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ECOSYSTEM ECOLOGY - ORIGINAL PAPER

The influence of environmental water on the hydrogen stable isotope ratio in aquatic consumers

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Abstract Aquatic food webs are subsidized by allochthonous resources but the utilization of these resources by consumers can be difficult to quantify. Stable isotope ratios of hydrogen (deuterium:hydrogen; δD) potentially distinguish allochthonous inputs because δD differs between terrestrial and aquatic primary producers. However, application of this tracer is limited by uncertainties regarding the trophic fractionation of δD and the contributions of H from environmental water (often called "dietary water") to consumer tissue H. We addressed these uncertainties using laboratory experiments, field observations, modeling, and a literature synthesis. Laboratory experiments that manipulated the δD of water and food for insects, cladoceran zooplankton, and fishes provided strong evidence that trophic fractionation of δD was negligible. The proportion of tissue H derived from environmental water was substantial yet variable among studies; estimates of this proportion, inclusive of lab, field, and literature data, ranged from 0 to 0.39 (mean 0.17 ± 0.12 SD). There is a clear need for additional studies of environmental water. Accounting for environmental water in mixing models changes estimates of resource use, although simulations suggest that uncertainty about the environmental water contribution does not substantially increase the uncertainty in estimates of resource use. As long as this uncertainty is accounted for, δD may be a powerful tool for estimating resource use in food webs.

Keywords Food web · Deuterium · Fish · Zooplankton · Insect

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Introduction

Inputs of allochthonous organic matter from terrestrial systems subsidize consumer respiration and production in aquatic ecosystems (Birge and Juday 1927; Vannote et al. 1980; Polis et al. 1997). These subsidies support consumers, stabilize food web interactions, and link aquatic and terrestrial ecosystems (Cole et al. 1994; Huxel and McCann 1998; Pace et al. 2004). As a result, ecologists are increasingly interested in quantifying allochthonous subsidies to consumers.

Allochthony, defined as the proportion of an aquatic consumer's biomass that is derived from terrestrial organic matter, is difficult to quantify. Some researchers have used natural abundance stable isotope ratios of carbon (δ^{13}C) to trace allochthonous organic matter through food webs (Grey et al. 2001; Karlsson et al. 2003). In many systems, however, insufficient separation between the δ^{13}C of terrestrial and aquatic primary production hinders this approach. An alternative approach manipulates the δ^{13}C of aquatic primary producers by adding inorganic ^{13}C to the water, creating a large separation in δ^{13}C between aquatic and terrestrial organic material (Cole et al. 2002). While

this approach is powerful and has been applied successfully in several systems, it may overestimate allochthony for consumers linked to detrital autochthonous pathways; furthermore, the high cost of ^{13}C enrichment limits the size and number of systems in which it can be applied (Carpenter et al. 2005; Solomon et al. 2008). Thus there has been considerable interest in identifying alternative methods for quantifying allochthony in aquatic systems.

Hydrogen stable isotope ratios (δD) are potentially a useful tracer for quantifying allochthonous subsidies to aquatic consumers (Doucett et al. 2007). The two most common isotopes of hydrogen are deuterium (D) and hydrogen (H) (^{2}H and ^{1}H, respectively). Because D is twice as heavy as H, physical and physiological processes that fractionate isotopes often discriminate strongly against D, creating strong contrasts in the δD, denoted in standard notation relative to the international standard Vienna standard mean ocean water (VSMOW). In terrestrial primary producers but not aquatic ones, kinetic fractionation during evapotranspiration alters the δD of the water available for incorporation into organic matter via photosynthesis (Smith and Ziegler 1990). For this and other reasons, aquatic primary producers tend to have much lower δD than terrestrial plants from the same location (Doucett et al. 2007).

Hydrogen stable isotope ratios have been used for many years in studies of hydrology, plant physiology, animal migration, and other topics (Ehleringer et al. 1993; Kendall and McDonnell 1998; Hobson and Wassenaar 2008; Sharp 2008). Yet until recently their use as a food web tracer was limited by methodological challenges (DeNiro and Epstein 1981; Schimmelmann and DeNiro 1993). Recent technological and methodological advances have overcome some of these challenges (Wassenaar and Hobson 2000; Wassenaar and Hobson 2003). As a result, interest in using δD as a food web tracer has been increasing rapidly. A recent investigation in streams of the desert southwest (USA) demonstrated that the δD of terrestrial and aquatic organic matter differed substantially, and that consumer δD fell between these end members, suggesting reliance on a mixture of these resources (Doucett et al. 2007). Similar patterns have been observed in California streams and Wisconsin lakes (J. C. Finlay, University of Minnesota, unpublished data; C. T. Solomon, unpublished data). These early studies suggest that δD may be a powerful tool for quantifying allochthonous contributions to aquatic food webs.

Applying any stable isotope ratio as a food web tracer requires knowing how well the isotope ratio in the diet is reflected in the consumer. Early measurements of δD in food webs found that δD of consumers was often different than that of their prey or diet (Stiller and Nissenbaum 1980; Macko et al. 1983; Malej et al. 1993; but see Estep and Dabrowski 1980). At least two mechanisms might explain such differences (Birchall et al. 2005; Doucett et al. 2007). First, H atoms from ambient water that enter a consumer either in water consumed with the diet or by diffusion can potentially be incorporated during biosynthesis into the non-exchangeable H in consumers' tissues. Even small contributions of this "environmental" or "dietary" water to tissue H can substantially alter tissue δD, because δD of water is typically much more enriched in D than that of terrestrial or aquatic primary producers (for instance, δD in the Colorado River is approximately -150 to $-160‰$ for terrestrial primary producers, $-290‰$ for aquatic primary producers, and $-110‰$ for water; Doucett et al. 2007). Second, the δD value of a consumer's tissues may differ from that of its diet due to isotopic fractionation during biosynthesis (as occurs, for example, with nitrogen stable isotopes; Minagawa and Wada 1984).

Estimates of the magnitude of these two effects are scarce and variable. For aquatic consumers we are aware of only three published studies from which an estimate of the environmental water contribution (or the data to make such an estimate) is available, all of which were conducted in marine environments. In those three studies, which considered different organisms and used different methods, the environmental water contribution ranged from 0 to 35% of tissue H (see Literature review, below). Similarly, drinking water contributed ~20% of tissue H in birds (*Coturnix coturnix*) in a well-controlled laboratory experiment (Hobson et al. 1999). Trophic fractionation of δD appears to be substantial based on some observational studies (Schimmelmann and Deniro 1986; Malej et al. 1993; Birchall et al. 2005). However, none of these studies considered environmental water effects on consumer δD, which might also explain observed differences in δD between consumers and their diets.

Uncertainty about environmental water contributions and trophic fractionation of hydrogen stable isotopes complicates the use of δD data for anything more precise than a qualitative indication of allochthonous resource use. Better understanding of these effects is therefore essential in order to make well-constrained estimates of allochthonous subsidies using δD. In this paper we use multiple approaches to constrain these two sources of uncertainty. We present new results from both observational studies and controlled laboratory experiments, and synthesize estimates of environmental water from the literature. Our findings have important implications for researchers using δD in aquatic food web studies.

Materials and methods

Mosquito experiment

Twenty-four experimental containers (14 × 14 × 4-cm plastic containers with tight-fitting lids) were assigned to

one of four treatments in a 2 × 2 factorial design. The treatments manipulated the δD of the food (concentrated algal cells) and ambient water for mosquito larvae (*Aedes aegeptyi*). Containers were filled with 400 ml of either normal tap water or D-enriched tap water (750 µl of 99.9 atom% D_2O added to 20 l of tap water in a closed carboy). Eggs of *A. aegypti* were hatched under vacuum, and approximately equal numbers of larvae were placed into each experimental container. Containers were kept closed, in the dark, and at 28.3°C throughout the experiment. Each day, we changed the water in the experimental containers and added new food.

Larvae were fed concentrated algae with either natural abundance (normal) δD values or D-enriched δD values. We grew two cultures of *Scenedesmus* sp. (Chlorophyceae) in chemostats. These chemostats received the same nutrient drip except that one was spiked with 40 µl l^{-1} of 99.9 atom% D_2O. Several times per week we removed accumulated algae from an overflow flask attached to each chemostat, concentrated the cells with two centrifugations (15 min at 4,000–4,500 r.p.m.), and froze the concentrate. An aliquot of thawed concentrate was added to each experimental container after each water change. We froze algae before feeding them to mosquito larvae to preclude fixation of new photosynthate from the ambient water in the mosquito containers.

Throughout the experiment we regularly collected samples of the water ($n = 8$ and $n = 9$ for D-enriched and normal treatments, respectively) and of the algae ($n = 9$ each for D-enriched and normal treatments). In addition, for half of the containers we took a weekly sample of the "used" water that we poured out during the water change, to check whether the water δD changed during the 24 h that it was in the container. We filtered water samples through glass fiber filters (0.7-µm pore size) into scintillation vials, taking care that no air bubbles were present. These samples were stored in the dark at ~2°C until analysis. Algal samples were dried at 60°C, ground to a fine powder, and prepared for analysis.

Larvae were removed from the experiment after 5 weeks, or sooner if they reached their fourth instar (to insure that individuals were sampled before pupation). They were held in tap water for 1 day to evacuate their guts, then dried at 60°C and prepared for analysis.

The H isotopic composition of a consumer can be described by a two-end member mixing model that incorporates isotopic enrichment:

$$\delta D_{consumer} = \omega * (\delta D_{water} + \varepsilon_{water}) + (1 - \omega) * (\delta D_{food} + \varepsilon_{food}), \quad (1)$$

where δD_{water} is the mean observed water δD for the water treatment applied to a container, δD_{food} is the mean observed algae δD for the algae treatment applied to a container, ω is the proportion of tissue H derived from environmental water, and ε_{water} and ε_{food} are isotopic enrichment terms describing how H isotopes are fractionated as they are incorporated into tissues from the diet or water. Because mosquitoes were raised under the experimental conditions from the time they hatched, we assumed that they were in complete isotopic equilibrium with their food.

Note that by rearranging Eq. 1 it is possible to include both of the enrichments in one term that describes the net trophic enrichment (ε_H) between a consumer and its diet and water:

$$\delta D_{consumer} = \omega * \delta D_{water} + (1 - \omega) * \delta D_{food} + \varepsilon_H, \quad (2)$$

where $\varepsilon_H = \omega \times \varepsilon_{water} + (1-\omega) \times \varepsilon_{food}$. Besides estimating ω, our study design enabled us to estimate ε_H, which is of practical utility in food web tracer studies (but not ε_{water} and ε_{food}, which are also physiologically interesting parameters). We fit the mixing model given by Eq. 2 by least squares using an optimization routine in the R statistical package. We used the likelihood ratio test to test the null hypothesis that $\varepsilon_H = 0$, by comparing the negative log-likelihood from the fit to the full model (Eq. 2) to that from the fit to a reduced model in which ε_H was set equal to zero:

$$\delta D_{consumer} = \omega * \delta D_{water} + (1 - \omega) * \delta D_{food}, \quad (3)$$

After selecting the best model based on the likelihood ratio test, we estimated the uncertainties of the parameter estimates as the SDs from 1,000 bootstrap iterations of the model fit. In each bootstrap iteration the model residuals were randomly reassigned to fitted values within each treatment, and new values of δD_{water} and δD_{food} for each treatment were drawn from normal distributions described by the empirical means and SDs.

Zooplankton experiment

We conducted a similar experiment using *Daphnia galeata mendotae*, a cladoceran zooplankter. Twelve autoclaved experimental containers (4-l glass Mason jars with screw top lids) were randomly assigned to one of the four treatments in the 2 × 2 factorial experiment described above. An air line through the lid of each jar supplied 0.2 µm-filtered air to provide aeration and maintain slight positive pressure. Jars were filled with 1 l of normal deionized water or D-enriched deionized water (40 µl of 99.9 atom% D_2O l^{-1}). We added 50 adult zooplankton to each jar from a lab colony started 8 months earlier with wild-caught individuals from Lake Mendota, Wisconsin. We changed the water in experimental containers weekly in this experiment (instead of daily as with the mosquitoes) to minimize mortality associated with handling during water changes.

Zooplankton were fed 2–3 times per week with concentrated algae having either natural abundance or D-enriched δD values. We used *Nannochloropsis limnetica* (Eustigmatophyceae) instead of *Scenedesmus* sp. in this experiment because pilot studies demonstrated that *N. limnetica* was a superior food for *D. galeata*. *N. limnetica* was batch cultured on cold-filtered (0.2 μm) Chu-10 algal growth medium in sterile conditions under 16:8 h light:dark at 18°C (Chu 1942). We added 40 μl l^{-1} of 99.9 atom% D_2O to the Chu-10 for batches of D-enriched algae. Each batch was harvested near peak biomass, and cells were concentrated by centrifugation at 2,000 r.p.m. for 10 min. The algal pellet was resuspended in 5 ml of deionized water and then added to an experimental container. Because we did not kill algal cells in this experiment prior to feeding them to the organisms, the experiment was conducted in continuous darkness to prevent new photosynthesis by algae. Temperature was controlled at 18°C.

As in the mosquito experiment, we regularly collected samples of the water ($n = 18$ each for D-enriched and normal treatments) and of the algae ($n = 15$ each for D-enriched and normal treatments) throughout the experiment. Because we suspected that the δD of the water might change appreciably between the weekly water changes in this experiment, we sampled only the "used" water (see above) to develop a conservative estimate of the water δD. Methods for sampling water and algae were the same as those used for the mosquito experiment.

The experiment continued until two new generations of zooplankton had been observed (7 weeks), to ensure that sampled individuals had grown entirely under experimental conditions. At the conclusion of the experiment we placed zooplankton into deionized water and held them for 12 h to evacuate their guts, then dried them at 60°C and prepared them for analysis. We estimated ω and ε_H using Eqs. 2 and 3, as described above.

Fish study

We gathered samples of salmonid muscle tissue, fish feed, and ambient water from three fish hatcheries in order to estimate environmental water use by fish. Whereas the mosquito and zooplankton experiments manipulated the δD of both food and water, this study took advantage of a natural continental gradient in the δD of meteoric water (and did not manipulate the δD of food). The fish species that we sampled (with locations and mean ± SD total length) were: rainbow trout (*Oncorhynchus mykiss*; Cumming, Georgia; 218 ± 9 mm); rainbow trout (Anaconda, Montana; 63 ± 1 mm); brook trout (*Salvelinus fontinalis*; Old Forge, New York; 138 ± 18 mm); and Atlantic salmon (*Salmo salar*; Old Forge, New York; 110 ± 2 mm). A small portion of dorsal muscle tissue was removed from individual fish and rinsed with DI water. Fish feed and fish muscle tissue samples were dried at 60°C and then homogenized. All fish had been fed on a single feed formulation from soon after the end of the sac fry stage. Water samples were collected in scintillation vials directly from inlet pipes to the hatcheries, taking care not to introduce air bubbles to the vials. We estimated ω and ε_H using Eqs. 2 and 3, as described above.

Estimating environmental water use from field observations

Besides conducting controlled experiments to estimate environmental water use, we also used observational data to estimate it under field conditions. Because most organisms consume a variety of prey from multiple trophic levels, estimating the δD of their food (and therefore, calculating ω) is difficult using field data. We took two approaches to help resolve uncertainty related to diet.

First, we considered a consumer with a fairly restricted diet, so that we felt fairly confident calculating ω from the data. The phantom midge, *Chaoborus* spp. (Diptera), is an important zooplankton predator in the pelagic zone of many lakes (Wetzel 2001). During the summer of 2007, we measured the δD of *Chaoborus*, their dominant zooplankton prey, and the water in four lakes on the Wisconsin–Michigan border. *Chaoborus* and zooplankton were collected at night by oblique net tows through the upper mixed layer. Enough individual *Chaoborus* were picked live from the sample to provide adequate mass (about 350 μg dry weight) for δD determination. We also identified the dominant zooplankton species in these samples and picked live individuals in a similar manner. The dominant species in each lake were *Daphnia* (seven dates in Paul Lake and two dates in Peter Lake); *Holopedium gibberum* (one date in Peter Lake); *Bosmina* (four dates in Tuesday Lake), and *Leptodiaptomus minutus* (two dates in Crampton Lake). Water samples were collected from the upper mixed layer and filtered and stored as described above. Water samples were collected within 7 days (usually within 1 day) of *Chaoborus* and zooplankton collection. We estimated ω for *Chaoborus* as the average of the values calculated from each lake–date combination using Eq. 3 (assuming trophic fractionation was zero, see Results).

Second, we considered a wide range of consumers for which we could calculate an upper bound for ω (ω_{max}), even though we could not precisely determine the δD of their food. We assumed that the prey consumed by any consumer is ultimately supported by either aquatic or terrestrial primary production. Aquatic primary production is substantially depleted in δD compared to terrestrial inputs, and both are depleted compared to lake water. Thus a mixing model that assumes that a consumer obtains H and

D only from aquatic primary production or lake water gives us the absolute upper limit for ω. During the summer of 2007, we estimated the δD of zooplankton ($n = 31$), zoobenthos ($n = 42$), and fishes ($n = 22$), as well as water ($n = 22$), phytoplankton ($n = 22$), and periphyton ($n = 13$), from the four lakes described above. For phytoplankton this involved some experimentation, the details of which will be described elsewhere (C. T. Solomon et al. unpublished manuscript). For each taxon–lake combination we calculated ω_{max} using Eq. 3. We used the phytoplankton value as the end member for aquatic primary production because phytoplankton δD was always lower than periphyton δD (see Results) and thus results in the maximum possible estimate of ω_{max}. We also considered how estimates differed if we used periphyton instead of phytoplankton as the end member. We summarized the results by aggregating taxa based on guild (zooplankton, zoobenthos, or fish) and relative trophic level (low or high). Specifically, for zooplankton we sampled herbivorous cladocerans including *Bosmina* sp., *Daphnia* spp., *Holopedium gibberum*, and diaptomids (low trophic level) as well as *Chaoborus* spp. (high trophic level). For zoobenthos we sampled chironomid larvae (low trophic level) as well as anisopteran odonate larvae (high trophic level). All zoobenthos were collected at a depth of 1 m. Finally, for fish we sampled cyprinid and gasterosteid minnows including brook stickleback (*Culaea inconstans*), fathead minnow (*Pimephales promelas*), finescale dace (*Phoxinus neogaeus*), and golden shiner (*Notemigonous chrysoleucas*) (low trophic level) as well as centrarchid predators including largemouth bass (*Micropterus salmoides*), pumpkinseed (*Lepomis gibbosus*), and bluegill (*Lepomis macrochirus*) (high trophic level).

Determination of δD

Organic samples were weighed (approximately 350 μg) into silver capsules and pyrolyzed at 1,400°C to H_2 gas using a Thermo-Electron (Bremen, Germany) thermal-chemical elemental analyzer. Chromatographically purified H_2 gas was then introduced to a Thermo-Electron Delta Plus XL gas isotope-ratio mass spectrometer for δD measurement using an open-split interface (CONFLO II). Prior to measurement, all organic samples were oven-dried at 60°C for 48 h and then equilibrated using the bench-top procedure of Wassenaar and Hobson (2003) to correct for exchange of H atoms between samples and ambient water vapor (DeNiro and Epstein 1981). In this procedure, both samples and standards (with known δD values for non-exchangeable H) are exposed to water vapor in laboratory air for a period at least 2 weeks to allow all exchangeable H to obtain a constant δD value. Organic δD values were normalized on the VSMOW scale using known δD values for three standards donated by L. Wassenaar (Environment Canada, Saskatoon, SK). These standards were Chicken Feather (δD = −147‰), Cow Hoof (δD = −187‰), and Bowhead Whale Baleen (δD = −108‰). Based on results indicating very little variation in the proportion of exchangeable H among samples of very different chemical compositions (Wassenaar and Hobson 2000), we assumed that our organic samples possessed similar amounts of exchangeable and non-exchangeable H as these three standards. Repeated analyses of several internal organic standards showed that organic δD values were precise to within ±3.0‰ (SD), on average.

Water samples were analyzed for δD via cavity-ring-down laser spectroscopy on a Los Gatos Research DLT-100 liquid-water analyzer. Water δD values were normalized on the VSMOW-SLAP scale using internal water standards (that had been previously calibrated against VSMOW and SLAP). Repeated analyses of several internal water standards showed that water δD values were precise to within ±0.6‰ (SD), on average.

Literature review

We reviewed the literature for published estimates of ω or data from which we could calculate ω. We considered observational and experimental studies that focused on any aquatic (freshwater or marine) organism.

Results

Mosquito and zooplankton experiments

Our additions of D_2O successfully created large differences in δD between treatment levels of both food and water (Fig. 1). In the mosquito experiment, algal δD (mean ± SD) in the D-enriched and normal treatments was −3.2 ± 4.6‰ ($n = 9$) and −175.2 ± 20.6‰ ($n = 9$), respectively. The large SD in the normal algae treatment resulted from one outlying observation with δD = −228.9‰; excluding this point changes the value for this treatment to −168.5 ± 4.5‰. Water δD in the mosquito experiment was 181.9 ± 5.3 ($n = 9$) in the D-enriched treatment and −58.7 ± 0.8 ($n = 8$) in the normal treatment. These values are for the "new" water added to containers, which was not different from the "used" water removed from containers ($F_{2,56} = 2.4$, $P = 0.1$). There was also no difference in the δD of "used" water taken from the two food treatments ($F_{2,31} = 0.6$, $P = 0.6$). In the zooplankton experiment, δD in the D-enriched and normal treatments was, respectively, −58.5 ± 10.2‰ ($n = 15$) and −222.0 ± 17.7‰ ($n = 15$) for algae and 147.4 ± 18.1 ($n = 18$) and −59.4 ± 1.5 ($n = 18$) for "used" water.

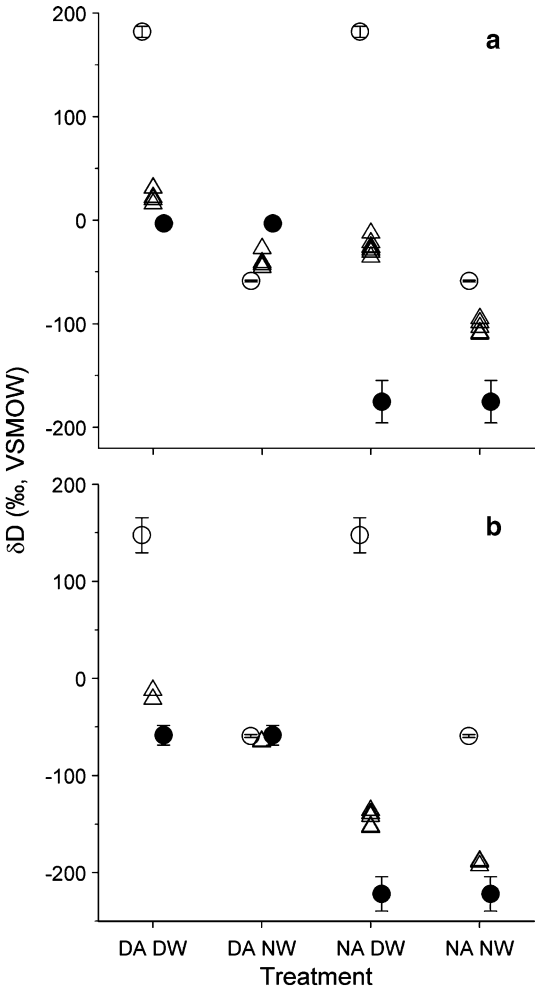

Fig. 1 Hydrogen stable isotope ratios (δD) relative to Vienna standard mean ocean water (*VSMOW*) of **a** mosquito larvae (*triangles*) and **b** zooplankton (*triangles*), their ambient water (*open circles*) and food (*filled circles*) in four treatments. For mosquitoes and zooplankton, *points* indicate composite samples of the individuals from a single replicate. For food and water, *points* indicate the mean ($\pm$1 SD) of multiple samples collected throughout the experiment (see text for details). Treatments are combinations of D-enriched (*DA*) or normal algae food (*NA*) and D-enriched (*DW*) or normal water (*NW*)

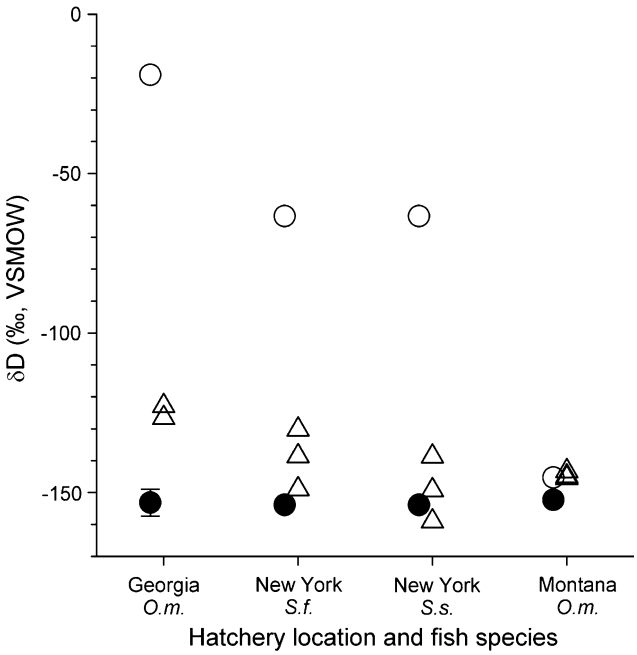

Fig. 2 δD relative to VSMOW, of muscle tissue (*triangles*) for several fish species collected from hatcheries spanning a natural continental gradient (Georgia, New York, Montana) in the δD of meteoric water. Also shown are the δD values of ambient water (*open circles*) and fish feed (*filled circles*; mean $\pm$ 1 SD, $n = 2$). *O.m. Oncorhynchus mykiss*, rainbow trout; *S.f. Salvelinus fontinalis*, brook trout; *S.s. Salmo salar*, landlocked Atlantic salmon

Consumer δD values fell between food and water δD for all of the treatments in both experiments, indicating that the H in consumer tissues was derived from both sources (Fig. 1). Organisms from replicate experimental containers within a given treatment had very similar δD values. There was no evidence that trophic fractionation was different from zero in either experiment (likelihood ratio test; mosquitoes, $\chi^2_1 = 1.1$, $P = 0.3$; zooplankton, $\chi^2_1 = 2.3$, $P = 0.1$), so we estimated ω in both experiments using Eq. 3. Estimates of ω ($\pm$ bootstrapped SD) were 0.391 ± 0.041 for mosquitoes and 0.205 ± 0.041 for zooplankton. The models provided good fits to the data, particularly for zooplankton. For mosquitoes, the apparent contribution of water was more variable among treatments, with apparently higher contributions in the normal water treatments than in the D-enriched water treatments (Fig. 1a). Consequently, the fitted model under-predicted δD in the normal algae-normal water treatment, and over-predicted δD in the D-enriched algae–D-enriched water treatment.

Fish study

As with mosquitoes and zooplankton, the δD of fish muscle tissue was intermediate between food and water δD (Fig. 2). Water δD differed considerably among the three hatcheries in this study (-19.1‰ to -145.3‰). There was very little difference in the δD of fish feed among the hatcheries, probably because all feeds were primarily composed of marine-based fish meal. The fish study therefore resembled a 1×3 experimental design, with one level of food δD and three levels of water δD. There was no evidence that trophic fractionation for these fishes was different from zero (likelihood ratio test; $\chi^2_1 = 1.3$, $P = 0.2$), so we estimated ω using Eq. 3. The estimate of ω in this experiment ($\pm$ bootstrapped SD) was 0.124 ± 0.024.

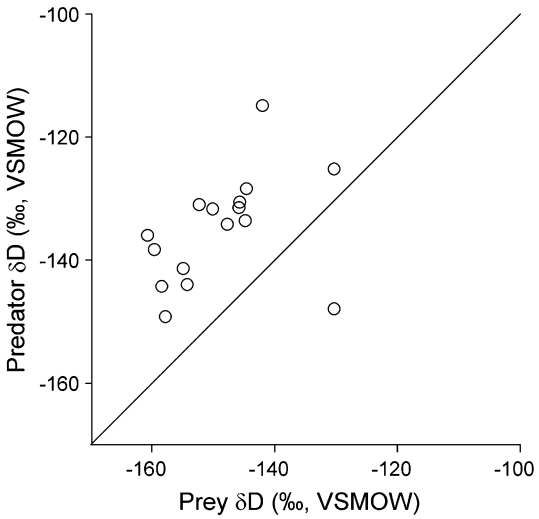

Fig. 3 δD relative to VSMOW, of *Chaoborus* spp. (planktonic predators on zooplankton) and their prey (the dominant zooplankton species; see text) on several dates between May and October 2007 in Peter, Paul, Tuesday, and Crampton Lakes. Most points lie above the 1:1 line, indicating a contribution of ambient water (δD $\sim$ −46‰) to tissue H in *Chaoborus*. Excluding the one observation below the 1:1 line, the mean (±SD) estimate from these data of the contribution of environmental water to tissue H (ω) is 0.148 ± 0.054

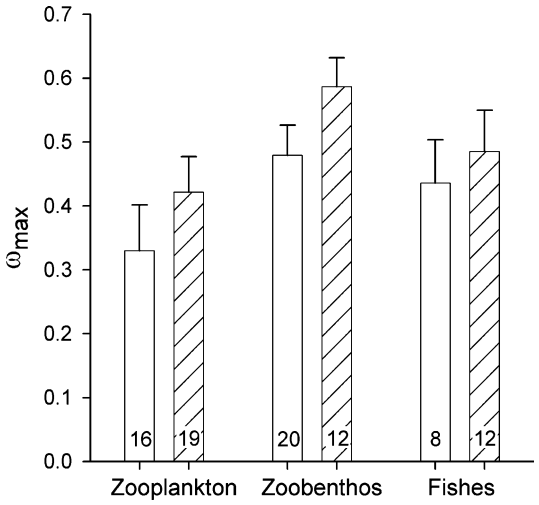

Fig. 4 Estimates of the maximum ω (ω_{max}; mean ± 1 SD) if consumers rely entirely on phytoplankton sources of primary production, for zooplankton, zoobenthos, and fishes from four lakes. Within each group, *open bars* show consumers with lower trophic level (herbivorous cladocerans, chironomids, and cyprinid minnows) and *shaded bars* show consumers with higher trophic levels (*Chaoborus* sp., odonates, and centrarchid fishes). Number of samples is shown at the *base of bars*

Field estimates of ω

Throughout the growing season in four lakes, the δD of *Chaoborus* was usually higher than that of its prey (Fig. 3). Water δD in these lakes ranged from −55 to −39‰ depending on the lake and the time of year. There was one observation for which *Chaoborus* δD was lower than prey δD and therefore outside the bounds of the mixing model end members. The mean (±SD) estimate of ω was 0.139 ± 0.064 if we set $\omega = 0$ for this observation, or 0.148 ± 0.054 if we excluded it altogether.

Estimates of the maximum possible environmental water contribution ($\omega_{\max}$) from field data varied depending on a consumer's trophic level and guild (Fig. 4). For individual consumer-lake combinations, estimates ranged from 0.24 to 0.69 and were approximately normally distributed. These values are based on calculations that use phytoplankton δD as the diet end member; if instead we assumed that consumers relied on periphyton-based pathways, estimates of $\omega_{\max}$ were lower (mean difference ± SD = 0.09 ± 0.02). Within each group (zooplankton, zoobenthos, or fishes), the mean $\omega_{\max}$ was higher for consumers that occupied higher trophic levels. Across groups, the mean $\omega_{\max}$ was higher for zoobenthos and fishes (the two groups for which the assumption of complete reliance on phytoplankton is least accurate) than for zooplankton. For Crampton, Paul, Peter, and Tuesday Lakes, the mean observed end member δD values (±SD) used in these calculations were: water, −40.1 ± 1.6‰, −44.1 ± 2.6‰, −43.9 ± 2.4‰, and −51.6 ± 2.6‰; periphyton, −164.8 ± 11.2‰, −172.6 ± 23.0‰, −187.4 ± 16.9‰, and −183.2 ± 5.6‰; and phytoplankton, −193.6 ± 8.7‰, −197.7 ± 8.9‰, −197.5 ± 8.9‰, and −205.1 ± 9.0‰.

Literature survey

We found or calculated ten estimates of ω for aquatic organisms from several phyla, including the estimates derived in this study (Table 1). These estimates were variable, ranging from 0 to 0.39 (mean 0.173 ± 0.122). Five estimates were derived from observational studies that did not control organisms' diets or even fully constrain the scope of possible diet δD. If these cases are excluded, the range of estimates is reduced to 0.12–0.39 (mean 0.212 ± 0.111) despite relatively little change in the taxonomic diversity represented. There were no consistent taxonomic patterns in the data; for instance, the three available estimates for insects were 0.06, 0.12, and 0.39.

Discussion

Trophic fractionation

Net trophic fractionation of δD between consumers and their diets and water was not significantly different from zero for mosquitoes, zooplankton, or fish. Furthermore, in two treatments in which the δD of food and water were

Table 1 Estimates of the proportion (ω) of tissue H derived from environmental water for aquatic consumers

Source	Consumer	Habitat	ω	ω SD	Notes
Estep and Dabrowski (1980)	Snail	Marine	0.00	–	a, b, c
Macko et al. (1983)	Amphipod	Marine	0.12	–	a, d
Malej et al. (1993)	Jellyfish	Marine	0.35	–	a, b, e
J. C. Finlay et al., unpubl.	Shredder insects	Freshwater	0.12	0.12	b, f
J. C. Finlay et al., unpubl.	Scraper insects	Freshwater	0.06	0.06	b, g
M. W. O'Neill et al., unpubl.	Trout	Freshwater	0.23	0.03	h
This study	Mosquito	Freshwater	0.39	0.04	–
This study	Zooplankton	Freshwater	0.20	0.04	–
This study	Fish	Freshwater	0.12	0.02	–
This study	*Chaoborus*	Freshwater	0.14	0.06	b

The SD of the estimate (ω *SD*) is shown when available. In cases where the authors did not explicitly estimate ω we calculated ω based on the δD of consumers, their diet, and their water; see notes and Eq. 3 (main text) for details. *unpubl.* Unpublished data

(a) δD of solid samples not corrected for exchangeable H

(b) Diet not observed explicitly; δD of diet determined from assumed diet

(c) δD of snail, diet, and water were respectively −111‰, −111‰, and 4.7‰

(d) δD of amphipod, diet, and water were respectively −118.6‰, −132.8‰, and −18.6‰. Note that in one experiment amphipods with initial δD = −118.6‰ showed only minimal change in δD (to −109.9‰) when exposed for 10 days to D-enriched sea water (δD = 94.1‰) and fed fresh *Ulva* (δD = −167.5‰) instead of detrital *Ulva* (δD = −132.8‰). Assuming that organisms continued to grow during that experiment, the results suggest a lower ω than estimated in the table

(e) δD of jellyfish, diet, and water were respectively −58.4‰, −92.1‰, and 4.6‰

(f) Mean value for 14 samples of *Lepidostoma* sp. and *Heteroplectron* sp. from four sites. For each sample, ω was calculated from a mixing model based on measured δD of insects and of food (CPOM; n = 1–3) and water (n = 1) at that site. Negative estimates of ω (n = 3) were set to zero. Data from J. C. Finlay (University of Minnesota), R. R. Doucett, and C. McNeeley (Eastern Washington University)

(g) Mean value for 14 samples of *Glossosoma* sp., *Neophylax* sp., and Psephenidae from three sites. For each sample, ω was calculated from mixing model based on measured δD of insects and of food (diatoms, n = 2–11) and water (n = 1) at that site. Negative estimates of ω (n = 2) were set to zero. Data from J. C. Finlay (University of Minnesota), R. R. Doucett, and C. McNeeley (Eastern Washington University)

(h) Value of ω calculated as the mean from four treatments in a 2 × 2 factorial experiment manipulating the δD of food and water. Sample sizes per treatment were: fish, n = 35; water, n = 7; food, n = 18. Data from M. W. O'Neill (Northern Arizona University) and R. R. Doucett

nearly identical, consumers also showed that same δD value (D-enriched algae–normal water treatment in zooplankton experiment and Montana samples in fish study). Previous observational studies have attributed differences in δD between consumers and their diets to trophic fractionation, but did not consider potential simultaneous effects of environmental water use on consumer δD (Schimmelmann and Deniro 1986; Malej et al. 1993; Birchall et al. 2005). Indeed, in the absence of an experimental manipulation it is impossible to distinguish between trophic fractionation and environmental water as potential explanations for differences in δD between consumers and their diets. Our results suggest that environmental water is a more likely explanation than trophic fractionation for the differences observed in prior studies between consumer and diet δD.

Environmental water contribution to tissue H

Our experiments, our field observations, and the literature indicate that environmental water generally contributes significantly (more than a few percent) to the non-exchangeable H in the tissues of aquatic consumers. This conclusion contrasts with some of the pioneering studies of δD as a trophic tracer, which concluded that environmental water was probably not significant (Estep and Dabrowski 1980; Macko et al. 1983). However, it agrees with recent work demonstrating a substantial (20–30%) contribution of environmental water to the non-exchangeable H in the tissues of birds (Hobson et al. 1999) and the hair of small mammals (Podlesak et al. 2008).

Perhaps the most striking pattern in the available estimates of ω is their variability (Table 1). Several factors probably contribute to this variability. First, given that the uncertainty around a single estimate of ω is fairly large (particularly for observational studies that have less control over or information about the δD of diet and water; Table 1), the variability among estimates may not be quite as large as it appears. By conducting replicated studies and seeking to maximize the contrast between different levels of food and water δD, we achieved fairly precise estimates of ω in our experiments and field estimates. Future efforts

to provide additional estimates of ω should consider similar steps, by creating strong gradients in food and water δD or by utilizing strong natural gradients.

Biochemical variation among organisms (or taxa) may introduce variability into measured δD values and, therefore, calculated ω estimates. For instance, both the lipids and the amino acids in an organism's tissues may be either synthesized de novo or derived from the diet. Synthesized lipids are known to be strongly depleted in D relative to cell water (Smith and Epstein 1970; Sessions et al. 1999), and synthesis of amino acids may also fractionate against D (Birchall et al. 2005). Furthermore, the proportion of diet-derived and synthesized molecules can vary among organisms (Birchall et al. 2005). Thus measured tissue δD reflects not only an organism's diet, but also the net influence of these various biochemical effects. Future experimental work to refine our understanding of these processes could potentially reduce the variability in measured δD values and lead to better-constrained estimates of ω.

One other factor that might contribute to variation in ω estimates is a compounding or accumulation of environmental water contributions that seems likely to occur in food chains. A simple example demonstrates this idea: imagine a fish that eats exclusively zooplankton that eat exclusively phytoplankton. Assume that the contribution of environmental water to tissue H is the same for the fish and the zooplankton (say, $\omega = 0.20$). The water-derived H in the fish's tissues should then include not only the 20% that it gets from its water, but also the 20% that the zooplankton gets from its water. Both field and laboratory studies may occasionally measure this total, compounded environmental water contribution rather than a per trophic-level estimate, if the water contribution is calculated using δD data for a resource that is more than a single trophic transfer removed from the consumer. For instance, our field-based estimates of the maximum possible environmental water contribution for secondary (and higher) consumers such as odonates and fish are estimates of ω_{compound}, because we calculated the environmental water contribution based on the δD of basal resources. In contrast, our estimate for *Chaoborus* is a true ω value, because we compared their δD to that of their diet.

Trophic compounding of environmental water contributions is likely to be an important consideration when using δD data in mixing models to estimate resource use. Most mixing model applications seek to determine the sources of production that ultimately support consumers, which requires tracing through multiple trophic transfers for anything but a primary consumer. How large will the total contribution of environmental water to consumer H be in these situations? Consider a simple linear food chain model, where each consumer eats only from the next lowest trophic level and where ω is a constant for all consumers. Then the total contribution of water-derived H to the tissues of a consumer is:

$$\omega_{\text{compound}} = 1 - (1 - \omega)^{\tau}, \quad (4)$$

where τ is difference in trophic level between the resource and the consumer. In reality, of course, most consumers are omnivorous with respect to both sources of primary production and trophic levels of prey; it is also possible, as discussed above, that ω might vary among taxa. Thus the assumptions that permit writing this simple algebraic relationship for ω_{compound} will generally be violated. Nonetheless, Eq. 4 provides a useful simple model for considering the scope of the issue. Using this model, if ω is near the mean value from Table 1 (0.17), then ω_{compound} approaches 0.40 for high-level consumers (Fig. 5).

It is also instructive to reverse this chain of thinking, and ask what can be learned about ω based on estimates of ω_{compound}. As described above, our field-based estimates of the maximum possible environmental water contribution are actually estimates of the maximum possible ω_{compound}. In discussing these estimates, it is important to recall that true values of ω_{compound} for these consumers are likely to be lower because the assumption that these consumers rely entirely on autochthonous resources is false in most cases (Carpenter et al. 2005; Pace et al. 2007; Solomon et al. 2008). For instance, we estimated that $\omega_{\text{max}} = 0.41$ for *Chaoborus* (Fig. 4), but that $\omega = 0.15$ (Fig. 3). Indeed, the observation that ω_{max} is higher for zoobenthos and fishes than for zooplankton is probably due, at least in part, to the greater reliance of benthos on allochthonous resources in

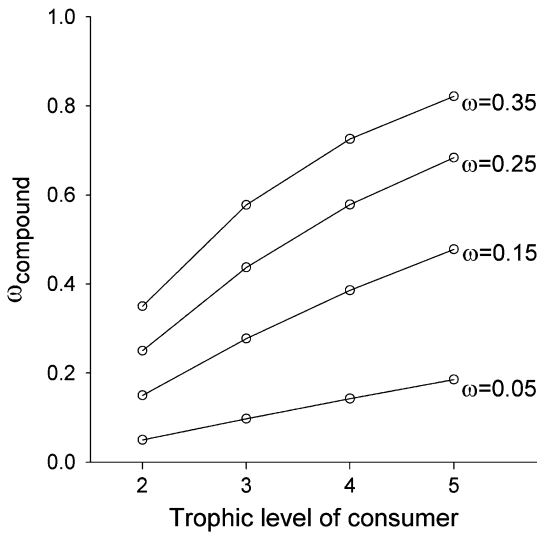

Fig. 5 The total contribution of environmental water to tissue H ($\omega_{compound}$) depends on the value of ω and on the trophic level of the consumer. Assuming a linear food chain where each consumer eats only from the next lowest trophic level and where ω is a constant for all consumers, $\omega_{\text{compound}} = 1-(1-\omega)^{\text{trophic level}}$, as shown here

these lakes. At any rate, our estimates of ω_{max} are rarely >0.50, even for consumers with fairly high trophic positions such as anisopteran odonate larvae and centrarchid fishes (mean $\omega_{max} = 0.50$ and 0.46, respectively; Fig. 4). We can speculate about how large ω could be given those values of ω_{max} if we assume a trophic position for these consumers (say, somewhere between 3 and 5) and compare to Fig. 5. This suggests that the average value of ω in these food chains cannot exceed $\sim$0.25 (and is probably lower).

Accounting for environmental water in mixing models

Our conclusion that ω is appreciable but also uncertain has important implications for δD-based mixing model estimates of allochthony. We explored these implications with simple simulations where we estimated the allochthonous contribution to the biomass of a grazing fish (trophic level = 2) from a desert stream, using data from Doucett et al. (2007). The contribution of terrestrial organic matter to fish biomass can be calculated from a mixing model:

$$\delta D_{fish} = \omega * \delta D_{water} + (1 - \omega) * (t * \delta D_{terrestrial} + (1 - t) * \delta D_{algae}), \quad (5)$$

where ω is the contribution of environmental water to the H in fish tissues, t is the allochthony of the fish (i.e., the proportion of its biomass that is derived from terrestrial organic matter), and the δD terms give the stable H isotope ratio for the various compartments. We calculated 10,000 Monte Carlo estimates of t, each time drawing δD values for each compartment from the normal distributions given by the observed data (Table 2) and drawing ω values from one of three distributions. Specifically, we considered these three scenarios for ω: (1) no contribution of water H to tissue H ($\omega = 0 \pm 0$); (2) a broadly constrained estimate that $\omega = 0.173 \pm 0.122$, as indicated by the data in Table 1; (3) a narrowly constrained estimate for ω with the same mean as indicated in Table 1 but a smaller SD ($\omega = 0.173 \pm 0.050$). In scenarios 2 and 3, randomly generated values of ω that were <0 were set to 0.

The results of these simulations are shown in Table 2. The point estimate of allochthony for scenario 1 is similar to that of Doucett et al. (2007), who also assumed that $\omega = 0$. The SD of the estimate for scenario 1 is larger than that given by Doucett et al. (2007), because the model that we used here considers more sources of uncertainty in calculating t than does the model that they used. As expected, setting the point estimate of ω at 0.170 (scenario 2 and 3) substantially decreases the point estimate for t. But the most interesting result of this analysis lies in the comparison of the SD of t among the three scenarios. While increasing the uncertainty about the true value of ω does contribute to uncertainty about t, this effect is relatively modest given the uncertainties inherent in calculating source contributions to a mixture when the stable isotope ratios of the sources and the mixture are all variable.

Table 2 Simulations demonstrating how uncertainty about ω affects uncertainty in consumer allochthony estimated by a mixing model

Ecosystem component	δD, mean ± SD (n)[a]
Water	−80.5 ± 0.4 (5)
Algae	−264.3 ± 11.5 (10)
Terrestrial organic matter	−151.3 ± 7.3 (5)
Fish	−181.6 ± 5.9 (9)
Scenario	**Proportion allochthony (t), mean ± SD[b]**
(1) $\omega = 0 \pm 0$	0.70 ± 0.23
(2) $\omega = 0.170 \pm 0.121$	0.50 ± 0.29
(3) $\omega = 0.170 \pm 0.050$	0.52 ± 0.27

[a] Observed δD from a desert stream for a grazing fish, stream water, and autochthonous and allochthonous end members (data from Doucett et al. 2007)

[b] Mixing model estimates of allochthony (mean ± SD of 10,000 Monte Carlo simulations incorporating the variability in the data and in the estimate of ω) for three scenarios in which the mean and SD of ω differ

The uncertainty analysis presented here is only an example. The effect of uncertainty in ω on uncertainty in allochthony estimates will vary depending on the variance of the δD of the consumer, the end members, and the water, and on the trophic level of the consumer. Nonetheless, this analysis suggests that useful inferences about allochthony can be made from δD data despite our currently limited understanding of ω. Studies that do use δD data to calculate allochthony estimates should include uncertainty analyses to understand the limitations of their estimates given uncertainty about ω and observed variability in the δD of ecosystem components.

Conclusion

The results presented here provide guidance for applications of δD as a food web tracer, and suggest avenues for further refinement of this tool. Undoubtedly the most important current limitation of the δD approach is the uncertainty that we have highlighted regarding the magnitude of environmental water contributions to tissue H. To make appropriate inferences about resource use from δD data, researchers will have to explicitly address the implications of this uncertainty. Further laboratory experiments and careful observational studies will add significantly to our understanding of environmental water contributions and how they compound across trophic levels. For now, the best available data suggest using a point estimate of $\omega = 0.173$

in mixing model calculations, but carrying the fairly large uncertainty in this estimate (SD = 0.122) through to estimates of the uncertainty in mixing model proportions.

Hydrogen stable isotope ratios can help identify the contribution of allochthonous subsidies to aquatic food webs, and the power of this approach will only increase as estimates of ω are refined. Thus hydrogen stable isotope ratios are likely to become an important tool for estimating allochthonous inputs and for other tracer applications in food webs. One promising approach might be to combine δD data with other stable isotope ratios to provide complementary information and constrain trophic relationships. Such approaches will open the door to new insights about pattern and process in aquatic food webs.

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