

## MEASURING TERRESTRIAL SUBSIDIES TO AQUATIC FOOD WEBS USING STABLE ISOTOPES OF HYDROGEN

RICHARD R. DOUCETT,<sup>1</sup> JANE C. MARKS,<sup>2</sup> DEAN W. BLINN,<sup>2</sup> MELANIE CARON,<sup>1</sup> AND BRUCE A. HUNGATE<sup>1,2,3</sup>

<sup>1</sup>Colorado Plateau Stable Isotope Laboratory, Northern Arizona University, Flagstaff, Arizona 86011 USA

<sup>2</sup>Department of Biological Sciences and Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, Arizona 86011 USA

**Abstract.** Understanding river food webs requires distinguishing energy derived from primary production in the river itself (autochthonous) from that produced externally (allochthonous), yet there are no universally applicable and reliable techniques for doing so. We compared the natural abundance stable isotope ratios of hydrogen ( $\delta\text{D}$ ) of allochthonous and autochthonous energy sources in four different aquatic ecosystems. We found that autochthonous organic matter is uniformly far more depleted in deuterium (lower  $\delta\text{D}$  values) than allochthonous: an average difference of  $\sim 100\%$ . We also found that organisms at higher trophic levels, including both aquatic invertebrates and fish, have  $\delta\text{D}$  values intermediate between aquatic algae and terrestrial plants. The consistent differences between leaves and algae in  $\delta\text{D}$  among these four watersheds, along with the intermediate values in higher trophic levels, indicate that natural abundance hydrogen isotope signatures are a powerful tool for partitioning energy flow in aquatic ecosystems.

**Key words:** *allochthonous; autochthonous; deuterium; hydrogen isotopes; mixing models; pyrolysis; stable isotopes.*

### INTRODUCTION

River food webs rely on two major energy sources: primary production within the river itself (autochthonous), and primary production on land (allochthonous), transferred to the aquatic habitat as leaf litter and dissolved organic carbon. These energy sources differ in quality (Thorpe and Delong 2002), support distinct groups of secondary producers (Vannote et al. 1980), vary in seasonality (Ram et al. 2003), and are likely to respond differently to environmental changes (Hungate and Marks 2002). For these reasons, distinguishing energy inputs to rivers originating from the river itself from that derived from the surrounding landscape is central to understanding the functioning of river food webs and their influences on river biogeochemistry.

Currently, there is no universally applicable technique for distinguishing these energy sources in river food webs. In some cases, stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) are sufficiently different in leaves and algae to trace their importance as energy sources in aquatic food webs (Junger and Planas 1994, Doucett et al. 1996b, Grey et al. 2001, Thorpe and Delong 2002). In others, however,  $\delta^{13}\text{C}$  values do not differ sufficiently between

leaves and algae, or variation is too high, usually because of variation in the  $\delta^{13}\text{C}$  signature of dissolved inorganic carbon and thus in the isotopic signature of algae (e.g., Doucett et al. 1996a, Finlay et al. 1999).

Here, we present data illustrating the potential for stable isotope ratios of hydrogen ( $\delta\text{D}$ ) to partition allochthonous and autochthonous energy sources in aquatic food webs. Differences among terrestrial plant species in  $\delta\text{D}$  have long been recognized (Smith and Ziegler 1990), and have been utilized in an effort to distinguish food sources (Smith and Epstein 1970, Estep and Dabrowski 1980, Macko et al. 1983), though not in the context of the application proposed here. Methodological challenges, however, prevented the widespread use of hydrogen isotopes to differentiate food sources. Specifically, researchers were concerned that there was incomplete recovery of water during combustion (Schimmelmann and DeNiro 1983) and that exchange between hydrogen in ambient water vapor with organic hydrogen in samples influenced analytical results (DeNiro and Epstein 1981). Many of these methodological challenges have been addressed, with the advent of automated pyrolysis systems coupled to isotope-ratio mass spectrometers (Tobias and Brenna 1997), and with standardization of equilibration techniques to correct for water vapor contamination (Wassenaar and Hobson 2000, 2003). With these advances, the potential use of  $\delta\text{D}$  as a natural tracer can now be more fully explored.

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<sup>3</sup> Corresponding author. E-mail: bruce.hungate.@nau.edu

TABLE 1. Hydrogen stable isotope values ( $\delta D$ ; mean  $\pm$  SE) for allochthonous and autochthonous energy sources and for water from four aquatic ecosystems, showing higher tissue  $\delta D$  for terrestrial plants compared to aquatic primary producers.

Site	Allochthonous			Autochthonous			Water	
	Energy source	$\delta D$ (‰)	<i>n</i>	Energy source	$\delta D$ (‰)	<i>n</i>	$\delta D$ (‰)	<i>n</i>
Ash Fork	grass	$-124.7 \pm 27.9$	2	cyanobacteria	$-245.5 \pm 3.1$	3	$-104.0 \pm 0.9$	2
	mesquite	$-142.1 \pm 1.4$	3	diatoms	$-231.2 \pm 6.3$	6		
	tamarisk	$-143.8 \pm 5.4$	3	filamentous algae	$-240.0 \pm 3.2$	6		
	willow	$-161.8 \pm 4.2$	6					
Colorado River	grass	$-157.7$	1	filamentous algae	$-291.6 \pm 4.7$	8	$-106.8 \pm 0.3$	9
	tamarisk	$-153.2 \pm 1.1$	3					
	willow	$-161.9 \pm 10.2$	2					
Devil's Hole	leaf litter	$-128.2 \pm 5.5$	6	cyanobacteria	$-244.6 \pm 4.2$	5	$-102.9 \pm 0.7$	3
				diatoms	$-214.3 \pm 9.7$	7		
Fossil Creek	leaf litter	$-151.3 \pm 6.5$	5	diatoms	$-251.4 \pm 4.1$	5	$-80.5 \pm 0.4$	5
				filamentous algae	$-277.3 \pm 23.5$	5		
Overall†		$-147.2 \pm 4.8$			$-245.5 \pm 8.4$			

† The overall mean is the average and standard error for category means across all ecosystems surveyed.

## METHODS

We sampled four aquatic ecosystems in the southwestern United States: Fossil Creek, Arizona (34°25' N, 111°34' W); Devil's Hole, Nevada (36°25' N, 116°17' W); the Colorado River, Arizona (36°05–07' N, 113°12–16' W), and Ash Fork, Nevada (36°25' N, 116°18' W). Representative water samples were collected at all sites, filtered to  $<1 \mu\text{m}$ , and stored in 60-mL glass vials without head space. At Devils Hole and in Fossil Creek, we sampled terrestrial leaf litter collected from the riparian area (in Fossil Creek) or collected in litter traps above the aquatic habitat (Devils Hole). In Ash Fork, we sampled senesced grass and litter from beneath live tamarisk and willow trees. Leaf material of the same species was collected from riparian vegetation in the Colorado River. We collected three classes of autochthonous material: cyanobacteria and diatoms were collected using epilithic scrapes; both were sampled from Ash Fork and Devils Hole, whereas only diatoms were collected from Fossil Creek. Filamentous green algae, primarily *Cladophora* spp., were collected from the Fossil Creek, Colorado River, and Ash Fork sites. The algae collected were the most abundant primary producers at each site during the time of collection. Benthic invertebrate samples were collected using a combination of surber, rock scrapes, and core samplers, and then sorted and identified to the lowest possible taxonomic level in the laboratory. Fish were sampled using a combination of electrofishing and netting techniques appropriate to the habitat. Fish sampled were rainbow trout (*Oncorhynchus mykiss*) in the Colorado River, Arizona, Devils Hole pupfish (*Cyprinodon diabolis*) in Devil's Hole, Nevada, mountain desert sucker (*Pantosteus clarki*) in Fossil Creek, Arizona, and Ash Meadows Amargosa pupfish (*Cyprinodon nevadensis mionectes*) in Point of Rocks Springs pool and Jack Rabbit Spring in Ash Meadows National Wildlife Refuge, Nevada.

Organic samples were oven-dried at 60°C for 24 hours and ground to a fine powder. Subsamples, about 350  $\mu\text{g}$ , were weighed into silver cups for isotopic analysis. As much as 12–22% of the hydrogen in complex organic molecules is freely exchangeable with ambient water vapor (Wassenaar and Hobson 2000). For this reason, accurate organic  $\delta D$  measurements require controlling for hydrogen isotope exchange (Bowen et al. 2005), especially when samples are analyzed from different geographical locations. To negate the effect of exchangeable hydrogen on bulk-tissue  $\delta D$  values, all samples and calibration standards were equilibrated with local water vapor according to Wassenaar and Hobson (2003). We used the chicken feather (CFS), cow hoof (CHS), and bowhead whale baleen (BWB) calibration standards obtained from L. Wassenaar to convert bulk  $\delta D$  (i.e., exchangeable H + non-exchangeable H) to  $\delta D$  of the non-exchangeable portion only (see Wassenaar and Hobson 2003). Organic samples and standards were pyrolyzed at 1400°C, producing H<sub>2</sub> and CO gases that were separated chromatographically, and the H<sub>2</sub> was analyzed for stable isotope composition using an isotope-ratio mass spectrometer (Thermo-Finnigan TC/EA and Delta<sup>PLUS</sup>-XL; Thermo Electron Corporation, Bremen, Germany; details of analysis available online).<sup>4</sup> Water samples were analyzed for  $\delta D$  by headspace equilibration with H<sub>2</sub> gas and a Pt catalyst using a Thermo-Finnigan Gas-Bench II and Delta<sup>PLUS</sup>-XL. Data on  $\delta^{13}\text{C}$  for Fossil Creek samples, used here for comparison with  $\delta D$ , were obtained from J. C. Marks (*unpublished data*). All  $\delta^{13}\text{C}$  and  $\delta D$  data are relative to VPDB and VSMOW respectively, and are expressed in per mil (‰) notation (Coplen 1996).

## RESULTS AND DISCUSSION

The  $\delta D$  values of water from three of the four habitats were similar, around  $-100\text{‰}$  (Table 1), and typical of

<sup>4</sup> (<http://www.isotope.nau.edu>)

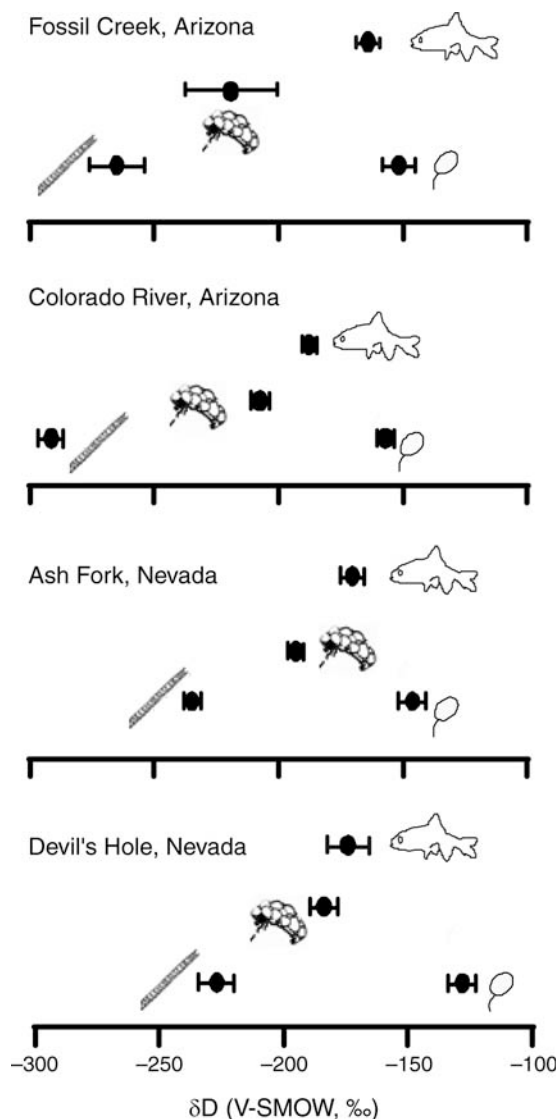


FIG. 1. Consistent differences in  $\delta D$  between algae (bottom left symbol) and leaf litter (bottom right symbol) in four aquatic ecosystems, showing intermediate status of benthic invertebrates and fish. Approximate trophic position is indicated by vertical position in each graph. Values are means  $\pm$  SE. V-SMOW (Vienna Standard Mean Ocean Water) is an international standard for the hydrogen isotopic composition of water.

surface water in the southwestern United States (Kendall and Coplen 2001). Water from Fossil Creek exhibited higher  $\delta D$  values, possibly reflecting both its springwater source and lower latitude (Kendall and Coplen 2001). For all sites, the  $\delta D$  of algae was much lower than the  $\delta D$  of leaves (Table 1). Tissue  $\delta D$  for allochthonous primary producers ranged from  $-125$  to  $-162\%$ , whereas  $\delta D$  of autochthonous primary producers was far lower, ranging from  $-214$  to  $-292\%$ . Within sites, categories of autochthonous primary producers

(cyanobacteria, diatoms, and filamentous algae) did not consistently differ. Some differences in allochthonous producers were evident: in the two sites where both were sampled, tamarisk  $\delta D$  was higher than willow  $\delta D$  for both Ash Fork and the Colorado River; however, these differences were far smaller (less than 10‰ in both cases) than differences between tissue  $\delta D$  values between allochthonous and autochthonous primary producers. Thus, our survey indicates that variation within these groups is far smaller than variation between them, setting the stage for using  $\delta D$  as a tracer for partitioning allochthonous from autochthonous energy flow in aquatic ecosystems.

There are several explanations for our finding that algae are strongly depleted in  $\delta D$  compared to terrestrial plants. First, algae may fractionate against D during photosynthesis to a greater extent than terrestrial  $C_3$  plants (Yakir 1992). Second, water in terrestrial plants is evaporated from leaves, causing isotopic enrichment of leaf water (Washburn and Smith 1934, Wershaw et al. 1970). This enrichment is highest where relative humidity and stomatal conductance are low (Bariac et al. 1989, Pendall et al. 2005); differences between terrestrial plants and algae may be less pronounced in more mesic watersheds. Third, higher lipid concentrations in algae compared to terrestrial plant leaves (e.g., Becker 1994, Kupferberg et al. 1994, Porter and Villar 1997) would cause lower  $\delta D$  signatures in algae, because lipids are depleted in deuterium (Smith and Epstein 1970, Sessions et al. 1999). These explanations are not mutually exclusive; any or all could contribute to the differences in tissue  $\delta D$  between algae and terrestrial plants. Whatever the mechanism, the consistent separation of algae and leaves in  $\delta D$  observed in four independent locations suggests that stable isotopes of hydrogen may be a generally useful tool to partition energy flow in aquatic ecosystems.

We found that tissue  $\delta D$  for insects and fish consumers, occupying the second and third trophic levels in these ecosystems, were intermediate between  $\delta D$  of algae and leaves (Fig. 1). This suggests combined reliance on these energy sources for consumer biomass production. Isotope mixing models could be used to calculate the relative contributions of algae and terrestrial leaves to consumer biomass, but doing so requires more information (or assumptions). Specifically, we must know (or assume to be negligible) both the contribution of drinking water to the tissue  $\delta D$  signature, and trophic fractionation; i.e., enrichment in  $\delta D$  up the food chain.

Drinking water can influence  $\delta D$  signatures of non-exchangeable hydrogen in animals, contributing to around 20% of the signature in quail (Hobson et al. 1999). For the aquatic organisms we sampled, it is not known whether this occurs, and if so, to what extent. Spatial variation in this influence is likely minimal in

TABLE 2. Hydrogen stable isotope values ( $\delta D$ ) for water collected along a 360-km reach of the Colorado River in June and September 2006 showing little spatial or seasonal change.

Distance below Glen Canyon Dam (km)	June $\delta D$ (‰)	September $\delta D$ (‰)
0	$-106.8 \pm 0.5$	$-107.4 \pm 0.4$
46	$-106.9 \pm 0.4$	$-106.2 \pm 0.1$
98	$-105.7 \pm 0.3$	$-106.4 \pm 0.9$
99	$-107.3 \pm 0.2$	$-106.6 \pm 0.1$
142	$-105.1 \pm 0.4$	$-104.9 \pm 0.1$
232	$-107.3 \pm 0.8$	$-105.7 \pm 0.9$
264	$-107.4 \pm 0.2$	$-107.1 \pm 0.5$
360	$-108.0 \pm 0.2$	$-104.4 \pm 0.4$

Notes: The  $\delta D$  values are means  $\pm$  SE. Samples were collected and analyzed in triplicate.

well mixed aquatic ecosystems, as indicated by our data from the Colorado River where, over a 360-km reach, we observed very little variation in the  $\delta D$  of river water; variation over time was also minimal (Table 2). In systems with more dynamic hydrology, seasonal and spatial differences may be more pronounced, such as systems with alternating reliance on summer vs. winter precipitation in the southwestern United States (Pendall 2000). Whether trophic transfers substantially alter hydrogen isotope signatures is unclear. Observations are mixed, with some suggesting enrichment (Malej et al. 1993, Birchall et al. 2005), whereas others indicate none (Estep and Dabrowski 1980). Experimental approaches indicate that trophic enrichment is negligible (Smith and Ziegler 1990, Hobson et al. 1999). In the cases we examined,  $\delta D$  values of top predators were higher than their likely prey (Fig. 1), yet prey signatures also varied, enough that it was not possible to ascribe variation in the  $\delta D$  signature of top predators to that due to putative trophic fractionation versus that caused by selecting prey of differing  $\delta D$  signature. Our results caution against using hydrogen isotope signatures to indicate trophic position (Birchall et al. 2005) or geographical location (Whitledge et al. 2006) in aquatic systems without carefully quantifying variation in the hydrogen

isotope signature of the food base, variation that can be quite large (Table 1, Fig. 1). On the other hand, if trophic fractionation and drinking water contributions to tissue  $\delta D$  are known (or are negligible), the large differences we observed in  $\delta D$  between algae and terrestrial plants could resolve contributions of these energy sources to aquatic food webs.

As an example, we compare energy partitioning calculations using  $\delta^{13}C$  with those for  $\delta D$  at one site in Fossil Creek. Because of unresolved issues of trophic fractionation and drinking water contributions, this example should be considered exploratory. At this site, algae are 7.7‰ depleted in  $\delta^{13}C$  compared to plant leaves, and mountain desert sucker, a grazing fish, is intermediate (Table 3). Assuming no trophic fractionation for  $\delta^{13}C$  (DeNiro and Epstein 1978), mass balance suggests that around a third of the carbon in this fish is derived from autochthonous primary production. Algae have lower  $\delta D$  signatures than leaf litter at this site, a difference of 113‰. As for  $\delta^{13}C$ , the mountain desert sucker is intermediate between these end members. Assuming no trophic fractionation for  $\delta D$  (Smith and Ziegler 1990, Hobson et al. 1999), mass balance indicates slightly less than one third of the organic hydrogen in this species originates from autochthonous algal productivity (Table 3). These estimates are remarkably similar. However, the 113‰ difference between algae and leaf litter in  $\delta D$  yielded greater resolution than the 7.7‰ difference in  $\delta^{13}C$ , resulting in two-fold narrower confidence intervals when using  $\delta D$  (Table 3). Furthermore, at other sites,  $\delta^{13}C$  values of algae and leaf litter are indistinguishable (J. C. Marks, unpublished data), but the differences in  $\delta D$  persist. While trophic fractionation and drinking water influences must still be resolved, this comparison suggests that mixing models based on  $\delta D$  may prove a powerful complement to those based on  $\delta^{13}C$  for distinguishing allochthonous and autochthonous sources of energy for higher trophic levels in aquatic ecosystems.

Our findings indicate that  $\delta D$ -based mixing models can shed light on energy flow in aquatic food webs where

TABLE 3. Agreement between energy source partitioning using  $\delta^{13}C$  and  $\delta D$  in Fossil Creek.

Source	$\delta^{13}C$			$\delta D$		
	Mean $\pm$ SE (‰)	CI	<i>n</i>	Mean $\pm$ SE (‰)	CI	<i>n</i>
Algae	$-35.1 \pm 0.6$		15	$-264.3 \pm 11.5$		10
Terrestrial	$-27.8 \pm 0.7$		6	$-151.3 \pm 7.3$		5
Fish	$-30.3 \pm 0.8$		5	$-181.6 \pm 5.9$		9
Algae	$33.9 \pm 11.1$	7.7–60.1		$26.9 \pm 5.7$	14.2–39.5	
Terrestrial	$66.1 \pm 11.1$	39.9–92.3		$73.1 \pm 5.7$	60.5–85.8	

Notes: The first three rows show values for carbon isotope ( $\delta^{13}C$ ) and hydrogen isotope ( $\delta D$ ) composition as means  $\pm$  SE, with sample size (*n*). The last two rows show results from isotope mixing model calculations using the Isoerror model (Phillips and Gregg 2001; <http://www.epa.gov/wed/pages/models/stableIsotopes/isotopes.htm>), where percentage contributions to fish tissue carbon and hydrogen are given (mean  $\pm$  SE in the first column; 95% CI in the second column).



PLATE 1. Typical flow and riparian zone of Fossil Creek, Arizona, USA, before (left panel) and after (right panel) return of full flow to the river following decommissioning of the 100-year old hydropower dam in June 2005 by Arizona Public Service, the power company that operated the hydropower facility. Stable isotope samples collected for this study were collected before decommissioning. Photos © Nick Berezenko, used by permission.

both allochthonous and autochthonous sources of productivity are present. Laboratory investigation of drinking water, trophic fractionation, and other possible influences on organic hydrogen isotopic composition will help refine this technique. The survey data we report here should also be expanded to include a broader range of terrestrial and freshwater algal species, and other watersheds outside of the southwestern United States, particularly in more mesic climates. Nevertheless, given the large differences we observed in  $\delta D$  between algae and terrestrial organic matter, we submit this technique holds considerable promise for addressing hypotheses about energy flow in aquatic ecosystems.

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