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BULK HYDROGEN STABLE ISOTOPE COMPOSITION OF SEAWEEDS: CLEAR SEPARATION BETWEEN ULVOPHYCEAE AND OTHER CLASSES[1]

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Little is known about the bulk hydrogen stable isotope composition (δ^2H) of seaweeds. This study investigated the bulk δ^2H in several different seaweed species collected from three different beaches in Brazil, Australia, and Argentina. Here, we show that Ulvophyceae (a group of green algae) had lower δ^2H values (between −94‰ and −130‰) than red algae (Florideophyceae), brown algae (Phaeophyceae), and species from the class Bryopsidophyceae (another group of green algae). Overall the latter three groups of seaweeds had δ^2H values between −50‰ and −90‰. These findings were similar at the three different geographic locations. Observed differences in δ^2H values were probably related to differences in hydrogen (H) metabolism among algal groups, also observed in the δ^2H values of their lipids. The marked difference between the δ^2H values of Ulvophyecae and those of the other groups could be useful to trace the food source of food webs in coastal rocky shores, to assess the impacts of green tides on coastal ecosystems, and to help clarify aspects of their phylogeny. However, reference materials for seaweed δ^2H are required before the full potential of using the δ^2H of seaweeds for ecological studies can be exploited.

Key index words: **Chlorophyta; green algae; hydrogen; intercontinental; macroalgae; seaweeds; stable isotopes; Ulvophyceae**

***Abbreviations*: D, deuterium; H, hydrogen; St., sampling station; δ^2H, hydrogen stable isotope composition; VMSOW, Vienna Standard Mean Ocean Water**

Seaweeds are important components of marine rocky shores, supplying food and shelter to animals that inhabit these ecosystems (Duggins et al. 1989, Lobban and Harrison 1994). However, in coastal zones the food source for herbivores can have multiple origins, including seaweeds, phytoplankton, seagrasses and terrestrial biomass. To estimate the relative importance of each source, biomarker and stable isotope techniques are commonly employed (Fredriksen 2003, Hanson et al. 2010, Smith et al. 2016).

Biomarkers like fatty acids appear to be more useful than stable isotopes in tracing the source of energy for consumers in ecosystems rich in seaweeds (Graeve et al. 2002, Hanson et al. 2010, Dethier et al. 2013). The fatty acid composition of different taxonomic groups of seaweed is very specific (Galloway et al. 2012).

In contrast to fatty acids, the stable isotopes of carbon and nitrogen in seaweeds are highly variable because they can be significantly influenced by

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environmental factors (Carvalho et al. 2008a, 2009, Marconi et al. 2011, Mackey et al. 2015). As a consequence, the use of C and N stable isotopes to elucidate the role of seaweeds as food sources in coastal food webs requires large sampling programs to cover temporal and spatial variations (Fredriksen 2003, Won et al. 2007). At the other extreme, sulfur stable isotopes of seaweeds vary in a very narrow range, similar to that of phytoplankton, and as such, are only useful to determine whether the material has a marine origin (Carvalho et al. 2008b).

Hydrogen stable isotopes can be very useful to trace the energy source of consumers in aquatic ecosystems, especially if employed together with other isotopes (Jardine et al. 2009, Babler et al. 2011, Cole et al. 2011). Hydrogen stable isotope composition (indicated by δ^2H) of Ulvophyceae, a class in Chlorophyta (green algae), tend to have lower δ^2H values than Bryopsidophyceae (another class in Chlorophyta), Florideophyceae (a class in Rhodophyta, red algae) and Phaeophyceae (a class in Ochrophyta, brown algae; Macko et al. 1983, Fenton and Ritz 1988, 1989). However, the low numbers of observations in these previous studies limit the broader extrapolation of the findings. Low δ^2H values in Ulvophyceae have also been observed for studies based on the analysis of individual biochemical compounds like lipids and cellulose (DeNiro and Epstein 1981, Sternberg et al. 1986).

Bulk seaweed δ^2H is the weighted average of individual hydrogen constituted in all forms (organic and inorganic) in the seaweed thallus. Through the life of a seaweed, such compounds are taken up, accumulated or discarded. In each of these processes, it is possible that there is a difference in the rate of reaction of hydrogen (H) and deuterium (D; the heavier H isotope, with an extra neutron), which is known as isotopic fractionation. Because of fractionation, seaweeds and each of their hydrogenated compounds have δ^2H values that differ from their main H source, the surrounding water. However, this difference is not random and for some specific compounds a very strong and clear relationship can be found (Sachse et al. 2012). Therefore, the δ^2H value of the surrounding water is likely to be the most important environmental factor determining seaweed δ^2H value in most cases. The seawater δ^2H value is fairly constant at around 0‰, but in fresh, brackish or subsurface water the δ^2H value can be considerably different from nearby seawater due to processes of evaporation and condensation (Hoefs 2009, Schmidt et al. 2011, Sachse et al. 2012).

For aquatic plants, which include seaweeds, factors that affect the ratio photosynthesis/respiration, like temperature, can have a significant effect on fractionation (Sachse et al. 2012). Salinity can also be a factor due to its potential effect on a wide range of metabolic processes in both phytoplankton and halophilic plants (Ladd and Sachs 2015a,b, Maloney et al. 2016, Sachs et al. 2016). Seaweeds in the littoral zone are subject to extremes of temperature and salinity (Dring 1982, Lobban and Harrison 1994), while those permanently submerged encounter a much more stable environment. Therefore, it is could be expected that these two groups of seaweeds tend to have different δ^2H values.

Despite the potentially strong influence of environmental factors on seaweed δ^2H values, for individuals of different species under similar environmental conditions (e.g., in a same tidal pool), it is likely that such environmental factors will be very similar. In these cases, phylogeny, and not environment, is expected to be the main driver of δ^2H. Because of the distinction between Ulvophyceae and other algal groups (Macko et al. 1983, Fenton and Ritz 1988, 1989), we hypothesized that Ulvophyceae would have a bulk δ^2H value different from other seaweed taxa in the same microhabitat irrespective of geographic location and environmental conditions. To test this hypothesis, we collected algae from different seaweed groups at three locations across the globe (Brazil, Australia and Argentina).

MATERIAL AND METHODS

Because our objective was to compare δ^2H values among different taxonomic groups, our sampling strategy was designed to collect specimens of different phylogenetic groups likely to be subject to the same micro–environmental conditions (i.e., collecting specimens very close to each other; within 1 m^2). To try to generalize our conclusions, we performed the sampling in distant locations subject to different climatic and macro-environmental conditions. This design made it possible to isolate the differences among taxonomic groups from the environmental effects.

The three distant locations chosen were Shelly beach, Ballina, Australia (28°51′54″ S, 153°35′38″ E; collection in May 2011); Sabiaguaba beach in Fortaleza, Brazil (3°46′25″ S, 38°25′57″ W; collection in April 2011), and Playa Parana beach, in Puerto Madryn, Argentina (42°48′36″ S, 64°54′24″ W; collection in June 2012; Fig. 1). The three locations represent three different climates: tropical (Brazil), subtropical (Australia) and temperate (Argentina). Different climatic regions are likely to present seaweeds with distinct environmental conditions.

Collection sites at each location were chosen according to their potential to present different environmental conditions for the seaweeds (Fig. 1). In Brazil, this was the degree of exposure to dryness or rain, which was determined by position relative to mean seawater level (tidal range: ~2 m). Although the Coco River mouth is at ~500 m to the west of the collection site, the prevalent coastal current moves from east to west, resulting in limited freshwater at the study site.

In Argentina, position relative to seawater level was also a criterion, but because all specimens were submerged, specimens collected from different depths were likely to have experienced differences in temperature and light exposure. No rivers exist close to the collection site.

In Australia, collections were made at two different positions relative to mean seawater level (tidal range: ~3 m), and also at two different positions relative to a nearby freshwater source (urban storm drain). Each collection site measured

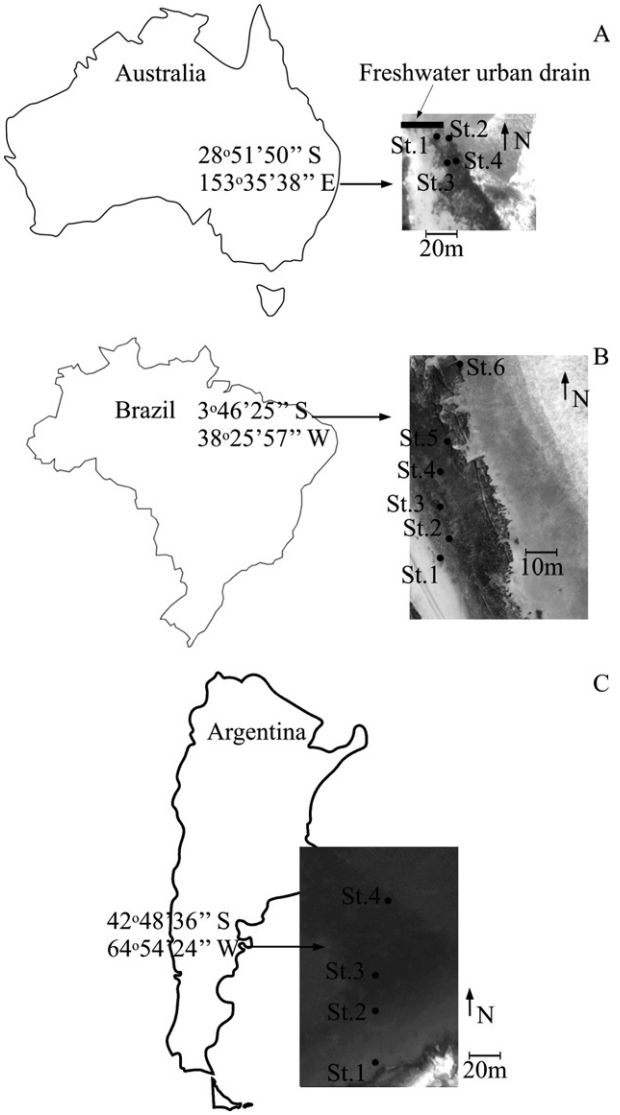

FIG. 1. Sampling points in Sabiaguaba beach (Brazil), Shelly beach (Australia) and Puerto Madryn (Argentina). Photos modified from maps.google.com.

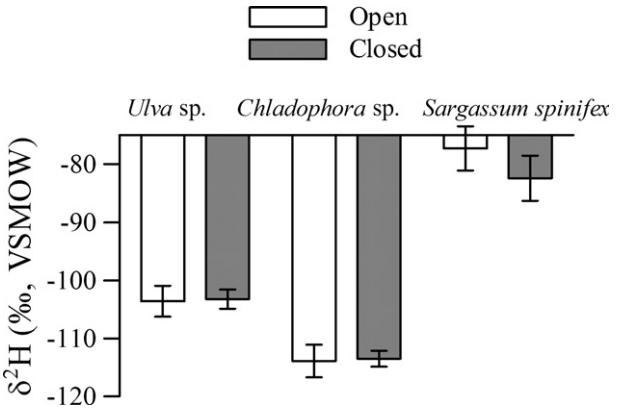

FIG. 2. Hydrogen stable isotope composition (δ^2H, ‰ Vienna Standard Mean Ocean Water) in seaweed material from three different species measured with (open), and without (closed), exposure to air for 2 weeks.

less than 1 m^2, to ensure that all individuals collected were exposed to very similar environmental conditions. All collections were done during low water, and were finished in a single day. The Richmond River's mouth is located ~1.5 km to the south of the collection site, and episodic flooding would influence the study site (Eyre 1997, Eyre and Pont 2003). However, all pools would have an equal freshwater influence.

At least three different thalli of each selected species were collected from each site, pooled together and treated as a single sample to obtain a potentially more representative value for each sampled species, and to avoid outlier variations. All samples were dried at 60°C and ground with a mortar and pestle. Australian samples were immediately stored in a desiccator until δ^2H measurement. Dried samples from Brazil and Argentina were stored in sealed plastic containers, sent to Australia and then left in a desiccator until analysis. Between 0.5 and 1.0 mg of individual samples were used for all measurements with a Thermo–Chemical Elemental Analyzer (Thermo Fisher) coupled to a Delta V Plus IRMS (Isotope Ratio Mass Spectrometer; Thermo Fisher, North Ryde, NSW, Australia) located at Southern Cross University, Australia. We corrected for the H^{3+} factor, which severely affects δ^2H measurements, by following the standard procedure suggested by the manufacturer, which was an automated daily calibration done solely for this purpose using a reference gas (H_2).

Samples and standards were left standing open to atmospheric air for 2 weeks, following the principle of identical treatment (Doucett et al. 2007, Carter et al. 2011, Meier-Augenstein et al. 2013). We used 2 keratin standards: kudzu horn ($\delta^2H = -54.1‰$ relative to VSMOW [Vienna Standard Mean Ocean Water]) and caribou horn ($\delta^2H = -197‰$ relative to VSMOW). In addition, we also used a synthetic standard, a polyethylene foil, NIST 8540, $\delta^2H = -100‰$ relative to VSMOW. This standard has no interaction with atmospheric water vapor, and thus would be representative for samples with low interaction with atmospheric water vapor. To determine whether our samples would be better compared to the keratin standards or to the polyethylene standard, we evaluated the change in δ^2H values after exposure to laboratory air for 2 weeks. Results showed no significant difference in the three samples (unpaired Student's *t*-test, $P > 0.25$ in all cases; Fig. 2). Therefore, we compared our results to the polyethylene sheet standard, and not to the keratin standards. If the keratin standards were used, all values would be shifted −103‰ (results not shown). However, because the correction would be the same for each and every sample, there would be no effect on the findings of this study. These uncertainties mean that the results obtained in this study cannot be considered absolute values because they were not compared to a known standard (currently unavailable). Errors in repeated standard measurements were less than 2‰, assessed by measuring the polyethylene standard every 15 samples in a sequence.

Student's *t*-tests and one-way ANOVA (followed by the Tukey–Kramer test when appropriate) were undertaken applying a significance level of 5% using the Kyplot 5.0 software (Kyenslab, Tokyo, Japan). Tests were done only when the number of replications was at least three.

RESULTS

A complete list of species collected at different sites and their δ^2H values is given in Table 1. The

hydrogen isotope composition (δ^2H) was not analyzed at the organism phylum, because it was obvious that Chlorophyta (green algae) values were heterogeneous (values for Ulvophyceae were very different from values for Bryopsidophyceae), and also because, for each of the other two phyla (Rhodophyta, red algae, and Ochrophyta, brown algae) there was only a single class, respectively Florideophyceae and Phaeophyceae.

The δ^2H values of Ulvophyceae (−94‰ and −135‰) were clearly lower than that of the other three algal groups (−43‰ and −94‰) for all cases except a single Phaeophyceae *Dictyota dichotoma*, which had a value of −102‰ at a single sampling station in Argentina (all results in this paragraph can be visualized in Fig. 3 or Table 1). In Brazil, Ulvophyceae δ^2H values (−112.5‰ ± 13.5‰) were significantly different ($t_{1,17} = 8.26$, $P = 10^{-7}$, unpaired Student's *t*-test) from Florideophyceae (−67.7‰ ± 10.0‰). In Australia, Ulvophyceae δ^2H values (−115.5‰ ± 9.9‰) were significantly different from those in Florideophyceae (−71.8‰ ± 11.5‰) and Phaeophyceae (−81.1‰ ± 3.9‰; one-way ANOVA followed by Tukey–Kramer test; $F_{2,18} = 43.9$, $P = 10^{-5}$ and 10^{-6} respectively). In Argentina, Ulvophyceae δ^2H values (−105.4‰ ± 8.2‰) were significantly different from those in Florideophyceae (−72.1‰ ± 10.5‰), Phaeophyceae (−74.0‰ ± 18.2‰), and Bryopsidophyceae (−57.4‰ ± 3.4‰; one-way ANOVA followed by Tukey–Kramer test, $F_{3,24} = 14.5$, $P = 10^{-4}$, 10^{-3} and 10^{-5} respectively). Ulvophyceae δ^2H values were not significantly different among algae collected from Brazil, Argentina, or Australia ($F_{2,18} = 0.9$, $P = 0.41$, one-way ANOVA).

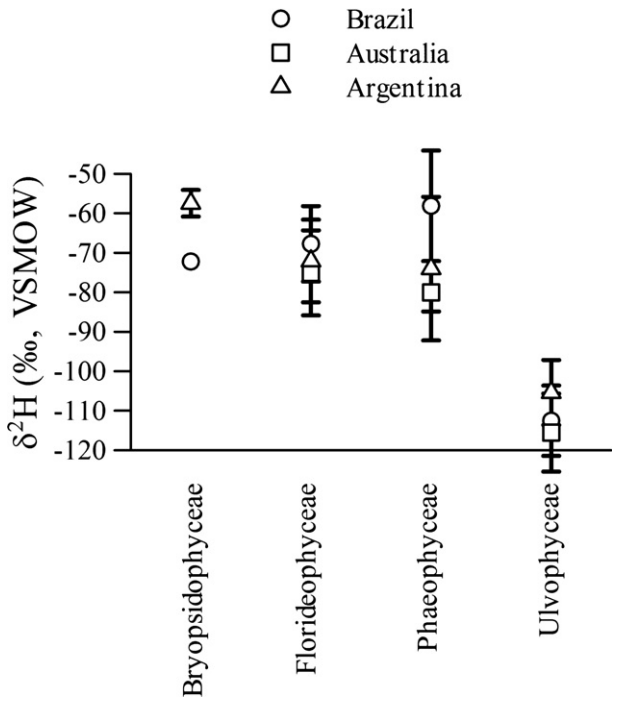

FIG. 3. Hydrogen stable isotope composition (δ^2H, ‰ Vienna Standard Mean Ocean Water) in seaweeds from four different classes (*x*-axis) collected from three different geographic origins (Brazil, Australia, and Argentina) separated by sampling site. Each sampling site consisted of an area <1 m^2. Symbols show average values, while bars show standard deviation.

Differences in δ^2H values amongst other groups were not as clear (Fig. 3). In Australia, Phaeophyceae and Florideophyceae were not statistically different ($F_{3,24} = 14.5$, $P = 0.13$, one-way ANOVA followed by Tukey–Kramer test). In Argentina, the δ^2H values of these two groups were also not significantly different ($F_{2,18} = 43.9$, $P = 0.77$, one-way ANOVA followed by Tukey–Kramer test), and Bryopsideophiceae δ^2H values were significantly different from Phaeophyceae, but not from Florideophyceae ($F_{2,18} = 43.9$, $P = 0.02$ and 0.09, respectively, one-way ANOVA followed by Tukey–Kramer test). Again comparing different beaches, δ^2H values of Phaeophyceae from Argentina and Australia were not significantly different (unpaired Student's *t*-test, $t_{1,15} = 1.1$, $P = 0.55$), while Florideophyceae δ^2H values were also not significantly different among the three different beaches ($F_{2,24} = 0.5$, $P = 0.69$, one-way ANOVA), even after removing the outlier ($F_{2,23} = 1.7$, $P = 0.19$) in Argentina, *Hymenena* sp. ($\delta^2H = -43$‰; Table 1).

There was only one instance where it was possible (more than three replicates) to compare different classes in a single site. At station 2, in Australia, δ^2H values in Phaeophyceae (−71.7‰ ± 12.2‰) and Florideophyceae (−81.9‰ ± 5.6‰) were not significantly different (unpaired Student's *t*-test, $t_{1,4} = 1.3$, $P = 0.26$).

Brazil δ^2H values for Ulvophyceae and Florideophyceae tended to be higher for stations lower in the littoral profile (Fig. 4), but this difference was not significant. Site effect on a single class was done only twice, due to limitations in replicates. In Australia, δ^2H values of Phaeophyceae from sites 2 (−81.9‰ ± 5.6‰) and 4 (−82.7‰ ± 0.5‰) were also not significantly different (unpaired Student's *t*-test, $t_{1,4} = 0.25$, $P = 0.82$; Fig. 4B). In Argentina, no significant difference was found between sites 1 (−75.8‰ ± 3.7‰) and 2 (−74.1‰ ± 1.9‰) for δ^2H values in Florideophyceae (unpaired Student's *t*-test, $t_{1,6} = 0.8$, $P = 0.45$; Fig. 4C).

We also investigated if there were differences among orders in a single class. Chladophorales and Ulvales (two Ulvophyceae orders) δ^2H values (−114.4‰ ± 16.1‰ and −110.1‰ ± 11.2‰ respectively) were not significantly different in Brazil ($t_{1,7} = 0.5$, $P = 0.66$ unpaired Student's *t*-test). Phaeophyceae could be divided into two groups in Argentina, with significantly different δ^2H values ($t_{1,6} = 2.5$, $P = 10^{-3}$, unpaired Student's *t*-test): Dictyotales (−91.8‰ ± 7.5‰) and Laminariales (−59.8‰ ± 7.0‰). Similarly, in Australia significant difference were found among Ectocarpales

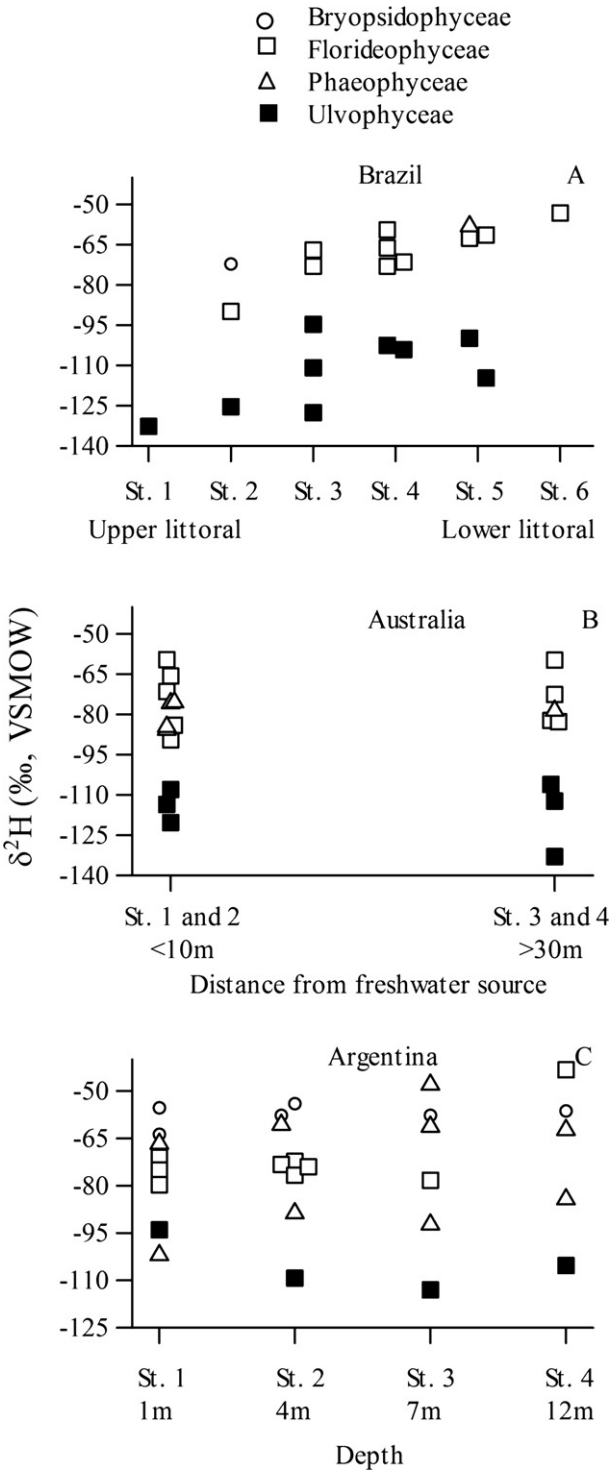

FIG. 4. Hydrogen stable isotope composition (δ^2H, ‰ Vienna Standard Mean Ocean Water) in seaweeds from four different classes grouped by sampling site (see Fig. 1 for details).

(−76.6‰ ± 1.7‰) and Fucales (−83.8‰ ± 1.6‰; $t_{1,5} = 5.7$, $P = 10^{-3}$, unpaired Student's *t*-test). Dictyotales in Argentina was not significantly different from Ulvophyceae, but only marginally ($t_{1,6} = 2.4$, $P = 0.051$, unpaired Student's *t*-test). Still among the Paheophyceae, it is worthy to mention the extreme discrepancy between *Padina* sp. from Brazil (−58‰) and from Australia (−83‰).

DISCUSSION

Low δ^2H values in Ulvophyceae. The main finding of this study was that Ulvophyceae δ^2H values could be clearly separated from δ^2H values in the other groups regardless of location (Fig. 3). In addition, although single sites covered less than 1 m^2, differences >70‰ between coexisting species were observed (Station Australia 3; Table 1). Such differences occurred in algae situated a few tens of centimeters from each other inside a same tidal pool. Variation in a single taxon could be large as well (Figs. 3 and 4), but it was clear that δ^2H was strongly driven by taxon. These results agree with previous findings based on compound-specific analysis (DeNiro and Epstein 1981, Sternberg et al. 1986). In these previous studies, it was argued that all algae had the same H source, and that differences among them would be due to metabolic processes between H assimilation in photosynthesis and compound formation. Therefore, if different groups of algae produce different compounds that tend to have different δ^2H values, this can be reflected in their bulk H isotopic composition.

Certain classes of algae including Ulvophyceae have lost the biosynthetic pathway to synthesize isoprenoids (triterpenoids) in the cytosol via the mevalonic acid pathway (Schwender et al. 2001, Vranova et al. 2013). They use the methylerythritol phosphate pathway exclusively (Schwender et al. 2001). This is expected to result in isoprenoid lipids in green algae that are ^{2}H–depleted relative to those in non–green algae. It has been found that Ulvophyceae can produce more isoprenoids than most other algal groups (Broadgate et al. 2004), which may also contribute to their low δ^2H values, as isoprenoid lipids are among the most ^{2}H-depleted biomolecules (Hayes 2001, Schmidt et al. 2003).

Another line of evidence suggesting that Ulvophyceae process H differently from other algal groups is the observation that Ulvophyceae are rich in the polyunsaturated fatty acids C18, and poor in the C20. In contrast, Florideophyceae and Phaeophyceae are rich in C20, and poorer in C18, a trend that has been observed in many different geographical locations (Johns et al. 1979, Fleurence et al. 1994, Graeve et al. 2002). Monounsaturated C20 fatty acid (20:1) had higher δ^2H values than C18:1 in green microalgae (Zhang and Sachs 2007). Hence, it is possible that the lower amount of relatively heavier C20 in Ulvophyceae is related to their lower δ^2H values observed here (Fig. 3).

It could also be speculated that the low δ^2H values in Ulvophyceae could reflect a larger lipid content in this group compared to other groups,

TABLE 1. Species collected at each sampling point in Brazil, Argentina and Australia. See Figure 1 for station description. The δ^2H values reported represent mean ± SD ($n = 3$ or 4); only the means were used for statistical analyses, as the repeated measurements were not true replications.

Station	Class	Species	δ^2H (‰ VSMOW)
Brazil 1	Ulvophyceae	*Chaetomorpha antennina*	−132.7 ± 3.4
Brazil 2	Bryopsidophyceae	*Derbesia marina*	−72.2 ± 2.4
	Florideophyceae	*Centroceras clavulatum*	−89.9 ± 4.9
	Ulvophyceae	*Ulva flexuosa*	−125.4 ± 2.6
Brazil 3	Florideophyceae	*C. clavulatum*	−73.0 ± 0.5
		Palisada perforate	−67.0 ± 8.3
	Ulvophyceae	*C. antennina*	−127.6 ± 1.2
		Rhizoclonium riparium	−94.7 ± 5.2
		Ulva lactuca	−110.9 ± 2.8
Brazil 4	Florideophyceae	*Bryocladia cuspidata*	−73.0 ± 2.1
		Gelidium sp.	−59.5 ± 6.2
		Gracilaria domingensis	−66.2 ± 2.4
		Hypnea musciformis	−71.5 ± 6.9
	Ulvophyceae	*R. riparium*	−102.5 ± 2.7
		U. lactuca	−104.1 ± 7.2
Brazil 5	Florideophyceae	*Bryothamnion seaforthii*	−62.6 ± 1.2
		Pterocladiella bartletti	−61.4 ± 4.2
	Phaeophyceae	*Padina* sp.	−58.1 ± 4.5
	Ulvophyceae	*Cladophora* sp.	−114.7 ± 2.4
		U. lactuca	−99.9 ± 4.4
Brazil 6	Florideophyceae	*Gracilaria* sp.	−53.2 ± 5.9
Australia 1	Florideophyceae	*Jania verrucosa*	−65.7 ± 7.4
		Laurencia sp.	−89.5 ± 3.1
	Phaeophyceae	*Petalonia fascia*	−75.8 ± 1.7
	Ulvophyceae	*Cladophora* sp.	−120.3 ± 3.1
		Ulva sp.	−108.0 ± 3.0
Australia 2	Florideophyceae	*J. verrucosa*	−59.6 ± 3.9
		Laurencia sp.	−84.0 ± 1.3
		Pterocladiella capillacea	−71.5 ± 3.4
	Phaeophyceae	*P. fascia*	−75.5 ± 2.6
		Sargassum fallax	−84.6 ± 3.3
		Sargassum spinifex	−85.7 ± 10.6
	Ulvophyceae	*Ulva* sp.	−113.5 ± 3.0
Australia 3	Florideophyceae	*Amphiroa anceps*	−72.6 ± 5.0
		J. verrucosa	−59.8 ± 7.8
	Phaeophyceae	*P. fascia*	−78.6 ± 4.0
	Ulvophyceae	*Cladophora* sp.	−133.0 ± 3.3
		Ulva sp.	−112.3 ± 7.7
Australia 4	Phaeophyceae	*Padina* sp.	−83.2 ± 0.8
		S. fallax	−82.3 ± 2.2
		S. spinifex	−82.7 ± 4.0
	Ulvophyceae	*Ulva* sp.	−106.1 ± 6.6
Argentina 1	Bryopsidophyceae	*Codium decorticatum*	−55.3 ± 5.1
		Codium vermilara	−63.7 ± 2.5
	Florideophyceae	*Anotrichium furcellatum*	−71.0 ± 0.0
		Ceramium vrigatum	−77.3 ± 4.2
		Heterosiphonia merenia	−79.7 ± 2.1
		Polysiphonia abcissa	−75.0 ± 1.7
	Phaeophyceae	*Dictyota dichotoma*	−102.0 ± 3.0
		Undaria pinnatifida	−66.7 ± 3.8
	Ulvophyceae	*Ulva rigida*	−94.0 ± 4.4
Argentina 2	Bryopsidophyceae	*C. decorticatum*	−54.0 ± 6.1
		C. vermilara	−57.7 ± 9.3
	Florideophyceae	*A. furcellatum*	−73.3 ± 1.2
		C. vrigatum	−72.3 ± 3.1
		Lomentaria clavellosa	−74.0 ± 4.6
		P. abscissa	−76.7 ± 2.5
	Phaeophyceae	*D. dichotoma*	−88.7 ± 0.6
		U. pinnatifida	−60.7 ± 2.9
	Ulvophyceae	*U. rigida*	−109.3 ± 1.2
Argentina 3	Bryopsidophyceae	*C. vermilara*	−57.7 ± 3.2
	Florideophyceae	*A. furcellatum*	−78.3 ± 4.0
	Phaeophyceae	*D. dichotoma*	−92.3 ± 3.5
		U. pinnatifida	−61.3 ± 3.2
	Ulvophyceae	*U. rigida*	−113.0 ± 3.5

(continued)

TABLE 1. (continued)

Station	Class	Species	δ^2H (‰ VSMOW)
Argentina 4	Bryopsidophyceae	*C. vermilara*	−56.3 ± 4.6
	Florideophyceae	*Hymenena* sp.	−43.3 ± 4.2
	Phaeophyceae	*D. dichotoma*	−84.3 ± 1.2
		U. pinnatifida	−62.3 ± 2.9
	Ulvophyceae	*U. rigida*	−105.3 ± 5.7

because lipids, as the most reduced biomolecules, tend to contain the highest proportion of hydrogen from NADPH (Hayes 2001, Schmidt et al. 2003). This hydrogen has a very low δ^2H value (ca. −250‰ to −600‰; Luo et al. 1991, Schmidt et al. 2003). So, a seaweed taxa that tends to be lipid rich would be expected to have a lower δ^2H value of bulk hydrogen than species that contain lower proportions of lipids. However, our results do not support this hypothesis: Ulvophyceae, which are the algae with lowest δ^2H, do not have more lipids than other algal groups (Fleurence et al. 1994).

Effects from photosynthesis or respiration could also have influence on the bulk δ^2H values of seaweeds. Available evidence is very scarce (Sachse et al. 2012), but indicates that H fractionation in photosynthesis is probably the only important process, as it can be very large (Yakir and DeNiro 1990, Luo et al. 1991), while respiration seems to be negligible (Estep and Hoering 1981). Studies about metabolic pathways that lead to lipid biosynthesis (Sachse et al. 2012), including controlled studies testing for the influence of factors such as growth rates and temperature (Zhang and Sachs 2007, Zhang et al. 2009) have demonstrated that the δ^2H values in some lipids can be affected by these factors.

Environmental influence on δ^2H. This study was not designed to investigate the influence of environmental factors on algal δ^2H. Still, our findings allow some preliminary discussions. Although not significant, the lower δ^2H values for shallower sites in Brazil (Fig. 4A) may reflect these sites being more exposed than those in the deeper sites to freshwater and to dryness. The larger exposure to surface freshwater means that it is possible that the δ^2H value of the H source for these organisms was different from that for organisms in the deeper sites (unless there was significant input of groundwater; Schmidt et al. 2011). The δ^2H value of rainwater, which was not measured, but probably ranges between −10‰ and −20‰ for the sampled areas (Bowen et al. 2005, Bowen 2017), is lower than that of the seawater δ^2H value, near to 0‰ (Hoefs 2009). Thus, the influence of freshwater from precipitation could explain the apparent trend of higher δ^2H values for deeper sites in Brazil. On top of the effects due to change in water source, a difference in salinity could also be partially responsible for higher δ^2H values for the deeper sites because higher salinity tends to give plants a higher δ^2H value (Sachse et al. 2012). The lack of clear pattern for algae collected in Australia regarding site of collection (Fig. 4B) reveals that the potential influence of freshwater from the urban drain (Fig. 1) was not important for seaweed δ^2H composition. It must be emphasized that these considerations are preliminary due to lack of direct water δ^2H measurements.

Dryness can affect algal δ^2H via transpiration, which tends to be faster for the light H isotope (Hoefs 2009). Thus, the effect of transpiration would be to increase the δ^2H value in the algal tissue (or the surrounding water, if in a tidal pool). If transpiration were important for algae in the intertidal zone, those algae at the upper littoral would have higher δ^2H values. This was the opposite of the apparent trend (Fig. 4A). Therefore, it seems that transpiration was not a strong factor determining algal δ^2H in Brazil.

Algae from Brazil came from near the Equator, where the seawater temperature was 27°C ± 0.5°C during the 4 months before collection (NOAA 2013). In Australia, algae were collected in May, which means they were growing (it is unlikely that the collected thalli were older than that, as they were mostly small specimens) during their last 4 months in water with temperatures of 25°C ± 1°C (NOAA 2013). In Argentina, algae came from waters with temperature of 15 ± 2°C (NOAA 2013) during the 4 months before algae were collected (these temperatures are for the open ocean nearby, but should be good approximations for coastal waters). There was no systematic difference among the δ^2H values of algae in a same class from different locations (Fig. 3), which suggests that a difference in temperature between 27°C and 15°C was not an important influence on δ^2H values in seaweeds. This temperature range can be even wider, however, because algae in the intertidal zone are subject to extremes of temperature (Dring 1982). In the present case, some algae from Brazil and Australia probably faced high temperatures approaching 40°C in the tidal pools.

δ^2H: a potential taxonomic tool?. The taxonomic control on δ^2H values could be employed for the study of the phylogeny of seaweeds (Sternberg et al. 1986). The δ^2H values in Ulvophyceae and Bryopsidophyceae were markedly different (Fig. 3), even though both are Chlorophyta. Here, we classified these two groups as two different classes, which is

an accepted practice (Guiry 2012). However, there are lines of evidence that suggest that Brypsidophyceae is an order (Bryopsidales) in the Ulvophyceae (Pröschold and Leliaert 2007, Cocquyt et al. 2010, Friedl and Rybalka 2012). Our results, although not the usual tool for taxonomists, could be used as an argument that it is more likely that Bryopsidophyceae are not in the same group of Ulvophyceae, as their physiologies for H are different. In fact, the Ulvophyceae, if considered as a class that comprises Bryopsidales among other groups, is probably not monophyletic (Zuccarello and Price 2009). This and the differences observed in δ^2H between orders of Phaeophyceae (Table 1) suggest that δ^2H might be useful in the same way to highlight differences among groups with confused taxonomy.

CONCLUSIONS

Ulvophyceae have lower δ^2H values than other algal groups in Argentina, Brazil and Australia (Fig. 3), the difference being likely due to fractionation in H metabolism, rather than environmental factors. The remarkable difference in δ^2H values between Ulvophyceae and other algal groups (Fig. 3), and also between other more specific algal groups (see text for details) indicates that δ^2H has a potential to become a tool to help resolve taxonomic questions for seaweeds. However, the full potential of bulk δ^2H measurements in seaweeds will only be fulfilled when measurements are undertaken using an absolute scale, which will require standards (Meier-Augenstein et al. 2013). Seaweed δ^2H will then have application to coastal food webs. For example, food web subsidy by green tides of Ulvophyceae (Fletcher 1996, Hu et al. 2010).

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