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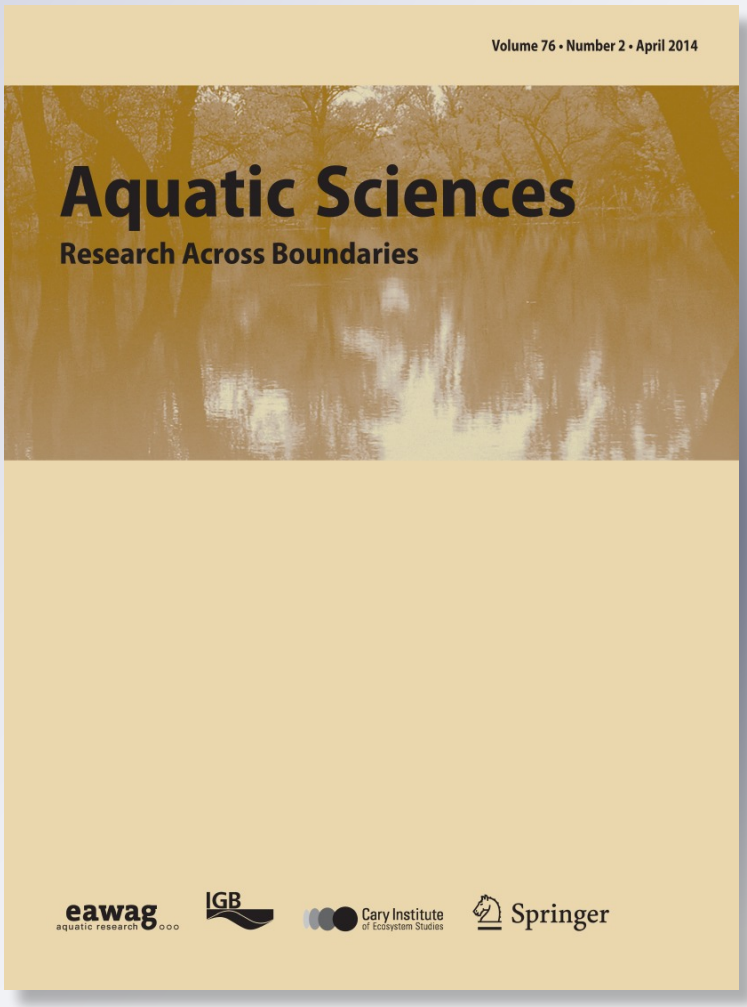

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RESEARCH ARTICLE

Hydrogen isotope discrimination in aquatic primary producers: implications for aquatic food web studies

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Abstract Large differences in δ^2H of primary producers between aquatic and terrestrial ecosystems are used to identify subsidies, discriminate organic matter sources, and reduce uncertainty in food web studies. Previous investigations of hydrogen isotope ratios suggest there may be predictable differences between the δ^2H of water and organic matter for different types of primary producers. We define the difference in the net isotopic discrimination between water and bulk organic matter (om) as: $\Delta_H = (\delta^2H_{om} - \delta^2H_{water}) \div (1 + \delta^2H_{water} \div 1{,}000)$. We summarized Δ_H values from published literature and we measured the δ^2H of water and primary producers in order to compare Δ_H among aquatic and terrestrial primary producers. Measurements were made from three water body types (lake, river, coastal lagoon) and their associated watersheds. Although we predicted a large and equivalent net isotopic discrimination for aquatic primary producers, we found considerable variability among groups of aquatic producers. Macroalgae, benthic microalgae, and phytoplankton had more negative Δ_H values (i.e. greater isotopic discrimination) than both aquatic macrophytes and terrestrial vegetation. The more positive δ^2H_{om} and hence lower Δ_H of terrestrial vegetation was expected due to relative increases in the heavier isotope, deuterium, during transpiration. However, the more positive values of δ^2H_{om} and relatively low Δ_H in aquatic macrophytes, even submerged species, was unexpected. Marine macroalgae had high variability in δ^2H_{om} as a group, but low variability within distinct species. Variability among types of primary producers in δ^2H_{om} and in Δ_H should be assessed when hydrogen is used in isotopic studies of food webs.

Keywords Deuterium · Macrophytes · Macroalgae · Hydrogen isotopes · Food webs · Lakes · Rivers · Coastal zone

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Introduction

Stable isotopes are powerful for analyzing trophic interactions in aquatic ecosystems. Differences in stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are frequently used to elucidate the diet of aquatic organisms and to describe food web structure (Peterson and Fry 1987). However, isotope analyses can produce ambiguous results when variability within food sources is large relative to differences between sources (Phillips and Gregg 2001; Fry 2006). Ratios of the stable isotopes of hydrogen relative to a standard (δ^2H) are a potentially powerful complement to other isotopes because the mass difference between protium and deuterium results in large differences in δ^2H (>100 ‰) between some sources (Doucett et al. 2007). However, the hydrogen isotope ratios of the bulk organic matter (δ^2H_{om}) among various aquatic primary producers including vascular plants and macro- and micro-algae has not been systematically evaluated to determine how variability in these producers might affect the use of hydrogen isotopes as food web tracers.

Hydrogen incorporated into the organic matter of aquatic photosynthetic autotrophs ultimately comes from the surrounding water. Based on published laboratory and field measurements, aquatic primary producers have δ^2H_{om} that is ~160–170 ‰ more negative than environmental water (Yakir and DeNiro 1990; Luo and Sternberg 1991; Luo et al. 1991; Doucett et al. 2007; Solomon et al. 2011). The very negative values of δ^2H_{om} are due to fractionation during photosynthesis. Plants that photosynthesize in air (terrestrial plants) experience the same theoretical fractionation during the photolysis of H_2O, but a second process, transpiration is also important. Thus, the deuterium of terrestrial vegetation is enriched relative to aquatic primary producers because of differential retention of deuterium relative to protium in plant water (Roden and Ehleringer 1999; Roden et al. 2000; Barbour et al. 2004; Cuntz et al. 2007). The consistent difference between δ^2H_{om} of algae and terrestrial plants provides a method to identify external organic matter support of consumers in aquatic food webs (e.g., Doucett et al. 2007; Solomon et al. 2011). However, other biophysical and biochemical processes also affect the relative abundance of hydrogen isotopes in organic matter including the effects of diffusion (Roden and Ehleringer 1999; Yakir et al. 1989; Flanagan et al. 1991; Shu et al. 2008), processes associated with heterotrophic carbon metabolism (Yakir 1992; Sessions 2006; Luo and Sternberg 1992), and variations in biochemical synthesis that lead to organismal differences in molecules like lipids (Yakir 1992; Sternberg et al. 1986; Yakir and DeNiro 1990; Sessions et al. 1999). These processes of diffusion, metabolism, synthesis, and storage may lead to relative enrichment or depletion in deuterium as represented diagrammatically in Fig. 1. Thus, the δ^2H_{om} of producers may not be a simple function of the net effects of the best-understood processes transpiration and photosynthesis. The magnitude of the non-photosynthetic effects that influence producer hydrogen isotope composition indicated in Fig. 1 is difficult to characterize for the bulk organic matter of primary producers. A recent review by Sachse et al. (2012) evaluates hydrogen fractionation particularly in the context of using lipid biomarkers and discusses current understanding of processes affecting fractionation.

Surface water δ^2H (δ^2H_{water}) varies geographically due to atmospheric partitioning in vapor and precipitation as influenced by temperature, altitude, latitude, humidity, and also because of processes like evaporation of surface water and groundwater inputs (Kendall and Coplen 2001; Bowen et al. 2005). The δ^2H_{om} value of a primary producer is a function of both climate and hydrological conditions that influence the hydrogen isotope ratio of water. The net difference between the ratios of deuterium to protium in producer tissue and the surrounding water is the net isotope discrimination value (Δ_H), which is calculated as the difference between the hydrogen stable isotope ratios of producer organic matter and water.

$$\Delta_H = \left(\delta^2H_{om} - \delta^2H_{water}\right) \div \left(1 + \delta^2H_{water} \div 1000\right) \quad (1)$$

Note that we refer to Δ_H as an isotopic discrimination factor, not as fractionation. Fractionation is the term for differential isotope changes in discrete mass balance chemical reactions (e.g., photosynthesis) or physical processes (e.g., evaporation) (Fry 2006; Emerson and Hedges 2008). In the case of bulk organic matter, which is our interest in the context of food web studies, the net isotopic difference reflects a number of possible processes (Fig. 1) rather than a simple set of fractionations.

Analysis of ecological process with hydrogen isotope ratios is not new (e.g., Macko et al. 1983), but recently a number of studies have used hydrogen isotope ratios to

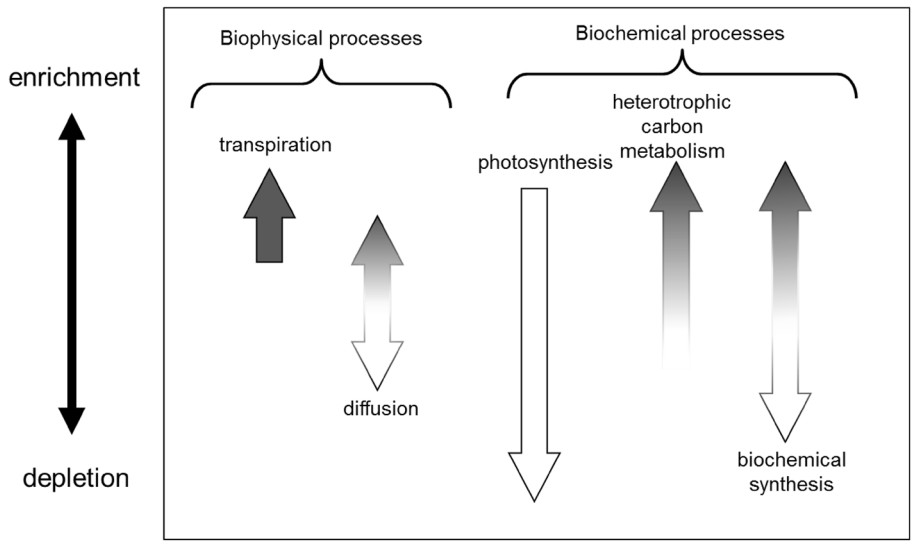

Fig. 1 Conceptual diagram of isotope discrimination and fractionation processes affecting Δ_H values between water and organic matter of aquatic primary producers. *Upward arrows* represent enrichment processes and *downward arrows* represent depletion processes. *Graded shading* represents uncertainty in magnitude. For example, transpiration is an enrichment process of known magnitude and heterotrophic carbon metabolism is an enrichment process of variable magnitude. See text for references that provide detail on the various processes

distinguish organic matter sources supporting aquatic food webs (e.g., Doucett et al. 2007; Jardine et al. 2009; Finlay et al. 2010; Caraco et al. 2010; Solomon et al. 2011; Cole et al. 2011; Babler et al. 2011; Karlsson et al. 2012; Batt et al. 2012; Cole and Solomon 2012; Wilkinson et al. 2013). Prior studies provide insight into the processes affecting isotopic composition of specific plant compounds (Cuntz et al. 2007; Hou et al. 2008). However, less is known about the processes affecting hydrogen isotope ratios of the bulk organic matter in the wide variety of aquatic primary producers that are studied in the context of food web analysis.

The variability in hydrogen isotopic discrimination (Δ_H) among primary producers is fundamental to assessing the application of this isotope in ecological studies. Understanding of patterns should improve the interpretation of models and inform sampling and experimental designs used to answer ecological questions. For example, reviews of the variability in isotopic ratios associated with trophic transfers of carbon and nitrogen (Vander Zanden and Rasmussen 2001; Post 2002; McCutchan et al. 2003; Vanderklift and Ponsard 2003; Bunn et al. 2013) provide average and group-specific isotopic discrimination values used in mixing models in hundreds of studies. Since δ^2H_{om} displays a range of values even for primary producers growing in the same location (e.g., DeNiro and Epstein 1981), the net effects of isotopic discrimination may vary based on both biological and environmental factors. Here, we examine patterns in δ^2H_{om} for different types of aquatic autotrophs relative to water and in comparison with terrestrial material. We summarized data from prior studies on aquatic primary producers. We also measured Δ_H for five categories of primary producers collected from three different aquatic ecosystems (lake, river, coastal) and their associated watersheds. We expected to find large differences in Δ_H between aquatic and terrestrial primary producers but also expected that the magnitude of difference might vary among types of ecosystems and aquatic primary producers.

Materials and methods

Isotopic data collection

Water, plant, and algal samples were collected in a coastal lagoon, a river, and several lakes. Terrestrial vegetation was collected from the watersheds of these systems. We measured δ^2H of water and autotroph organic matter and categorized primary producers into five groups: terrestrial vegetation, macrophytes, macroalgae, benthic microalgae, and phytoplankton. We considered plants that do not live in water for any part of the tidal cycle as terrestrial vegetation (TV). Vascular aquatic plants were categorized as macrophtyes (MP), and algae were categorized as either macroalgae (multicellular: MA) or microalgae (unicellular). Microalgae were further classified as either phytoplankton (PHY) or benthic microalgae (BMA) according to habitat and collection method. The coastal samples were from the Virginia Coast Reserve Long Term Ecological Research site (VCR–LTER), which comprises marshes, mudflats, lagoons and barrier islands off the Eastern Shore of Virginia. The river samples were from the Hudson River between river kilometers 45 and 175 where the southern tip of Manhattan Island, New York is river kilometer 0. The lake samples were from the University of Notre Dame Environmental Research Center (UNDERC) located near Land O'Lakes, Wisconsin.

We collected larger plants from both aquatic and terrestrial environments and sampled microalgae from benthic and pelagic environments. Multicellular seaweeds such as *Gracilaria vermiculaphylla* and *Ulva lactuca* were classified as macroalgae and comprised eight species, most of which were found in the coastal marine system. These macroalgae were collected from sub-tidal habitats and not exposed to air during any part of the tidal cycle. *Chara* sp., found in the lake system, is a green algae (Chlorophyta) but is also considered a developmental step between macroalgae and embryophytes. There was no morphological equivalent to macroalgae for the Hudson River system. Vascular aquatic plants were categorized as macrophytes and included 13 species ranging from seagrass (*Zostera marina*) to pondweeds (e.g. *Potamogeton pusillus*) and water lilies (e.g. *Nuphar variegata*). The growth patterns of macrophytes influence their exposure to air, so macrophytes were further classified as emergent, floating, or submerged. Emergent plants had variable exposure to air based on plant height and tides. Floating plants had leaves on the surface of water permanently exposed to air, while submerged plants grew underwater and were not exposed to air. In the river and lake systems we collected benthic microalgae as scrapings from tiles, natural rock, or wood substrate. High levels of sediment re-suspension made this method infeasible in the coastal system, so we used a modified version of the vertical migration technique (Riera and Richard 1996) to collect phototactic benthic diatoms. Phytoplankton were sampled from incubated laboratory cultures of native planktonic assemblages grown in filtered site water as per Caraco et al. (2010) as well as from algal net tows picked clean of non-algal material. Terrestrial vegetation sampled from the lake watershed comprised broadleaf deciduous and evergreen tree species, as well as moss (*Sphagnum* spp.) and a shrub (*Chamaedaphne calyculata*). Below, we refer only to terrestrial vegetation and macrophytes as plants, and we use primary producers to describe all autotrophs including plants and algae.

Field samples were rinsed thoroughly to remove any salts and epiphytic growth. Samples were dried at 60 °C for at least 24 h prior to grinding. Subsamples of about 350 µg were weighed into silver cups for isotopic analysis. All isotopic analyses were performed at the Colorado Plateau Stable Isotope Laboratory. All samples of organic materials were pyrolized to H_2 and the isotope ratio was measured on the H_2 gas (Doucett et al. 2007). Organic matter samples for δ^2H were analyzed with a Thermo-Finnigan TC/EA and Delta^{PLUS}-XL (Thermo Electron Corporation, Bremen, Germany). Water samples were analyzed for δ^2H with cavity ring down laser spectroscopy using Los Gatos Research Off-Axis Integrated Cavity Output coupled to a CTC LC-PAL liquid autosampler. All values are reported in per mil notation (‰) and are in relation to the international standard of Vienna Standard Mean Oceanic Water (δ^2H_{VSMOW}).

Several approaches were employed to assess the precision, accuracy, and reproducibility of the organic matter hydrogen ratio analyses. In this context, it is important to note that hydrogen in organic matter samples includes both an exchangeable and a non-exchangeable component. The analytical method measures variation in the non-exchangeable component after normalizing for the exchangeable component. A bench-top equilibration following Wassenaar and Hobson (2003) was used in all analyses wherein sample material was allowed to exchange with ambient water vapor prior to sample analysis. Variability among materials reflects differences in the non-exchangeable component as long as the proportion of exchangeable to non-exchangeable material is relatively similar among samples and standards. Relative to the primary producer material analyzed in this study, the most significant standard used in the analysis was a filamentous algae —*Cladophora* sp. from the Colorado River. This material was representative of the aquatic primary producers and has a δ^2H more negative than −200 ‰. Further, the exchangeable to non-exchangeable ratio of *Cladophora* is likely similar at least to the other algae used in this study and possibly similar to the higher plants. The *Cladophora* standard provided good reproducibility in day to day sample runs and a highly linear relationship between expected and observed values (M. Caron, personal communication).

Water samples were collected at approximately the same location and time as the organic matter samples for an accurate representation of the surrounding hydrogen pool. For samples with no corresponding water value in the Hudson River data, δ^2H_{water} was estimated from the location of the sampling site based on an empirical relationship consistent with the rainout effect between the measured river water δ^2H values and the distance of the sampling site from the mouth of the Hudson River ($R^2 = 0.94$).

We also conducted a literature search for published values of δ^2H from the total organic matter of aquatic plants and algae by using the keywords: hydrogen, deuterium, delta D, and H-2 in the Web of Science. We calculated hydrogen isotope discrimination (Δ_H) from these data when environmental water isotope ratios were also reported. Note we excluded published data from Solomon et al. (2011); Cole et al. (2011); and Batt et al. (2012) because these were part of our field survey data.

Uncertainties in isotopic discrimination

Isotopic discrimination (Δ_H) is calculated from Eq. 1 above and is the sum of all possible processes:

$$\Delta_H = \sum(\varepsilon_{lw} + \varepsilon_{bio} + \Delta_x) \quad (3)$$

where ε_{lw} is biophysical discrimination in leaf water due to uptake, transport, and transpiration; ε_{bio} is biochemical fractionation due to photosynthesis, biosynthesis of lipids, and heterotrophic carbohydrate metabolism; and Δ_x represents all other possible processes that influence the difference in hydrogen isotopic ratios (Fig. 1). Separate measurements of these discrimination and fractionation processes were beyond the scope of this study, but their implications are discussed below. Since organic matter samples were always more depleted in deuterium than water ($\delta^2H_{om} < \delta^2H_{water} < 0$), Δ_H values are negative following Eq. 1 (i.e. there is a negative isotope discrimination relative to source water). Relative to a constant δ^2H_{water}, a highly negative Δ_H indicates a greater depletion in deuterium relative to protium and more negative δ^2H_{om}; a less negative Δ_H value indicates a less negative δ^2H_{om}.

Exchangeable hydrogen could potentially affect estimates of Δ_H. In the bench-top equilibration conducted prior to sample analysis, the water molecules that exchange with organic hydrogen are more enriched in deuterium and thus shift the measured δ^2H_{om}. Direct assessments of hydrogen exchange suggest exchangeable H is on the order of 10 % for keratin, hair, and meat (Chesson et al. 2009). The key issues for our analyses are: (1) how far off are the calculated Δ_H values given hydrogen exchange, and (2) how comparable are different measures of Δ_H given potentially different levels of exchange among different producer bulk organic matter samples? To explore the sensitivity of Δ_H to this problem, we first assumed values of δ^2H from our lake study site: −220 ‰ for algal material, −140 ‰ for vascular plant material, and −45 ‰ for water. These values resemble pre-exchange measurements if H-exchange is about 10 % (Solomon et al. 2011; Cole et al. 2011). Thus, Δ_H values are −183 ‰ and −99 ‰ for the algal and vascular plant material, respectively. When the organic materials equilibrate at the analysis laboratory (δ^2H_{water} at CPSIL ∼−90 ‰), the measured values are more positive

depending on H-exchange. Assuming H-exchange in the range of 5–20 %, Δ_H values would be −174 ‰ at 5 % and −147 ‰ at 20 % for algae, and −99 ‰ at 5 % and −90 ‰ at 20 % for higher plants. In this example, the worse scenario for comparison would be algae with 20 % H-exchange having a Δ_H of −147 ‰ relative to terrestrial material with a 5 % H-exchange and Δ_H of −90 ‰. This difference in Δ_H is still large and easy to distinguish in data. Detecting differences in Δ_H is more problematic when Δ_H values are closer, especially if materials have different H-exchange. For the comparisons we make below, the effect of H-exchange on the Δ_H values is likely small (<10 %) with the possible consequence that differential H-exchange among data categories obscure detections of some differences in statistical tests (see next section).

Statistical tests

In order to evaluate if hydrogen isotope ratios differ among primary producers, non-parametric Kruskall–Wallis tests were performed to evaluate differences in Δ_H among categories of producers (test statistic reported as *K*). Non-parametric tests were used because our data did not meet normality or homogeneity of variances assumptions based on Kolmogorov–Smirnov tests. We used Δ_H values instead of hydrogen isotope ratios (δ^2H_{om}) to eliminate variability associated with δ^2H_{water} among the different sites. Differences were evaluated with post hoc exacted Wilcoxon Mann–Whitney rank sum tests with Bonferroni corrections (Sokal and Rohlf 2012). For the Kruskall–Wallis and Mann–Whitney tests we required that a test statistic have a probability value <0.05 to assign significance. It is possible that some significant effects were not detected (type 2 error) due to H-exchange that obscured true differences in Δ_H values (see prior section). This problem is most likely where Δ_H values are relatively similar for the primary producers being compared. In cases where significant differences were observed, the differences encompass any variability associated with H-exchange and are robust. Statistical tests were performed in R version 2.15.1 (R Development Core Team 2012, URL http://www.R-project.org).

Results

Aquatic primary producer hydrogen discrimination: literature survey

Variability in reported δ^2H of aquatic primary producers is substantial within and among locations (Table 1). Plotting δ^2H_{water} versus δ^2H_{om} suggests a general relationship between these two variables (Fig. 2), but not the relatively constant isotopic discrimination that would be expected if δ^2H_{om} were simply the result of photosynthetic fractionation. Further, the literature data suggest possible differences among major groups of aquatic producers (Fig. 2). Macrophyte Δ_H values appear smaller than the other groups, but we could only find data for four species. Benthic algae had larger values of Δ_H relative to macrophytes (Table 1). Macroalgal δ^2H_{om}, for which there was the greatest number of measurements, varied substantially (Fig. 2), with Δ_H values ranged from −213 to −38 ‰ (Table 1). We did not test the literature data for differences among groups because of the heterogeneity of methods used by researchers.

Aquatic primary producer hydrogen discrimination: field survey

All of the plant and algal samples in our field survey were depleted in deuterium relative to water, with δ^2H_{om} values ranging from 38 to 220 ‰ more negative than corresponding δ^2H_{water} (Fig. 3). As with the literature data, δ^2H_{water} values were somewhat related to δ^2H_{om}, but there was a wide variation, even among producers at the same site. For example, in the coastal system, δ^2H_{water} was consistent throughout all seasons (−9.99 ‰ ± 0.9), but δ^2H_{om} varied from −230 to −85 ‰ among different marine producers (Fig. 3). In comparison with the coastal system, δ^2H_{water} for the freshwater systems varied more in space and time. In the Hudson River, δ^2H_{water} ranged from −43 to −71 ‰ among sampling locations, which was partly a function of oligohaline conditions in the most downstream sites. Samples from 18 different lakes had δ^2H_{water} values that varied between −40 and −75 ‰. Temporal variation in the lakes was low based on a few of the lakes we previously sampled over the May to September period (Solomon et al. 2011; Batt et al. 2012). For example, in Peter Lake the mean (±standard deviation) of δ^2H_{water} was −43.9 ‰ ± 2.4.

We calculated Δ_H for the 253 producer samples from our survey and classified these into five categories based on taxa, morphology, and sampling method (Table 2). The five categories were terrestrial vegetation, macrophytes, macroalgae, benthic microalgae, and phytoplankton (Table 2). Primary producers with the lowest Δ_H values have the most negative δ^2H_{om} values relative to water. Phytoplankton and benthic algae in the lake and river system had the most negative Δ_H values while terrestrial vegetation and aquatic macrophytes had the least negative values (Table 2). The category of primary producers with the most variability was macroalgae (s.d. of all observations: 55 ‰). Most species of macroalgae had low variation within ranges comparable to other groups (s.d. ~15 ‰), but as with the literature data, there

Table 1 Hydrogen isotope discrimination (Δ_H, ‰) calculated from literature data for aquatic primary producers where both producer organic matter δ^2H_{om} and environmental water isotope δ^2H_{water} were reported (units ‰)

Producer Group	References	Producer	δ^2H_{om}	δ^2H_{water}	Δ_H
Macroalgae	Fenton and Ritz (1989)	*Acrocarpia paniculata*	−88	6	−93
	Schlieg and Vogel (1970)	Brown seaweed	−95	0	−95
	Estep and Dabrowski (1980)	*Chondrus crispus*	−84	0	−84
	Estep and Dabrowski (1980)	*Chondrus crispus*	−90	12	−101
	Estep and Dabrowski (1980)	*Chondrus crispus*	−103	2	−105
	Finlay et al. (2010)*	*Cladophora*	−195	−48	−153
	Finlay et al. (2010)*	*Cladophora*	−214	−48	−174
	Finlay et al. (2010)*	*Cladophora*	−229	−46	−192
	Finlay et al. (2010)*	*Cladophora*	−242	−46	−205
	Smith and Epstein (1970)	*Corallina chilense*	−47	−9	−38
	Fenton and Ritz (1989)	*Eklonia radiata*	−39	6	−45
	Estep and Dabrowski (1980)	*Enteromorpha clathrata*	−174	0	−174
	Smith and Epstein (1970)	*Enteromorpha marginata*	−72	−12	−61
	Doucett et al. (2007)*	Filamentous algae	−240	−104	−152
	Doucett et al. (2007)*	Filamentous algae	−292	−107	−207
	Doucett et al. (2007)*	Filamentous algae	−277	−81	−213
	Estep and Dabrowski (1980)	*Fucus vesiculosus*	−102	2	−104
	Estep and Dabrowski (1980)	*Fucus vesiculosus*	−116	0	−116
	Estep and Dabrowski (1980)	*Fucus vesiculosus*	−116	12	−126
	Smith and Epstein (1970)	*Gigartina cristata*	−88	−9	−86
	Fenton and Ritz (1989)	*Gigartina sp.*	−41	6	−47
	Smith and Epstein (1970)	*Grateloupia setchellii*	−94	−9	−86
	Schiegl and Vogel (1970)	Green seaweed	−57	0	−57
	Schiegl and Vogel (1970)	Green seaweed	−103	0	−103
	Fenton and Ritz (1989)	*Heterozostera tasmanica*	−83	3	−86
	Fenton and Ritz (1989)	*Hormosira banksii*	−31	3	−34
	Finlay et al. (2010)*	*Lemanea*	−136	−48	−92
	Finlay et al. (2010)*	*Lemanea*	−138	−46	−96
	Smith and Epstein (1970)	*Macrocystis pyrifera*	−70	−9	−62
	Schiegl and Vogel (1970)	Mosslike alga	−166	0	−166
	Finlay et al. (2010)*	*Nostoc*	−184	−48	−143
	Finlay et al. (2010)*	*Nostoc*	−196	−47	−156
	Finlay et al. (2010)*	*Nostoc*	−218	−48	−179
	Finlay et al. (2010)*	*Nostoc*	−226	−46	−189
	Estep and Dabrowski (1980)	*Ulva lacutca*	−166	2	−168
	Estep and Dabrowski (1980)	*Ulva lacutca*	−180	12	−190
	Fenton and Ritz (1989)	*Ulva spathulata*	−107	3	−110
	Fenton and Ritz (1989)	*Ulva taeniata*	−70	6	−76
	Group mean				−120 ± 53
Macrophytes	Smith and Epstein (1970)	*Frankenia grandifolia*	−61	−12	−50
	Smith and Epstein (1970)	*Phyllospadix torreyi*	−17	−9	−8
	Smith and Epstein (1970)	*Salicornia bigelovii*	−82	−12	−71
	Smith and Epstein (1970)	*Zostera marina*	−76	−10	−67
	Group mean				−49 ± 29

Table 1 continued

Producer Group	References	Producer	δ^2H_{om}	δ^2H_{water}	Δ_H
Benthic algae	Doucett et al. (2007)*	Diatoms	−214	−103	−124
	Doucett et al. (2007)*	Diatoms	−231	−104	−142
	Doucett et al. (2007)*	Diatoms	−251	−81	−185
	Finlay et al. (2010)*	Diatoms	−146	−48	−103
	Finlay et al. (2010)*	Diatoms	−185	−46	−146
	Finlay et al. (2010)*	Diatoms	−190	−48	−149
	Group mean				−141 ± 27

Hydrogen isotope discrimination was calculated following Eq. 1 in the text. Note values of Δ_H are negative with more negative values indicating greater isotope discrimination relative to water. For each primary producer group means ± one standard deviation are presented. An asterisk (*) indicates studies that reported using bench-top equilibration and high temperature pyrolysis in isotopic measurements

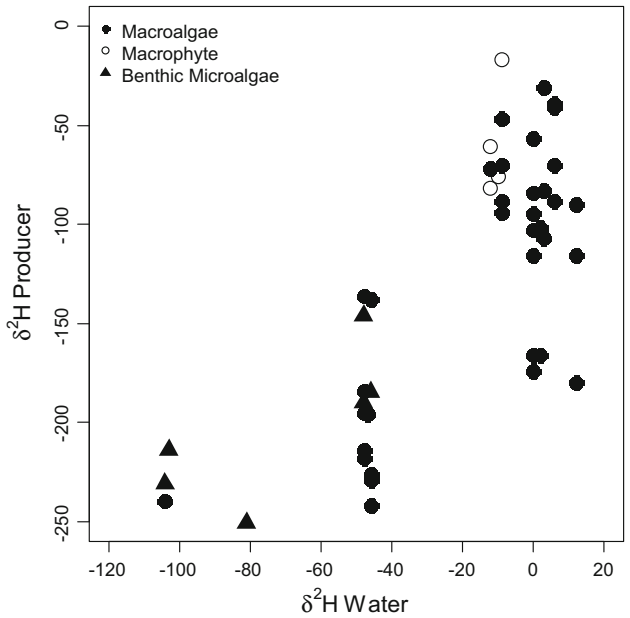

Fig. 2 δ^2H_{water} and δ^2H_{om} of producers based on data in the literature. *Symbols* represent the four categories of producers: macroalgae (*closed circles*), macrophytes (*open circles*), and benthic microalgae (*closed triangles*)

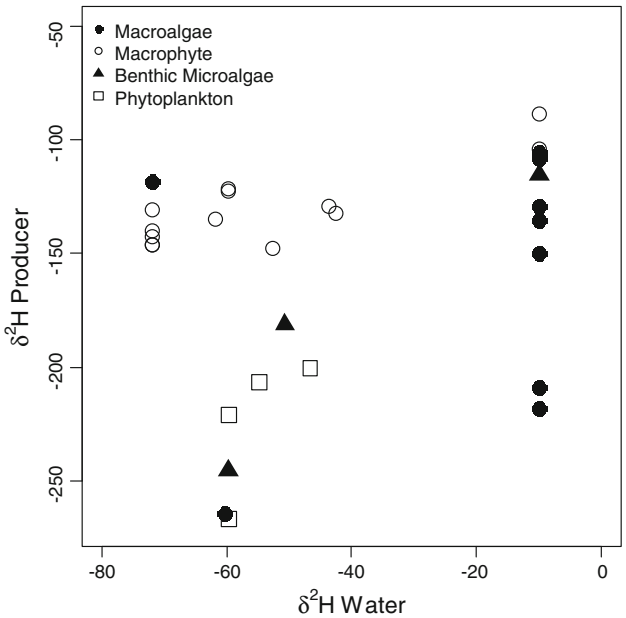

Fig. 3 δ^2H_{water} and δ^2H_{om} of producers based on data collected in this study. *Symbols* represent the four categories of producers: macroalgae (*closed circles*), macrophytes (*open circles*), benthic microalgae (*closed triangles*), and phytoplankton (*open squares*)

was a wide variation among species within this group (Table 2). For example, Δ_H values varied from −96 ‰ for *Codium fragile* to −201 ‰ for *Ulva lactuca*. Freshwater filamentous green algae, which we classified as macroalgae, also had a low (highly negative) mean Δ_H that was similar to *Ulva lactuca* and *Enteromorpha* sp. (Table 2). Vascular plants including both terrestrial and aquatic macrophytes were relatively enriched in deuterium compared to algae and had less negative Δ_H values. Even fully submerged species of macrophytes such as the rooted plants *Zostera marina* and *Valisneria americana* were enriched in deuterium compared to algae in the same systems.

Comparison of plant groups

We first contrasted the differences in Δ_H among groups of primary producers when data for all three systems (i.e. lake, river, coastal) were combined (Fig. 4a). There were significant differences in Δ_H among the five types of primary producers (Kruskal–Wallis test: $K = 122$, $p < 0.001$; $n = 253$). Post hoc tests revealed significant differences between phytoplankton and benthic algae ($p < 0.001$). Phytoplankton had the lowest average values ($\Delta_H = -173$ ‰ ± 26). Macroalgae were not significantly different from benthic algae, but were significantly different from both macrophytes ($p < 0.0001$) and terrestrial vegetation ($p < 0.0001$). Terrestrial vegetation and macrophytes

Table 2 Mean hydrogen isotope discrimination values (Δ_H, ‰) between organic matter and water grouped by primary producer types considered in this study

	Description	$\Delta_H \pm$ s.d.	N	System
Benthic microalgae	Vertical migration	−106.4 ± 19.8	16	Coast
	Rock scrapings	−194.7 ± 9.2	7	River
	Tile scrapings	−116.3 ± 35.3	19	Lake
	All benthic microalgae	−126 ± 41	42	
Macroalgae	*Agardhiella subulata*	−98.9 ± 14.3	8	Coast
	Chara sp.	−50.3 ± 14.0	5	Lake
	Codium fragile	−96.4 ± 13.2	8	Coast
	Ectocarpus siliculosus	−141.4	1	Coast
	Enteromorpha flexuosa	−210.3	1	Coast
	Filamentous algae	−216.4 ± 32.2	5	Lake
	Gracilaria vermicuphylla	−126.7 ± 15.4	14	Coast
	Scytosiphon lomentaria	−120.5 ± 9.1	2	Coast
	Ulva lactuca	−201.0 ± 16.1	18	Coast
	All macroalgae	−143 ± 55	62	
Macrophytes	*Brasenia schreberi*	−73.4 ± 20.7	6	Lake
	Isoetes lacustris	−44.0 ± 5.5	3	Lake
	Myriophyllum spicatum	−92.0	1	River
	Myriophyllum fawellii	−100.5	1	Lake
	Najas flexilis	−63.7	1	Lake
	Nuphar variegata	−80.2 ± 9.6	5	Lake
	Nymphaea odorata	−76.9 ± 8.1	5	Lake
	Pontederia cordata	−77.5 ± 3.9	2	Lake
	Potamogeton pusillus	−78.0 ± 8.3	7	Lake
	Potomogeton crispus	−95.3	1	River
	Sparganium angustifolium	−95.7 ± 10.1	3	Lake
	Spartina alterniflora	−94.8 ± 12.4	13	Coast
	*Trapa natans***	−57.90 ± 16.5	6	River
	Vallisneria americana	−69.0 ± 7.6	5	River
	Zostera marina	−79.4 ± 8.6	22	Coast
	All macrophytes	−79 ± 16	81	
Phytoplankton	Algal net tow	−187.4 ± 29.9	10	River
	Algal net tow	−160.9 ± 19.8	10	Lake
	Regrowth cultures*	−161.0 ± 0.03	4	Lake
	All phytoplankton	−173 ± 26	24	
Terrestrial vegetation	Broadleaf/deciduous	−109.0 ± 11.5	3	Coast
	Pine needles/evergreen	−90.8 ± 1,7	2	Coast
	Broadleaf/deciduous	−77.0 ± 6.3	13	Lake
	Pine needles/evergreen	−92.1 ± 7.9	11	Lake
	Broadleaf/deciduous	−56.9 ± 8.1	5	River
	Pine needles/evergreen	−62.9 ± 31.4	2	River
	Sphagnum spp.	−81.8 ± 10.1	4	Lake
	Chamaedaphne calyculata	−119.1 ± 7.4	4	Lake
	All terrestrial vegetation	−85 ± 19	44	

Benthic microalgae were collected by scrapping surfaces or using a photo-tactic vertical migration technique. Phytoplankton samples were collected by net tows or by culturing in filtered lake water

* From Solomon et al. (2011)

** Some values from Caraco et al. (2010)

had the least negative Δ_H values (−85 ‰ ± 19 and −79 ‰ ± 16, respectively), and these groups were not significantly different. Emergent, floating, and submerged types of macrophytes were not significantly different from each other ($K = 2.9$, $p > 0.2$).

When each system was considered independently, Δ_H values of the groups displayed similar patterns (Fig. 4b–d). In the Hudson River, terrestrial vegetation and macrophytes had the least negative values of Δ_H and were not significantly different (Fig. 4b). Phytoplankton Δ_H values

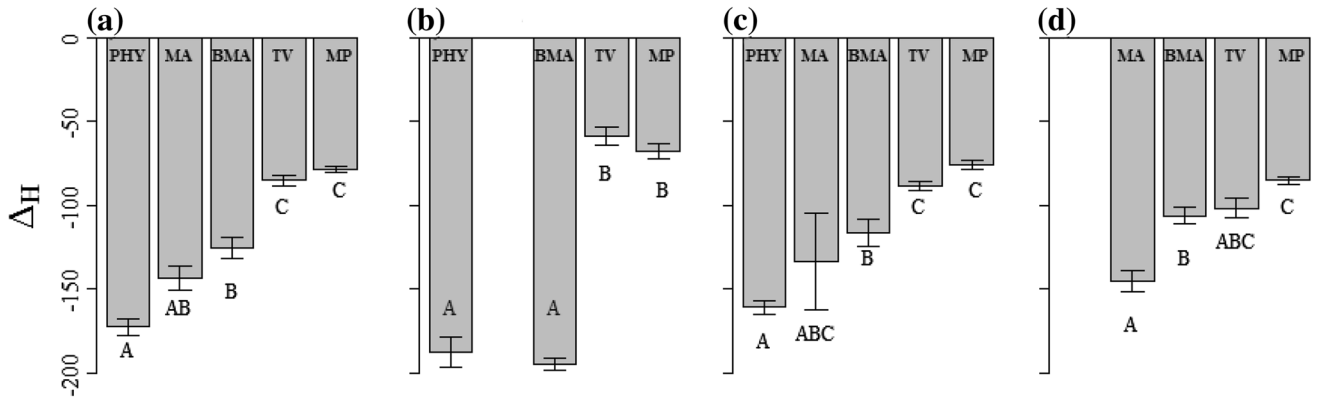

Fig. 4 Mean Δ_H values based on the differences between hydrogen stable isotope ratios of water (δ^2H_{water}) and organic matter (δ^2H_{om}) for 5 categories of primary producers from three different watershed types. Panels are **a** data combined from all three systems, **b** river only, **c** lake only, **d** coastal system only. Categories are abbreviated as follows: *MA* macroalgae, *MP* macrophytes, *BMA* benthic microalgae, *PHY* phytoplankton, *TV* terrestrial vegetation. *Error bars* are standard errors; *letters* reflect significant differences between categories based on post hoc exacted Wilcoxon Mann–Whitney rank sum tests with Bonferroni corrections. Categorization of primary producers is discussed in the text

were most negative (−187 ‰ ± 30) and significantly different than both groups of higher plants ($p < 0.001$). Benthic algae collected from the river had Δ_H values (−195 ‰ ± 9) similar to phytoplankton.

In lakes, both groups of higher plants (terrestrial and aquatic macrophytes) had less negative values of Δ_H than either type of microalgae (phytoplankton and benthic microalgae). However, macroalgae were not significantly different from any of the other groups (Fig. 4c). Macroalgae in the lakes included both *Chara* sp. and filamentous epilithic green algae. *Chara* was highly enriched in deuterium resulting in a much less negative Δ_H relative to filamentous green algae (*Chara* $\Delta_H = -50$ ‰ ± 14; filamentous algae $\Delta_H = -216$ ‰ ± 32).

Values of Δ_H for primary producers in the coastal system were also significantly different among groups ($K = 49$, $p < 0.001$). The aquatic primary producer groups were significantly different from each other but all overlapped with terrestrial vegetation (Fig. 4d). Pairwise comparisons between genera revealed significant differences between several types of macroalgae. For example, *Ulva lactuca* had a much more negative Δ_H value than all the other macroalgae ($p < 0.05$, note *Enteromorpha* sp. was excluded because $n = 1$ for this taxa). Within the macrophtye group, the submerged seagrass, *Zostera marina,* was not significantly different from the emergent marsh grass *Spartina alterniflora*.

The ordering of Δ_H between groups was relatively consistent across all three systems with algae having the most negative values and terrestrial vegetation and macrophytes having the least negative values (Fig. 4). However, when individual groups were tested for differences among systems (i.e., differences of a group among the lake, river, and coastal sites), all groups differed significantly except for macroalgae. For example, macrophytes exhibited differences between sites ($K = 12$, $p < 0.01$). The coastal macrophytes (*Spartina alternaflora* and *Zostera marina*) had significantly less negative Δ_H values than the river or lakes based on post hoc comparisons ($p < 0.05$). Values of Δ_H for benthic algae were significantly different among the three sites ($K = 17$, $p < 0.001$). The most negative Δ_H values were in the river and the least negative were observed in the coastal system. Benthic algal Δ_H values in the river were significantly different from those in both the lake ($p < 0.01$) and coastal ($p < 0.01$) system. Phytoplankton Δ_H values were also different among systems ($K = 7$, $p < 0.01$) with the river having a more negative value than the lakes ($p < 0.01$).

Terrestrial vegetation Δ_H values were also significantly different ($K = 20$, $p < 0.001$) as would be expected given differences in conditions (e.g., transpiration) among the sites. Plants from the coastal system had the most negative Δ_H values while plants from the river system had the least negative values. Plants sampled in the lake watersheds had Δ_H values that were significantly different from those in the coastal ($p < 0.05$) or river ($p < 0.001$) system.

Discussion

We found a consistent pattern of hydrogen isotope discrimination for groups of primary producers in three types of aquatic systems, supporting the use of hydrogen isotope ratios to distinguish energy sources in aquatic food webs. Our results are consistent with other observations (Epstein et al. 1976; Doucett et al. 2007; Finlay et al. 2010; Caraco et al. 2010) that algae are strongly depleted in deuterium compared to terrestrial plants. However, some of the other

variability we observed cannot yet be explained with current understanding of stable hydrogen isotopes. Our results indicate that Δ_H can be used to differentiate among some, but not all groups, of aquatic primary producers. The organic matter of primary producers is not simply a function of δ^2H_{water} and a fixed Δ_H but tends to vary among sites and producer types. In freshwater systems, terrestrial vegetation can be distinguished from algal primary producers but not from vascular aquatic plants. Application of hydrogen isotopes may, therefore, be complicated in lakes or rivers where macrophytes are a significant part of the food web.

Variability of δ^2H_{water}

Climate and hydrologic conditions influence the isotopic composition of meteoric, surface, and ground- water (Schiegl and Vogel 1970; Kendall and Coplen 2001; Bowen et al. 2005); therefore hydrogen isotope values vary across systems. Water in the lake and river systems we considered was predictably more depleted in deuterium than the coastal marine system (Kendall and Coplen 2001). Intense mixing and exchange with the ocean as well as low freshwater input maintains more constant values in the coastal lagoons we sampled, for which the δ^2H_{water} was near mean ocean water values of 0 ‰. Variability in δ^2H_{water} among lakes and along the Hudson River (upriver to downriver) is consistent with seasonal, continental, precipitation, and evaporation effects that influence these freshwater systems (Kendall and Coplen 2001). The range of these differences (lake to lake, upriver to downriver) for our study sites was on the order of 30 ‰. For lakes, evaporation will vary with surface area to volume ratios, thus differences in lake morphometry are likely related to differences in δ^2H_{water}. Also, the relative inputs of groundwater versus surface water to lakes may differentially influence their δ^2H_{water} (Krabbenhoft and Webster 1995). The gradient of δ^2H_{water} observed in the Hudson River is likely related to inputs of freshwater sources, evaporation, mixing within the tidal freshwater estuary, and mixing of oligohaline water at the most downstream end of the system.

Variability in δ^2H_{water} among systems or within a system influences the δ^2H of plant organic matter (Figs. 2, 3). This water-driven variability in δ^2H_{om} can transfer up the food web and complicate interpretation of mixing model results for organisms with long tissue turnover times. Comparisons of hydrogen isotopes ratios from different systems should account for isotopic differences in the δ^2H of water, especially between water bodies that vary in size, salinity, latitude, elevation, and precipitation. Since environmental parameters can affect δ^2H of organic matter by changing δ^2H_{water}, environmental variation would most strongly affect δ^2H of organic matter in primary producers with high turnover rates such as algae. However, temporal variability in δ^2H_{water} was low in the lake, river, and lagoon systems we studied.

In the context of food web analyses, published hydrogen ratios should only be used from a separate location or study if differences in initial water hydrogen isotope ratios are taken into account, especially for freshwater ecosystems. The development of a global database of water isotope values (GNIP, administered by the International Atomic Energy Association and the World Meteorological Organization, http://www-naweb.iaea.org/napc/ih/IHS_resources_gnip.html) is making hydrogen isotope ratio data more available. As the geographic coverage of this database grows, it should be possible to pair δ^2H_{water} values with primary producer measurements from similar sites to constrain estimates of hydrogen isotope discrimination (Δ_H).

Biophysical influences on Δ_H

We observed smaller Δ_H values in terrestrial plants relative to those of most aquatic primary producers, consistent with transpiration effects (e.g. differential evaporation of protium relative to deuterium in leaf water). However, aquatic macrophytes including completely submerged species had Δ_H values similar to terrestrial plants. This was an unexpected result. These data suggest that deuterium enrichment in leaf water of terrestrial plants due to transpiration is not the only explanation for differences between δ^2H in aquatic and terrestrial plants since functional stomata are not a sole predictor of deuterium enrichment. While some emergent and floating-leafed macrophytes do transpire, stomata on most submerged aquatics are considered non-functional because of wax occlusions (Sculthorpe 1967), which prevent transpiration even if exposed to air. The observed similarities in Δ_H for macrophytes and terrestrial vegetation in our data may reflect different enrichment and depletion processes for aquatic versus terrestrial plants. In aquatic plants for which there is no transpiration, deuterium enrichment in leaf water (especially for submerged plants) should be negligible and plant tissue hydrogen stable isotopes might be expected to simply reflect the fractionation associated with photosynthesis. For example, Yakir and DeNiro (1990) found this expected photosynthetic fractionation values in *Lemna* plants of about −170 ‰ relative to water. However, they also measured post-photosynthetic fractionation (about +150 ‰) related to metabolism (Yakir and DeNiro 1990). Hence, for *Lemna* the δ^2H_{om} was not simply the result of photosynthesis but reflected the consequences of other processes. For our data, the similarity of Δ_H in comparing macrophytes with terrestrial plants is unresolved and requires further research.

In addition to transpiration, other biophysical influences on leaf water are unlikely to explain the enrichment we found in aquatic macrophytes. Aquatic macrophtyes (Rascio 2002) and unicellular green algae (Yakir 1992) both have high levels of exchange between photosynthetic cells and environmental water. A direct pathway between water uptake and photosynthesis minimizes leaf/cell water heterogeneity. The differences we observed in Δ_H values between the three systems may be related to differences in environmental conditions like relative humidity or physiological differences between the autotrophs found at each location.

Biochemical influences on Δ_H

We found Δ_H values in phytoplankton, some macroalgae, and some benthic algae in the range of −160 to −170 ‰ that were approximately consistent with a large depletion in deuterium due to photosynthesis. The δ^2H_{om} of these primary producers relative to water suggests that most of the organic matter is produced with little depletion or enrichment of deuterium beyond what occurs as a consequence of photosynthesis. The most deuterium-depleted primary producers we observed were some microalgae and filamentous macroalgae. In a few cases, these Δ_H values were even more negative than the range of −160 to −170 ‰ expected from photosynthesis. Since lipid biosynthesis strongly fractionates against deuterium (e.g., Sessions et al. 1999), $\Delta_H < -170$ ‰ may be an indication of species where lipids are a substantial component of producer biomass. However, the highest lipid contents we found reported in the literature for the most deuterium-depleted algae (relative to water) in our data were only 6.4 and 9 % for species of *Ulva* and *Enteromorpha*, respectively (Wahbeh 1997). If lipids in these macroalgae are 100 ‰ depleted relative to the value for the whole alga (Smith and Epstein 1970), a simple mixing model where 90 % of the mass is −170 ‰ and 10 % of the mass is −270 ‰ for the lipid fraction results in a final tissue δ^2H_{om} of −180 ‰. Lipid content would need to be near 30 % for these algae to achieve a tissue isotopic value similar to the values we measured for *Ulva* and *Enteromorpha*. Thus, these macroalgae as well as some filamentous algae and cyanobacteria are more depleted in deuterium than expected based on net isotopic discrimination due to photosynthesis and reasonable assumptions about lipid content (Fig. 2). This conclusion, however, must be tempered by uncertainty about expected fractionations related to photosynthesis and lipid synthesis as well as the δ^2H of non-lipid organic matter in these and other algae.

Variability in Δ_H for benthic microalgae across our systems could be related to differences in community composition of the organisms we sampled. Although microscopic inspection suggested algae dominated our samples, other chemosynthetic and heterotrophic microbes were present. These organisms could affect measured values of δ^2H especially in cases where microbes (e.g. methane oxidizers) rely on sources of hydrogen that are highly depleted in deuterium (Deines et al. 2009).

Terrestrial succulent plants use Crassulacean Acid Metabolism (CAM) as an adaption to water stress—a condition not expected in the aquatic environment. However, observations of substantial diel hydrogen ion changes (5–290 mmol H^+ kg^{-1}) in aquatic species indicate the operation of an acid metabolism (Keeley 1998). Separation of carbon uptake and reduction during "aquatic" acid metabolism (Rascio 2002) may provide a competitive advantage for carbon acquisition in the aquatic environment where diffusion of carbon is many times lower than in air. These aquatic producers use CO_2 as a carbon source. In the absence of aquatic acid metabolism, most aquatic primary producers use bicarbonate. Bicarbonate uptake in aquatic plants and algae is widely observed including in many of the genera included in this study (e.g., *Potamogeton*: Sand-Jensen et al. 1992). However, we also measured the isotopic composition of genera for which there is evidence of CO_2 use and acid metabolism (e.g., *Pontedaria*: Pagano and Titus 2007). These macrophytes and macroalgae did not have an H-isotopic composition distinct from those that use HCO_3^-.

The high variability we found for Δ_H in macroalgae may be a promising area to investigate for a more complete understanding of drivers of aquatic producer δ^2H. *Chara*, a submerged macroalgae that grows unattached in open water, was the most deuterium-enriched macroalgae in the lake system. *Chara* has traits that make it intermediate between macroalgae and embryophytes, and is considered the closest algal relative of higher plants. Other branched and highly structured macroalgae like the coastal seaweeds *Agardhiella subulata* and *Codium fragile* were also enriched in deuterium relative to more simply structured *Ulva lactuca*, which grows as sheets only two cells thick. These differences in structure may be related to differences in composition that influence δ^2H.

Conclusions

We found large and consistent differences in hydrogen isotope discrimination among major groups of aquatic primary producers across freshwater and marine ecosystems. Relative to water deuterium was depleted in microalgae, variable in macroalgae, and relatively enriched in both terrestrial vegetation and aquatic macrophytes. These differences can aid in partitioning energy sources in many aquatic ecosystems, particularly in distinguishing

algal and terrestrial sources. Based on our results, hydrogen isotopes are less useful in distinguishing sources in aquatic food webs where both macrophytes and terrestrial vegetation are important, but $\delta^{13}C$ and $\delta^{15}N$ are often distinct for these sources (e.g., Batt et al. 2012). Food web studies using hydrogen isotopes should either measure the $\delta^{2}H$ of primary producers directly, or where this is infeasible (e.g. phytoplankton), account for the variability in isotope discrimination relative to water thereby providing a means to estimate $\delta^{2}H$ of producer biomass. Finally, while Δ_H was relatively constant within most producers groups for a given system, the causes of the large variability of Δ_H in macroalgae require further investigation.

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