers have high spatial and temporal complexity compared with most other ecosystems. Fluvial ecosystems are characterized by strong longitudinal gradients in environmental conditions from headwaters to mouth, heterogeneity at all spatial scales, and considerable temporal variation.

Two features of rivers are particularly important to the study of food webs. First, drainage networks contain both allochthonous organic matter, derived primarily from terrestrial sources, and autochthonous organic matter derived from production within rivers. Five major types of living and dead organic matter (OM) are available to stream consumers (Figure 10.1), including two allochthonous sources (terrestrial plant detritus and soils), two autochthonous sources (aquatic macrophytes and algae), and aquatic heterotrophic bacteria and fungi, that may rely on a mix of the other sources.

The River Continuum Concept (RCC; Figure 10.2) predicts that the relative contribution of these sources to total organic carbon varies with stream size (Vannote et al. 1980). In this model, allochthonous terrestrial carbon is expected to be important for food webs in small forest streams because overhanging trees block sunlight, greatly reducing algal productivity, and adding large amounts of detritus to streams. With increasing stream size, algal productivity (i.e. periphyton in small streams, transitioning to planktonic forms in large rivers) becomes a more important source of carbon as canopy shading decreases. As turbidity increases in large or disturbed rivers, light limits autotrophic production again; hence, allochthonous forms of carbon are expected to dominate energy flow in food webs in large rivers as well as in headwater streams. However, several recent studies have shown that some large rivers (e.g., the Mississippi River) have appreciable amounts of algal productivity (Kendall et al., 2001; Wissel & Fry, 2005; Delong & Thorp 2006). While the RCC describes broad patterns in the relative abundance of

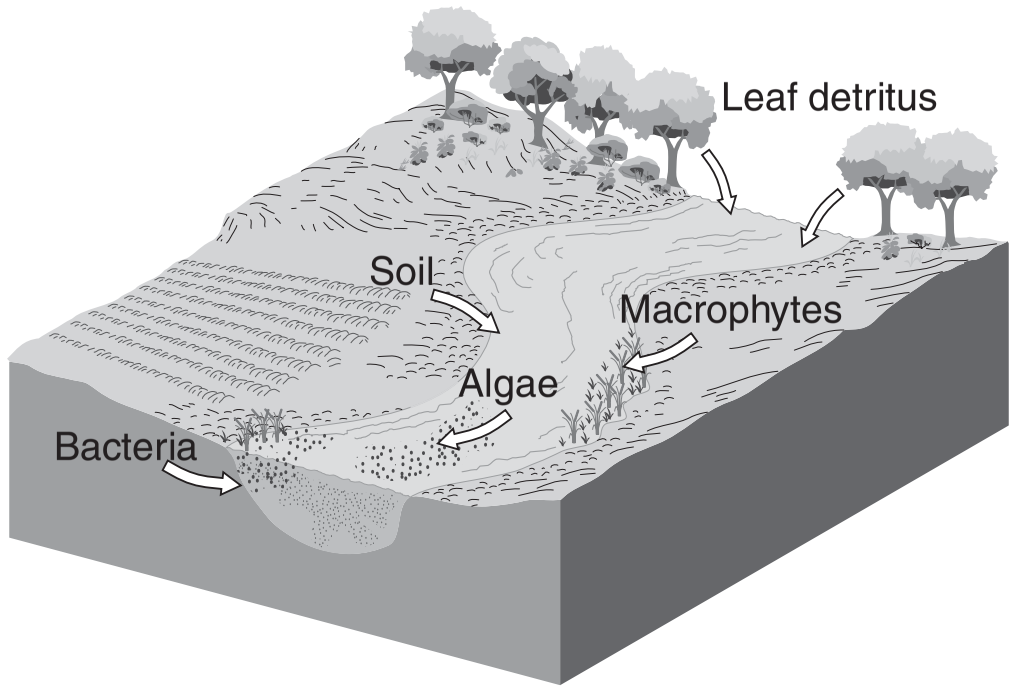

Figure 10.1 The five main sources of organic matter to stream ecosystems. "Bacteria" = both benthic and planktonic heterotrophs, and "algae" = benthic and planktonic algae and cyanobacteria.

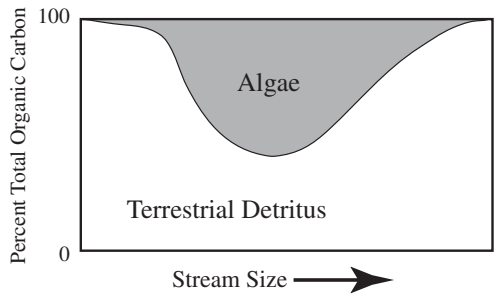

Figure 10.2 General predictions of the relative contribution of terrestrial and autotrophic organic matter to rivers, as inferred from the River Continuum Model of Vannote et al. (1980). Some recent publications have found that a large percent of the seston in large rivers in temperate climates is algal derived. (e.g., Kendall et al. 2001; Wissel & Fry 2005; Delong & Thorp 2006.)

organic matter sources in river networks, these sources vary greatly widely in nutritional quality; hence, there is little correlation between the amount of organic resources and its availability to higher trophic levels. As described later, stable isotopes have played a large role in quantifying sources of production supporting stream consumers, greatly improving our understanding of energy sources to river consumers.

A second key feature of river ecosystems is that their diverse habitats are hydrologically connected throughout the drainage network. Hence, both fluvial transport of resources and movement of organisms play important and often definitive roles in food web structure and productivity (Vannote et al. 1980; Power & Dietrich 2002; Woodward & Hildrew 2002). As a consequence of the variable hydrologic linkages among habitats, streams contain a complex mixture of organic matter types, and food web interactions are difficult to constrain by traditional approaches.

Natural abundance stable isotopes are well suited to studying processes and interactions in river food webs. Carbon and nitrogen stable isotope ratios of organic matter sources in rivers vary widely, providing the basis of the stable isotope tools that have provided extensive insight about transfer of energy and nutrients through these food webs. When key assumptions are satisfied, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ may be used to trace the source of productivity fueling food webs (i.e. the habitat, resource type, or in some cases, the specific taxa), the origin of organisms, and the trophic position of consumers. Such information is essential for understanding the dynamics of food webs as well as for detecting responses to environmental and human-driven change.

As discussed below in detail, $\delta^{13}C$ values are useful because of the wide range of $\delta^{13}C$ of algae at the base of food webs. The other major source

Table 10.1 Potential locations and mechanisms for stable isotope variation in organic matter sources in rivers and riparian zones.

Habitats or sources	Stable isotope	Primary mechanism	Conditions or locations observed	Examples
Longitudinal				
Pool–riffle	$\delta^{13}C$, $\delta^{15}N$	Fractionation	High algal growth, low CO_2	Finlay et al. (1999); Trudeau & Rasmussen (2003)
River confluence	$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$	Source	Mixing of chemically distinct waters	Figure 10.8
Upstream–downstream	$\delta^{13}C$, $\delta^{15}N$	Source and fractionation	Small or spring fed streams, point source nutrient inputs	Kennedy et al. (2005)
Within site Terrestrial–aquatic	$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$	Source and fractionation	Widespread	Finlay (2001)
Benthic–planktonic	$\delta^{13}C$	Fractionation	Rivers with attached and planktonic algae	Debruyn & Rasmussen (2002); Delong & Thorp (2006)
River–riparian interface	$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$	Source and fractionation	Large streams, rivers	Bastow et al. (2002); Kato et al. (2004)
Marine–river	$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$	Source	Coastal streams an rivers	Chaloner et al. (2002); MacAvoy et al. (2000)

of energy in river food webs, terrestrial detritus, has a much better constrained $\delta^{13}C$, so that these two carbon sources are often isotopically distinct (Finlay 2001). Recent literature reviews confirm that there are very minor changes in $\delta^{13}C$ during trophic transfer of organic carbon following fixation by plants (Vander Zanden & Rasmussen 2001; McCutchan et al. 2003) and minimal changes during decomposition. These features often make $\delta^{13}C$ the most effective tracer of organic C sources and energy flow in aquatic ecosystems.

In rivers, investigators have identified a growing number of ways that variation in $\delta^{13}C$ may be used to distinguish carbon sources in food webs (Table 10.1). Variation in $\delta^{13}C$ of sources has been applied toward basic research to study energy sources for riparian and aquatic consumers (e.g. Finlay 2001; Bastow et al. 2002; Finlay et al. 2002; Huryn et al. 2002; McCutchan & Lewis 2002), parasitism (Doucett et al. 1999a), and origins of juvenile progeny (e.g. Doucett et al. 1999b; McCarthy & Waldron 2000). $\delta^{13}C$ have also been used to examine applied issues in river food webs, including

land-use change (e.g. Hicks 1997; England & Rosemond 2004), invasive species (e.g. Kennedy et al. 2005), contaminant transfer (e.g. Berglund et al. 2005), and eutrophication (e.g. Debruyn & Rasmussen 2002).

Nitrogen stable isotopes are a powerful tracer of the nitrogen cycle and food web interactions in freshwater ecosystems. $\delta^{15}N$ natural abundance techniques are primarily used to study nitrogen sources and cycling (see Kendall et al., this volume, pp. 375–449), as an integrative measure of food chain length (Finlay et al. 2002; Jepsen & Winemiller 2002), and to quantify energy or nutrient sources in food webs when differences in $\delta^{15}N$ among organic matter or prey sources are very large (Table 10.1). $\delta^{15}N$ may be used to estimate trophic position because there is a consistent increase in $\delta^{15}N$ of consumers that is related to trophic position in food webs. Reviews of controlled feeding studies and comparisons with gut content analyses confirm that $\delta^{15}N$ yield quantitative information regarding trophic position of consumers (Vander Zanden et al. 1997), although fractionation is variable and poorly understood for basal consumers that have N-poor diets (see McCutchan et al. 2003; Vanderklift & Ponsard 2003). However, $\delta^{15}N$ offers many advantages over alternate techniques to measure trophic structure in ecosystems. In particular, isotope-based estimates of trophic position are spatially and temporally integrated, in contrast to observational approaches. However, when prey $\delta^{15}N$ vary among habitats or energy sources, measurements of $\delta^{13}C$ or other tracers are necessary to accurately estimate trophic position from $\delta^{15}N$ (see Post 2002).

As with $\delta^{13}C$, $\delta^{34}S$ values are useful for determining food sources because of the wide range in the $\delta^{34}S$ of organic matter at the base of food webs, especially those of coastal environments or ecosystems with strong gradients in redox conditions, and because of the apparent minimal fractionation during trophic transfer of S. Relatively little is known about trophic fractionation, however. A recent review of controlled-diet studies found that organisms fed high-protein diets had higher $\delta^{34}S$ values than when fed low-protein diets, with fractionation ranging from −0.5 to +2‰ (McCutchan et al. 2003).

As briefly summarized here, natural abundance stable isotope studies are increasingly integrated into food web studies in rivers and elsewhere. However, use of these techniques depends on key issues such as distinct end-members, and limited or well characterized temporal and spatial variation of potential organic matter or prey sources. Thus, the extent of isotopic variation at the base of the food web determines the usefulness of these tools for studies of both energy and material flow, and trophic structure. While predictive understanding of such variation is limited in rivers and other aquatic ecosystems, this situation is changing. Below, we describe spatial and temporal variations in the isotopic compositions of organic matter at the base of food webs, synthesize recent progress in understanding mechanisms driving these patterns, and provide recent examples of new or improved applications of stable isotope methods to basic and applied research in rivers.

Table 10.2 Typical compositional values of major organic matter sources; the ranges of observed values are in parentheses. The data are gleaned from the references cited in text. For a much more detailed list of C:N values of potential contributors to stream seston, see Rostad et al. (1997) and Sterner & Elser (2002).

Organic matter source	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	C : N (at.)
Heterotrophic bacteria	Similar to substrate	−15 to +20	−15 to +20	4 to 8
Freshwater autotrophs		−15 to +20	−10 to +33	5 to 12
Periphyton	−35 to −18 (−47 to −8)			5 to 8
Phytoplankton	−32 to −23 (−42 to −19)			
Macrophytes*	−27 to −20	−15 to +20	−10 to +33	10 to >50
Soil organic matter		0 to +5	0 to +5	8 to >25
C3	−27 (−32 to −22)		(−30 to +35)	
C4	−13 (−16 to −9)			
Terrestrial plants		−3 to +7	0 to +5	15 to >50
C3	−27 (−32 to −22)	(−10 to +10)	(−10 to +20)	
C4	−13 (−16 to −9)			

* Excluding bryophytes.

Stable isotope ratios of organic matter sources in stream ecosystems

The main types of organic matter in rivers shown in Figure 10.1 have widely variable $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values (Table 10.2). Examination of the ranges of $\delta^{13}C$ and $\delta^{15}N$ in rivers (Figure 10.3) shows substantial overlap among the various sources. However, isotopic ranges for organic matter sources are much smaller in a specific river than found in a global literature survey, and sources often have distinct compositions. For example, the typical range of $\delta^{13}C$ and $\delta^{15}N$ values of organic matter sources in the San Joaquin River, a major agricultural river in California (USA), shows that algae (phytoplankton) often have distinct isotope ratios compared with other sources (Figure 10.4). Moreover, other tracers, in particular elemental ratios, also vary among sources (Figures 10.3 & 10.4) and may be used to complement isotope approaches for determining the sources of organic matter. For example, the C : N of seston (suspended particulate organic matter – POM) is usually more useful than $\delta^{13}C$ and/or $\delta^{15}N$ for determining the dominant source of organic matter to major rivers (Kendall et al. 2001), and lower C : N values generally correlate with higher quality of organic matter for consumers.

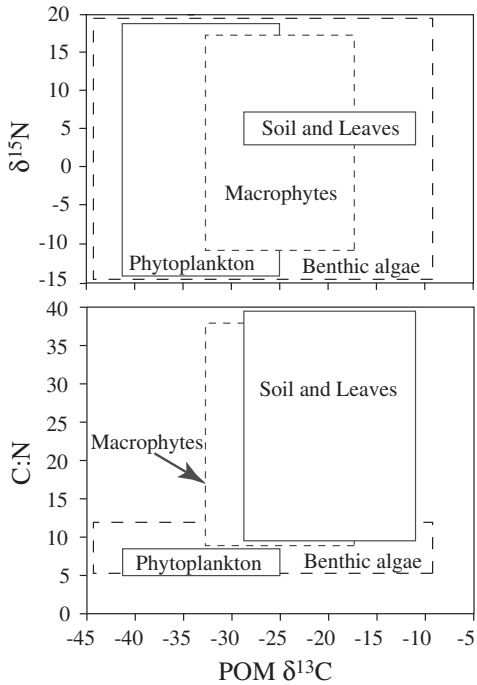

Figure 10.3 Typical ranges in $\delta^{15}N$, $\delta^{13}C$, and C : N (atomic) values of different particulate organic matter (POM) sources to rivers, based on a literature survey.

Understanding the environmental and physiological controls of stable isotope ratios in food webs is a first step for effective use of these tracers in rivers. Natural isotopic variation in the plant sources of organic materials is determined by two general factors:

1 the isotope ratio of inorganic **source** elements (i.e., C, N, S), either of dissolved species (such as NO_3^-, HCO_3^-, or SO_4^{-2}) or gaseous compounds (such as CO_2, N_2, or H_2S);
2 their subsequent **fractionation** during assimilation (uptake) by terrestrial or aquatic plants.

A well-known model of carbon isotope variation in terrestrial plants (Farquhar et al. 1982) provides a useful framework for discussing controls of isotopic variation. The model states that $\delta^{13}C$ of plants is described by the following equation:

$$\delta^{13}C_{CO2} - a - (b - a)c_i/c_e$$

where a is the discrimination due to slower diffusion of $^{13}CO_2$, b is the discrimination against $^{13}CO_2$ by Rubisco (ca. 27‰), c_i is the intercellular CO_2

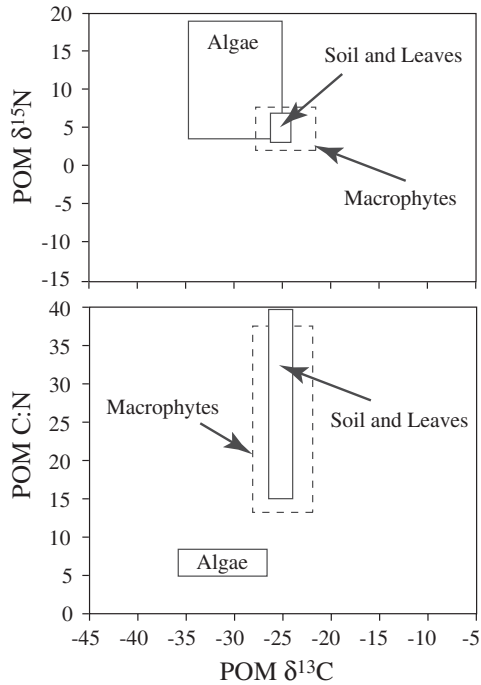

Figure 10.4 Typical compositions of different particulate organic matter (POM; seston) sources to the San Joaquin River, California, USA. Note that the ranges for different organic matter sources are usually much less than shown in Figure 10.3.

concentration (≈ growth rate), and c_e is the external CO_2 concentration. The discrimination factor b is equivalent to the more commonly used term ε, or fractionation factor, and is almost always >0. Similar principles apply to understanding variation in plant $\delta^{15}N$ and $\delta^{34}S$ because they also are influenced by both the isotope ratio of the inorganic source and fractionation during uptake, but far less is known about these isotopes in freshwater ecosystems.

The influence of source and fractionation effects varies widely within and between terrestrial and aquatic ecosystems. Because both environments are significant sources of organic matter to river and riparian food webs (Figure 10.1), we must consider the controls on the isotopic compositions of food sources in both terrestrial and freshwater ecosystems. Below we briefly review isotopic variation within the five major sources of organic matter in rivers, with greater emphasis on aquatic producers because of larger, but less well known, isotopic variation in freshwaters.

We also consider briefly C:N ratios because of their utility in describing the source and quality of organic matter in ecosytems. C:N ratios can be reported as either atomic (at.) or mass (wt.) ratios. Mass ratios (the normal

output of mass spectrometer analysis of %C and %N in OM) can be converted to atomic ratios by multiplying by 14/12. We use atomic ratios in this chapter.

Terrestrial plants

Detailed reviews of controls of terrestrial plant stable isotope ratios may be found elsewhere (Dawson et al. 2002 and references therein) and are only briefly described here. A more thorough discussion of this topic is found in Garten et al., this volume, pp. 61–82 and Evans, this volume, pp. 83–98.

Controls on $\delta^{13}C$ of plants

For $\delta^{13}C$, terrestrial plants fall into two main categories, based on different photosynthetic pathways for uptake of carbon: C4 plants, of which corn and some grasses are the most important members, and C3 plants, which include deciduous and coniferous trees. The average $\delta^{13}C$ value of C3 plants is around −27‰ (Bender 1968; Smith & Epstein 1971; Troughton 1972), with a total range of about 15‰. In semi-arid regions, water limitation promotes use of the C4 photosynthetic pathway, which enhances water-use efficiency and lowers isotopic fractionation (as discussed more thoroughly in Marshall et al., this volume, pp. 22–60 and Evans, this volume, pp. 83–98). As a consequence, the average $\delta^{13}C$ value of C4 plants is about −13‰, with a smaller range of values (Figure 10.3). The $\delta^{13}C$ values of C4 plants are higher than most other organic sources to rivers, except for attached algae and macrophytes under some conditions (Hillaire-Marcel 1986).

Terrestrial plants use a single form of inorganic carbon (CO_2) derived from a large, well-mixed atmospheric reservoir ($\delta^{13}C$ of −8‰); thus, source effects on $\delta^{13}C$ are relatively minor. Isotopic variation for terrestrial plants is thus related to fractionation during CO_2 assimilation, and fractionation is primarily related to plant growth rates and water use. The variation that arises for C3 and C4 plants is generally small compared with freshwater autotrophs (aquatic photosynthetic plants), although the presence of both C3 and C4 plants in a watershed expands the potential $\delta^{13}C$ range of the terrestrial end-member substantially (Figure 10.3).

Controls on $\delta^{15}N$ of plants

Nitrogen metabolism also distinguishes plants into two general categories: N-fixing plants (e.g., legumes and certain grasses, notably alfalfa) that assimilate atmospheric N_2, and non-N-fixing plants that use only other forms of plant-available N. These forms include inorganic N (NH_4^+, NO_3^-) as well as organic N (e.g. amino acids), that may have a wide range in $\delta^{15}N$ values depending on environmental conditions (Figure 10.3). However, most terrestrial plants have $\delta^{15}N$ values in the range of −6 to +5‰ (Fry 1991). Plants

fixing atmospheric N_2 have a more restricted $\delta^{15}N$ range of about −3 to +1‰ (Fogel & Cifuentes, 1993), close to the $\delta^{15}N$ value of atmospheric N.

Most investigations have concluded that there is negligible fractionation during terrestrial plant uptake (assimilation) in most natural N-limited systems (Nadelhoffer & Fry 1994; Högberg 1997); nevertheless, tree tissues and litter typically have slightly lower $\delta^{15}N$ values than the source of nitrogen. Under higher nutrient conditions, preferential uptake of ^{14}N by plants results in a few per mil fractionation between plants and dissolved inorganic N (DIN). Many processes (e.g., volatilization, nitrification, and denitrification) can alter the $\delta^{15}N$ values of the ammonium or nitrate that is ultimately utilized by the plant (Handley et al. 1999), as will be discussed in more detail below. As a result of these complications, plants with $\delta^{15}N$ values < −10‰ or > +10‰ are not unusual, especially when they are growing near streams or in wetlands where $\delta^{15}N_{DIN}$ is high because of denitrification or eutrophic conditions.

Controls on $\delta^{34}S$ of plants

Sulfur isotopes ($\delta^{34}S$) are less commonly used than those of either C or N for determining sources of organic matter, primarily because the analytics are more complicated but also because much less is understood about sources of variability in ecosystems. Nevertheless, they have been used when a dual (C, N) isotope approach has proved unsuccessful (Peterson et al. 1985; Loneragan et al. 1997; Connolly et al. 2003), especially in marine or coastal environments where plants are likely to be isotopically labeled with the high $\delta^{34}S$ of marine sulfate (about +20‰).

Terrestrial plants and soils have $\delta^{34}S$ values that average +2‰ (Table 10.2), but soils have a much larger range (Krouse et al. 1991). The wide range of $\delta^{34}S$ values for soils and terrestrial plants has been attributed to atmospheric inputs, local mineral sources and reduction processes in anaerobic environments (Krouse et al. 1991). In areas with low atmospheric inputs of sulfur, the $\delta^{34}S$ values of vegetation generally reflect those of the soil sulfate. However, in areas with high atmospheric S inputs, the $\delta^{34}S$ value of vegetation may also reflect direct incorporation of S as sulfur dioxide through the stomata as well as possible enrichment in ^{34}S due to biogenic emission of reduced sulfur gases depleted in ^{34}S (Krouse et al. 1991; Wadleigh & Blake 1999). An intriguing study of the $\delta^{34}S$ of lichens across the island of Newfoundland (Canada), which showed an 11‰ gradient in $\delta^{34}S$ related to distance from the coast and proximity to local S emission sources, beautifully illustrates how the spatial distribution of atmosphere $\delta^{34}S$ sources is reflected in the $\delta^{34}S$ of plants (Wadleigh & Blake 1999).

C:N values

Terrestrial plant species vary widely in their nutritional quality for growth of stream consumers. C:N provides a general indication of their quality for

heterotrophs. The C:N of fresh and senesced leaves vary widely, with a mean value around 20 (Sterner & Elser 2002). Litter with low C:N can be a significant food source to local stream consumers. However, OM with high C:N (e.g., woody debris, soil organic matter, and litter of nutrient-limited plants) is unlikely to be very nutritive to consumers without substantial microbial processing. In general, the C:N of terrestrial litter exceed those of aquatic production, and there is insufficient N to meet growth demands of many stream heterotrophs. However, decomposers colonize leaves and immobilize N, thus decreasing bulk C:N and improving its nutritional quality for invertebrate consumers (see 'Microbial heterotrophs', below).

Soil organic matter

The isotopic composition of soil organic matter largely reflects the isotopic compositions of the plants growing on them. Soil organic matter $\delta^{13}C$ values of about −27‰ and −13‰ are expected in areas dominated by C3 and C4 plants, respectively (Boutton 1996). Most soils have $\delta^{15}N$ values of +2 to +5‰ (Broadbent et al. 1980). On a regional and global scale, soil and plant $\delta^{15}N$ values systematically decrease with increasing mean annual precipitation and decreasing mean annual temperature (Amundson et al. 2003). Most soils have organic matter $\delta^{34}S$ values in the range of 0 to +5‰. The $\delta^{34}S$ of the organic matter is strongly affected by the $\delta^{34}S$ of atmospheric inputs. For example, organic matter in soils in New Zealand has $\delta^{34}S$ values approaching that of seawater (ca. +20‰), suggesting that the oceanic spray is the primary sulfur source (Kusakabe et al. 1976). Some soils in Alberta (Canada) and California (USA) with low $\delta^{34}S$ values (ca. −30‰) have been encountered and it has been suggested that the sulfur was derived from the weathering of sulfide minerals and/or organic sulfur in shales (Krouse et al. 1991).

Median C:N ratios of organic matter in the top 15 cm of arable soils range from 10 to 12, with most ratios in the range of 8 to 25 (Brady 1990; Aitkenhead & McDowell 2000). The ratios are higher in humid than arid areas, and higher in colder than warmer areas (Brady 1990). Most soil microorganisms have ratios between 4 and 9 (Brady 1990; Rostad et al. 1997). The much lower C:N values of soils compared with terrestrial plants reflect the cycling of plant material during decomposition (Brady 1990).

Aquatic plants

Macrophytes and algae have a wide range of $\delta^{13}C$ and $\delta^{15}N$ values (Figure 10.3); the same is true of $\delta^{34}S$ but much less data are available. These ranges are so large and have such extensive overlap with terrestrial plants and soils that one might wonder how contributions from different sources

of organic matter can be distinguished. The ranges of autotroph $\delta^{13}C$ and $\delta^{15}N$ values from any specific river, however, are substantially smaller than the total literature range (Figure 10.4), often making quantitative separation with two or more tracers feasible. In addition, macrophytes (and bryophytes) have restricted distributions in river networks, and are usually quantitatively unimportant in fueling food webs because they are inedible for most herbivores.

The isotopic compositions of aquatic plants, and the organic matter derived from them, are more variable and less predictable than terrestrial plants and detritus (Figure 10.3), mainly because of (i) the large variation in the concentrations and stable isotope ratios of dissolved inorganic carbon (DIC), nitrogen (DIN), and sulfur (DIS) in freshwater systems, and (ii) the physiological diversity of aquatic autotrophs. The effects of some of the physical and biogeochemical processes that cause the large variations in the isotopic compositions of the dissolved inorganic species, and ultimately lead to variability in the isotopic compositions of aquatic plants, are illustrated in schematic form in Figure 10.5.

Because of their complexity, the *controls* on the carbon, nitrogen, and sulfur isotope biogeochemistry of aquatic plants are described separately below, in greater detail than for the other sources considered in this chapter.

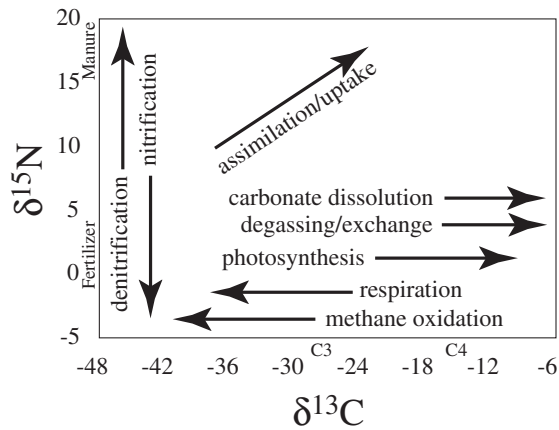

Figure 10.5 Conceptual model showing the main biogeochemical processes that control the $\delta^{13}C_{DIC}$ and the $\delta^{15}N_{NO_3}$, and consequently the $\delta^{13}C$ and $\delta^{15}N$ of aquatic plants and particulate organic matter (POM). The arrows indicate the usual effect of an increased amount of the specified process on the $\delta^{13}C_{DIC}$ and/or $\delta^{15}N_{NO_3}$, the $\delta^{13}C$ and/or $\delta^{15}N$ of the aquatic plants growing in the ecosystem, and ultimately the food webs based on these plants. For example, increased amounts of NO_3^- formed by nitrification of NH_4^+ probably causes decreases in $\delta^{15}N_{NO_3}$ (but usually minimal affect on $\delta^{13}C_{DIC}$), and assimilation causes significant increases in both $\delta^{13}C_{DIC}$ and $\delta^{15}N_{NO_3}$. The approximate $\delta^{13}C$ and $\delta^{15}N$ values of important C and N sources are also shown. (e.g., C3 plants and nitrate from manure, respectively.)

Furthermore, each section is subdivided into separate discussions of "source effects" and "fractionation effects." The section on source effects is intended to provide an overview of:

1 the biogeochemistry and isotopic compositions of the major dissolved inorganic species (e.g., HCO_3^-, NO_3^-, SO_4^{2+}) that affect the isotopic compositions of aquatic plants;
2 how different watershed and in-stream processes affect the isotopic compositions of the dissolved species;
3 the typical ranges of fractionation factors for dissolved species caused by these processes;
4 how these processes vary seasonally.

The section on fractionation effects focuses only on fractionations during assimilation (uptake), not on fractionations that only control the isotopic compositions of the dissolved species.

Controls on $\delta^{13}C$ of aquatic plants and DIC

Reported values for $\delta^{13}C$ of freshwater aquatic plants and algae range from about −47 to −8‰, with values typically falling in the range of −30 to −20‰ (LaZerte & Szalados 1982; Hamilton & Lewis 1992; Angradi 1993, 1994; Schlacher & Wooldridge 1996; Thorp et al. 1998; Cloern et al. 2002; Finlay 2004). Macrophytes have a more restricted $\delta^{13}C$ range, perhaps mostly because of their limited geographic distribution within drainage networks, i.e., lentic (still-water) habitats of larger rivers. Planktonic (free living, suspended) and periphytic (attached) algae are widely distributed in drainage networks, with periphyton dominating small streams and rivers, and planktonic forms more important in larger rivers. Where these forms co-occur, attached forms should have higher $\delta^{13}C$ than planktonic forms as discussed in detail below.

Source effects

In contrast to terrestrial plants, aquatic autotrophs derive inorganic carbon from DIC. Depending on the pH of the stream, the DIC may be comprised mostly of aqueous CO_2 (at pH < 6.4), HCO_3^- (pH 6.4–10.3), or CO_3^{-2} (pH > 10.3). The $\delta^{13}C$ of DIC has an observed range of ca. 30‰ due to variation in sources and subsequent physical (e.g. mixing) and biological (e.g. respiration, photosynthesis) processes, as discussed below. However, $\delta^{13}C_{DIC}$ values of −12 to −8‰ are most commonly observed in temperate rivers (Mook & Tan 1991; Kendall 1993; Bullen & Kendall 1998; Finlay 2003).

Variation in the sources and sinks for DIC control $\delta^{13}C_{DIC}$ in rivers. The main sources of DIC in fresh waters are atmospheric CO_2, carbonate rock dissolution, and respiration, and each has a distinct $\delta^{13}C$. The DIC produced by carbonic acid (i.e., H_2CO_3) dissolution of marine carbonates ($\delta^{13}C$ values = ca. 0‰) generally produces $\delta^{13}C$ values in the range of −15 to −5‰ depending mainly on whether the source of the C in the carbonic acid is C3

or C4 plants. Addition of respired CO_2, which has a $\delta^{13}C$ value similar to the organic carbon substrate (e.g., –27‰ for C3 plants) lowers $\delta^{13}C_{DIC}$; respiration has a large effect when DIC is low and less influence when DIC is high (Fry & Sherr, 1984). The main sinks affecting DIC in rivers are photosynthetic uptake by plants, degassing of CO_2 to the atmosphere, recharge to groundwater, and carbonate precipitation.

The $\delta^{13}C_{DIC}$ is affected by both in-stream and watershed-scale processes. The main watershed-scale processes that affect the $\delta^{13}C_{DIC}$ are:

1 dominance of C3 vs. C4 plants in the watershed;
2 presence of carbonate minerals in the bedrock and soil;
3 the relative proportions of groundwater vs. surface runoff contributing to streamflow.

As summarized in Figure 10.5, the main in-stream processes that affect $\delta^{13}C_{DIC}$ in rivers are:

1 degassing and CO_2 exchange with the atmosphere;
2 dissolution/precipitation of carbonate minerals in the stream;
3 discrimination during photosynthesis, which leaves the residual DIC pool enriched in ^{13}C;
4 respiration.

Other processes, including oxidation of methane produced in anoxic stream sediments, soils, or bedrock may also affect the $\delta^{13}C_{DIC}$. The relative strength of these processes affecting stream $\delta^{13}C_{DIC}$ varies spatially with stream size and productivity (Finlay 2003), and with geology and hydrology (Bullen & Kendall 1998). The acidity of the stream, which is a function of both in-stream and watershed-scale processes, also has a profound affect on $\delta^{13}C_{DIC}$ because this controls whether the dominant form of DIC is CO_2, HCO_3^-, or CO_3^{-2}.

Streams and rivers typically show large seasonal changes in $\delta^{13}C_{DIC}$, caused by both variation in contributions of groundwater vs. soil runoff to the river and changes in stream metabolism. For example, Figure 10.6 shows seasonal variation in discharge, alkalinity (≈ DIC), and $\delta^{13}C_{DIC}$ in a small stream in Maryland, USA. Hydrology strongly affects the $\delta^{13}C_{DIC}$, with the oscillations in $\delta^{13}C$ during storm events reflecting shifts in the relative proportion of flow from soil water vs. groundwater flowpaths contributing to discharge. The broad seasonal changes in alkalinity (Figure 10.6c) reflect differences in the proportions of DIC derived from calcite and soil CO_2 (Figure 10.6d). Riverine CO_2 concentrations often decrease while the $\delta^{13}C_{DIC}$ increases during the summer because of photosynthesis, whereas CO_2 concentrations often increase and $\delta^{13}C_{DIC}$ decrease during the late fall as photosynthesis declines and in-stream decay and respiration increases (Kendall 1993; Atekwana & Krishnamurthy 1998; Finlay 2003). Episodic algal blooms in rivers can cause the $\delta^{13}C$ of the seston to oscillate as the DIC pool is drawn down by photosynthesis and then replenished by respiration and transport of DIC from upstream (Figure 10.7).

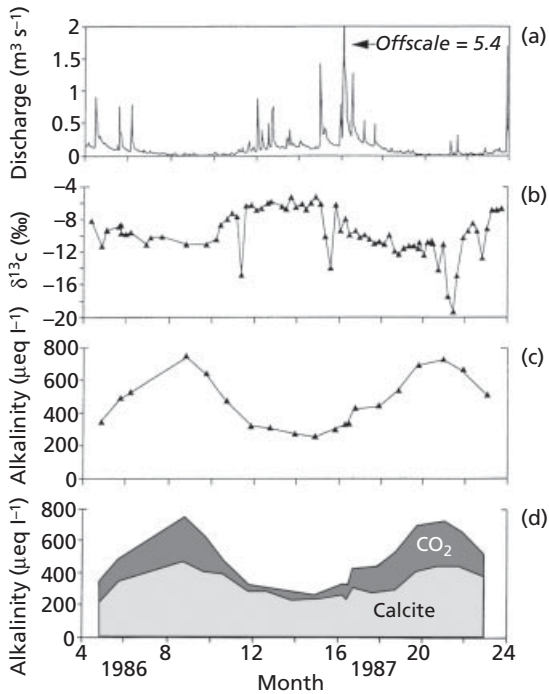

Figure 10.6 Seasonal changes at Hunting Creek in the Catoctin Mountains, MD, 1986–87. (a) Discharge. (b) $\delta^{13}C$ of stream dissolved inorganic carbon (DIC) collected weekly. (c) Alkalinity. (d) Estimation of the relative contributions of carbon from CO_2 and calcite to stream alkalinity using $\delta^{13}C$ of calcite = −5‰ and $\delta^{13}C$ of CO_2 = −21‰; shaded areas show the relative proportions of carbon sources. (Modified from Kendall 1993.)

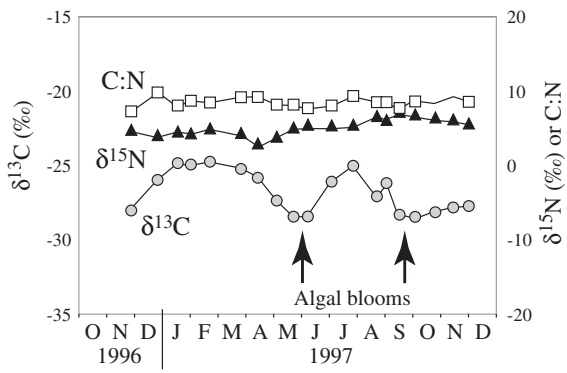

Figure 10.7 Temporal changes in the $\delta^{13}C$, $\delta^{15}N$, and C:N (at.) of particulate organic matter (POM) in the Yazoo River (USA) due to successive algal blooms. The average C:N is 8.5, indicating that the POM is largely algal; the almost constant C:N shows that the proportion of terrestrial vs. algal POM shows little seasonal variability. The oscillations in $\delta^{13}C$ while the $\delta^{15}N$ remains almost constant reflect the relative effects of the small dissolved inorganic carbon (DIC) and large NO_3^- pools on the fractionations caused by assimilation. (Modified from Kendall et al. 2001.)

An additional important source of variation in algal $\delta^{13}C$ is that aquatic plants commonly use two species of DIC (i.e. dissolved CO_2 and HCO_3^-). The $\delta^{13}C$ of these species are controlled by temperature-dependent equilibrium reactions, with the $\delta^{13}C$ of aqueous CO_2 being 6–11‰ lower than the $\delta^{13}C$ of HCO_3^- for temperatures of 35–0°C, respectively (Mook et al. 1974). Although rigorously studied for only a few taxa, many lotic (flowing water) autotrophs, including diatoms and green algae, apparently switch from CO_2 to HCO_3^- when CO_2 supply is insufficient (Raven & Beardall 1981). Some exceptions include cyanobacteria that have specialized inorganic carbon pumps to concentrate DIC, resulting in very little fractionation (Goericke et al. 1994), and red algae and bryophytes that are obligate CO_2 users (Raven & Beardall 1981; Glime & Vitt 1984). Thus, $\delta^{13}C_{DIC}$ and differences in the form of DIC used by autotrophs can contribute considerably to variation in the $\delta^{13}C$ of autotrophs in freshwaters.

Fractionation effects in aquatic plants

Fractionation during carbon uptake and assimilation also varies more widely in aquatic compared with terrestrial plants (Keeley & Sandquist 1992), contributing to the wide range of observed algal $\delta^{13}C$ in streams and lakes. The isotopic fractionations during photosynthesis are dependent on several factors, including aqueous CO_2 concentration and diffusion rate, and plant growth rate (Fry & Sherr 1984; Fogel & Cifuentes 1993; Hecky & Hesslein 1995; Laws et al. 1995). In general, when the availablility of dissolved CO_2 is high and/or when the growth rate is low (i.e., little CO_2 is required), the fractionation between the $\delta^{13}C_{DIC}$ and plants is greatest. However, when there is a scarcity of CO_2 and/or when growth rates are high, plants discriminate less against $^{13}CO_2$ and, as described above, may shift to use of HCO_3^- (Fry & Sherr 1984; Mariotti et al. 1984; Fogel & Cifuentes 1993; Laws et al. 1995; Barth et al. 1998). This mechanism may explain the typical increase in algal $\delta^{13}C$ during spring and summer when primary production is highest and the concentration of CO_2 is lowest because of uptake. An additional mechanism affecting oscillations in $\delta^{13}C$ of algae during successive algal blooms is that as photosynthesis consumes more and more of the DIC pool, the residual DIC becomes progressively ^{13}C-enriched due to fractionation, resulting in a corresponding increase in the $\delta^{13}C$ of algae (Figure 10.7).

Although there is a large range of growth rates for algae in rivers due to variable light and nutrient limitation, much more variation in ε appears to be related to effects of CO_2 supply (Finlay 2004). CO_2 availability is strongly influenced by the physical constraints of gas diffusion in aquatic ecosystems. CO_2 diffuses four times more slowly through water than air, and this has two important consequences for DIC use and isotope fractionation by freshwater autotrophs. First, the thickness of the diffusive (boundary) layer around aquatic plants varies according to water velocity or turbulence. Thick boundary layers lead to a reduced "apparent fractionation" because $^{13}CO_2$ that is

discriminated against is effectively trapped within the boundary layer and partially assimilated before it can diffuse away, resulting in algae with higher $\delta^{13}C$. While the actual kinetic isotope fractionation factor for assimilation may be unaffected by diffusion, comparison of the $\delta^{13}C$ of the algae and $\delta^{13}C_{DIC}$ of water (beyond the boundary layer) shows a smaller difference in their $\delta^{13}C$ values than when the boundary layer is thinner. This effect has been consistently demonstrated in lakes and open canopied streams with low CO_2 concentrations (e.g., Hecky & Hesslein 1995; Finlay et al. 1999).

A second consequence of slow CO_2 diffusion is that the dissolved CO_2 pool (and consequently the entire DIC pool) equilibrates very slowly with the atmosphere, resulting in large deviations from atmospheric concentrations when rates of photosynthesis and respiration are not balanced, or when groundwater inputs of DIC are high. CO_2 concentrations in rivers thus range from levels well below atmospheric saturation to as much as 20 fold higher or more (Duarte & Agusti 1998; Finlay 2003). Therefore, in contrast to terrestrial plants, isotopic fractionation of DIC by algae often is influenced by multiple factors in aquatic environments.

Controls on $\delta^{15}N$ of aquatic plants and DIN

Values for $\delta^{15}N$ of freshwater aquatic plants range from about −15 to +20‰ (Hamilton & Lewis, 1992; Angradi 1993, 1994; Thorp et al. 1998; Cloern et al. 2002; and unpublished U.S. Geological Survey data from the Everglades, Florida). The extreme $\delta^{15}N$ values are often associated with human disturbances of various kinds, with $\delta^{15}N$ values in the range of −1 to +7‰ perhaps typical of undisturbed riverine and marsh ecosystems. In a study of >1000 aquatic plants in the Everglades, algae (periphyton and epiphyton) had the same average $\delta^{15}N$ as macrophytes (+2‰), and both had $\delta^{15}N$ values as high as +15‰; however, macrophytes (e.g. lilypads) had values as low as −13‰ whereas the lowest algae $\delta^{15}N$ values were −7‰ (unpublished U.S. Geological Survey data). The same principles of source and fractionation effects described above for $\delta^{13}C$ also apply to $\delta^{15}N$. The $\delta^{15}N$ of aquatic plants is controlled by the type of DIN utilized, its $\delta^{15}N$ value, and the fractionations associated with discrimination against ^{15}N during uptake of N that may vary by plant species and environmental conditions. As for $\delta^{13}C$, fractionations between the $\delta^{15}N$ of DIN and the plant are greatest when the pool sizes are large and/or growth rates low, and smallest when DIN is scarce and/or growth rates are high.

Controls of $\delta^{15}N$ of basal resources in food webs have been less well studied than for $\delta^{13}C$ in aquatic ecosystems, in part because of the complexity of the nitrogen cycle, and in part because of the analytical challenges in measuring the $\delta^{15}N_{DIN}$ and of organic matter compounds with low %N. Recent improvements in natural abundance methods for analyzing NH_4^+ and NO_3^- are rapidly

increasing the available data (Sigman et al. 1997, 2001; Holmes et al. 1998; Sebilo et al. 2004), but there are still few studies of the effect of different DIN species on the $\delta^{15}N$ of plants and detritus in rivers.

Source effects

The main N sources for non-fixing aquatic plants are NH_4^+ and NO_3^- (i.e. DIN). The $\delta^{15}N_{DIN}$ range widely in the environment (see Kendall et al., this volume, pp. 375–449), from typical low values (−10 to +5‰) for N derived from N fixation or fossil fuel combustion to over +30‰ for N derived from animal waste or that has undergone intensive denitrification (Heaton 1986). Variation in the sources and sinks for DIN control $\delta^{15}N_{DIN}$ in rivers. The main sources of DIN in fresh waters are atmospheric deposition, mineralization of organic matter (from soil, terrestrial plant detritus, sediments, and N-fixing aquatic plants), and fertilizer and animal waste. The main sinks are assimilation, degassing of N_2 and other gases to the atmosphere via denitrification and nitrification pathways, and recharge to groundwater.

Like $\delta^{13}C_{DIC}$, $\delta^{15}N_{DIN}$ are affected by both in-stream and watershed-scale processes. In addition, the $\delta^{15}N_{DIN}$ and the $\delta^{15}N$ of plants are both strongly affected by the form of the DIN (i.e. NH_4^+ and NO_3^-). The first control is briefly described here, and explored much more thoroughly in Kendall et al., this volume, pp. 375–449. The main watershed-scale influences on $\delta^{15}N_{DIN}$ are: (i) land use and extent of human disturbance in the watershed and airshed; (ii) the relative proportions of groundwater vs. surface runoff contributing to streamflow; (iii) the prevalence of N-fixing plants (such as alders and legumes) in the watershed; (iv) the presence of reducing conditions in groundwater, the riparian and hyporheic zones, and sediments; and (v) climate (e.g., temperature, rain amount).

The main processes that affect $\delta^{15}N_{DIN}$ in rivers are (i) assimilation, (ii) nitrification, (iii) denitrification, and (iv) mineralization. Redox conditions in the stream, a function of both in-stream and watershed-scale processes, also have a profound affect on $\delta^{15}N_{DIN}$ because it controls whether the dominant form of DIN is NO_3^-, NO_2^-, or NH_4^+. All of these processes influence $\delta^{15}N_{DIN}$ because the residual reactants in the water column, sediments, or soils become enriched in ^{15}N (Figure 10.5). The fractionations associated with microbial N transformation that control, in part, $\delta^{15}N_{DIN}$ are discussed here, while plant fractionation of $\delta^{15}N_{DIN}$ during N uptake is discussed in the following section.

The $\delta^{15}N_{DIN}$ is strongly affected by watershed-scale processes and cycling of N in streams. Mineralization usually causes only a small fractionation (ca. 1‰) between organic matter and ammonium. The extent of fractionation during nitrification is dependent on the size of the substrate pool (reservoir). In N-limited systems, the fractionation associated with nitrification is usually

minimal and depends on which step is rate determining. Because the oxidation of nitrite to nitrate is generally rapid in natural systems, this is often not the rate determining step, and most of the N fractionation is probably caused by the slow oxidation of ammonium. Uptake of NO_3^- by algae may also be a major control on in-stream variation in $\delta^{15}N_{NO_3}$, particularly in eutrophic, nitrate-rich rivers.

In pristine basins, DIN from watersheds is derived mainly through mineralization of organic matter and subsequent nitrification in soils and groundwater; nitrate is the main form of DIN transported to streams. In such streams, transport of dissolved organic N (DON) may equal or exceed DIN inputs to streams; much less is known about $\delta^{15}N_{DON}$. The $\delta^{15}N$ of NO_3^- leaving terrestrial ecosystems is often low in such settings (Kendall et al., this volume, pp. 375–449). However, given that it is estimated that half the nitrogen added to the biosphere currently is from anthropogenic sources (Vitousek et al. 1997), it is likely that much of the N supplied by watersheds to river systems is from agricultural fertilizer applications, animal feed lots, atmospheric deposition, and sewage. In many situations, nitrate from fertilizer can be distinguished from nitrate from animal waste because most synthetic fertilizers are generated from air nitrogen and therefore possess $\delta^{15}N$ values within a few per mil of zero, whereas nitrate derived from waste usually has $\delta^{15}N$ values between +10‰ and +20‰ (Heaton 1986; Kendall 1998; Kendall et al., this volume, pp. 375–449).

Biologically mediated reduction of nitrate to N_2 and other gases (i.e. denitrification) in low-O_2 groundwater, soils, and sediments can have a large influence on $\delta^{15}N_{DIN}$ moving from land to water, described in greater detail in Kendall et al., this volume, pp. 375–449. Briefly, denitrification in groundwater and riparian zones causes the $\delta^{15}N$ of the residual nitrate to increase exponentially as nitrate concentrations decrease. For example, denitrification of fertilizer nitrate ($\delta^{15}N$ of +0‰) can yield residual nitrate with much higher $\delta^{15}N$ values (e.g., +15 to +30‰) that are within the range of compositions expected for nitrate from manure or septic-tank sources. Measured enrichment factors (apparent fractionations) associated with denitrification range from −40 to −5‰ (Kendall 1998).

Denitrification in the water column in rivers is rare but common in benthic sediments. Benthic denitrification has been shown to cause small fractionations (ranging from −1.5 to −3.6‰) in the $\delta^{15}N_{NO_3}$ in the overlying waters. These fractionations are much smaller than expected for groundwater denitrification because nitrate diffusion through the water–sediment interface, which causes minimal fractionation, is the rate determining step (Sebilo et al. 2003; Lehman et al. 2004). The isotopic fractionation caused by diffusive transport of DIN may influence algal $\delta^{15}N$, and small effects have been observed under experimental conditions (Macleod & Barton 1998; Trudeau & Rasmussen 2003).

The final source effects we consider are isotopic differences between the two main forms of DIN available to aquatic plants, NH_4^+ and NO_3^-. NH_4^+ is

usually the more preferred form of DIN, so isotopic differences between these forms or their relative availability may have a large influence on plant $\delta^{15}N$. Unlike DIC, the speciation of NH_4^+ and NO_3^- is not controlled by equilibrium chemical reactions; there is no a priori reason for their $\delta^{15}N$ values to be similar or related. Instead, the relative proportion of these species is strongly affected by redox conditions, with NO_3^- the more abundant species in well-oxygenated waters and NH_4^+ and NO_2^- more abundant in low-O_2 waters. The concentration of NO_2^- can be more than 10 times that of NO_3^- in highly eutrophic waters (e.g., organic-rich sediments or animal waste lagoons). Many studies have observed large and variable differences in the $\delta^{15}N$ values of NH_4^+ and NO_3^-, particularly in disturbed or eutrophic ecosystems (Kendall 1998); rapid and complete cycling of N may result in relatively consistent steady-state $\delta^{15}N$ values among the various N pools.

Fractionation effects in aquatic plants

As for carbon, aquatic plant fractionation of $\delta^{15}N$ is also influenced by the supply relative to demand for DIN, but far less is known about the fractionations for freshwater algae than for terrestrial or marine plants. Under nitrogen-limited conditions, uptake of DIN by terrestrial and aquatic plants causes little or no fractionation of $\delta^{15}N_{DIN}$ but this situation changes rapidly in the presence of excess N (Fogel & Cifuentes 1993; Pennock et al 1996; Casciotti et al. 2002; Granger et al. 2004; Needoba et al. 2004). A conceptual model developed in the Great Lakes (USA) describes that the $\delta^{15}N$ of seston is controlled by the balance between NH_4^+ uptake and degradative processes that increase the $\delta^{15}N$, and NO_3^- uptake that decreases the $\delta^{15}N$ of seston (McCusker et al. 1999).

The $\delta^{15}N$ of algae in rivers in the USA is generally about 4–5‰ lower than the $\delta^{15}N$ of the associated NO_3^- (Battaglin et al. 2001a, 2001b; Kratzer et al. 2004). Data from a longitudinal sampling of the San Joaquin River (USA) illustrates this pattern (Figure 10.8). Seston samples collected in the riverine part of this transect were > 90% algae. In this section, NH_4^+ concentrations were too low to analyze for $\delta^{15}N$, and NO_3^- is believed to be the main source of N for algal growth. The downstream increase in NO_3^- concentration, $\delta^{15}N_{NO3}$, and $\delta^{15}N_{POM}$ in the riverine section are thought to reflect increases in groundwater (with higher $\delta^{15}N$ values suggestive of animal waste) downstream (Kratzer et al. 2004).

Controls on $\delta^{34}S$ of aquatic plants and DIS

As described for terrestrial plants, $\delta^{34}S_{DIS}$ may be influenced by atmospheric or local mineral sources, and the isotopic fractionations associated with S cycling that alter the $\delta^{34}S$ of sulfate or sulfide. The $\delta^{34}S$ of aquatic plants are controlled primarily by the $\delta^{34}S$ of DIS, and are little affected by plant fractionation. A survey of the $\delta^{34}S$ of aquatic plants from the Everglades

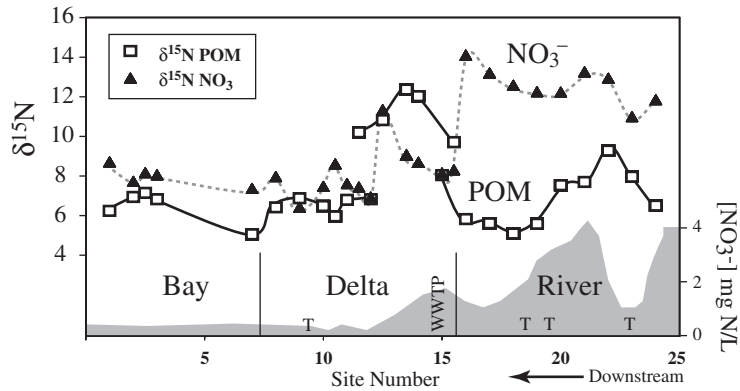

Figure 10.8 Spatial changes in nitrate concentrations, nitrate $\delta^{15}N$, and suspended particulate organic matter (POM; which is predominantly algal) $\delta^{15}N$ in the San Joaquin River, CA, due to downstream changes in inputs of NO_3^- from groundwater, waste-water treatment plants (WWTPs), and other inputs. The river, delta, and bay reaches, as well as the locations of major tributaries (T) and WWTPs are marked. The transect spans a distance of >100 km, from the confluence with Mud Slough (site 25) to the Golden Gate Bridge (site 1). The drop in NO_3^- at site 23 and the slightly downstream peaks in $\delta^{15}N$ are a result of a large algal bloom at the confluence with the Merced River; the $\delta^{13}C$ values of POM also oscillate near the confluence owing to algal blooms due to mixing of waters. (C. Kendall, unpublished U.S. Geological Survey data.)

found values of +5 to +33‰ (Kendall, unpublished U.S. Geological Survey data), similar to the range of $\delta^{34}S_{SO4}$ observed in another study in the Everglades (Bates et al. 2002). The highest plant $\delta^{34}S$ values were found in anoxic areas affected by sulfate reduction (Kendall et al. 2000). More commonly, anoxic areas in streams and bogs result in organic matter with lower than normal $\delta^{34}S$ (Trust & Fry 1992). For example, mangroves can have $\delta^{34}S$ values as low as −20‰ (Fry et al. 1982).

Aquatic plants are less commonly analyzed for $\delta^{34}S$ than for either $\delta^{13}C$ or $\delta^{15}N$ because the $\delta^{34}S$ analytics are more complicated, many aquatic plants and seston have low %S, and also much less is understood about sources of S variability in ecosystems. However, part of the explanation for the scarcity of $\delta^{34}S$ data for organics is the fear (mostly unfounded) that analysis will cause contamination of the mass spectrometer. Recent improvements in natural abundance methods for analyzing organic samples are rapidly increasing the available data (e.g., Fry et al. 2002; Yun et al. 2005), but there are still very few studies of the effect of different DIS species on the $\delta^{34}S$ of plants and detritus in rivers. Despite these problems, $\delta^{34}S$ of aquatic plants has been used when a dual (C, N) isotope approach has proved unsuccessful (Peterson et al. 1985; Loneragan et al. 1997; Connolly et al. 2003), especially in marine or coastal environments where plants are

likely to be isotopically labeled with the high $\delta^{34}S$ of marine sulfate (about +20‰).

Source effects

A literature review of $\delta^{34}S$ values in aquatic ecosystems found that the $\delta^{34}S_{SO_4}$ in lakes ranged from +3.5 to +87‰, with the low values found in deep waters, and the $\delta^{34}S_{H_2S}$ ranged from −32 to −11‰ (Nriagu et al. 1991). The $\delta^{34}S_{SO_4}$ in rivers also show a wide range (−20 to >+30‰) with mean values for rivers in North America, Europe, and globally of +4, +6, and +7‰, respectively (Nriagu et al. 1991). Marine sulfate has a $\delta^{34}S$ of ca. +20‰, and atmospheric $\delta^{34}S_{SO_4}$ values are typically in the range of −5 to +25‰ (Wadleigh et al. 1996; Krouse & Mayer 2000).

Like $\delta^{13}C_{DIC}$ and $\delta^{15}N_{DIN,}$ the $\delta^{34}S_{DIS}$ is affected by both in-stream and watershed processes. In addition, the $\delta^{34}S_{DIS}$ and the $\delta^{34}S$ of plants are both strongly affected by the form of the DIS (i.e. SO_4^{2-} or H_2S). The main watershed characteristics that affect $\delta^{34}S_{DIS}$ are: (i) land use and extent of human disturbance in the watershed and airshed; (ii) the presence of sulfate on soil exchange sites or sulfide minerals in soils, sediments, or bedrock; (iii) the relative proportions of groundwater vs. surface runoff contributing to streamflow; and (iv) the presence of reducing conditions in groundwater, the riparian and hyporheic zones, and sediments.

The main processes that affect $\delta^{34}S_{DIS}$ in rivers are (i) sulfate reduction and (ii) sulfide oxidation. Redox and pH conditions in the stream, which are a product of both in-stream and watershed-scale processes, also have a profound affect on $\delta^{34}S_{DIS}$ because this controls whether the dominant form of DIS is SO_4^{2-} or H_2S. For good discussions of watershed and biogeochemical controls on the $\delta^{34}S$ of sulfate and sulfides, see Mitchell et al. (1998) and Trust & Fry (1992), respectively.

As described for terrestrial plants, $\delta^{34}S_{DIS}$ may be influenced by the isotopic fractionations associated with S cycling that alter the $\delta^{34}S$ of sulfate or sulfide. Bacterial reduction of sulfate is the primary source of the variability of $\delta^{34}S$ observed in freshwater systems because sulfide oxidation involves minimal fractionation of S. During sulfate reduction, the bacteria produce H_2S gas that has a $\delta^{34}S$ value ca. 25‰ lower than the sulfate source (Clark & Fritz 1997). Consequently, the residual pool of sulfate becomes progressively enriched in ^{34}S. Thus, long-term anaerobic conditions ought to be reflected in the $\delta^{34}S$ values of sulfate, and consequently $\delta^{34}S$ of plants, that are significantly higher than that of the original sulfate source.

Sulfate-reducing bacteria use dissolved sulfate as an electron acceptor during the oxidation of organic matter. Since this process is accompanied by oxidation of organic matter, there is a corresponding shift in $\delta^{13}C_{DIC}$ toward that of the organic source due to the production of CO_2. Under conditions of low concentrations of reactive organic matter, re-oxidation of mineral

sulfides can lead to constant recycling of the dissolved sulfur pool. In such cases, increases in $\delta^{34}S$ do not occur in conjunction with reaction progress since the sulfide concentration in solution is held approximately constant (Fry 1986; Spence et al. 2001).

Fractionation effects in aquatic plants

Sulfate is the dominant DIS species used by plants, especially in environments where sulfide concentrations are low. However, in environments where sulfide concentrations are significant, H_2S inhibits the uptake of sulfate (Brunold & Erismann 1975). In most cases, assimilatory sulfate reduction does not lead to significant fractionations; for aquatic plants, typical fractionations range from 0 to 3‰ (Nriagu et al. 1991). The unusually low $\delta^{34}S$ values of mangroves have been attributed to assimilation of H_2S by plant roots in the mud (Fry et al. 1982).

Microbial heterotrophs

The last organic matter "source" for rivers we consider, heterotrophic bacteria and fungi, are potential consumers of the four other types of organic matter that we have considered (Figure 10.1). However, because of their ubiquity and potential to modify isotope ratios of other sources, we consider them separately from other consumers in food webs.

The $\delta^{13}C$ of heterotrophic bacteria growing on organic carbon substrates are typically the same or slightly higher than the $\delta^{13}C$ of the organic matter used (Coffin et al. 1989). However, $\delta^{15}N$ relationships are considerably more complicated. Since N availability often limits terrestrial plant growth, terrestrial detritus inputs to freshwaters initially have high C : N ratios (Sterner & Elser 2002), with insufficient N to meet microbial growth demands. Subsequent microbial immobilization of DIN by bacteria can cause significant changes in the $\delta^{15}N$ of bacteria, as well any organic particles they are adhered to, if the $\delta^{15}N$ of DIN used by bacteria are different from the $\delta^{15}N$ of the original organic matter (Caraco et al. 1998). Such effects on $\delta^{15}N$ would be minimal where microbial incorporation of N does not occur (e.g. low C : N detritus). However, terrestrial plant litter has high C : N and frequently has lower $\delta^{15}N$ than aquatic DIN, leading to conditions that favor changes in bulk $\delta^{15}N$ during decomposition of terrestrial organic matter in aquatic ecosystems.

To illustrate this point, we compare $\delta^{15}N$ of oak leaves decomposing in a large eutrophic river (Hudson River, NY) and a small pristine stream (Fox Creek, CA; Figure 10.9). In Fox Creek, $\delta^{15}N_{DIN}$ is apparently similar to that of leaves, so that minor temporal changes are observed during decomposition. In contrast, $\delta^{15}N_{DIN}$ in the nitrate-rich Hudson is much higher than $\delta^{15}N$ of riparian plants, leading to rapid increases in the $\delta^{15}N$ of decomposing leaves.

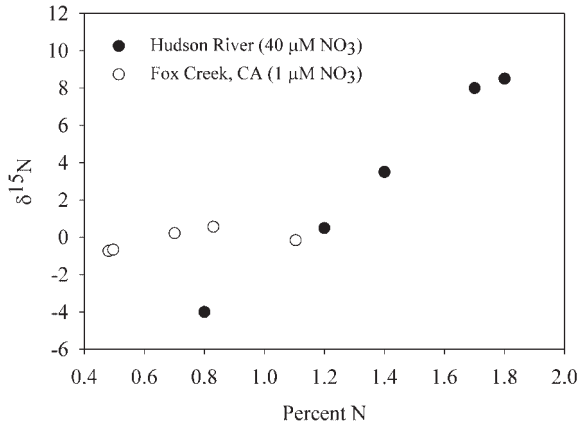

Figure 10.9 Change in $\delta^{15}N$ in terrestrial leaf litter (*Quercus* spp. for both sites) in a pristine stream (Fox Creek, CA; Finlay, unpublished data) and eutrophic river (Hudson River, NY; Caraco et al. 1998) during *in situ* decomposition and microbial incorporation of external N from river water.

There is little known about the $\delta^{15}N$ and $\delta^{34}S$ of bacteria in natural settings, mainly because of the difficulty of isolating pure samples. A recent study of different fractions of organic matter in the Ohio River (USA) showed that the colloidal (<0.2 μm) fraction, which contains a large proportion of heterotrophic bacteria, had $\delta^{13}C$ and $\delta^{15}N$ values of about −30‰ (±2‰) and ca. 0‰ (±2‰), respectively. These values were ca. 2‰ lower than the values for terrestrial OM and 7–12‰ lower than the $\delta^{15}N$ of both algal and detrital OM (Delong & Thorp 2006). Low $\delta^{15}N$ values have previously been reported for bacteria. For example, Wissel & Fry (2005) suggested that the low $\delta^{15}N$ of seston (to −6‰) collected in the winter from river sites near the mouth of the Mississippi River (USA) were due to large inputs of bacteria. This explanation was consistent with the low C:N (ca. 5.5) and the low chlorophyll levels. This might be the explanation for $\delta^{15}N$ values seen at other large river sites in the USA, where changes in seston $\delta^{15}N$ values as low as −16‰ have been observed (Kendall et al. 2001; unpublished U.S. Geological Survey data for these same sites). Bacteria growing on organic-rich bottom sediments may be affected by the low $\delta^{34}S$ of H_2S produced by sulfate reduction in such reducing environments.

C:N ratios

Data for C:N (at.) ratios of freshwater aquatic vegetation exclusive of phytoplankton are sparse. The reported range of C:N ratio for freshwater

phytoplankton is about 5–8, averaging close to the Redfield ratio of 6.6 for marine phytoplankton (Redfield 1958; LaZerte 1983; Harris 1986). Thorp et al. (1998) reported C:N values ranging from about 8 to 10 for benthic algae and from about 11 to 12 for aquatic macrophytes in the Ohio River (USA). Hamilton & Lewis (1992) report C:N ratios of different size-fractions of algal-derived seston from the Orinoco River floodplain (Venezuela) between about 6 and 9. The higher and more variable C:N ratios of lacustrine plankton compared with marine plankton appear to be caused by variations in N and P nutrient limitations in the lakes (Hecky et al. 1993). Riverine microorganisms from the Mississippi River (USA) have C:N values in the range of 5–15 (Rostad et al. 1997). Seston and algae samples collected from some 70 small-river sites in the upper Mississippi River basin during a dry period in August 1998 (unpublished U.S. Geological Survey data, C. Kendall) showed the following average C:N values: seston was 9.4 (±1.8, n = 68), handpicked *Spirogyra* and *Cladophora* was 11.7 (±2.3, n = 68), and periphyton on woody snags was 14.6 (±3.5, n = 42). Various submerged macrophytes (n = 490) and periphyton (n = 640) from the Everglades (USA) have C:N ratios with median values of 22 and 16, respectively; C:N values for old, woody, and/or partially degraded Everglades aquatics can have C:N values > 80 (unpublished U.S. Geological Survey data, C. Kendall). A study of ca. 900 plants in the fresh, brackish, and salt water in the San Francisco Bay ecosystem showed C:N (wt.) ratios of 10 to >100 (Cloern et al. 2002). In summary, there can be a wide range in C:N ratio for various types of aquatic plants (Figure 10.3), but generally plankton has C:N values (5–8) that are lower than periphyton and macrophytes (10–30), and the C:N values of terrestrial and aquatic plants show considerable overlap.

C, N, and S isotopic variability and its applications in river ecology

Variation in stable isotope ratios among organic materials in rivers provides the basis of many important applications in rivers (Table 10.1). As we have seen, stable isotope ratios are highly varied in rivers and often influenced by multiple biogeochemical, physical, and physiological processes throughout watersheds. When well understood and predictable, this variation is useful; however, isotope tracers may be much less efficiently used if the signals are unexpectedly variable, or if source separation is ultimately not possible. Environmental conditions and resource type and abundance show substantial seasonal and spatial variability. These changes directly influence $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in resources and consumers, and thus determine when, where, and how stable isotopes may be applied in food web studies. Below we identify the major spatial and temporal patterns in stable isotope ratios in river

food webs, identify predictive relationships between controlling variables, and show how and where this variation can serve as a useful tracer for the study of river ecology.

Spatial patterns and applications of stable isotope tracers

Recent studies in watersheds ranging from meters to basin scales have shown three types of strong spatial patterns in the isotopic compositions of organic matter sources that are helpful in the planning and design of stable isotope studies: longitudinal, water velocity gradients, and anthropogenic. These patterns reflect spatial variability in the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of dissolved inorganic species, which is integrated into the isotopic compositions of organisms living in the river.

Longitudinal effects on $\delta^{13}C$

The percent of the organic C that is derived from allochthonous (terrestrial) sources vs. autotrophic (in stream) sources varies systematically from headwaters to mid-size streams to major rivers (Figure 10.2; Vannote et al. 1980). $\delta^{13}C$ can help determine the origin of organic matter in river networks and its contribution to production in food webs, but only when terrestrial detritus and autothonous OM have distinct $\delta^{13}C$ values. The terrestrial detritus that often dominates organic matter pools in rivers has a well-constrained mean $\delta^{13}C$ value of $-28.2 \pm 0.2‰$ (± standard error; C3 plants only), with no consistent trend with stream size (Figure 10.10; Finlay 2001) because the aquatic detrital pool integrates terrestrial plant $\delta^{13}C$ through time and space. In contrast, many rivers show strong longitudinal (i.e., downstream) changes in the $\delta^{13}C$ of DIC, algae, seston, and consumers. For example, in small temperate watersheds, algal $\delta^{13}C$ are highly varied, from as low as −47‰ in headwater streams and springs to values as high as −8‰ in moderate-size rivers (Figure 10.10).

Longitudinal changes in algal $\delta^{13}C$ are due to combined effects of physical and biogeochemical processes in streams and their watersheds. Examples of physical processes that can cause longitudinal changes in $\delta^{13}C_{DIC}$ and consequently in $\delta^{13}C$ of algae include variable percentages of water in the stream derived from groundwater vs. soil water with watershed scale (Bullen & Kendall 1998), and degassing of CO_2 from springs and groundwater that cause a gradual increase in $\delta^{13}C_{DIC}$ (Kendall & Doctor 2004; Finlay 2004; Doctor et al. in press). The main biological processes causing longitudinal changes in $\delta^{13}C_{DIC}$ and algal $\delta^{13}C$ are the balance of total ecosystem respiration and photosynthesis (Figure 10.5). These physical and biological processes may be occurring simultaneously along the stream reach.

In temperate forested rivers, strong longitudinal patterns in algal $\delta^{13}C$ are caused by downstream changes in $\delta^{13}C_{DIC}$ and algal fractionation, which appear to be strongly linked to stream CO_2 (Finlay 2004). In a study within a

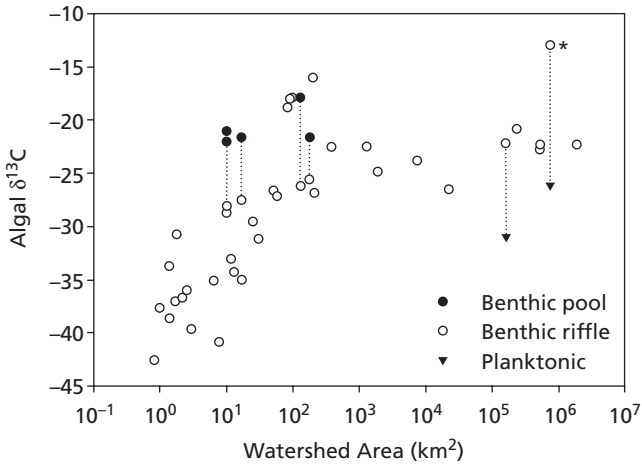

Figure 10.10 Spatial variation in $\delta^{13}C$ of autotrophic organic carbon sources to temperate river and riparian food webs during summer. $\delta^{13}C$ data representing the most edible algal form (usually epilithic diatoms or periphyton) within a site were used for the plot. Considerable variation exists between autotrophs within many of these sites, but most of these forms (bryophytes, rhodophyta, and filamentous chlorophytes) are inedible or edible only by specialist grazers. In some cases, data for herbivores with primarily algal diets were used instead of direct measurements. Data for other biomes (e.g. alpine, arctic, desert) were not included. Dotted lines indicate data for pool and riffle or benthic and planktonic algae pairs within a location. The * indicates data from the same river (St Lawrence, Canada) but different sampling sites and dates. (Data are updated and expanded from Finlay (2001) to include additional sources: Delong & Thorp (2006), Barnard et al. (2006), Finlay (2004), Huryn et al. (2002), deBruyn & Rasmussen (2002), McCutchan & Lewis (2002), Delong et al. (2001), and Thorp et al. (1998).)

single watershed, Finlay (2004) found that 90% of variation in benthic algal $\delta^{13}C$ in riffles was explained by CO_2 concentration since it directly affects fractionation. However, algal $\delta^{13}C$ also showed correlations with $\delta^{13}C_{DIC}$ and algal growth rates. Thus, measurements of CO_2 or its correlates (e.g. pH, O_2) may provide a predictive tool for identification of useful spatial variation in $\delta^{13}C$.

Longitudinal patterns in CO_2 concentrations in river networks appear to control $\delta^{13}C$ differences between algae and terrestrial OM. In the headwater streams, CO_2 is high due to inputs from soil and groundwater, and $\delta^{13}C_{DIC}$ and algal growth rates are low. These conditions result in algal $\delta^{13}C$ values that are lower than the $\delta^{13}C$ of terrestrial detritus. With increasing stream size, algal $\delta^{13}C$ increase because of the combined effects of several processes, including lower fractionation due to low CO_2 and high algal growth rates and biomass level, and higher $\delta^{13}C_{DIC}$ due to uptake of DIC and perhaps exchange with atmospheric CO_2 and degassing. In large rivers, algal $\delta^{13}C$ might then

decrease if the rivers become strongly heterotrophic, as observed in the Amazon River (Araujo-Lima et al. 1986). However, some recent publications have found that a large percent of the seston in large rivers in the USA is algal derived (e.g., Kendall et al. 2001; Wissel & Fry 2005; Delong & Thorp 2006).

Algal $\delta^{13}C$ are thus most different from those of terrestrial detritus in headwater streams, lentic habitats of small rivers, and some large, heterotrophic rivers (Figure 10.10; Araujo-Lima et al. 1986; Finlay 2004). Overlap between algal and terrestrial $\delta^{13}C$ is most often observed in fast flowing, well-mixed riffles of small rivers (Figure 10.10). It is important to note, however, that substantial variation may exist for a given stream size. In addition, considerable $\delta^{13}C$ variation exists between autotroph taxa; if diverse autotroph growth forms are present, and if specialist herbivores that consume macroalgae, bryophytes, or macrophytes are important to the food web under consideration, these sources must be accounted for.

Longitudinal gradients in $\delta^{13}C_{DIC}$ and consequently algal $\delta^{13}C$ can also be caused by physical, biogeochemical, or anthropogenic factors that create variations in chemical conditions over short spatial scales. Some examples may include springs or groundwater upwelling zones (e.g., Rounick & James 1984; Hoffer-French & Herman 1989; Pentecost 1995; Finlay 2003, 2004; Kennedy et al. 2005); and junctions with other rivers, lakes, and impoundments that contribute nutrients and cause algal blooms. For example, Angradi (1993) interpreted variations in $\delta^{13}C$ and $\delta^{15}N$ of seston collected at intervals downstream from a dam on a Rocky Mountain (USA) river to reflect changes in the proportions of local source (e.g. riverine) materials that were rapidly overprinting the reservoir (e.g lacustrine) signature.

Water velocity gradient effects on $\delta^{13}C$

Downstream changes in stream channel morphology caused by the combined effects of differences in bedrock geology, basin slope, and sediment transport create alternating pools and riffles that have very different mean water velocities. These changes in stream gradient can have large differences in algal fractionation between habitats, as discussed below, and may also influence $\delta^{13}C_{DIC}$ by affecting groundwater–surface-water interactions. Newly introduced subsurface water usually has a lower $\delta^{13}C_{DIC}$ than the streamwater because of inputs of respiratory CO_2 (see Figure 10.6). However, the effect of the groundwater inputs may be masked by increases in $\delta^{13}C_{DIC}$ caused by rapid degassing of this CO_2-rich groundwater in the turbulence of the riffle (Doctor et al. in press).

Local water velocity conditions influence algal $\delta^{13}C$ through a negative relationship between flow rate and algal fractionation of $\delta^{13}C$. Slower waters

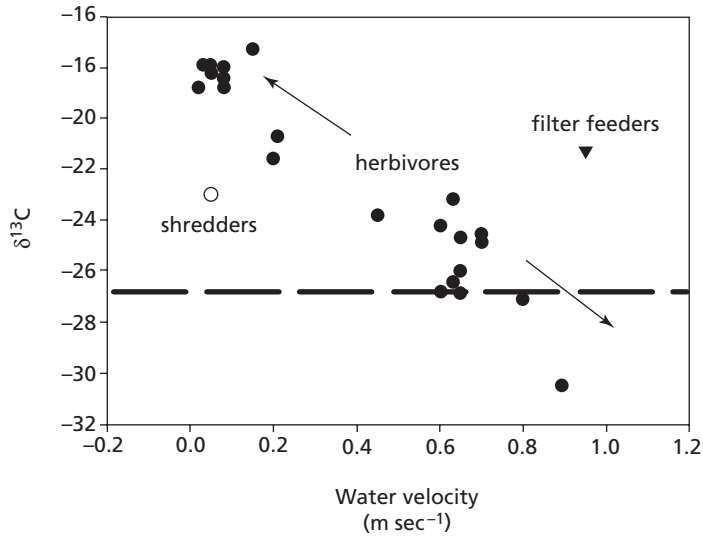

Figure 10.11 Relationship of water velocity with primary invertebrate functional feeding groups in the South Fork Eel River, CA. Herbivores included both collector-gathers and scraper taxa; herbivore $\delta^{13}C$ were strongly correlated with algal $\delta^{13}C$ at the site. The dashed line represents mean $\delta^{13}C$ for terrestrial organic carbon in the river. (Modified from Finlay et al. 2002.)

in pools have thicker benthic boundary layers, dominated by molecular diffusion, than the faster waters in riffles. The thicker diffusive layers inhibit diffusion of CO_2 and consequently cause more build-up of $^{13}CO_2$ near benthic algae in pools than found in riffles. The higher $\delta^{13}C_{DIC}$ in pools causes $\delta^{13}C$ of algae to be higher in pools than in riffles (Figure 10.11). This pattern is more likely observed in streams with pool–riffle geomorphology, where contrasts in water velocity are large, and where CO_2 supply is low and/or photosynthesis rates are high, than in deeper streams or ones with a more constant stream gradient. Thus water velocity effects are not obvious in small shaded streams where CO_2 concentrations are high and algal growth low (Macleod & Barton 1998; Finlay et al. 1999).

The higher $\delta^{13}C$ of algae in pools compared with riffles can impart a characteristic isotopic signature to a patch or habitat, thus providing a useful tool for food web analyses. Specifically, water velocity effects spatially label diet sources of consumers over a short distance along a stream. This label can then be traced through food webs, thus providing new information on the source and scale of resource use by herbivores (e.g., Finlay et al. 2002). For example, in the South Fork Eel River (USA), herbivorous invertebrates clearly rely on local sources of algal production, while presumed leaf-eaters (shredders) were found to consume an equal mix of leaves and algae within their pool habitats (Figure 10.11). Diet sources of

filter-feeding taxa could not be completely resolved with $\delta^{13}C$ because there are three potential sources of suspended particles available to them: suspended terrestrial detritus, and algae transported from both pools and riffles.

The presence of multiple organic matter sources within a site with similar isotope ratios, or isotopic variation over small spatial scales, creates conditions where a single isotope diet tracer is not sufficient to address the full complexity of trophic interactions. Such conditions appear to be most common in large rivers (Delong & Thorp 2006), and potentially at junctions with other rivers, lakes and impoundments (Table 10.1). In some cases, careful separation and isotopic analysis of mixed organic materials can help determine the $\delta^{13}C$ and/or $\delta^{15}N$ of individual sources (Hamilton et al. 2005; Delong & Thorp 2006). Other measurements, such as gut content analyses or C : N ratios, may also help eliminate some potential sources for consumers. However, for situations where there are more than two OM sources present, the best solution to resolving all sources is to use a second tracer, most often $\delta^{15}N$, that shows good isotopic separation among the potential sources to rivers.

Anthropogenic and other spatial effects on $\delta^{15}N$ of algae and consumers

Natural variation and human alterations for the N cycle sometimes create large differences in $\delta^{15}N$ among sources. Of the potential causes of spatial variation in $\delta^{15}N$ in streams, human perturbations of the nitrogen cycle are probably the main cause of large-scale spatial changes in biota $\delta^{15}N$ in watersheds.

Longitudinal changes in nitrate concentrations and $\delta^{15}N_{NO_3}$ often result in longitudinal changes in the $\delta^{15}N$ of algae. There are many potential causes of longitudinal gradients in $\delta^{15}N$ in streams, including anthropogenic contaminants (e.g. fertilizer, animal waste, emissions from power plants), proximity to the ocean (e.g., marine-derived nutrients), redox chemistry (e.g. denitrification, ammonium vs. nitrate as the dominant DIN form), and site-specific natural labeling of DIN pools. In particular, the $\delta^{15}N$ of aquatic biota can be affected by inputs of water from waste water treatment plants (WWTPs) and combined sewage overflows (CSOs) in urban areas, or confined animal feeding operations (CAFOs) and agricultural runoff in farming areas.

Several of these causes of longitudinal gradients in $\delta^{15}N$ are observed in data from a transect along the San Joaquin River (USA). Figure 10.8 shows spatial variation in the $\delta^{15}N$ of nitrate and seston (largely algae) in the San Joaquin River, from the headwaters, through the deltaic wetlands, and to the mouth of the San Francisco Bay. In the riverine part where nitrate concentrations are high, probably because of inputs from agricultural activities, the $\delta^{15}N$ of seston is 4–5‰ lower than $\delta^{15}N_{NO_3}$. In contrast, in the bay part where nitrate concentrations are low and DIN may be limiting photosynthesis

(Wankel et al. 2006), there is little difference between the $\delta^{15}N$ of nitrate and seston. The relationship between nitrate and seston $\delta^{15}N$ is more complex in the delta area because of tidal mixing, multiple channels in the delta, and ammonium inputs to the river from insufficiently treated (oxidized) waste water. Nitrification of the ammonium causes decreases in nitrate $\delta^{15}N$; the subsequent uptake of the now ^{15}N-enriched residual ammonium by algae, causes increases in the $\delta^{15}N$ of the seston.

Sites with higher N concentrations or loads typically have much higher $\delta^{15}N$ compared with pristine watersheds (Mayer et al. 2002). Several watershed studies have shown that the high $\delta^{15}N$ value of DIN derived from sewage (+10‰ to +25‰; Heaton 1986) can be traced in aquatic food webs influenced by urban development (e.g., Cabana & Rasmussen 1996; McClelland & Valiela 1998; Lake et al. 2001). Other studies have shown that the low $\delta^{15}N$ of seston derived from domestic wastes can result in whole food webs having lower $\delta^{15}N$ in impacted sites than in non-impacted sites (e.g., Van Dover et al. 1992; Tucker et al. 1999; DeBruyn & Rasmussen 2002). In agricultural areas where animal waste is often used as a fertilizer, $\delta^{15}N$ in aquatic organisms have been shown to be positively correlated with the percent of agriculture in the watershed (Harrington et al. 1998; Hebert & Wassenaar 2001; Udy & Bunn 2001; Anderson & Cabana 2005). For example, a detailed analysis of longitudinal changes in the $\delta^{15}N$ values of primary consumers (and organisms of higher trophic levels) in nested watersheds in the St Lawrence River Basin (Canada) showed that the $\delta^{15}N$ closely tracked spatial differences in land use, and that the percent of the watershed devoted to agricultural explained 69% of the total variation in $\delta^{15}N$ (Anderson & Cabana, 2005). These increases in the $\delta^{15}N$ of organisms and DIN with increasing N loads, watershed size, and percent agricultural land use in the watershed may be caused by increased amounts of denitrification in the groundwater flowing into the stream. Kinetic fractionation during denitrification can cause substantial increases in the $\delta^{15}N$ of the residual nitrate in streams (see Kendall et al., this volume, pp. 375–449). As a consequence, autotroph $\delta^{15}N$ varies with factors such as land use or amount of sewage input, and not watershed area *per se*.

Other environmental effects can cause spatial variations in $\delta^{15}N$ in rivers. For example, marine organic matter and organisms often have higher $\delta^{15}N$ values than oligotrophic freshwater ecosystems (France 1995; Bilby et al. 1996), creating a useful isotope separation for detecting marine-derived nutrients or prey in freshwater food webs. In addition, terrestrial organic matter appears to be often ^{15}N-depleted relative to aquatic autotrophs (e.g. Harrington et al. 1998; Mulholland et al. 2000; Delong & Thorp 2006), perhaps due to intensive denitrification in riparian and aquatic sediments (Ostrom et al. 2002). At sites where there are large $\delta^{15}N$ differences between autotrophic and detrital food web components, $\delta^{15}N$ are proving useful for source separation, especially when used with advanced statistical techniques (e.g., Delong & Thorp 2006). Finally, local changes in redox chemistry due

to processes occurring in dams or wetlands can also cause spatial changes in $\delta^{15}N$ of biota (Angradi 1993; Kendall et al. 2000, 2001). Much less is known about the behavior of $\delta^{15}N$ at the base of food webs, however, suggesting some caution in its application particularly under highly eutrophic conditions, as discussed below.

Spatial effects on $\delta^{34}S$ of biota

There is much less known about spatial distributions in the $\delta^{34}S$ of aquatic resources and organisms. We identify three potential causes of longitudinal gradients in $\delta^{34}S$ in streams: proximity to the ocean, bedrock geology, and redox chemistry. Sulfur isotopes are an effective tracer of organic matter at the land–ocean margin (Connolly et al. 2003) because ^{34}S-enriched sulfate can be transported up to hundreds of kilometers inland. For example, the $\delta^{34}S$ of marine sulfate and vegetation near the ocean are ca. +20‰ but decreases to +6‰ over ca. 100 km (Wadleigh et al. 1996; Wadleigh & Blake 1999). Geology may influence $\delta^{34}S$ where rivers cross geologic units with large concentrations of sulfide minerals. Oxidation of the sulfide minerals can cause decreases in the $\delta^{34}S$ of riverine sulfate, and presumably aquatic plants, as observed in the McKenzie River system (Hitchon & Krouse 1972). Last, reducing conditions typical of wetlands, bogs, and the deep waters of some lakes lead to low $\delta^{34}S$ values because sulfate reduction produces H_2S with low $\delta^{34}S$ values, which then may be assimilated into plants (Nriagu et al. 1991).

Along a river reach with variable contributions of organic matter derived from wetlands, the $\delta^{34}S$ of stream organic matter and consequently consumers would be expected to vary. For example, in the Everglades the $\delta^{34}S$ of sulfate in canals and marshes increases as agricultural sulfate from near Lake Okechobee is progressively reduced as water flows to the south (Bates et al. 2002). In contrast to the normal $\delta^{34}S$ pattern expected for redox gradients (e.g., lower $\delta^{34}S$ of biota with increasing amount of sulfate reduction to ^{34}S-depleted H_2S), the $\delta^{34}S$ of algae and fish increase with increasing extent of eutrophication and sulfate reduction in the Everglades (Kendall et al. 2000).

Probably the main use of $\delta^{34}S$ in food web studies is differentiating terrestrial versus marine sources of organic matter in near coastal environments, because of the large contrast in $\delta^{34}S$ values at the land–ocean margin (Connolly et al. 2003). There are two main types of studies: ones that use $\delta^{34}S$ to determine terrestrial plant contributions to coastal ecosystems (e.g., Peterson & Howarth 1987; Peterson & Fry 1987), and ones that use $\delta^{34}S$ to determine marine-derived nutrient contributions to near-coast watersheds (e.g., MacAvoy et al. 1998). Because of the extremely sharp gradient in $\delta^{34}S$ values between marine and terrestrial ecosystems, such studies have a high likelihood of producing quantitative results. When two isotope tracers are employed, the combination of $\delta^{34}S$ and $\delta^{13}C$ separates more producers than

other isotope tracer combination (Connolly et al. 2003), despite the high within-producer variability of $\delta^{34}S$ in coastal areas.

Temporal patterns and applications of stable isotope tracers

Implicit in the preceding discussion of source identification is the assumption that consumers are in approximate "isotopic equilibrium" with their food sources. This assumption was not widely tested in many previous applications of stable isotope methods, but temporal variation is increasingly evident in time series of $\delta^{13}C$ and $\delta^{15}N$ in consumers and resources (Kendall et al. 2001; McCutchan & Lewis 2001). Temporal variation in the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of dissolved species and consequently algae has the potential to greatly influence isotope-based inferences of food web relations (e.g. O'Reilly 2002), and should ideally be assessed prior to study design since it determines sampling frequency and the nature of other data necessary to interpret results. Thus, prediction of temporal variation and methods for dealing with it are important for the use of stable isotopes in food web studies.

Seasonal variation in $\delta^{13}C$ of algae

For reasons discussed previously, terrestrial plants show limited temporal variation in $\delta^{13}C$ relative to aquatic plants (e.g. Garten & Taylor 1992; Leffler & Evans 1999; McCutchan & Lewis 2002). While some minor variation exists among terrestrial detrital fractions during decomposition in streams (Hicks & Laboyrie 1999; Finlay 2001), allochthonous detritus has a comparatively constant $\delta^{13}C$ relative to autotrophic $\delta^{13}C$.

In contrast, autotrophs in streams and wetlands can show considerable seasonality in $\delta^{13}C$. In small unproductive streams, $\delta^{13}C_{DIC}$ values often change seasonally in response to watershed processes. In particular, seasonal variation in DIC sources to the stream (e.g., DIC derived from soil respiration vs. weathering reactions), causes higher $\delta^{13}C_{DIC}$ in the winter when DIC concentrations are low, and lower $\delta^{13}C_{DIC}$ in the summer when DIC concentrations are higher (Figure 10.6; Kendall 1993; Bullen & Kendall 1998). The algal $\delta^{13}C$ track the seasonality in $\delta^{13}C_{DIC}$ and CO_2 concentrations (Finlay 2004).

In larger streams and rivers, biological effects (e.g., the effects of algal productivity and/or respiration on CO_2 concentrations and $\delta^{13}C_{DIC}$), can overprint the effects of seasonality in watershed influences on $\delta^{13}C_{DIC}$. For example, when algal productivity is the main control on CO_2 or even DIC concentration, maximum algal $\delta^{13}C$ are typically observed during summer baseflow when productivity is greatest and CO_2 concentrations are lowest (McCutchan & Lewis 2001; Finlay 2004). Successive algal blooms can cause multiple maxima in the $\delta^{13}C$ of algae, as shown in Figure 10.7, where there is a 5‰ oscillation in the $\delta^{13}C$ of predominately algal POM in the Yazoo River (USA) due to algal blooms. Alternately, if respiration dominates ecosystem metabolism,

such as in streams with high detrital inputs, algal $\delta^{13}C$ values tend to be lower in the summer when respiration rates are highest (Figure 10.6).

The effect of this variation on stable isotope studies depends on whether it occurs as gradual (i.e. seasonal) environmental changes or due to more stochastic processes such as floods. Seasonal changes in environmental conditions may alter factors influencing algal $\delta^{13}C$ (e.g. productivity, DIC availability, and $\delta^{13}C_{DIC}$) slowly, producing predicable patterns and long periods of stable signals under baseflow conditions (McCutchan & Lewis 2002; Finlay 2004). This type of situation is more amenable to straightforward use of stable isotope methods. If consumer growth and $\delta^{13}C$ are measured, temporal variation in resource $\delta^{13}C$ may be incorporated into biomass models (McCutchan & Lewis 2002). However, the fast turnover of autotroph biomass causes rapid isotopic responses to environmental changes that affect $\delta^{13}C$. Thus, under eutrophic conditions or when discharge is highly varied (e.g. Singer et al. 2005), frequent sampling for both $\delta^{13}C$ and growth of consumers is necessary to account for temporal changes in autotrophic $\delta^{13}C$.

Given the strong effect of discharge on parameters influencing $\delta^{13}C$ in streams (e.g. nutrients, water velocity, DIC), flow data should provide a useful indicator of temporal variation, and inform sampling decisions and overall study design. For example, Kendall (1993) found a strong inverse relation between discharge and DIC, and between $\delta^{13}C_{DIC}$ and discharge (Figure 10.6). Finlay (2004) also found strong inverse relationships between DIC and discharge, and discharge determined much of the temporal variation in algal $\delta^{13}C$ observed in streams and rivers of the watershed. In a eutrophic stream with thick periphyton, Singer et al. (2005) found that flow history was a better predictor than instantaneous water velocity in explaining spatial and temporal variation in algal $\delta^{13}C$. This finding makes sense considering that algal $\delta^{13}C$ integrate the effects of temporal and spatial variations in local conditions, whereas instantaneous velocity does not.

Seasonal variability in $\delta^{15}N$ of algae

Streams commonly show seasonal variability in $\delta^{15}N_{DIN}$ due to temporal changes in DIN sources and their $\delta^{15}N$ values, flow, and in-stream biogeochemical processes (see Kendall et al., this volume, pp. 375–449). Temporal variation of algae $\delta^{15}N$ is less well known. In their study of four watersheds of differing elevations and sizes on North St Vrain Creek in the Rocky Mountains (USA), McCutchan & Lewis (2002) found that only one site (the montane site) showed seasonality in the $\delta^{15}N$ of algae, with $\delta^{15}N$ values that were highest just before snowmelt and decreased over the summer. Several large rivers in the USA showed minor seasonal variations in the $\delta^{15}N$ of algae-dominated POM (Kendall et al. 2001). Subsequent multi-year data from these same 40 river sites shows that many rivers showed significant seasonal variability, with $\delta^{15}N$ oscillations frequently correlated with discharge changes (C. Kendall,

unpublished U.S. Geological Survey data for these same rivers, 1998–2005). In some cases, DIN concentrations were so high, and DIN sources sufficiently constant, that even massive algal blooms did not cause enough drawdown of the DIN pool to cause changes in the algal $\delta^{15}N$ (Figure 10.7).

Investigations of temporal changes in the $\delta^{13}C$ and $\delta^{15}N$ of algae in marshes in the Everglades (USA) have shown that the $\delta^{13}C$ values show a strong inverse relation to $\delta^{15}N$ values; spatial data also show this same inverse relation. There are several coupled reactions that could cause this pervasive temporal and spatial pattern: respiration and denitrification, respiration and mineralization, and methane formation and then oxidation and denitrification. When water levels are low in the marshes, the $\delta^{13}C$ of algae are lower than when the water levels are higher, consistent with respiration effects exceeding atmospheric exchange effects in the dry season. The coincidence of low $\delta^{13}C$, high $\delta^{15}N$, and high $\delta^{34}S$ values with areas of increased eutrophication supports the theory that seasonal and spatial changes redox chemistry is a major control on the isotopic compositions of biota in this environment (Figure 10.12; Kendall et al. 2000).

Temporal changes in macrophytes related to growth and senescence cycles

Riparian plants can show significant seasonal variations in $\delta^{13}C$, $\delta^{15}N$, and C : N. In a study of wetland plants in the San Francisco Estuary (USA), the most common seasonal pattern was low C : N of foliage in spring when leaves/shoots first emerged, a gradual increase in C : N during the growth season, and a rapid increase in C : N in the fall when the new biomass died (Cloern

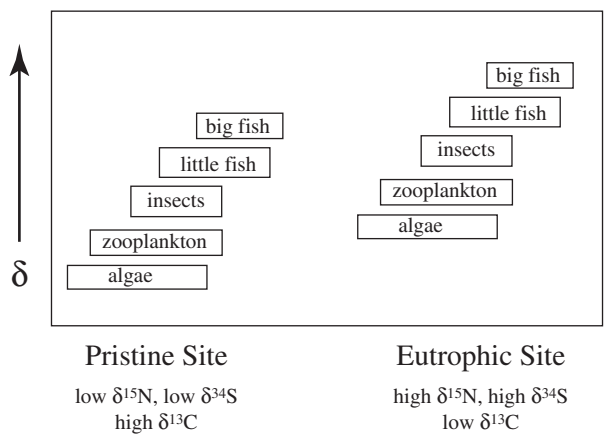

Figure 10.12 Cartoon showing how the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of primary producers at oligotrophic (pristine) vs. eutrophic wetlands sites in the Everglades (USA) affect the isotopic compositions of the entire food web. These site-specific shifts in δ values are due to different suites of biogeochemical reactions that affect the δ values at the base of the food webs.

et al. 2002); however, while some species (e.g., *Salix*, *Typha*) showed strong seasonality in C:N, others (e.g., *Spartina*) did not. Different species also showed different $\delta^{13}C$ and $\delta^{15}N$ patterns, with the isotopic compositions of specific species of plants declining, increasing, or showing no consistent pattern during the growth season (Cloern et al. 2002). There was no consistent relation between $\delta^{15}N$ and C:N of plants during the growth–death cycle; high C:N dead biomass showed high $\delta^{15}N$ values for *Spartina* and low $\delta^{15}N$ for *Typha*. These species-specific patterns of seasonal variability in wetland plants are similar to those reported in a study of terrestrial vegetation by Handley & Scrimgeour (1997).

Temporal changes in isotopic composition caused by degradative processes

Once terrestrial detritus enters streams, its $\delta^{15}N$ may be changed substantially during decomposition as N is immobilized onto litter. The degree of modification during decomposition should depend on the amount of N immobilized and on the contrast between the $\delta^{15}N$ of DIN and the terrestrial plants. High $\delta^{15}N_{NO_3}$ is commonly observed when NO_3 concentrations are high; hence, the strongest effects should be expected in eutrophic streams. This is illustrated by a comparison of $\delta^{15}N$ of decomposing oak leaves in high and low NO_3^- ecosystems (the Hudson River, NY, and Fox Creek, CA, respectively). Work of Caraco et al. (1998) shows that changes in $\delta^{15}N$ during decomposition may be substantial; however, such effects are more subtle under low NO_3^- conditions, as observed for the oligotrophic stream (Figure 10.9). Overall, these observations suggest that use of $\delta^{15}N$ as a source tracer will be least complicated in undisturbed, low DIN streams and lakes. However, separation of aquatic vs. terrestrial sources is usually greater in human-impacted rivers.

Temporal changes in seston composition

Nutrient transport and food webs in larger rivers are often dominated by suspended organic matter so they are considered separately from the preceding sections which dealt primarily with benthic OM and organisms. Few studies in major rivers have had sufficient temporal or spatial coverage for adequate assessment of seasonal or hydrologic changes in seston composition. However, detailed studies in the Amazon River (Brazil) have found seston compositions to be nearly constant over substantial time periods, distances, hydrologic fluctuations, and size fractions (Hedges et al. 1986; Quay et al. 1992). In contrast, seston in the St Lawrence River (Canada) was dominated by *in situ* photosynthesis during warm seasons, and terrestrial detritus during colder periods and storm surges (Barth et al. 1998). Seston in the Sanaga River (Cameroon) and Congo River also varied with discharge, with high proportions of seston derived from C4 plants in savannas during high

discharge periods, and high proportions of seston derived from C3 plants from the riverbanks during low discharge periods (Mariotti et al. 1991; Bird et al. 1998). A study of monthly seston samples from 40 sites on major rivers in the USA found that plankton is the dominant source of seston at many sites, with lower $\delta^{13}C$ and higher percentages of plankton during algal blooms in the spring and summer and downstream of reservoirs (Kendall et al. 2001). Particulate organic matter with unusually low C:N and low $\delta^{15}N$ values at a Mississippi River site was attributed to a bacterial bloom in the winter (Wissel & Fry 2005). These and other studies involving seston are just beginning to elucidate the origin, transport, and cycling of seston in major rivers, and the role it plays in riverine food webs.

Diel changes in algal $\delta^{13}C$ and $\delta^{15}N$

Daily cycles of temperature, redox potential, photosynthesis, and respiration cause cyclic changes in many important aquatic constituents including pH, DIC, conductivity, dissolved gases such as O_2 and CO_2, major elements, and trace metals. Very little is known about diel variations in the isotopic compositions of dissolved constituents in streams. However, recent studies show substantial diel changes in $\delta^{18}O_{O_2}$ and $\delta^{13}C_{DIC}$ that may eventually provide new insight into biogeochemistry of freshwaters. For example, in a recent study in a small stream in Montana (USA), there was a 14‰ change in $\delta^{18}O_{O_2}$ and 2‰ change in $\delta^{13}C_{DIC}$ (Parker et al. 2005), with minimum $\delta^{18}O$ values found at mid-day and minimum $\delta^{13}C$ values found in at dawn. A recent multi-isotope investigation of diel changes in the San Joaquin River (USA) showed that the $\delta^{13}C$ and $\delta^{15}N$ of algae, the $\delta^{15}N$ and $\delta^{18}O$ of nitrate, and $\delta^{13}C_{DIC}$ varied by about 2‰ while the $\delta^{18}O_{O_2}$ varied by >10‰. Recent work by Venkiteswaran et al. (in press) has shown that the temporal "shape" of the diel $\delta^{18}O$ curve can be explained by a small number of parameters, including photosynthesis to respiration ratio, uptake fractionation, temperature, and gas exchange rate. Hence, the natural diel change in the $\delta^{18}O$ of O_2 (and probably the isotopic compositions of the other constituents) provides a powerful biogeochemical "signal" in aquatic systems.

Isotopic indicators of ecosystem processes

In the preceding sections, we have mainly considered isotopes as tracers of organic matter, nutrients, or trophic interactions. Because stable isotopes of inorganic and organic materials are intricately linked to biogeochemical cycles, they are increasingly useful as indictors or predictors of various types of ecosystem processes. For example, algal $\delta^{13}C$ values appear to be strongly linked to the overall ecosystem metabolism of rivers or lakes (e.g. Finlay 2004). Metabolic processes such as photosynthesis and respiration strongly indicate or influence the parameters that control algal $\delta^{13}C$ such as algal growth rates, CO_2, and $\delta^{13}C_{DIC}$. Thus, algal $\delta^{13}C$ could potentially be used to

provide an integrated measure of the carbon balance of aquatic ecosystems (Finlay 2004).

Nitrogen isotopes in food webs appear to provide robust, integrated measurements of nitrogen loading and cycling in aquatic ecosystems. For example, $\delta^{15}N$ of estuarine plants and river consumers are well-predicted from N loads and land use (Cole et al. 2004; Anderson & Cabana 2005). Anderson & Cabana (2005) conclude that in the absence of important point-source N inputs, $\delta^{15}N$ in aquatic organisms appear to increase in a predictable pattern, following the increasing amounts of agricultural N inputs. The cause of the relationship between $\delta^{15}N$ of biota and agricultural land use or N loads is a subject of some debate, since it could be caused by increased denitrification, N cycling, or use of manure and/or septic waste in larger watersheds.

Stable isotopes may be used to detect "hot spots" of intense biogeochemical activity (McClain et al. 2003) in aquatic ecosystems and at their interfaces with terrestrial environments. Eutrophic or hypoxic environments may cause large decreases in $\delta^{13}C_{DIC}$ because of methane formation and subsequent oxidation, increases in $\delta^{15}N_{NO_3}$ because of denitrification, and either decreases or increases in $\delta^{34}S_{SO_4}$ because of sulfate reduction – with consequent incorporation of these biogeochemical signatures of redox conditions into local biota (Figure 10.12).

Probably the best example of use of stable isotope indicators to detect environmental change is for detecting effects of sewage. Sewage inputs can cause local "hot spots" of labile, organic matter with distinct isotopic signatures compared with other sources. For example, a study of municipal sewage and pulp-mill inputs to rivers in Canada showed that the waste material contained materials with $\delta^{15}N$ and $\delta^{34}S$ values that were distinct from upstream background conditions in the receiving environment (Wayland & Hobson 2001). In another sewage waste study on tributaries of the St Lawrence River (Canada), point-source N inputs from human waste lowered the $\delta^{15}N$ of biota collected below the points of discharge at the scale of kilometers (Anderson & Cabana 2006). When domestic waste was diverted from the stream, the $\delta^{15}N$ of small consumers changed by >4‰ over the next year, showing that streams impacted by large point-source urban discharges can show rapid recovery when the discharge is entirely eliminated. The study suggested that biogeochemical indicators such as stable isotopes may reveal recovering changes in the ecosystem nutrient metabolism, even before a complete taxonomic recovery of the community is observed (Anderson & Cabana 2006).

Alternates for complex and variable situations: other approaches and isotopic tracers

Ongoing research on variability in $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of dissolved species, plants, and consumers will undoubtedly continue to refine their use. Given

the widely varied environmental conditions present throughout drainage networks, it is clear that stable isotope values reported in one field study cannot always be applied to the next without thorough examination of the biogeochemical conditions. A careful attempt must be made to sample the spatial and temporal variability in the potential sources and in the organisms of interest, at scales relevant to the questions or organisms present.

Under many conditions, overlap of isotope signals or high temporal variation decrease the efficacy of isotopes as tracers of organic matter or trophic position. Improvements in statistical analyses of mixing models offer increasingly sophisticated means to separate sources in situations of variable or partially overlapping isotope ratios and to make use of multiple tracers (Phillips & Koch 2002; Phillips & Gregg 2003; Benstead et al. 2006).

High variability in the isotopic compositions of primary sources may sometimes be dealt with by using consumers to integrate small-scale spatial and temporal variation. For example, as we have seen, $\delta^{15}N$ of plants, microbes, and detritus are highly varied. To address this variability, investigators may use the $\delta^{15}N$ of a basal consumer instead of the primary resource to base (i.e., normalize) trophic position calculations (Cabana & Rasmussen 1996; Post 2002) or to assess the contribution of a specific habitat (i.e. not the specific resource type) to a predator's diet (e.g. Vander Zanden & Vadeboncoeur 2002). Since the diet of primary consumers integrates temporal and spatial variability in the isotopic compositions of resources, using primary consumers removes a considerable amount of environmental noise. Furthermore, as illustrated in Figure 10.12, use of primary consumers to normalize subsequent calculations of trophic position is advantageous since it allows comparisons of food web structure across ecosystems that may have very different $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ at the base of the food web due to differences in environmental conditions and nutrient cycling (Cabana & Rasmussen 1996; Post 2002).

In many cases, however, additional tracers are necessary to fully resolve food webs isotopically. Fortunately, a number of alternate isotope tracers are available and are increasingly used to enhance natural abundance techniques. For example, purposeful enrichment of the aquatic environment with ^{15}N-DIN, ^{13}C-DIC, and ^{13}C-DOC is relatively inexpensive, and such isotopic labeling techniques are increasingly used to study N and C cycling and energy flow in food webs. Isotope enrichment is expensive compared with natural abundance approaches, and equilibration between food sources may not occur, requiring sophisticated modeling approaches to interpret food web results. However, these approaches may be effectively coupled with natural abundance measurements to gain new insight into stream food web ecology (e.g. Mulholland et al. 2000; Hamilton et al. 2004).

Other stable isotope tracers are less well developed but have the potential to resolve issues associated with limitations of $\delta^{13}C$ and $\delta^{15}N$. As noted, applications of $\delta^{34}S$ are rapidly increasing. Strontium isotopes ($^{87}Sr/^{86}Sr$) have been

found to be extremely useful for studies of bird migration because the different Sr isotopic ratios of different geologic materials become incorporated in the plants growing on these materials (e.g. Chamberlain et al. 1997). The $^{87}Sr/^{86}Sr$ values are transferred up the food chain from the soil-exchange pool to leaves and then to small consumers without modification (Blum et al. 2000). The oxygen ($\delta^{18}O$) and hydrogen ($\delta^{2}H$) isotopes of butterfly wings and bird feathers have been found extremely useful for tracking migration paths (Wassenaar & Hobson 1998; Hobson et al. 1999) because of the strong environmental gradients in the $\delta^{18}O$ and $\delta^{2}H$ of surface waters (Kendall & Coplen 2001) and precipitation (Dutton et al. 2005) in North America. Furthermore, $\delta^{2}H$ and radiocarbon (^{14}C) difference among diet sources for consumers observed decades ago (e.g., Estep & Dabrowski (1980) and Broecker & Walton (1959) respectively) are only now being more thoroughly explored for their potential as routine tracers. Finally, compound-specific isotope ratio mass spectrometry (Evershed et al., this volume, pp. 480–540) has the potential for much more accurate determinations of food web relations than possible with the more conventional analysis of bulk solid samples by tracing specific organic molecules from diet to consumer.

Stable isotope techniques are always best used in combination with other methods and complementary data. This is particularly true when it is not possible to unequivocally separate sources or estimate trophic position in food webs with stable isotopes alone. For example, gut content analyses may allow an investigator to eliminate potential sources to consumers, and allow greater use of isotope techniques. Cation ratios (Sr/Ca and Ba/Ca) were found to decrease at each successive trophic level, suggesting that these ratios can be used to identify the trophic level at which an organism is primarily feeding (Blum et al. 2000). The measurement of resource availability, in terms of edibility or abundance, may also help constrain the number and variability of end members that must be considered for use of stable isotope tracers.

Conclusions

Spatial and temporal isotopic variation among organic matter sources is pervasive and is much less well understood compared with other factors that affect use of natural abundance approaches, such as trophic fractionation. Such variation determines when, where, and how isotope techniques may be applied in food web studies. Thus, assessment of the inherent environmental variability in organic matter or prey sources should be the first step in any attempt to use stable isotope methods in food web studies. Accurate assessments of extent of source variation is especially critical for studies of energy flow in food webs, but also influences use of $\delta^{13}C$ and $\delta^{15}N$ to migration of animals as well as trophic position estimation, since $\delta^{15}N$ often vary by habitat or organic matter source.

Physical and chemical conditions of rivers are characterized by spatial gradients, patchiness at all scales (e.g. Vannote et al. 1980; Frissell et al. 1986), and temporal variability. Natural abundance stable isotope tools are increasingly used to study basic and applied aspects of river food webs. Isotope tools are particularly useful because the physical and biogeochemical diversity of rivers creates variation in isotope ratios that is useful to test hypotheses and examine global change effects. Isotopic variation often complicates the simplest applications of these methods (e.g. sampling on one habitat or date to characterize a river food web); however, this variation can be used as a natural "signal" for carefully designed studies. As spatial and temporal complexity of food webs increase, measurements of multiple source tracers, consumer growth rates, movement rates of predators and prey, and modeling approaches are increasingly necessary. Recognition of sources of variation affecting stable isotope ratios enhances the usefulness and efficiency of isotope techniques to understand freshwater food web ecology.

Acknowledgments

JCF was supported while preparing this manuscript by the National Science Foundation via support to the National Center for Earth-surface Dynamics (STC EAR 0120914) and via a Division of Environmental Biology grant (DEB 0315990). CK was supported by the National Research Program of the U.S. Geological Survey.

References

Aitkenhead, J.A. & McDowell, W.H. (2000) Soil C : N ratio as a predictor of annual riverine DOC flux at local and global scales. *Global Biogeochemical Cycles*, **14**, 128–138.

Amundson, A., Austin, A.T., Schuur, E.A.G., et al. (2003). Global patterns of the isotopic composition of soil and plant nitrogen. *Global Biogeochemical Cycles*, **17**, 31-1–31-10.

Anderson, C. & Cabana, G. (2005) Delta N-15 in riverine food webs: effects of N inputs from agricultural watersheds. *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 333–340.

Anderson, C. & Cabana, G. (2006) Does $\delta^{15}N$ in river food webs reflect the intensity and origin of N loads from the watershed? *Science of the Total Environment*, **367**, 968–978.

Angradi, T.R. (1993) Stable carbon and nitrogen isotope analysis of seston in a regulated rocky mountain river, USA. *Regulated Rivers: Research and Management*, **8**, 251–270.

Angradi, T.R. (1994) Trophic linkages in the lower Colorado River: multiple stable isotope evidence. *Journal of the North American Benthological Society*, **13**, 479–495.

Araujo-Lima, C.A.R.M., Forsberg, B.R., Victoria, R.L. & Martinelli, L. (1986) Energy sources for detritivorous fishes in the Amazon. *Science*, **234**, 1256–1259.

Atekwana, E.A. & Krishnamurthy, R.V. (1998) Seasonal variations of dissolved inorganic carbon and $\delta^{13}C$ of surface waters: application of a modified gas evolution technique. *Journal of Hydrology*, **205**, 265–278.

Barnard, C., Martineau, C., Frenette, J.J., Dodson, J.J. & Vincent, W.F. (2006) Trophic position of zebra mussel veligers and their use of dissolved organic carbon. *Limnology and Oceanography*, **51**, 1473–1484.

Barth, J.A.C., Veizer, J. & Mayer, B. (1998) Origin of particulate organic carbon in the upper St. Lawrence: isotopic constraints. *Earth and Planetary Science Letters*, **162**, 111–121.

Bastow, J.L., Sabo, J.L., Finlay, J.C. & Power, M.E. (2002) A basal aquatic-terrestrial trophic link in rivers: algal subsidies via shore-dwelling grasshoppers. *Oecologia*, **131**, 261–268.

Bates, A.L., Orem, W.H., Harvey, J.W. & Spiker, E.C. (2002) Tracing sources of sulfur in the Florida Everglades. *Journal of Environmental Quality*, **31**, 287–299.

Battaglin, W.A., Kendall, C., Chang, C.C.Y., et al. (2001a) Chemical and isotopic composition of organic and inorganic samples from the Mississippi River and its tributaries, 1997–98. *U.S.Geological Survey Water Resources Investigation Report*, **01-4095**, 57 pp.

Battaglin, W.A., Kendall, C., Chang, C.C.Y., et al. (2001b) Chemical and isotopic evidence of nitrogen transformation in the Mississippi River, 1997–98. *Hydrological Processes*, **15**, 1285–1300.

Bender, M.M. (1968) Mass spectrometric studies of carbon-13 variations in corn and other grasses. *Radiocarbon*, **10**, 468–472.

Benstead, J.P., March, J.G., Fry, B., Ewel, K.C. et al. (2006) Testing isosource: Stable isotope analysis of a tropical fishery with diverse organic matter sources. *Ecology*, **87**, 326–333.

Berglund, O., Nystrom, P. & Larsson, P. (2005) Persistent organic pollutants in river food webs: influence of trophic position and degree of heterotrophy. *Canadian Journal of Fisheries and Aquatic Sciences*, **62**, 2021–2032.

Bilby, R.E., Fransen, B.R. & Bisson, P.A. (1996) Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams: evidence from stable isotopes. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 164–173.

Bird, M.I., Giresse, P. & Ngos, S. (1998) A seasonal cycle in the carbon-isotopic composition of organic carbon in the Sanaga River, Cameroon. *Limnology and Oceanography*, **43**, 143–146.

Blum, J.D., Taliaferro, E.H., Weisse, M.T. & Holmes, R.T. (2000) Changes in Sr/Ca, Ba/Ca and $^{87}Sr/^{86}Sr$ ratios between trophic levels in two forest ecosystems in the northeastern U.S.A. *Biogeochemistry*, **49**, 87–101.

Boutton, R.W. (1996) Stable carbon isotope ratios of soil organic matter and their use as indicators of vegetation and climate change. In: *Mass Spectrometry of Soils* (Eds T.W. Boutton & S. Yamasaki), pp. 47–82. Marcel Dekker, New York.

Brady, N.C. (1990) *The Nature and Properties of Soils*. Macmillan, New York, 621 pp.

Broadbent, F.E., Rauschkolb, R.S., Lewis, K.A. & Chang, G.Y. (1980) Spatial variability in nitrogen-15 and total nitrogen in some virgin and cultivated soils. *Soil Science Society of America Journal*, **44**, 524–527.

Broecker, W.S. & Walton, A. (1959) The geochemistry of C14 in fresh-water systems. *Geochemica et Cosmochemica Acta*, **16**, 15–38.

Brunold, C. & Erismann, K.H. (1975) H_2S as sulfur sources in *Lemna minor* L.: direct incorporation in to cystein and inhibition of sulfate assimilation. *Experientia*, **31**, 508–510.

Bullen, T.D. & Kendall, C. (1998) Tracing of weathering reactions and water flowpaths: a multi-isotope approach. In: *Isotope Tracers in Catchment Hydrology* (Eds C. Kendall & J.J. McDonnell), pp. 611–646. Elsevier, Amsterdam.

Cabana, G. & Rasmussen, J.B. (1996) Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences*, **93**, 10844–10847.

Caraco, N.F., Lampman, G., Cole, J.J., Limburg, K.E., Pace, M.L. & Fischer, D. (1998) Microbial assimilation of DIN in a nitrogen rich estuary: implications for food quality and isotope studies. *Marine Ecology – Progress Series*, **167**, 59–71.

Casciotti, K.L., Sigman, D.M., Hastings, M.G., et al. (2002) Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. *Analytical Chemistry*, **74**, 4905–4912.

Chaloner, D.T., Martin, K.M., Wipfli, M.S., et al. (2002) Marine carbon and nitrogen in southeastern Alaska stream food webs: evidence from artificial and natural streams. *Canadian Journal of Fisheries and Aquatic Science*, **59**, 1257–1265.

Chamberlain, C.P., Blum, J.D., Holmes, R.T., et al. (1997) The use of isotope tracers for identifying populations of migratory birds. *Oecologia*, **109**, 132–141.

Clark, I.D. & Fritz, P. (1997) *Environmental Isotopes in Hydrogeology*. CRC Press, Boca raton, FL.

Cloern, J.E., Canuel, E.A. & Harris, D. (2002) Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. *Limnology and Oceanography*, **47**, 713–729.

Coffin, R.B., Fry, B., Peterson, B.J. & Wright, R.T. (1989) Carbon isotopic compsitions of estuarine bacteria. *Limnology and Oceanography*, **34**, 1305–1310.

Cole, M.L., Valiela, I., Kroeger, K.D., et al. (2004) Assessment of a delta N-15 isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. *Journal of Environmental Quality*, **33**, 124–132.

Connolly, R.M., Guest, M.A., Melville, A.J. & Oakes, J.M. (2003) Sulfur stable isotopes separate producers in marine food-web analysis. *Oecologia*, **138**, 161–167.

Dawson, T.E., Mambelli, S., Plamboeck, A.H., et al. (2002) Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics*, **33**, 507–559.

DeBruyn, A.M H. & Rasmussen, J.B. (2002) Quantifying assimilation of sewage-derived organic matter by riverine benthos. *Ecological Applications*, **12**, 511–520.

Delong, M.D. & Thorp, J.H. (2006) Significance of instream autotrophs in trophic dynamics of the Upper Mississippi River. *Oecologia*, **147**, 76–85.

Delong, M.D., Thorp, J.H., Greenwood, K.S. & Miller, M.C. (2001) Responses of consumers and food resources to a high magnitude, unpredicted flood in the upper Mississippi River basin. *Regulated Rivers-Research and Management*, **17**, 217–234.

Doctor, D.H., Kendall, C., Sebestyen, S.D., et al. (in press) Carbon isotope fractionation of dissolved inorganic carbon (DIC) due to outgassing of CO2 from a headwater stream. *Hydrological Processes.*

Doucett, R.R., Giberson, D.J. & Power, G. (1999a) Parasitic association of *Nanocladius* (Diptera : Chironomidae) and *Pteronarcys biloba* (Plecoptera : Pteronarcyidae): insights from stable-isotope analysis. *Journal of the North American Benthological Society*, **18**, 514–523.

Doucett, R.R., Hooper, W. & Power, G. (1999b) Identification of anadromous and nonanadromous adult brook trout and their progeny in the Tabusintac River, New Brunswick, by means of multiple-stable-isotope analysis. *Transactions of the American Fisheries Society*, **128**, 278–288.

Duarte, C.M. & Agusti, S. (1998) The CO_2 balance of unproductive aquatic ecosystems. *Science*, **281**, 234–235.

Dutton, A., Wilkinson, B.H., Welker, J.M., et al. (2005) Spatial distribution and seasonal variation in $^{18}O/^{16}O$ of modern precipitation and river water across the conterminous USA. *Hydrological Processes*, **19**(20), 4121–4146.

England, L.E. & Rosemond, A.D. (2004) Small reductions in forest cover weaken terrestrial-aquatic linkages in headwater streams. *Freshwater Biology*, **49**, 721–734.

Estep, M.F. & Dabrowski, H. (1980) Tracing food webs with stable hydrogen isotopes. *Science*, **209**, 1537–1538.

Farquhar, G.D., O'Leary, M.H. & Berry, J.A. (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology*, **9**, 121–137.

Finlay, J.C. (2001) Stable-carbon-isotope ratios of river biota: Implications for energy flow in lotic food webs. *Ecology*, **82**, 1052–1064.

Finlay, J.C. (2003) Controls of streamwater dissolved inorganic carbon dynamics in a forested watershed. *Biogeochemistry*, **62**, 231–252.

Finlay, J.C. (2004) Patterns and controls of lotic algal stable carbon isotope ratios. *Limnology and Oceanography*, **49**, 850–861.

Finlay, J.C., Power, M.E. & Cabana, G. (1999) Effects of water velocity on algal carbon isotope ratios: Implications for river food web studies. *Limnology and Oceanography*, **44**, 1198–1203.

Finlay, J.C., Khandwala, S. & Power, M.E. (2002) Spatial scales of carbon flow in a river food web. *Ecology*, **83**, 1845–1859.

Fogel, M.L. & Cifuentes, L.A. (1993) Isotope fractionation during primary production. In: *Organic Geochemistry* (Eds M.H. Engel & S.A. Macko), pp. 73–94. Plenum Press, New York.

France, R. (1995) Stable nitrogen isotopes in fish: Literature synthesis on the influence of ecotonal coupling. *Estuarine Coastal and Shelf Science*, **41**, 737–742.

Frissell, C.A., Liss, W.J., Warren, C.E. & Hurley, M.D. (1986) A hierarchical framework for stream habitat classification: viewing streams in a watershed context. *Environmenal Management*, **10**, 199–214.

Fry, B. (1986) Stable sulphur isotopic distributions and sulphate reduction in lake-sediments of the Adirondack Mountains, New York. *Biogeochemistry*, **2**(4), 329–343.

Fry, B. (1991) Stable isotope diagrams of freshwater foodwebs. *Ecology*, **72**, 2293–2297.

Fry, B. & Sherr, E.B. (1984) ^{13}C measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions to Marine Science*, **27**, 13–47.

Fry, B., Joern, A. & Parker, P.L. (1978) Grasshopper food web analysis: use of carbon isotope ratios to examine feeding relationships among terrestrial herbivores. *Ecology*, **59**, 498–506.

Fry, B., Scalan, R.S., Winters, K. & Parker, P.L. (1982) Sulphur uptake by salt grasses, mangroves and seagrasses in anaerobic sediments. *Geochimica et Cosmochimica Acta*, **46**, 1121–1124.

Fry, B., Silva, S.R., Kendall, C. & Anderson, R.K. (2002) Oxygen isotope corrections for online $\delta^{34}S$ analysis. *Rapid Communications in Mass Spectrometry*, **16**, 854–858.

Garten, C.T., Jr. & Taylor, G.E., Jr. (1992) Foliar $\delta^{13}C$ within a temperate deciduous forest: spatial, temporal and species sources of variation. *Oecologia*, **90**, 1–7.

Glime, J.M. & Vitt, D.H. (1984) The physiological adaptations of aquatic Musci. *Lindbergia*, **10**, 41–52.

Goericke, R., Montoya, J.P. & Fry, B. (1994) Physiology of isotope fractionation in algae and cyanobacteria. In: *Stable Isotopes in Ecology and Environmental Science* (Eds K. Lajtha & B. Michener), pp. 199–233. Blackwell Scientific Publications, Oxford.

Granger, J., Sigman, D.M., Needoba, J.A. & Harrison, P.J. (2004) Coupled nitrogen and oxygen isotope fractionation of nitrate during assimilation by cultures of marine phytoplankton. *Limnology and Oceanography*, **49**(5), 1763–1773.

Hamilton, S.K. & Lewis, Jr., W.M. (1992) Stable carbon and nitrogen isotopes in algae and detritus from the Orinoco River floodplain, Venezuela. *Geochimica et Cosmochimica Acta*, **56**, 4237–4246.

Hamilton, S.K., Tank, J.L., Raikow, D.E., Siler, E.R., et al. (2004) The role of instream vs allochthonous N in stream food webs: modeling the results of an isotope addition experiment. *Journal of the North American Benthological Society*, 23, 429–448.

Hamilton, S.K., Sippel, S.J. & Bunn, S.E. (2005) Separation of algae from detritus for stable isotope or ecological stoichiometry studies using density fractionation in colloidal silica. *Limnology and Oceanography, Methods*, **3**, 149–157.

Handley, L.L. & Scrimgeour, C.M. (1997) Terrestrial plant ecology and N-15 natural abundance: The present limits to interpretation for uncultivated systems with original data from a Scottish old field. *Advances in Ecological Research*, **27**, 133–212.

Handley, L.L., Austin, A.T., Robinson, D., et al. (1999) The ^{15}N natural abundance ($\delta^{15}N$) of ecosystem samples reflects measures of water availability. *Australian Journal of Plant Physiology*, **26**, 185–199.

Harrington, R.R., Kennedy, B.P., Chamberlain, C.P., et al. (1998) ^{15}N enrichment in agricultural catchments: Field patterns and applications to tracking Atlantic salmon (Salmo. Salar). *Chemical Geology*, **147**, 281–294.

Harris, G.P. (1986) *Phytoplankton Ecology*. Chapman and Hall, London.

Heaton, T.H.E. (1986) Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. *Chemical Geology*, **56**, 87–102.

Hebert, C.E. & Wassenaar, L.I. (2001) Stable nitrogen isotopes in waterfowl feathers reflect agricultural land use in western Canada. *Environmental Science and Technology*, **35**, 3482–3487.

Hecky, R.E. & Hesslein, R.H. (1995) Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *Journal of the North American Benthological Society*, **14**, 631–653.

Hecky, R.E., Campbell, P. & Hendzel, L.L. (1993) The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. *Limnology and Oceanography*, **38**, 709–724.

Hedges, J.L., Clark, W.A., Quay, P.D., et al. (1986) Compositions and fluxes of particulate organic material in the Amazon River. *Limnology and Oceanography*, **31**, 717–738.

Hershey, A.E. & Peterson, B.J. (1996) Stream food webs. In: *Methods in Stream Ecology* (Eds G.A. Lamberti & F.R. Hauer), pp. 511–530. Academic Press, New York.

Hicks, B.J. (1997) Food webs in forest and pasture streams in the Waikato region, New Zealand: A study based on analyses of stable isotopes of carbon and nitrogen, and fish gut contents. *New Zealand Journal of Marine and Freshwater Research*, **31**, 651–664.

Hicks, B.J. & Laboyrie, J.L. (1999) Preliminary estimates of mass-loss rates, changes in stable isotope composition, and invertebrate colonisation of evergreen and deciduous leaves in a Waikato, New Zealand, stream. *New Zealand Journal of Marine and Freshwater Research*, **33**, 221–232.

Hillaire-Marcel, G. (1986) Isotopes and food. In: *Handbook of Environmental Isotope Geochemistry*, Vol. 2 (Eds P. Fritz & J.Ch. Fontes), pp. 507–548. Elsevier Science, Amsterdam.

Hitchon, B. & Krouse, H.R. (1972) Hydrogeochemistry of the surface waters of the Mackenzie River drainage basin, Canada – III. Stable isotopes of oxygen, carbon and sulphur. *Geochimica et Cosmochimica Acta*, **36**, 1337–1357.

Hobson, K.A., Wassenaar, L.I. & Taylor, O.R. (1999) Stable isotopes (δD and $\delta^{13}C$) are geographic indicators of natal origins of monarch butterflies in eastern North America. *Oecologia*, **120**, 397–404.

Hoffer-French, K.J. & Herman, J.S. (1989) Evaluation of hydrological and biological influences on CO_2 fluxes from a karst stream. *Journal of Hydrology*, **108**, 189–212.

Högberg, P. (1997) ^{15}N natural abundance in soil-plant systems. *New Phytologist*, **137**, 179–203.

Holmes, R., McCleland, M.J., Sigman, W., et al. (1998) Measuring $^{15}N–NH_4^+$ in marine, estuarine and fresh waters: An adaptation of the ammonia diffusion method for samples with low ammonium concentrations. *Marine Chemistry*, **60**, 235–243.

Huryn, A.D., Riley, R.H., Young, R.G., et al. (2002) Natural-abundance stable C and N isotopes indicate weak upstream-downstream linkage of food webs in a grassland river. *Archiv für Hydrobiologie*, **153**, 177–196.

Jepsen, D.B. & Winemiller, K.O. (2002) Structure of tropical river food webs revealed by stable isotope ratios. *Oikos*, **96**, 46–55.

Kato, C., Iwata, T. & Wada, E. (2004) Prey use by web-building spiders: stable isotope analyses of trophic flow at a forest-stream ecotone. *Ecological Research*, **19**, 633–643.

Keeley, J.E. & Sandquist, D.R. (1992) Carbon: freshwater plants. *Plant, Cell and Environment*, **15**, 1021–1035.

Kendall, C. (1993) *Impact of isotopic heterogeneity in shallow systems on stormflow generation*. PhD dissertation, University of Maryland, College Park, 310 pp.

Kendall, C. (1998) Tracing nitrogen sources and cycling in catchments. In: *Isotope Tracers in Catchment Hydrology* (Eds C. Kendall & J.J. McDonnell), pp. 519–576. Elsevier, Amsterdam.

Kendall, C. & Coplen, T.B. (2001) Distribution of oxygen-18 and deuterium in river waters across the United States. *Hydrological Processes*, **15**, 1363–1393.

Kendall, C. & Doctor, D.H. (2004) Stable isotope applications in hydrologic studies. In: *Treatise on Geochemistry*, Vol. 5, *Surface and Ground Water, Weathering, and Soils* (Ed. J.I. Drever), pp. 319–364. Elsevier, Amsterdam.

Kendall, C., Silva, S.R., Chang, C.Y., et al. (2000) Spatial changes in redox conditions and foodweb relations at low and high nutrient sites in the Everglades. *U.S. Geological Survey Open File Report*, **00-449**, 61–63.

Kendall, C., Silva, S.R. & Kelly, V.J. (2001) Carbon and nitrogen isotopic compositions of particulate organic matter in four large river systems across the United States. *Hydrological Processes*, **15**, 1301–1346.

Kennedy, T.A., Finlay, J.C. & Hobbie, S.E. (2005) Eradication of invasive *Tamarix ramosissima* along a desert stream increases native fish density. *Ecological Applications*, **15**, 2072–2083.

Kratzer, C.R., Dileanis, P.D., Zamora, C., et al. (2004) *Sources and transport of nutrients, organic carbon, and chlorophyll-a in the San Joaquin River upstream of Vernalis, California, during summer and fall, 2000 and 2001*. WRI 03-4127, U.S. Geological Survey. Online: http://water.usgs.gov/pubs/wri/wri034127/.

Krouse, H.R. & Mayer, B. (2000) Sulphur and oxygen isotopes in sulphate. In: *Environmental Tracers in Subsurface Hydrology* (Eds P.G. Cook & A.L.Herczeg), pp. 195–231. Kluwer Academic Publishers, Boston.

Krouse, H.R., Legge, A. & Brown, H.M. (1984) Sulphur gas emissions in the boreal forest: the West Whitecourt case study V: stable sulfur isotopes. *Water, Air and Soil Pollution*, **22**, 321–347.

Krouse, H.R., Stewart, J.W.B. & Grinenko, V.A. (1991) Pedosphere and biosphere. In: *Stable Isotopes: Natural and Anthropogenic Sulphur in the Environment* (Eds. H.R. Krouse and V.A. Grinenko), pp. 267–306. SCOPE 43, John Wiley and Sons, Chichester.

Kusakbe, M., Rafer, J.D., Stout, J. & Colle, T.W. (1976) Isotopic ratios of sulphur extracted from some plants, soils and related materials. *New Zealand Journal of Science*, **I9**, 433–440.

Lake, J.L., McKinney, R.A., Osterman, F.A., et al. (2001) Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems. *Canadian Journal of Fisheries and Aquatic Science*, **58**, 870–878.

Laws, E.A., Popp, B.N., Bidigare, R.R., et al. (1995) Dependence of phytoplankton isotopic composition on growth rate and $[CO_2]_{aq}$: theoretical considerations and experimental results. *Geochimica et Cosmochimica Acta*, **59**, 1131–1138.

LaZerte, B.D. (1983) Stable carbon isotope ratios: Implications for the source sediment carbon and for phytoplankton carbon assimilation in Lake Memphremagog, Quebec. *Canadian Journal of Fisheries and Aquatic Science*, **40**, 1658–1666.

LaZerte, B.D. & Szalados, J.E. (1982) Stable carbon isotope ratio of submerged freshwater macrophytes. *Limnology and Oceanography*, **27**(3), 413–418.

Leffler, A.J. & Evans, A.S. (1999) Variation in carbon isotope composition among years in the riparian tree *Populus fremontii*. *Oecologia*, **119**, 311–319.

Lehmann, M.F., Sigman, D.M. & Berelson, W.M. (2004) Coupling the $^{15}N/^{14}N$ and $^{18}O/^{16}O$ of nitrate as a constraint on benthic nitrogen cycling. *Marine Chemistry*, **88**, 1–20.

Loneragan, N.R., Bunn, S.E. & Kellaway, D.M. (1997) Are mangroves and seagrasses sources of organic carbon for penaeid prawns in a tropical Australian estuary? A multiple stable-isotope study. *Marine Biology*, **130**(2), 289–300.

MacAvoy, S.E., Macko, S.A. & Garman, G.C. (1998) Tracing marine biomass into tidal freshwater ecosystems using stable sulfur isotopes. *Naturwissenschaften*, **85**, 544–546.

MacAvoy S.E., Macko S.A., McIninch S.P. & Garman G.C. (2000) Marine nutrient contributions to freshwater apex predators. *Oecologia*, **122**(4), 568–573.

Macleod, N.A. & Barton, D.R. (1998) Effects of light intensity, water velocity, and species composition on carbon and nitrogen stable isotope ratios in periphyton. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 1919–1925.

Mariotti, A., Lancelot, C. & Billen, G. (1984) Natural isotopic composition of nitrogen as a tracer of origin for suspended organic matter in the Scheldt estuary. *Geochimica et Cosmochimica Acta*, **48**, 549–555.

Mariotti, A., Gadel, F., Giresse, P. & Mouzeo, K. (1991) Carbon isotope composition and geochemistry of particulate organic matter in the Congo River (Central Africa): Application to the study of Quaternary sediments off the mouth of the river. *Chemical Geology*, **86**, 345–357.

Mayer, B., Boyer, E.W., Goodale, C., et al. (2002) Sources of nitrate in rivers draining sixteen watersheds in the northeastern US: Isotopic constraints, *Biogeochemistry*, **57**, 171–197.

McCarthy, I.D. & Waldron, S. (2000) Identifying migratory Salmo trutta using carbon and nitrogen stable isotope ratios. *Rapid Communications in Mass Spectrometry*, **14**, 1325–1331.

McClain, M.E., Boyer, E.W., Dent, C.L., et al. (2003) Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. *Ecosystems*, **6**, 301–312.

McClelland, J.W. & Valiela, I. (1998) Linking nitrogen in estuarine producers to land-derived sources. *Limnology and Oceanography*, **43**, 577–585.

McCusker, E.M., Ostrom, P.H., Ostrom N.E., et al. (1999) Seasonal variation in the biogeochemical cycling of seston in Grand Traverse Bay, Lake Michigan. *Organic Geochemistry*, **30**, 1543–1557.

McCutchan, J.H. & Lewis, W.M. (2001) Seasonal variation in stable isotope ratios of stream algae. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie*, **27**, 3304–3307.

McCutchan, J.H. & Lewis, W.M. (2002) Relative importance of carbon sources for macroinvertebrates in a Rocky Mountain stream. *Limnology and Oceanography*, **47**, 742–752.

McCutchan, J.H., Lewis, W.M., Kendall, C. & McGrath, C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, **102**, 378–390.

Mitchell, M.J, Krouse, H.R., Mayer, B., et al. (1998) Use of stable isotopes in evaluating sulfur biogeochemistry of forest ecosystems. In: *Isotope Tracers in Catchment Hydrology* (Eds C. Kendall & J.J. McDonnell), pp. 489–518. Elsevier, Amsterdam.

Mook, W.G. & Tan, F.C. (1991) Stable carbon isotopes in rivers and estuaries. In: *Biogeochemistry of Major World Rivers* (eds E. Degens, S. Kempe & J. Richey), pp. 245–264. J. Wiley & Sons, Chichester.

Mook, W.G., Bommerson, J.C. & Staverman, W.H. (1974) Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. *Earth and Planetary Science Letters*, **22**, 169–176.

Mulholland, P.J., Tank, J.L., Sanzone, D.M., et al. (2000) Food resources of stream macroinvertebrates determined by natural-abundance stable C and N isotopes and a N-15 tracer addition. *Journal of the North American Benthological Society*, **19**, 145–157.

Nadelhoffer, K.J. & Fry, B. (1994) Nitrogen isotope studies in forest ecosystems. In: *Stable Isotopes in Ecology and Environmental Science* (Eds K. Lajtha & R.M. Michener), pp. 22–44. Blackwell Scientific Publishers, Oxford.

Needoba, J.A., Sigman, D.M. & Harrison, P.J. (2004) The mechanism of isotope fractionation during algal nitrate assimilation as illuminated by the N-15/N-14 of intracellular nitrate. *Journal of Phycology*, **40**, 517–522.

Nriagu, J.O., Rees, C.E., Mekhtiyeva, V.L., et al. (1991) Hydrosphere. In: *Stable Isotopes: Natural and Anthropogenic Sulphur in the Environment*, SCOPE 43 (Scientific Committee on Problems of the Environment) (Eds H.R. Krouse & V. Grinenko), pp. 177–265. J. Wiley & Sons, Chichester.

O'Reilly, C.M., Hecky, R.E., Cohen, A.S. & Plisnier, P.D. (2002) Interpreting stable isotopes in food webs: Recognizing the role of time averaging at different trophic levels. *Limnology and Oceanography*, **47**, 306–309.

Ostrom, N.E., Hedin, L.O., Von Fischer, J.C. & Robertson, G.P. (2002) Nitrogen transformations and NO_3 removal at a soil–stream interface: A stable isotope approach. *Ecological Applications*, **12**, 1027–1043.

Parker, S.R., Poulson, S.R., Gammons, C.H. & DeGrandpre, M.D. (2005) Biogeochemical controls on diel cycling of stable isotopes of dissolved O2 and dissolved inorganic carbon in the Big Hole River, Montana. *Environmental Science and Technology*, **39**, 7134–7140.

Pennock, J., Velinsky, D., Ludlam, J., et al. (1996) Isotopic fractionation of ammonium and nitrate during uptake by Skeletonema costatum: Implications for $\delta^{15}N$ dynamics under bloom conditions. *Limnology and Oceanography*, **41**(3), 451–459.

Pentecost, A. (1995) Geochemistry of carbon dioxide in six travertine-depositing waters of Italy. *Journal of Hydrology*, **167**, 263–278.

Peterson, B.J. & Fry, B. (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, **18**, 293–320.

Peterson, B.J. & Howarth, R.W. (1987) Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. *Limnology and Oceanography*, **32**, 1195–1213.

Peterson, B.J., Howarth, R.W. & Garrett, R.H. (1985) Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science*, **227**, 1361–1363.

Phillips, D.L. & Gregg, J.W. (2003) Source partitioning using stable isotopes: coping with too many sources. *Oecologia*, **136**, 261–269.

Phillips, D.L. & Koch, P.L. (2002) Incorporating concentration dependence in stable isotope mixing models. *Oecologia*, **130**, 114–125.

Post, D.M. (2002) Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, **83**, 703–718.

Power, M.E. & Dietrich, W.E. (2002) Food webs in river networks. *Ecological Research*, **17**, 451–471.

Quay, P.D., Wilbur, D.O., Richey, J.E., et al. (1992) Carbon cycling in the Amazon River: Implications from the ^{13}C compositions of particles and solutes. *Limnology and Oceanography*, **37**, 857–871.

Raven, J.A. & Beardall, J. (1981) Carbon dioxide as the exogenous inorganic carbon source for Batrachospermum and Lemanea. *British Phycological Journal*, **16**, 165–175.

Redfield, A.C. (1958) The biological control of chemical factors in the environment. *American Scientist*, **46**, 205–221.

Rostad, C.E., Leenheer, J.A. & Daniel, S.R. (1997) Organic carbon and nitrogen content associated with colloids and suspended particules from the Mississippi River and some of its tributaries. *Environmental Science and Technology*, **31**, 3218–3225.

Rounick, J.S. & James, M.R. (1984) Geothermal and cold springs faunas: inorganic carbon sources affect isotopic values. *Limnology and Oceanography*, **29**, 386–389.

Rounick, J.S., Winterbourn, M.J. & Lyon, G.L. (1982) Differential utilization of allochthonous and autochthonous inputs by aquatic invertebrates in some New Zealand streams: a stable carbon isotope study. *Oikos*, **39**, 191–198.

Sebilo, M., Billen, G., Grably, M. & Mariotti, A. (2003) Isotopic composition of nitrate-nitrogen as a marker of riparian and benthic denitrification at the scale of the whole Seine River system. *Biogeochemistry*, **63**, 35–51.

Sebilo, M., Mayer, B., Grably, M., et al. (2004) The use of the "ammonium diffusion" method for $\delta^{15}N–NH_4^+$ and $\delta^{15}N–NO_3^-$ measurements: comparison with other techniques. *Environmental Chemistry*, **1**, 99–103.

Sigman, D.M., Altabet, M.A., Micheneer, R., et al. (1997) Natural abundance-level measurements of the nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. *Marine Chemistry*, **57**, 227–242.

Sigman, D.M., Casciotti, K.L., Andreani, M., et al. (2001) A bacterial method for the nitrogen isotopic analyses of nitrate in seawater and freshwater. *Analytical Chemistry*, **73**, 4145–4153.

Singer, G.A., Panzenbock, M., Weigelhofer, G., et al. (2005) Flow history explains temporal and spatial variation of carbon fractionation in stream periphyton. *Limnology and Oceanography*, **50**, 706–712.

Schlacher, T.A. & Wooldridge, T.H. (1996) Origin and trophic importance of detritus – evidence from stable isotopes in the benthos of a small, temperate estuary. *Oecologia*, **106**, 382–388.

Smith, B.N. & Epstein, S. (1971) Two categories of $^{13}C/^{12}C$ ratios for higher plants. *Plant Physiology*, **47**, 380–384.

Spence, M.J., Bottrell, S.H., Thornton, S.F. & Lerner, D.N. (2001) Isotopic modelling of the significance of bacterial sulphate reduction for phenol attenuation in a contaminated aquifer. *Journal of Contaminant Hydrology*, **53**, 285–304.

Sterner, R.W. & Elser, J.J. (2002) *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton University Press, Princeton, NJ.

Thorp, J.H., Delong, M.D., Greenwood, K.S. & Casper, A.F. (1998) Isotopic analysis of three food web theories in constricted and floodplain regions of a large river. *Oecologia*, **117**, 551–563.

Troughton, J.H. (1972) Carbon isotopic fractionation in plants. *Proceedings of the 8th Conference on Radiocarbon Dating*, Lower Hutt, 18–25 October, Vol. 2, 39–57.

Trudeau, W. & Rasmussen, J.B. (2003) The effect of water velocity on stable carbon and nitrogen isotope signatures of periphyton. *Limnology and Oceanography*, **48**, 2194–2199.

Trust, B.A. & Fry, B. (1992) Stable sulphur isotopes in plants: a review. *Plant, Cell, and Environment*, **15**, 1105–1110.

Tucker, J., Sheats, N., Giblin, A.E., et al. (1999) Using stable isotopes to trace sewage-derived material through Boston Harbor and Massachusetts Bay. *Marine Environmental Research*, **48**, 353–375.

Udy, J.W. & Bunn, S.E. (2001) Elevated $\delta^{15}N$ values in aquatic plants from cleared catchments: why? Marine and Freshwater Research, **52**, 347–351.

Vander Zanden, M.J. & Rasmussen, J.B. (1999) Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology, 80, 1395–1404.

Vander Zanden, M.J. & Rasmussen, J.B. (2001) Variation in delta N-15 and delta C-13 trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, **46**, 2061–2066.

Vander Zanden, M.J. & Vadeboncoeur, Y. (2002) Fishes as integrators of benthic and pelagic food webs in lakes. *Ecology*, **83**(8), 2152–2161.

Vander Zanden, M.J., Cabana, G. & Rasmussen, J.B. (1997) Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (delta N-15) and literature dietary data. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 1142–1158.

Vanderklift, M.A. & Ponsard, S. (2003) Sources of variation in consumer-diet delta N-15 enrichment: a meta-analysis. *Oecologia*, **136**, 169–182.

Van Dover, C.L., Grassle, J.F., Fry, B., et al. (1992) Stable isotope evidence for entry of sewage-derived organic material into a deep-sea food web. *Nature*, **360**, 153–155.

Vannote, R.L., Minshall, G.W., Cummins, K.W., et al. (1980) The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, **37**, 130–137.

Venkiteswaran, J.J., Wassenaar, L.I. & Schiff, S.L. (In press) Dynamics of dissolved O_2 isotopic ratios: a transient model to quantify primary production, community respiration, and air-water exchange in aquatic ecosystems. *Oecologia*.

Vitousek, P.M., Mooney, H.A., Lubchenco, J. & Melillo, J. (1997) Human domination of Earth's ecosystems. *Science*, **277**, 494–499.

Wadleigh, M.A. & Blake, D.M. (1999) Tracing sources of atmospheric sulphur using epiphytic lichens. *Environmental Pollution*, **106**, 265–271.

Wadleigh, M.A., Schwarcz, H.P. & Kramer, J.R. (1996) Isotopic evidence for the origin of sulphate in coastal rain. *Tellus*, **48B**, 44–59.

Wankel, S.D., Kendall, C., Francis, C.A. & Paytan, A. (2006) Nitrogen sources and cycling in the San Francisco Bay Estuary: A nitrate dual isotopic composition approach. *Limnology and Oceanography*, **51**(4), 1654–1664.

Wassenaar, L.I. & Hobson, K.A. (1998) Natal origins of migratory monarch butterflies at wintering colonies in Mexico: New isotopic evidence. *Proceedings of the National Academy of Sciences, USA*, **95**, 15436–15439.

Wayland, M. & Hobson, K.A. (2001) Stable carbon, nitrogen, and sulfur isotope ratios in riparian food webs on rivers receiving sewage and pulp-mill effluents. *Canadian Journal of Zoology*, **79**, 5–15.

Wissel, B. & Fry, B. (2005) Sources of particulate matter in the Mississippi River, USA. *Large Rivers. Archiv für Hydrobiologie, Supplementband*, **15**(1–4), 105–118.

Woodward, G. & Hildrew, A.G. (2002) Food web structure in riverine landscapes. *Freshwater Biology*, **47**, 777–798.

Yun, M., Mayer, B. & Taylor, S.W. (2005) $\delta^{34}S$ measurements on organic materials by continuous flow isotope ratio mass spectrometry. *Rapid Communications in Mass Spectrometry*, **19**, 1429–1436.