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Deuterium as a food source tracer: Sensitivity to environmental water, lipid content, and hydrogen exchange

Grace M. Wilkinson,*[1] Jonathan J. Cole,[2] Michael L. Pace[1]
[1]Department of Environmental Sciences, University of Virginia, Charlottesville, Virginia
[2]Cary Institute of Ecosystem Studies, Millbrook, New York

Abstract

Hydrogen stable isotopes (δ^2H) are used for quantifying resources supporting food webs. However, application of δ^2H in mixing models requires; (1) correction for environmental water (ω) in consumer tissues, (2) consideration of differential fractionation among biochemical constituents, and (3) consideration of differential H-exchange among samples and standards. We present data and sensitivity analyses addressing each of these issues and provide recommendations for future isotope food web studies. First, we determined from field data that maximum ω for aquatic consumers averaged 0.23 ± 0.03, similar to the median ω from a survey of published values (0.22 ± 0.02). Resource use estimates based solely on δ^2H data were sensitive to the selected ω value. Second, to quantify the potential bias in bulk tissue analysis from differential tissue fractionation, we calculated the change in whole organism δ^2H before and after lipid extraction for 61 aquatic samples. The average change in consumers' δ^2H after lipid extraction was a positive shift of 11.8‰ relative to the pre-extraction value. This shift resulted in a minor change in resource use estimates when correcting for lipids. Finally, we evaluated the impact of correcting for H-exchange in samples using standards with dissimilar H-exchange portions. The impact of the correction factor for H-exchange on resource use estimates could be large if suitable standards are not used for comparison. From these analyses we conclude that despite these complicating factors, analysis of resource use is possible using whole organisms' δ^2H, especially in combination with cautionary sensitivity analysis.

Recently, hydrogen isotope ratios (δ^2H) have been used to quantify trophic resources in marine and freshwater consumers (e.g., Bortolotti et al. 2014; Berggren et al. 2014; Hondula et al. 2014). One of the key applications of hydrogen (H) isotopes in aquatic ecosystems has been to assess the degree to which aquatic consumers use organic matter from the surrounding watershed, known as allochthony (e.g., Cole et al. 2011; Karlsson et al. 2012; Kelly et al. 2014). There is a large separation in δ^2H values between allochthonous and autochthonous organic matter (material originating within the system) as well as among some aquatic primary producers such as different species of macroalgae and macrophytes (Hondula et al. 2014). This large separation in end members (organic matter sources) allows for more robust estimates of consumer resource use. However, applying H isotopes to estimate consumer resources requires: (1) correcting for environmental water δ^2H in consumer tissues, (2) evaluating the influence of differential fractionation among biochemical constituents used for sources and end-members due largely to varying lipid content, and (3) properly accounting for H-exchange during laboratory analysis.

*Correspondence: gmw6yy@virginia.edu

Environmental water, sometimes referred to as "dietary water," is the H in a consumer's tissue that is derived directly from water in the surrounding aquatic environment and not from food resources (Hobson et al. 1999; Solomon et al. 2009). Incorporation of environmental water into consumer tissues is useful for applications such as reconstructing animal and human migrations using δ^2H precipitation gradients (Hobson et al. 2004; O'Grady et al. 2012). For studies of aquatic consumer resource use dietary water is problematic. The environmental water correction (ω), formalized by Solomon et al. (2009), varies by consumer and increases with increasing trophic level (Birchall et al. 2005). The influence of environmental water on consumer δ^2H is calculated as a mixing model

$$\delta^2\mathrm{H}_{\mathrm{Consumer}} = (\omega_{\mathrm{tot}} \times \delta^2\mathrm{H}_2\mathrm{O}) + \left((1-\omega_{\mathrm{tot}}) \times \delta^2\mathrm{H}_{\mathrm{Resources}}\right) \quad (1)$$

where ω_{tot} is the trophically compounded value of ω (ω = ω_{tot} for primary consumers), the environmental water correction parameter (bounded between 0 and 1), and $\delta^2\mathrm{H}_{\mathrm{Consumer}}$, $\delta^2\mathrm{H}_2\mathrm{O}$, and $\delta^2\mathrm{H}_{\mathrm{Resources}}$ are the H isotope ratio values of the consumer, water, and end members, respectively (Solomon

et al. 2009). Surface water δ^2H_2O is considerably less negative than organic matter resources (Stiller and Nissenbaum 1980; Kendall and Coplen 2001), particularly in the case of aquatic primary producers (Hondula et al. 2014; Yang et al. 2014). As such, not correcting for the influence of environmental water on consumer tissue δ^2H underestimates consumer use of autochthonous sources. Conversely, over correcting for the influence of environmental water can cause corrected consumer δ^2H to become so negative that the value falls outside the bounds of the end members.

Biosynthesis of different tissue types (e.g., lipids) discriminates against the incorporation of deuterium, the heavier H isotope (Soto et al. 2013). Thus, the bulk value of δ^2H can be influenced by the tissue composition of the consumer in ways that are unrelated to food sources. In particular, lipids are highly depleted in ^2H making them substantially more negative (in del units) than other tissue constituents such as protein (Smith and Epstein 1970; Sessions et al. 1999). This fractionation in lipid synthesis also occurs for carbon isotopes (DeNiro and Epstein 1977; Bodin et al. 2007). In order to correct for this potential bias, lipids can be extracted from the samples before analysis (Folch et al. 1957; Arrington et al. 2006), or in the case of carbon isotopes, a correction can be applied to the whole organism (hereafter bulk) isotope values based on the carbon to nitrogen (C : N) ratio of the sample (Post et al. 2007), a proxy for lipid content. Few studies have evaluated the influence of lipids on bulk consumer δ^2H (Jardine et al. 2009; Soto et al. 2013). Those few that have indicate that lipids can influence bulk tissue δ^2H values for aquatic consumers, however, the magnitude of the influence of lipids on bulk tissue δ^2H has not been thoroughly evaluated and a correction has not been formulated.

The lipid bias is further complicated for bulk δ^2H measurements as the H in lipids is nonexchangeable while some other constituents undergo H-exchange with ambient water vapor (Soto et al. 2013). For example, approximately 20% of the H in proteins exchanges (Chesson et al. 2009). As such, tissue composition (e.g., percent lipid, protein, carbohydrate) influences the overall bulk tissue H-exchange value for the sample and can lead to differential exchange values among sample types. This differential exchange can be accounted for by correcting measured values after laboratory analyses. A common method for accounting for H-exchange is the comparative equilibration method (CEM; Wassenaar and Hobson 2003). CEM analyzes calibrated organic standards along with similar, but unknown samples after allowing exchange of both standards and samples with ambient water vapor to occur. The unknown sample values are then corrected based on the regression between measured vs. expected values for the standard (Doucett et al. 2007; Kelly et al. 2009). The common assumption is that the sample tissue composition is similar enough to the standards in the lab that assuming a single value of H-exchange for the material (based on the standards) is sufficient. If the standard and unknown samples have different H-exchange values, this assumption may lead to erroneous corrections which would in turn impact mixing model calculations of consumer resource use.

The goal of this article is to assess the impact of ω value selection, potential lipid bias, and H-exchange assumptions during analysis on estimates of aquatic consumer resource use. Recent publication of δ^2H data from a variety of aquatic ecosystems and consumers provided a unique opportunity to empirically evaluate the maximum likely value of ω and the impact that ω value selection has on consumer resource use estimates. Additionally, we evaluated the effect of lipids on bulk consumer δ^2H values by measuring both lipid-extracted and bulk samples for numerous aquatic consumers and end members. Finally, we performed a sensitivity analysis of the influence on mixing model estimates of resource use by applying realistic H-exchange fractions to known δ^2H values and then correcting those values for nonexchangeable H based on standards. From these analyses and a review of reported ω values, we synthesized recommendations for future research using δ^2H to estimate consumer resource use.

Materials and Procedures

Literature review of aquatic consumer ω values

We searched published papers to expand on the summary of ω_{tot} values reported in Solomon et al. (2009). Using Web of Science and the key terms hydrogen, deuterium, aquatic, and consumer, we included studies that either directly estimated the environmental water parameter through controlled laboratory experiments or inferred ω_{tot} from field sampling and a well constrained, known diet. Only studies that measured the nonexchangeable H fraction were considered as this is currently the most common analysis that is widely available. Additional information such as the trophic position of the consumer, habitat, tissue type sampled, and the environmental water estimation method were also recorded. If trophic position was not stated explicitly, it was inferred from the information provided in the study. These variables were then used to discern if there were any patterns in ω values among consumers, trophic levels, or study types.

Empirically deriving the maximum value of ω_{tot}

We also searched the literature for data on aquatic consumers, resources, and water δ^2H values (δ^2H_2O) for multiple systems. We corrected the reported raw values of consumer δ^2H for environmental water using the system specific δ^2H_2O and values of ω_{tot} ranging between 0 and 0.5, by increments of 0.01. We used ω_{tot}, the final ω value after trophic compounding was accounted for (Solomon et al. 2009), to make the analysis comparable across consumers. Consumers were characterized by the lowest taxonomic level provided in the study and were analyzed by taxonomic group. The number of corrected consumer δ^2H values that fell outside the bounds of the study-specific end members after correcting

Table 1. δ^2H isotope mean values and standard deviations (s.d.) for the three sensitivity analyses. *Daphnia* were assumed to be primary consumers for this analysis.

Parameter	Notation	Value (‰)	s.d. (‰)
Algae	δ^2H_A	−220	17.0
Terrestrial	δ^2H_T	−120	15.2
Daphnia A	δ^2H_{Cons}	−140	0.5
Daphnia B	δ^2H_{Cons}	−160	0.5
Daphnia C	δ^2H_{Cons}	−180	0.5
Daphnia D	δ^2H_{Cons}	−200	0.5
Environmental water correction	*(a)* ω	0.22*	2.0
Lake water	δ^2H_2O	−45	1.0

*Unless otherwise noted for the analysis

for each ω_{tot} value between 0 and 0.5 was tabulated and used to assess the empirical maximum of ω_{tot}. The most autochthonous consumers were the most likely to be pushed outside the bounds of the mixing model, thereby minimizing any confusion between terrestrial and water isotopic values in highly allochthonous consumers.

Impact of ω on estimates of consumer resource use

We evaluated the impact of the environmental water parameter on estimates of consumer resource use using two different datasets. First, we examined the impact of environmental water value selection on estimates of *Daphnia* allochthony from a δ^2H mixing model. As the δ^2H of environmental water (δ^2H_2O) is more similar to the δ^2H of allochthonous material than autochthonous material, the dietary water correction can potentially have a large impact on the estimate of consumer allochthonous resource use. Using the following two-source, δ^2H Bayesian mixing model

$$\delta^2H_{Cons} = \left((\phi_T \times \delta^2H_T) + (\phi_A \times \delta^2H_A)\right) \times (1-\omega) + \omega \times \delta^2H_2O$$
$$1 = \phi_T + \phi_A \quad (2)$$

described in Wilkinson et al. (2013), where δ^2H_T and δ^2H_A are the isotope end members for terrestrial and aquatic organic matter, respectively, and δ^2H_{Cons} is the isotope value of the aquatic consumer. We used three different ω values (0.1, 0.2, and 0.3) in the model and estimated allochthonous resource use. The isotope data used in the model are presented in Table 1.

Second, we evaluated the impact of environmental water value selection on macroalgal resource use by cultured hard clams (*Mercenaria mercenaria*) in a Virginian coastal bay using a multi-isotope mixing model with three potential clam resources. Specifically, we used the δ^2H, δ^{13}C, and δ^{15}N data from Hondula and Pace (2014). The potential resources for clams included in the model were macrophytes (MP; *Zostera marina* and *Spartina alterniflora*), macroalgae (MA; *Codium fragile*, *Gracillaria vermicuphylla*, *Agardhiella subulata*, and *Ulva letuca*), and microalgae (MI; phytoplankton and benthic algae) based on the analysis in Hondula and Pace (2014). Distributions for the clams and end member data comprised all available data from all sampling periods. Using the following 3-source, 3-isotope Bayesian mixing model

$$\delta^2H_{Clam} = \left((\phi_{MP} \times \delta^2H_{MP}) + (\phi_{MA} \times \delta^2H_{MA}) + (\phi_{MI} \times \delta^2H_{MI})\right) \times (1-\omega) + \omega \times \delta^2H_2O$$
$$\delta^{13}C_{Clam} = (\phi_{MP} \times \delta^{13}C_{MP}) + (\phi_{MA} \times \delta^{13}C_{MA}) + (\phi_{MI} \times \delta^{13}C_{MI}) + \Delta_C$$
$$\delta^{15}N_{Clam} = (\phi_{MP} \times \delta^{15}N_{MP}) + (\phi_{MA} \times \delta^{15}N_{MA}) + (\phi_{MI} \times \delta^{15}N_{MI}) + \Delta_N$$
$$1 = \phi_{MP} + \phi_{MA} + \phi_{MI} \quad (3)$$

in which Δ_C and Δ_N are the trophic enrichment values (1.05‰ ± 0.75‰ and 3.42‰ ± 0.83‰, respectively), ω was varied between 0.1 and 0.3 and the resulting source portions (φ_{MP}, φ_{MA}, and φ_{MI}) were recorded. Additionally, the same analysis was performed using only the δ^2H and δ^{15}N data and the δ^2H and δ^{13}C data in order to determine if the removal of an isotope tracer would increase the influence of environmental water value selection. The model was also run with just the δ^{13}C and δ^{15}N data to determine if the δ^2H, and therefore, potentially ω value, were substantially influential on the resource use posterior distributions.

The *Daphnia* and clam mixing models were written in R (R Core Development Team) using JAGS (Just Another Gibbs Sampler). The distributions of the source portions (φ_A and φ_T or φ_{MP}, φ_{MA}, and φ_{MI}) were center log transformed as these distributions were bounded between zero and one (Semmens et al. 2009). The median value of *Daphnia* percent allochthony (φ_T) and clam percent macroalgal use (φ_{MA}) are reported and discussed throughout, however, the quantiles of the full posterior distributions are provided in the figures. Changes in the median values between model runs are reported as changes in percentage points (i.e., absolute changes in the median values of resource use).

Change in δ^2H value from lipid extraction of bulk tissues

During the ice-free season, three consumer groups (zoobenthos, zooplankton, and fishes) and two end members (periphyton and terrestrial vegetation) were collected from four lakes on the University of Notre Dame's Environmental Research Center's property. Zooplankton were collected at night via oblique tows of a conical net (80 μm for zooplankton, 153 μm for *Chaoborus*) and separated by taxa under a dissecting microscope. Zoobenthos were collected using an Eckman dredge and separated from the sediments. A sample of dorsal muscle tissue was excised from fishes collected via minnow trapping, electrofishing, and angling. Periphyton was scraped from tiles suspended above the sediments of the lake in the littoral zone and terrestrial leaf samples were collected from trees within the watershed.

Table 2. Literature review of dietary water (ω) values ("muscle" = protein, "bulk" = whole organism). Assumed isotope diet composition is inferred for studies where the diet was not directly observed yet reasonably well known, whereas known isotope diet composition is from lab reared animals fed a known diet.

Reference	Consumer	Aquatic habitat	Aquatic zone	ω	s.d.	Trophic position	Tissue type	Diet
Estep and Dabrowski (1980)	Snail	Marine	Benthic	0.00	NA	2	Muscle	Assumed
Macko et al. (1983)	Amphipod	Marine	Pelagic	0.12	NA	2	Bulk	Known
Hondula and Pace (2014)	Clam	Marine	Benthic	0.15	0.09	2	Muscle	Known
Malej et al. (1993)	Jellyfish	Marine	Pelagic	0.34	0.12	3	Bulk	Assumed
Finlay et al. (2010)	Shredder insects	Freshwater	Benthic	0.12	0.12	2	Bulk	Assumed
Finlay et al. (2010)	Scraper insects	Freshwater	Benthic	0.06	0.06	2	Bulk	Assumed
Solomon et al. (2009)	Mosquito	Freshwater	Pelagic	0.39	0.04	2	Bulk	Known
Solomon et al. (2009)	Zooplankton	Freshwater	Pelagic	0.20	0.04	2	Bulk	Known
Wilkinson et al. (2013)*	Zooplankton	Freshwater	Pelagic	0.29	0.04	2	Bulk	Assumed
Berggren et al. (2014)**	Cyclopoids	Freshwater	Pelagic	0.16	0.03	3	Bulk	Assumed
Berggren et al. (2014)**	Calanoids	Freshwater	Pelagic	0.16	0.05	3	Bulk	Assumed
Berggren et al. (2014)**	Cladocerans	Freshwater	Pelagic	0.29	NA	2	Bulk	Assumed
Wang et al. (2009)	Chironomids	Freshwater	Benthic	0.31	0.03	2	Bulk	Known
Bortolotti et al. (2013)	Snails	Freshwater	Benthic	0.21	0.03	2	Bulk	Assumed
Nielson and Bowen (2010)	Brine shrimp	Saline lake	Pelagic	0.38	NA	2	Chitin	Known
Babler et al. (2011)	Gizzard Shad	Freshwater	Benthic	0.08	0.04	2	Muscle	Assumed
Solomon et al. (2009)	*Chaoborus*	Freshwater	Pelagic	0.14	0.06	3	Bulk	Assumed
Wilkinson et al. (2013)	*Chaoborus*	Freshwater	Pelagic	0.13	0.03	3	Bulk	Assumed
Jardine et al. (2009)	Water Strider	Freshwater	Neustic	0.25	NA	3	Bulk	Known
Solomon et al. (2009)	Fish	Freshwater	Pelagic	0.12	0.02	3	Muscle	Assumed
Jardine et al. (2009)	Trout	Freshwater	Pelagic	0.30	NA	3	Muscle	Known
Graham et al. (2014)	Atlantic Salmon	Mixed	Pelagic	0.36	0.05	3	Muscle	Known
Graham et al. (2014)	Arctic Char	Mixed	Pelagic	0.35	0.05	3	Muscle	Known
Solomon et al. (2009)	Trout	Freshwater	Pelagic	0.23	0.03	3–4	Muscle	Known

*Calculated from the difference in zooplankton and phytoplankton δ^2H from the three systems with the most autochthonous consumers
**Calculated from zooplankton samples that were assumed to be autochthonous based on a δ^{13}C mixing model

Consumers and organic matter samples were also collected from nine sampling sites along the Hudson River estuary. Organic matter samples included cyanobacteria, filamentous algae, various submerged and floating aquatic vegetation, and terrestrial leaf material. Dorsal muscle tissue was collected from 10 fish species. Zoobenthos from the soft sediments were sampled using a Ponar sampler at midchannel. Zebra mussels (*Dreissena polymorpha*) were collected midchannel at depths of 0.5–4 m. All of the materials for isotope analysis were dried at 60°C and ground to a fine powder.

Individual consumer samples from the Hudson River and the lakes were divided and half of the sample underwent lipid extraction and the other portion remained unchanged. Lipids were extracted with a 50 : 50 methanol–chloroform solution following the procedure of Folch et al. (1957). Isotope values were measured at the Colorado Plateau Stable Isotope Laboratory (CPSIL) using the benchtop equilibration method for exchangeable H described in Doucett et al. (2007). CPSIL uses a number of standards, one of which is *Cladophora* spp., a chlorophyte alga. All values are expressed in relation to the international standard of Vienna Standard Mean Oceanic Water (VSMOW). The change in δ^2H between lipid extracted and bulk samples ($\Delta\delta^2$H) is reported here using the per mil (‰) notation and as a percent of the pre-extraction bulk value. We compared $\Delta\delta^2$H to consumer C : N values as an indicator of consumer lipid content where a higher C : N implies greater relative lipid composition.

Impact of lipid correction on estimates of consumer resource use

Average lipid correction values ($\Delta\delta^2$H) calculated from the samples described above were applied to end member and *Daphnia* δ^2H values (Table 1) in a two-source (terrestrial material and algae) Bayesian mixing model

$$\delta^2\mathrm{H}_{\mathrm{Cons}} = \Big(((\phi_{\mathrm{T}} \times (\delta^2\mathrm{H}_{\mathrm{T}} + \Delta\delta^2\mathrm{H}_{\mathrm{T}})) + (\phi_{\mathrm{A}} \times (\delta^2\mathrm{H}_{\mathrm{A}} + \Delta\delta^2\mathrm{H}_{\mathrm{A}})) + \Delta\delta^2\mathrm{H}_{\mathrm{Cons}})\Big) \times (1-\omega) + (\delta^2\mathrm{H}_2\mathrm{O} \times \omega)$$
$$1 = \phi_{\mathrm{T}} + \phi_{\mathrm{A}} \quad (4)$$

where the sample specific corrections (subscripts the same as Eq. 2) are applied before the environmental water correction (ω_{tot} = 0.2 for this analysis). The model was run with sample-specific lipid corrections and without lipid corrections ($\Delta\delta^2H = 0$) to compute the difference in the terrestrial fraction (φ_T) between the two model runs.

Estimating realistic H-exchange values

An H-exchange value for *Daphnia pulicaria* of 0.074 was calculated based on reported tissue composition (Ventura and Catalan 2005) and the H-exchange values for those tissue types (Culebras and Moore 1977; Chesson et al. 2009). The likely H-exchange value for algae of 0.20 was calculated based on the average tissue composition (Becker 1994) of 17 algal species and the H-exchange values for those tissue types. The likely H-exchange for terrestrial material was assumed to be 0.23 which is the H-exchange value for cellulose (Chesson et al. 2009).

For the H-exchange sensitivity analysis, the isotope data in Table 1 was considered the true but unmeasured δ^2H values of the consumer and end members (δ^2H_{True}). The realistic H-exchange values, reported above, were used in this simple linear mixing model

$$\delta^2H_{Measured} = (\phi_{Exchangeable} \times \delta^2H_{Vapor}) + (\phi_{Nonexchangeable} \times \delta^2H_{True}) \quad (5)$$

to calculate what the measured δ^2H ($\delta^2H_{Measured}$) values of the samples would be if ambient water vapor (δ^2H_{Vapor}) was $-90‰$ (the approximate value at CPSIL). A keratin standard with an H-exchange value of 0.16 (Chesson et al. 2009) was used to correct ($\delta^2H_{Corrected}$) the measured values ($\delta^2H_{Measured}$) using the following equation

$$\delta^2H_{Corrected} = 1.19 \times \delta^2H_{Measured} + 17.143 \quad (6)$$

the coefficients of which have been empirically determined from a regression analysis of measured δ^2H of the keratin standard exchanging with the same water vapor (δ^2H_{vapor}) as the samples (independent variable) vs. the expected δ^2H of the nonexchangeable portion of the keratin standard based on an assumed del value. The $\delta^2H_{Corrected}$ values and the true δ^2H values were then used in the two-source δ^2H mixing model (Eq. 2) and the posterior distributions of φ_T were compared.

Assessment

Values of ω from literature and field data

In total, we found or calculated ω_{tot} values for 24 consumers from both freshwater and marine habitats (Table 2). There is not a significant difference in mean ω_{tot} values among trophic positions, between habitats, between muscle and bulk tissues, between aquatic habitats, or between assumed and known diet. Excluding the snail which has a reported ω of 0.0, ω_{tot} ranged from 0.06 to 0.39. The mean and standard deviation are 0.22 and 0.02, respectively (Fig. 1).

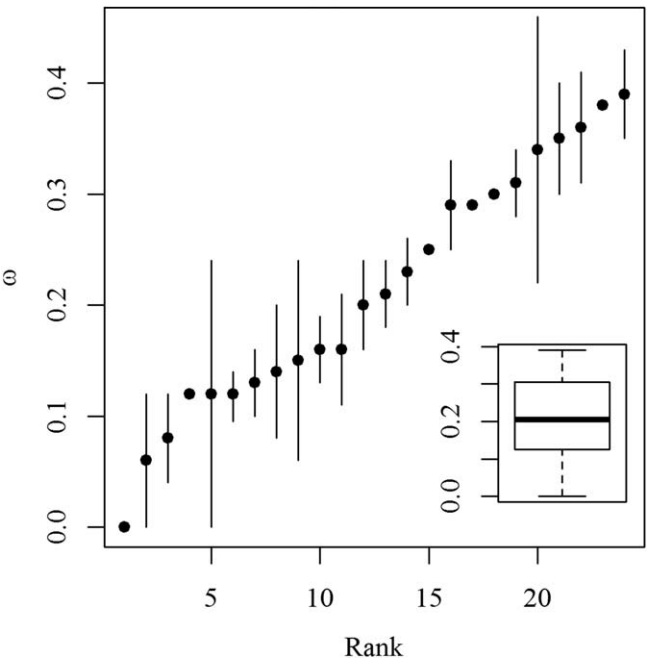

Fig. 1. Rank plot of ω values presented in Table 2. The error bars for each point are standard deviation, when reported. The inset is the distribution of the mean ω values reported in the literature.

For the field data of aquatic consumer δ^2H, all consumers remained within the bounds of the study and system specific mean end members at an ω_{tot} value of 0.15 and below (Fig. 2). All consumers across all studies fell outside the bounds of the end members when ω_{tot} was 0.50. The consumers in some groups such as calanoid copepods (Figs. 2A,G) and *Mesocyclops* (Fig. 2C) fell outside the bounds of the end members when ω_{tot} was below 0.50. Using the criteria that 90% of the consumers must fall within the end members, the empirical maximum for ω_{tot} is 0.23 ± 0.03 averaged across all consumers and studies in Figure 2.

While no clear pattern emerged from the ω_{tot} values and auxiliary data collected from the literature, there does appear to be an upper threshold for bulk tissue ω_{tot} of approximately 0.40 for aquatic consumers. The analysis of zooplankton consumer δ^2H corroborated this, indicating that ω_{tot} could not exceed 0.50 as all consumers fell outside the bounds of the end members when ω_{tot} was this high. Together, these results suggest an upper limit for bulk tissue ω_{tot} of 0.40–0.50 which is in agreement with the maximum value of approximately 0.40 put forth by Solomon et al. (2009). Using more stringent criteria that 90% of consumers remain within the bounds of the end members, we determined that the maximum ω_{tot} value for consumers using the δ^2H field data was 0.23, which is very similar to the median of the literature values (0.22). Although the field data are

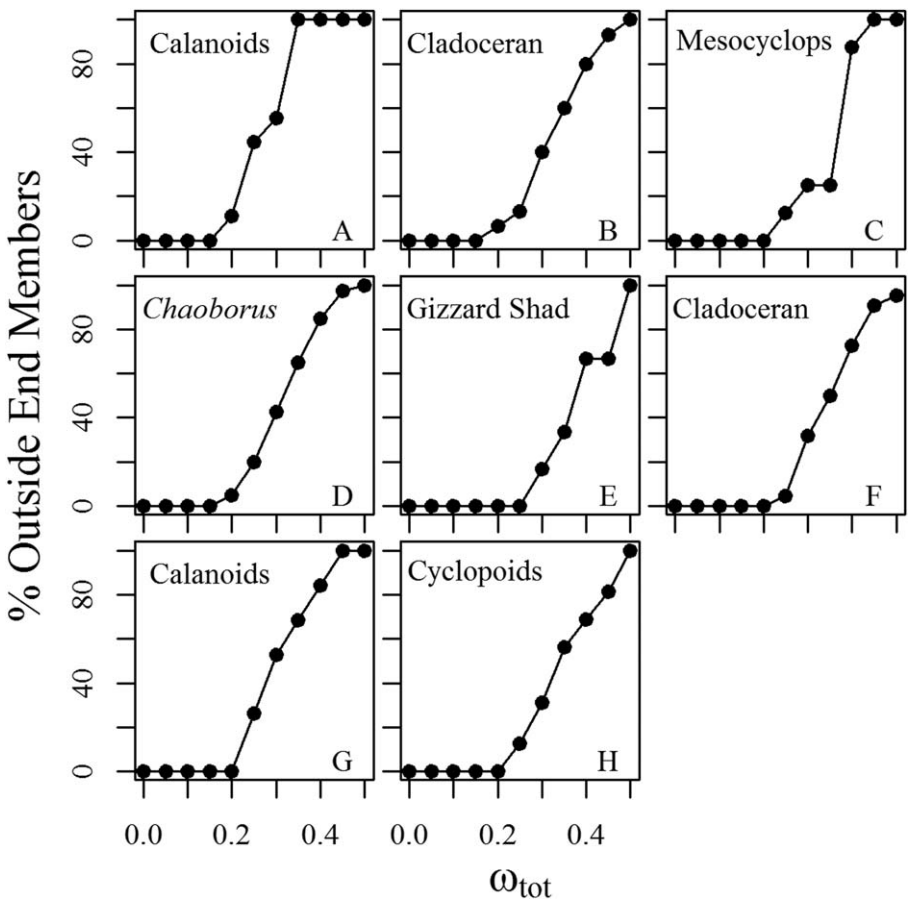

Fig. 2. The percent of consumers that fall outside the bounds of the end members after correcting for dietary water (ω_{tot}) using different values (increments of 0.05 shown though analysis was more refined). (A–D) Zooplankton from Wilkinson et al. (2013), E) fish from Babler et al. (2011), F–H) zooplankton from Berggren et al. (2014).

limited to freshwater consumers, they encompass more than 60 temperate ecosystems and are surprisingly consistent between studies. The literature median and survey-derived maximum are also very similar to previous compilations (Solomon et al. 2009) and modeled ω values (Cole and Solomon 2012). Considering the multiple lines of evidence, a reasonable assumed ω value is approximately 0.20 for aquatic consumers in the absence of other data.

Influence of ω value selection on resource use estimates

The *Daphnia* data from Table 1 was used to assess the impact of ω on estimates of consumer allochthonous resource use (φ_T) in a δ^2H-based model. The median of *Daphnia* φ_T ranged from 7% to 87% allochthonous when ω was 0.10 (Fig. 3A). In general, as ω increased, *Daphnia* φ_T decreased. There was a decelerating non-linear decrease in the median φ_T with increasing ω. The range in median φ_T also decreased at higher ω. For example, the range in median φ_T for ω of 0.30 was only 7–69% allochthonous (Fig. 3C).

The data on cultured hard clams from Hondula and Pace (2014) was used to assess the impact of ω on estimates of consumer resource use in a multi-isotope modeling approach with more than two potential resources. In all three versions of the model analysis that included δ^2H, the median values of clam resource use (φ_{MP}, φ_{MA}, and φ_{MI}) did not change by more than two percentage points for each value of ω_{tot} evaluated (Fig. 3D–F). Instead, the resource use posterior distributions were much more sensitive to the isotopes used in the model. The model runs using δ^2H + δ^{15}N, δ^{15}N + δ^{13}C, and δ^2H + δ^{13}C + δ^{15}N all had median φ_{MA} values between 63 and 69%. The exclusion of δ^{15}N in the δ^2H + δ^{13}C model run yielded substantially different results (data not shown), with a median φ_{MA} of 31%. However, even in the δ^2H + δ^{13}C analysis, changing ω between 0.1 and 0.3 did not change the posterior distribution of φ_{MA}. From these analyses, we conclude that there can be a substantial influence of ω value selection on resource use estimates when δ^2H is the only tracer in the mixing model. With the addition of another isotope tracer and another resource, the influence of ω value selection was greatly diminished. It is difficult to tease apart the confounding effect of the addition of isotope tracers and additional resources in the model as the

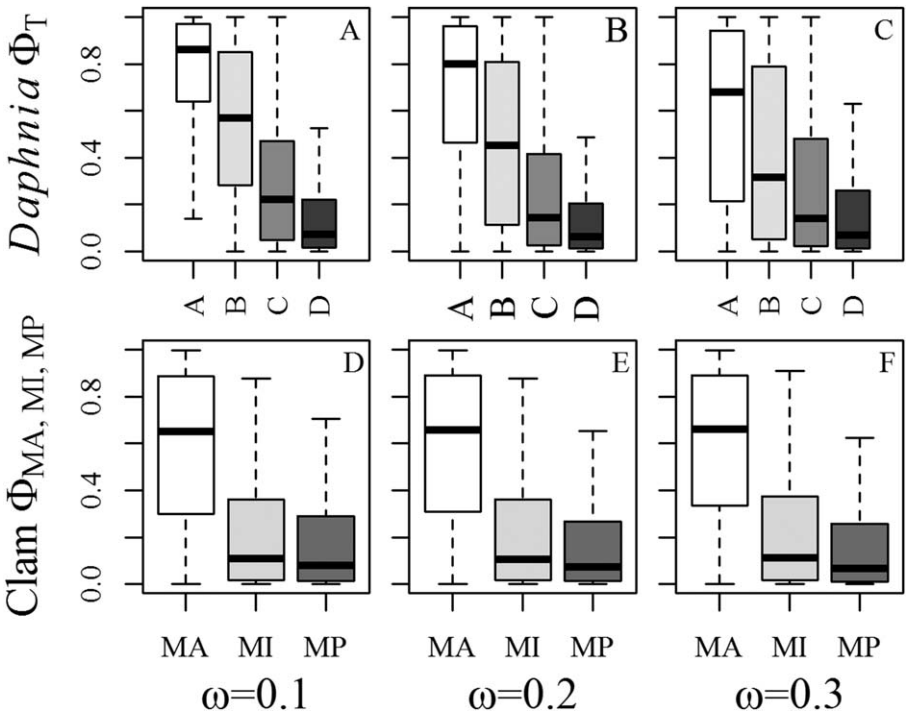

Fig. 3. The sensitivity of resource use estimates (φ_T or φ_{MA}, φ_{MI}, φ_{MP}) for *Daphnia* and clams (*Mercenaria mercenaria*) assuming different values of ω in the analysis. (A–C) The posterior distributions of φ_T for the four *Daphnia* in Table 1 using different values of ω in a Bayesian mixing model (Eq. 2), and (D–F) The posterior distributions of φ_{MA}, φ_{MI} and φ_{MP} for clams for the three-source, three-isotope mixing model (Eq. 3).

inclusion of either necessarily further constrains the influence of environmental water in the mixing model compared to the one-isotope, two-resource model.

Influence of lipid correction on resource use estimates

In total, 61 samples of lipid extracted consumer and end member tissue were analyzed (Table 3). The values reported are the shift, in per mil units (‰), from the bulk sample. A positive shift is a less negative number; that is the lipid-free sample is less negative than the bulk sample. The range in the difference in δ^2H before and after extraction ($\Delta\delta^2$H) was from −0.82‰ (algae) to 18.43‰ (zooplankton) and the average $\Delta\delta^2$H across all samples was 11.8‰ (Table 3). Only the algal samples were more negative than the bulk samples after extraction. All other consumer and end member samples were less negative after extraction consistent with expectation relative to the effect of removing lipids. Variation among groups was significant (α = 0.05) in $\Delta\delta^2$H (ANOVA, $F_{6,54}$ = 2.42, p-value = 0.03). A post hoc Tukey test revealed that algae and zooplankton $\Delta\delta^2$H were significantly different. There were no significant relationships between consumer C : N and $\Delta\delta^2$H or bulk δ^2H when all consumers were pooled or for individual consumer groups and end members.

Table 3. The change in δ^2H after lipid extraction ($\Delta\delta^2$H = lipid free δ^2H − bulk tissue δ^2H) and C : N values for three groups of consumers and four groups of end members collected from lakes in Upper Peninsula of Michigan and the Hudson River estuary. SAV and FLAV stand for submerged aquatic vegetation and floating leaf aquatic vegetation, respectively.

Sample type	Tissue type	*n*	Mean $\Delta\delta^2$H (‰)	s.d. $\Delta\delta^2$H (‰)	Mean C : N	s.d. C : N	$\Delta\delta^2$H as a% of bulk δ^2H
Algae	Bulk	5	−0.82	14.05	7.50	1.54	0.3
Periphyton	Bulk	4	+8.29	3.37	14.04	6.19	9.3
SAV and FLAV	Bulk/leaf	4	+9.21	5.52	13.66	1.68	3.5
Terrestrial vegetation	Leaf	8	+13.39	11.98	na	na	7.0
Fish	Muscle	19	+13.43	5.39	3.80	0.22	10.8
Zoobenthos	Bulk/muscle	13	+11.20	10.30	5.22	0.76	8.3
Zooplankton	Bulk	8	+18.43	9.15	5.84	0.87	13.0

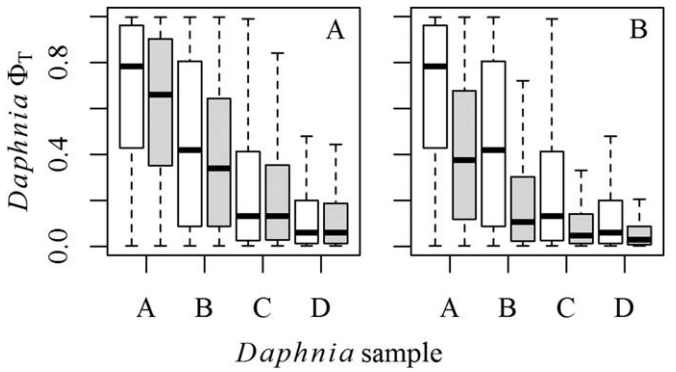

Fig. 4. The posterior distributions of φ_T for four *Daphnia* samples (a) with (gray) and without (white) δ^2H lipid correction and (b) with the true (white) and H-exchange corrected (gray) δ^2H values.

The lipid correction values for zooplankton, algae, and terrestrial material were applied in Eq. 4 to the *Daphnia* samples in Table 1 to determine the influence of lipid correction on estimates of consumer resource use. In this case, the median terrestrial resource use (φ_T) decreased between bulk and lipid corrected estimates (Fig. 4A). For all *Daphnia* samples, applying the lipid correction decreased the median estimate of φ_T by 0 to 14 percentage points with the largest difference in the most allochthonous *Daphnia* (*Daphnia* A) and the smallest difference in the most autochthonous *Daphnia* (*Daphnia* D). The small shifts in δ^2H after lipid extraction for the consumer group with the largest $\Delta\delta^2$H had a minimal impact on mixing model results indicating that lipids do not have a substantial influence aquatic δ^2H values.

Unlike the predictable relationship between lipids and carbon isotope values (Post et al. 2007), there was no pattern in the difference in bulk and lipid extracted values ($\Delta\delta^2$H) and the C : N ratio of the tissue. The lack of a relationship between these two variables in this dataset could be due, in part, to the difference in exchangeable H content of bulk and lipid-free samples and the standard materials. As there is no relationship between $\Delta\delta^2$H and tissue C : N, and lipid content was not measured, a mathematical correction similar McConnaughey and McRoy (1979) for δ^{13}C cannot be formulated for δ^2H at this time.

Influence of H-exchange on resource use estimates

The bias in the estimate of the terrestrial fraction of *Daphnia* due to correcting samples with a nonlike standard ranged from −9 percentage points to +44 percentage points (Fig. 4B). The bias increased as *Daphnia* were less terrestrial and became more algal. While this was a simple exercise with only one set of estimated H-exchange values used and many assumptions, it does illustrate the consequence of using standards during analysis that are not similar to the unknown samples. Development of more specific standards would improve the accuracy of mixing model calculations. However, this exercise demonstrates that previous δ^2H mixing model results with values corrected using the CEM method are not invalid even though more appropriate standards could be developed.

Discussion

From the environmental water sensitivity analyses, it is clear that the selection of ω impacts the magnitude of resource use estimates. For the one-isotope, δ^2H *Daphnia* allochthony model, the largest changes in allochthony estimates for consumers occurred between the lowest values of ω. The approximately 40% decrease in consumer allochthony estimates observed in this study by increasing ω_{tot} from 0.10 to 0.20 was equivalent in magnitude to the decrease in allochthony observed by Solomon et al. (2009) for fish, Babler et al. (2011) for gizzard shad, and Berggren et al. (2014) for zooplankton using similar increasing intervals of ω_{tot}. Interestingly, there was not a substantial change in the variability of zooplankton allochthony with an increase in ω_{tot} from 0.10 to 0.20. However, at very high values of ω_{tot}, the consumers that fell outside the bounds of the end members were forced to 0% allochthony by the model specifications. This explains the pattern of decreasing change in consumer allochthony with increasing values of ω_{tot}.

For the two- and three-isotope clam resource use models, changing ω had very little impact on the final source portion distributions. The medians of the source portion distributions changed by no more that 1–2 percentage points over a wide range of ω values and the width of the distributions remained constant. The clam sensitivity analysis is an example of the diminishing influence of ω value selection with more isotope data and/or another resource further constraining the environmental water parameter. Although all consumer resource use models are not likely to be as insensitive to the ω value selection as the hard clam example evaluated here, additional information in the model in the form of multiple tracers or another resource is likely to dampen the influence of ω on the model outcome compared to models based solely on δ^2H (such as the *Daphnia* example).

As demonstrated by the zooplankton ω sensitivity analysis, the selected ω value can have a large influence on the resultant allochthony estimate. Many previous studies of zooplankton allochthony have used lower ω values (in the range of 0.13–0.17) than the literature mean reported in this study (Babler et al. 2011; Cole et al. 2011; Wilkinson et al. 2013; Berggren et al. 2014). Even though the lower environmental water estimate in these previous studies leads to a higher consumer allochthony estimate, the difference is generally not substantial. A change in ω from 0.15 to 0.22 for primary consumers results in a change in the allochthony estimate of approximately 10%. In other words, a consumer estimated to be 40% allochthonous when ω = 0.15 would decrease to ~30% allochthonous when ω = 0.22. While an ecological interpretation of a 10% decrease is context

dependent (i.e., it may be perceived as a much larger change if the original estimate was 20% allochthony vs. 80% allochthony), it is not a substantial decrease in many cases. Additionally, while increasing ω decreases consumer allochthony estimates, the patterns in consumer allochthony observed among systems in many studies (Babler et al. 2011; Wilkinson et al. 2013; Berggren et al. 2014) would remain unchanged.

The exclusion of lipids in bulk hydrogen isotope samples complicates the estimate of consumer resource use when the change in δ^2H after lipid extraction differs between the consumer and the end members. If all of the end member and consumer δ^2H values shifted equally after lipid extraction, the distance between the end members and consumers would not change, resulting in a similar resource use estimate as using bulk δ^2H values. Our data indicate that the shift in δ^2H after lipid extraction differs between end members and some consumer groups, for example, zooplankton and algae. Therefore, not correcting for lipids (via lipid extraction) may result in a biased estimate of consumer resource use, although this bias is likely to be smaller than not properly correcting for environmental water or properly defining the end members (Yang et al. 2014). The values that we obtained for the influence of lipids on consumer δ^2H are also substantially lower than values calculated from controlled lab feeding experiments (Jardine et al. 2009) possibly because organisms in natural environments that could be experiencing periods of starvation or low resource concentrations tend to have lower lipid content relative to well fed lab-reared organisms (Hirche and Kattner 1993).

Bulk tissues are the most commonly used in tracer analyses measuring food web resource partitioning. Researchers are, in general, interested in the food resources that support an entire consumer rather than some of its tissue. Further, for many small invertebrates (e.g., *Daphnia*) whole organism analysis is the only practical way to measure isotope ratios. In the case of estimating consumer allochthony, the increase in consumer δ^2H after lipid extraction indicates that not correcting for the influence of lipids will lead to an underestimation of allochthonous resource use. However, correcting for the influence of lipids had a minimal impact on the mixing model results. When there is a large difference in the isotope values of the potential food web resources, the assumption that bulk tissue is adequate for the tracer analysis is valid.

A correction for H-exchange based on nonsimilar standards can have a large impact on final δ^2H values of consumers and end members used in mixing models. The goal of the exercise presented here is to demonstrate the potential impact of H-exchange; however, the analysis is not intended to serve as a correction but simply as an illustration that H-exchange fractions could feasibly differ from the standard material used in the analysis. If CEM remains popular and widely available, more suitable standards may need to be developed and evaluated. An alternative to CEM is the steam equilibration method which allows steam of known isotopic composition to exchange completely with exchangeable hydrogen in the sample (Meier-Augenstein et al. 2013). This technique still requires standards with a similar organic matrix and exchangeable fraction as the sample but it eliminates other problems associated with the CEM method such as unaccountable H-exchange due to water adsorption. Steam equilibration is currently not a technique widely used for aquatic samples of the nature described here, though it may become more available in the future.

Recommendations

When possible, a species and system specific measurement of ω for the consumer of interest is desirable. As environmental conditions, consumer, and prey species could influence the value of ω, estimates derived from controlled laboratory feeding experiments (Jardine et al. 2009; Wang et al. 2009), field measurements from aquaculture operations (Solomon et al. 2009; Hondula and Pace 2014) or well constrained estimates of diet composition in the field (Finlay et al. 2010; Wilkinson et al. 2013) are preferable. If a direct estimate is not feasible, a value from the literature or the mean of ω values for a similar habitat, zone, or feeding strategy of the consumers reported in Table 2 could be used. When using a literature value to correct consumer δ^2H for environmental water, a sensitivity analysis similar to the one presented in Figure 3 should be performed to evaluate the impact of the ω value selection on the final estimate of consumer resource use. Additionally, if a Bayesian mixing model is used to estimate consumer resource use, uncertainty in the ω value can be estimated in the model as well (Solomon et al. 2011).

Finally, further research is needed to define a more universal lipid correction procedure for hydrogen isotope measurements. However, if the consumer and end members of interest are similar to those listed in Table 3, the $\Delta\delta^2$H values could be applied in a sensitivity analysis to explore the influence of lipids on the final estimate of consumer resource use. Finally, the simple example chosen to illustrate the potentially large influence of standard material on H-exchange corrections demonstrates the need for standards to be further evaluated for their suitability for the types of investigations evaluated here.

Many stable isotope studies of resource use are motivated by questions where a relative answer is sufficient. For example, are aquatic consumers significantly (say $>$ 10%) supported by terrestrial materials? In these ecological studies, application of hydrogen and other stable isotopes can provide a sufficient answer as indicated by the examples in this article despite complicating factors related to various types of fractionations and physiological processes (e.g., water use). We conclude that important ecological insights are obtainable using whole organisms' isotope values and

mixing model methods especially when buttressed with cautionary sensitivity analysis.

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