Using multiple stable isotopes including deuterium (δ^2H) to trace organic matter in a complex near-shore lagoon

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ABSTRACT

Stable isotopes are a powerful way to describe and quantify trophic relationships in aquatic systems. Evaluating the ratios of carbon and nitrogen isotopes of consumers and organic matter sources in aquatic systems can answer key ecological questions about the flow of energy between producers and higher trophic levels. However, this method is only feasible when sources have distinct combinations of isotopic ratios. This thesis evaluates the potential for deuterium to improve food web models in aquatic systems based on the large differences in isotopic ratios between primary producers.

Evaluation of hydrogen isotopic ratios from plants and algae collected from marine and freshwater settings revealed differential incorporation of deuterium into plant organic matter. Most aquatic plants were relatively depleted in deuterium, except for macrophytes such as seagrass, which were enriched similar to many types of terrestrial vegetation. This result suggests that interpretations of allochthony from deuterium signatures alone would be complicated in a system where macrophytes are abundant. Large differences were found in hydrogen isotope ratios between different species of macroalgae that cannot usually be differentiated using other isotopes.

Including hydrogen isotopic ratios in a food web model along with carbon and nitrogen revealed sources of organic matter support to *Mercenaria mercenaria* grown in aquaculture pens in the Virginia Coast Reserve. A Bayesian model found that macroalgae was the leading source of organic matter to clam diets, and that hydrogen isotope ratios improved model performance relative to models based only on carbon and nitrogen. The ecological implications to clam feeding are discussed in the context of potential changes to primary production in the Virginia Coast Reserve.

Results indicate that hydrogen isotope ratios can improve food web analysis in coastal and marine systems when used in combination with carbon and nitrogen ratios. However, the hydrogen isotopic composition of primary producers needs to be measured and not assumed based on source water measurements since the variability in hydrogen isotopic ratios among species is not yet predictable. The application of hydrogen isotopes to marine food web studies warrants further research because it has the potential to be a powerful and reliable tool for food web analysis in aquatic systems.

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INTRODUCTION

Stable isotopes are a powerful way to describe and quantify trophic relationships in aquatic systems. Evaluating the ratios of carbon and nitrogen isotopes of consumers and organic matter sources in aquatic systems can answer key ecological questions about the flow of energy between producers and higher trophic levels. However, this method is only feasible when sources have distinct combinations of isotopic ratios. Shallow coastal lagoons often have complex food webs with many possible sources of organic matter that may be difficult to distinguish using the most commonly measured isotopes of carbon and nitrogen. In these ecosystems, additional information is needed to draw conclusions about basal resource use and trophic dynamics. The overall goal of this thesis was to evaluate the potential for hydrogen isotopes to improve food web models in aquatic systems. Hydrogen isotopes have proven useful in distinguishing among primary producers in freshwater systems but have not yet been evaluated in coastal or marine systems.

In the first chapter, I evaluate the ability of hydrogen isotopes to distinguish among primary producers in aquatic ecosystems. The isotope deuterium is incorporated into plant organic matter via photosynthesis. Because of differential loss of the lighter isotope (protium) due to evaporation in stomates, terrestrial plants are enriched in deuterium relative to aquatic plants where such evaporative losses do not occur. Significant differences between sources of interest is key for models to produce unambiguous results and consequently for isotopic data to be useful in food web studies. For example, large differences between hydrogen isotopic ratios of terrestrial and pelagic primary producers can identify the use of allochthonous resources (allochthony) in lakes and rivers. I compared values of hydrogen isotopic ratios from marine plants and algae to those from freshwater plants from rivers and lakes. Although I expected to

find all aquatic plants to be relatively depleted in deuterium, I found that macrophytes such as seagrass were equally enriched similar to many types of terrestrial vegetation. This result suggests that interpretations of allochthony from deuterium signatures alone would be complicated in a system where macrophytes are abundant. I also found large differences in hydrogen isotope ratios between different species of macroalgae that cannot usually be differentiated using other isotopes.

The second chapter uses hydrogen isotopic ratios in a food web model along with carbon and nitrogen to differentiate the organic matter sources supporting a consumer in a marine coastal lagoon. *Mercenaria mercenaria* (L.), the hard clam, is raised in aquaculture growout pens within the Virginia Coast Reserve. This species is economically and ecologically significant for the Eastern Shore of Virginia, where environmental and anthropogenic pressures have the potential to significantly alter the quality and quantity of primary producers that are the ultimate source of food for clams. I used ratios of three stable isotopes in a Bayesian framework to model source contributions to clam diets from eight potential sources of primary production. I found that macroalgae was the leading source of organic matter to clam diets, and that hydrogen isotope ratios improved model performance relative to models based only on carbon and nitrogen.

The results of this thesis indicate that hydrogen isotope ratios can improve food web analysis in coastal and marine systems when used in combination with carbon and nitrogen ratios. In the case of hydrogen the isotopic composition of primary producers needs to be measured and not assumed based on source water measurements and set values of photosynthetic fractionation. The variability in hydrogen isotopic ratios among species is not yet predictable. However with measurement, the source contributions of a variety of primary producers can be determined as in the case of *M. mercenaria* in a Virginia coastal lagoon. I provide strong evidence that clams

selectively feed on high quality macroalgal detritus and describe the ecological implications to clam feeding of potential changes in the Virginia Coast Reserve. This thesis indicates that the application of hydrogen isotopes to marine food web studies warrants further research because it has the potential to be a powerful and reliable tool for food web analysis in all aquatic systems.

CHAPTER 1

Hydrogen isotope fractionation in aquatic primary producers: implications for aquatic food web studies[1]

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Abstract

Large differences in δ^2H of primary producers between aquatic and terrestrial ecosystems can be used to identify subsidies, discriminate between organic matter sources, and reduce uncertainty in carbon flow models. Previous investigations of hydrogen isotope signatures suggest there may be predictable differences between water and organic matter δ^2H for different types of primary producers, but this variability has not been systematically reviewed. We surveyed aquatic and terrestrial primary producers from three water body types (lake, river, coastal lagoon) to compare net fractionation values between water δ^2H and organic matter δ^2H. Although theory predicts large and equivalent fractionation for aquatic vegetation, we found considerable variability among groups of aquatic primary producers. Macroalgae, benthic microalgae, and phytoplankton were more depleted in δ^2H than both aquatic macrophytes and terrestrial vegetation. The terrestrial vegetation enrichment was expected due to fractionation during evapotranspiration; however, the relative enrichment of δ^2H in aquatic macrophytes, even submerged species, was unexpected. Marine macroalgae had high variability in δ^2H signatures as a group, but low variability within distinct species. Variability in fractionation between primary producers should be assessed when hydrogen is used in isotopic approaches to distinguish sources in energy flow models.

Key words: Deuterium, macrophytes, macroalgae, delta D, allochthony

Introduction

Stable isotopes are powerful tools for analysis of trophic dynamics 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 in ecosystems. However, isotopes can produce ambiguous results when the amount of variability within food source signatures is large relative to differences between source signatures. $\delta^{2}H$ may be a powerful complement to other isotopic signatures in mixing models because the relative mass difference between its stable isotopes is the greatest and often results in large differences (>100‰) between sources. The variability in $\delta^{2}H$ among plant and algal types has not been systematically reviewed to determine broader applicability to aquatic food web studies.

Hydrogen incorporated into the organic matter of aquatic plants ultimately comes from hydrogen ions in the surrounding water. Aquatic primary producers are predicted to have $\delta^{2}H$ approximately 170‰ more depleted than environmental water (Yakir and DeNiro 1990; Doucett et al. 2007; Solomon et al. 2011) due to preferential use of ^{1}H during photosynthesis. Theory predicts enriched $\delta^{2}H$ for terrestrial vegetation relative to aquatic primary producers due to biophysical fractionation during evapotranspiration (Roden and Ehleringer 1999; Roden et al. 2000; Barbour et al. 2004; Cuntz et al. 2007). The consistent difference between $\delta^{2}H$ signatures of both planktonic and benthic microalgae and terrestrial plants is used to help identify external organic matter support (hereafter allochthony) in river and lake food webs (e.g. Doucett et al. 2007; Solomon et al. 2011; Cole et al. 2011). However, diffusion of leaf water throughout plants and synthesis of biochemicals also affect the relative abundance of hydrogen isotopes in organic matter (Figure 1), and the magnitude of separation due to evapotranspiration may not be significant in mesic environments (Jardine et al. 2009). Variability in reported $\delta^{2}H$ of aquatic

primary producers is substantial within and between locations (Table S1), but has not been systematically reviewed. Understanding patterns in this variability across groups of aquatic primary producers will inform the design of food web studies and help determine potential applications of hydrogen isotope investigations by clarifying which sources can be reliably distinguished using hydrogen.

Water δ^2H varies geographically due to partitioning in vapor and precipitation (Bowen et al. 2005). The δ^2H value of organic matter is a function of both climate and hydrological conditions that influence environmental water as well as biophysical processes that affect leaf water pools and biochemical pathways like photosynthesis. The net difference between the ratios of deuterium (^2H) to protium (^1H) in plant tissue and the surrounding water is a fractionation value (ε) that can be used to estimate the hydrogen isotope signature (δ^2H) of water or of organic matter (δ^2H_{OM}) when the other value is known such that $\delta^2H_{water} = \delta^2H_{OM} + \varepsilon$, where ε = the sum of all possible hydrogen fractionation processes.

Although hydrogen isotope ratios have long been in use in the analysis of ecological processes (e.g. Macko et al. 1983), recent analytical and technical advances have allowed for improved precision and reliability in measurements of δ^2H (Wassenaar and Hobson 2000) that account for isotopic exchange with ambient water vapor during sample analysis. An emerging focus has been to use deuterium measurements to trace carbon and energy flows through 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). These studies have supported the usefulness of δ^2H as a single or a complementary tracer of organic matter sources in aquatic food webs. Although prior studies have provided insight into the fractionation of hydrogen isotopes in specific plant compounds as well as the processes associated with fractionation (Cuntz et al. 2007; Hou et al.

2008), less is known about hydrogen isotope signatures of bulk organic matter in the context of food web analysis for natural systems.

The variability in isotopic fractionation is fundamental to assessing the utility of natural abundance isotope applications in ecological studies. An understanding of patterns observed in nature should improve the interpretation of models and inform experimental designs used to answer ecological questions. Variability in fractionation processes such as trophic enrichment have been examined for carbon and nitrogen (e.g., Vander Zanden and Rasmussen 2001; Post 2002; McCutchan et al. 2003; Vanderklift and Ponsard 2003). The average and group-specific fractionation values resolved in these analyses have informed mixing models in hundreds of isotopic studies. With the emergence of deuterium as a useful isotope for discriminating organic matter sources, a greater understanding of its fractionation variability in aquatic primary producers is needed both from a theoretical and an empirical perspective. Since δ^2H displays a range of values even for primary producers growing in the same location (e.g. DeNiro and Epstein 1981), the net effects of fractionation may vary based on both biological and environmental factors. Here, we examine those biological and environmental patterns in fractionation for different types of aquatic autotrophs relative to water and in comparison with terrestrial material. We compare values of ε determined for five categories of primary producers collected from three different aquatic ecosystems to evaluate the potential of hydrogen to discriminate between organic matter sources in food web studies. We expected to find large differences in ε between aquatic and terrestrial primary producers but also that the magnitude of difference might vary by location and physiology.

Materials and methods

Isotopic data collection

Water, plant, and algae samples were collected in three types of aquatic ecosystems (coastal lagoon, river, and lakes) for separate investigations of organic matter support to aquatic food webs. We measured δ^2H of these water and tissue samples and categorized primary producers into five ecological and taxonomic groups: terrestrial vegetation, macrophytes, macroalgae, benthic algae, 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 non-vascular aquatic species were categorized as either macroalgae (MA) or microalgae based on multi-cellularity. 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 lagoons and barrier islands off the Eastern Shore of Virginia. The river samples were from the Hudson River between Nyack and Cheviot, New York. The lake samples were from the University of Notre Dame Environmental Research Center (UNDERC) located near Land O'Lakes, Wisconsin.

We collected larger plants as grab samples from both aquatic and terrestrial environments and sampled microalgae from benthic and pelagic environments. Multicellular seaweeds such as *Gracilaria* and *Ulva* were classified as macroalgae and comprised eight species, most of which were found in the coastal marine system. These macroalgae were subtidal and not exposed to air during any part of the tidal cycle. *Chara,* found in the lake system, is taxonomically classified as green algae but also considered a developmental step between macroalgae and embryophytes. There was no morphological equivalent to macroalgae sampled in the Hudson River. Vascular

aquatic plants were categorized as macrophytes and included thirteen species of plants such as seagrass, pondweeds, and water lilies. Macrophytes were also classified according to their habitat as emergent, floating, or submerged in order to evaluate the influence of exposure to air. Emergent plants had variable exposure to air based on height of the plant or due to the tidal cycle; floating plants had leaves on the surface of water permanently exposed to air; and submerged plants only grew underwater. In the two freshwater systems we collected benthic microalgae as scrapings from tiles, natural rock, or wood substrate. High levels of sediment resuspension made this method not feasible 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. Terrestrial vegetation comprised broadleaf deciduous and evergreen species, and also moss *(Sphagnum)* and shrub *(Chamaedaphne)* in the lake system. Below, we refer only to terrestrial vegetation and macrophytes as plants, and we use primary producers to describe the polyphyletic grouping that includes algae.

Field samples were rinsed thoroughly to remove any salts and dried at 60°C for at least 24 hours 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 and isotope ratio values are reported in the standard del (‰) notation relative to Vienna Standard Mean Ocean Water (VSMOW). Sample values were calibrated to local water vapor according to Wassenaar and Hobson (2003) and compared to a suite of normalization reference standards including powdered caribou hoof and powdered kudo horn (Doucett et al.

2007). 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).

Environmental 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 dataset, δ^2H was estimated from the location of the sampling site based on a logarithmic relationship consistent with the rainout effect between the 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 Web of Science. We calculated hydrogen fractionation from these data when environmental water isotope signatures were also reported.

Calculating fractionation

We define the fractionation term ε as the difference between the organic matter and environmental water calculated as: $\varepsilon = \Delta\delta^2H = \delta^2H_{water} - \delta^2H_{OM}$. Fractionation ($\varepsilon$) is the sum of all possible hydrogen fractionation processes: $\varepsilon = \sum(\varepsilon_{lw} + \varepsilon_{bio} + \varepsilon_{?})$, where ε_{lw} is biophysical fractionation in leaf water due to uptake, transport, and evapotranspiration; ε_{bio} is biochemical fractionation due to photosynthesis, biosynthesis of lipids, and heterotrophic carbohydrate metabolism, and $\varepsilon_{?}$ represents all other possible hydrogen fractionation processes (Figure 1). Measurements of these separate fractionation values were not within the scope of this study but their implications are discussed. Since organic matter samples were always more depleted in deuterium than water ($\delta^2H_{OM} < \delta^2H_{water} < 0$), ε values are positive. A large ε indicates a greater

depletion in deuterium and more negative δ^2H_{OM}; smaller ε values indicate relatively enriched organic matter and a less negative δ^2H_{OM}. The fractionation term α can be calculated from ε as $\alpha = \varepsilon/1000 + 1$.

Statistical tests

In order to determine utility of hydrogen signatures in separating organic matter sources in food web studies, non-parametric Kruskall–Wallis tests were performed to test for significant differences in fractionation between categories of producers at $\alpha=0.05$ (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 fractionation values instead of hydrogen signatures to eliminate variability associated with environmental water signatures among the different sites. Differences were evaluated with post hoc exacted Wilcoxon Mann–Whitney rank sum tests with Bonferroni corrections (Sokal and Rohlf 1995). Statistical tests were performed in R (R Development Core Team 2011, URL http://www.R-project.org).

Results

Isotope data

We calculated ε for 248 plant samples that were classified into five categories based on morphology and sampling method (Table 1). Environmental water signatures in the coastal system were relatively consistent throughout all seasons (-9.99 ± 0.9‰). Water signatures from freshwater systems varied more in space and time. Water from the Hudson River varied between

-43‰ and -71‰ between sampling locations. Samples were collected from 18 different lakes that had δ^2H values that varied between -40‰ and -75‰.

All of the plant and algal samples were depleted in deuterium relative to water, ranging from 38–220‰ more depleted than corresponding water signatures (Table 1). Primary producers with the largest fractionation values have the most negative (i.e. depleted) δ^2H values. Phytoplankton and benthic algae in the lake and river system had the largest fractionation values while terrestrial vegetation and aquatic macrophytes had the smallest values. The category of primary producers with the most variability was marine macroalgae (s.d. of 47‰). Most species of macroalgae had variation within ranges comparable to other groups (s.d. ~15‰), but across all species within this group, the ε values varied from 95‰ for *Codium* to 200‰ for *Ulva* and *Enteromorpha*. We also observed large fractionation values in filamentous green algae in the lake system. Both terrestrial plants and aquatic macrophytes, even submergent species of macrophytes such as the submergent rooted grasses like *Zostera marina* and *Valisneria americana*, were relatively enriched in deuterium compared to algae.

Comparison of Plant Groups

We first contrasted the differences in fractionation between groups of primary producers when data for all three systems (i.e. lake, river, coastal) were combined. Differences in fractionation indicate that hydrogen isotope signatures vary among sources of organic matter (Figure 2a). A non-parametric Kruskall–Wallis test revealed a significant effect of primary producer type on fractionation values ($K = 129.1$, $p < 0.001$; $n = 248$). Post hoc tests revealed significant differences between phytoplankton and benthic algae ($p < 0.001$). Phytoplankton had the greatest average fractionation values (164.36‰ ± 24.3). Macroalgae were not significantly

different from benthic algae or phytoplankton, but were significantly different from both macrophytes ($p < 0.0001$) and terrestrial vegetation ($p < 0.0001$). Terrestrial vegetation and macrophytes had the lowest fractionation values (80.97‰ ± 18.3 and 78.31‰ ± 13.7) and these groups were not significantly different. Emergent, floating, and submergent types of macrophytes were not significantly different from each other ($K = 2.9098$, $p > 0.2$).

When each system was considered independently, fractionation values of the groups we considered displayed similar patterns (Figure 2b, c, d). In the lake system, both groups of higher plants had smaller values of ε than either type of microalgae, but macroalgae were not significantly different than any of the other groups (Figure 2b). The group of samples categorized as macroalgae in this system comprised both muskgrass *(Chara sp.)* and filamentous epilithic green algae. Hydrogen signatures of *Chara* were highly enriched in deuterium (ε = 46.62‰ ± 13.0) relative to those of the filamentous green algae (ε = 204.26‰ ± 27.9). When *Chara* were either excluded from the macroalgae group or classified as macrophytes, macroalgal fractionation values were significantly different than each of the other plant types ($p < 0.01$). When *Chara* was included in the macrophyte group, macrophyte fractionation values were significantly different from terrestrial plants ($p < 0.05$).

In the river system, terrestrial vegetation and macrophyte had the smallest values of ε (55.24‰ ± 12.8; 70.29‰ ± 13.1) and were not significantly different (Figure 2c). Phytoplankton fractionation values were higher (180.98‰ ± 25.4) and significantly different than both of groups of higher plants ($p < 0.001$). Benthic algae collected from the river had similarly large fractionation values (185.61‰ ± 11.6), but had a low sample size ($n = 4$), and were not significantly different from phytoplankton, macrophytes, or terrestrial vegetation.

Fractionation values of primary producers in the coastal system were significantly different among groups ($K = 49.05$, $p < 0.001$). Each of the aquatic primary producer groups was significantly different but none was significantly different from terrestrial vegetation (Figure 2d). Terrestrial vegetation was also not significantly different from any other groups when macroalgal and macrophyte genera were each considered separately. Post hoc pairwise comparisons between genera revealed significant differences between several types of macroalgae. *Ulva* was significantly different from each of the other macroalgae ($p < 0.05$). Within the group of macrophtyes, the submerged seagrass *Zostera marina* was not significantly different from the emergent marsh grass *Spartina alterniflora*.

Although patterns of fractionation between groups were relatively consistent across all three systems, fractionation values of each group differed significantly among systems for all of the groups except for macroalgae. Kruskall–Wallis tests revealed significant differences between sites for groups of macrophytes $K = 11.48$, $p < 0.01$). The coastal macrophytes sampled comprised an emergent marsh grass, *Spartina alterniflora,* and a submerged seagrass, *Zostera marina*. Post hoc comparisons showed that fractionation values of these marine macrophytes were significantly larger than macrophytes found in either of the freshwater systems ($p < 0.05$).

Fractionation values of benthic algae were significantly different among the three sites ($K = 16.48$, $p < 0.001$). The largest values were in the river system and the smallest were observed in the coastal system. Benthic algal fractionation values in the lake system were significantly different than those in both the river ($p < 0.01$) and coastal ($p < 0.01$) system. Phytoplankton fractionation values were also significantly larger in the river system than in the lake system ($K = 10.76$, $p < 0.01$).

Terrestrial vegetation fractionation values were also significantly different ($K = 20.00$, $p < 0.001$) but displayed the opposite pattern across systems. Plants from the coastal system had the largest fractionation values and plants from the river system had the smallest fractionation values. Trees sampled in the lake ecosystem had fractionation values significantly different than those in the coastal ($p < 0.05$) or river ($p < 0.001$) system.

Discussion

We found a consistent pattern of hydrogen isotope fractionation for groups of primary producers in three types of aquatic systems, supporting the utility of hydrogen as a useful tool to distinguish energy sources in aquatic food webs. Our results are consistent with other observations (Epstein et al. 1976; Doucett et al. 2006; Finlay et al. 2010; Caraco et al. 2010) that algae are strongly depleted in δ^2H compared to terrestrial plants, however some of the other variability we observed is not yet accounted for in empirical or theoretical studies using hydrogen isotopes. Our results indicate that ε can be used to differentiate between groups of aquatic primary producers but not to predict source δ^2H with precision. In freshwater systems, terrestrial vegetation can be distinguished from aquatic primary producers except for vascular aquatic plants. Identification of allochthony via δ^2H may therefore be complicated in lakes or rivers where macrophytes are a significant part of the food web. With a more complete understanding of the environmental and physiological influences on hydrogen fractionation, problems associated with using estimates from literature of water or plant δ^2H to constrain ε may become surmountable without introducing a large amount of uncertainty into isotopic models.

Water δ^2H variability

Climate and hydrologic conditions influence the isotopic signature of meteoric, surface, and ground- water (Schiegl and Vogel 1970; Bowen et al. 2005) and therefore hydrogen isotope values vary across systems. The freshwater lake and river systems we considered were predictably more depleted in deuterium than the coastal marine system due to the long residence time of seawater (Kendall and Coplen 2001). Variability in δ^2H between lakes and along the Hudson River suggests that seasonal, continental, and precipitation effects influence these freshwater systems (Kendall and Coplen 2001). Differences in enrichment due to evaporation may vary across lakes with different surface area to volume ratios, while intense mixing and exchange with the ocean maintains more constant values in the coastal system.

Variability in water δ^2H influences the δ^2H of plant organic matter and could mask otherwise consistent fractionation in plants. This water-driven temporal variability in source δ^2H can transfer up the food web and complicate interpretation of mixing model results for organisms with long tissue turnover times. Comparisons of hydrogen signatures from different systems should account for isotopic differences in environmental 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 water δ^2H, a difference in consumer δ^2H could be interpreted as either a difference in resource use or as a difference in environmental conditions affecting the same resource. Environmental variation would affect δ^2H of organic matter the most for primary producers with high rates of tissue turnover.

In the context of food web analyses, published hydrogen isotope signatures should only be used from a separate location or study if differences in initial water signatures are taken into account, especially for freshwater ecosystems. In conjunction with the global database of water isotope values, (GNIP, administered International Atomic Energy Association and the World

Meteorological Organization), this could be possible in the future with a growing dataset of hydrogen isotope signatures from similar algae and plant types across systems to constrain estimates of hydrogen fractionation values.

Biophysical influences on ε

We observed smaller fractionation values in terrestrial plants relative to those of most aquatic primary producers, consistent with evaporative enrichment in leaf water. However, aquatic macrophytes had similar fractionation to terrestrial plants, even for submerged species. These data suggest that isotopic enrichment in leaf water of terrestrial plants is not the main explanation for differences between δ^2H in algae and terrestrial plants since functional stomata are not a sole predictor of hydrogen enrichment. While some emergent and floating-leafed macrophytes do evapotranspire, stomata on most submerged aquatics are considered non-functional because of wax occlusions (Sculthorpe 1967), which prevent evapotranspiration even if exposed to air. The observed similarities in fractionation for macrophytes and terrestrial vegetation in our data may reflect differences in the components and magnitudes of ε_{lw} and ε_{bio} for aquatic and terrestrial plants. In aquatic plants where there is no evapotranspiration ε_{lw} is negligible, but an enrichment of the same magnitude due to processing of carbohydrates in the formation of starches and other storage materials would result in the same net difference from water. Fractionation during uptake from soil water to roots or during transport to leaves could occur in water prior to evapotranspiration and incorporation into leaf tissue. Although uptake by roots is not traditionally considered as a fractionation process (Walker and Richardson 1991), depletion up to 9‰ during root uptake of water has been observed in some halophytes (Ellsworth

and Williams 2007) and may therefore account for some of the fractionation we observed in rooted plants.

In addition to evapotranspiration, other biophysical influences in 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 water heterogeneity and, consequently, the influence of ε_{lw} on leaf water at the site of metabolism. The differences we observed in fractionation values between the three systems may be related to differences in environmental conditions like relative humidity or physiological differences between the plants found at each location.

Biochemical influences on ε

We found large fractionation values consistent with 170‰ depletion due to photosynthesis in phytoplankton, some macroalgae, and benthic algae in the river system. The δ^2H of these primary producers relative to water suggest that most of the organic matter in these organisms is starch produced directly from chloroplasts in photosynthesis. The most deuterium-depleted primary producers we observed were microalgae and filamentous macroalgae. These fractionation values were greater than the 170‰ difference expected from photosynthesis. Since lipid biosynthesis pathways strongly fractionate against deuterium (e.g. Sessions et al. 1999), $\varepsilon >$ 170‰ may be an indication of species where lipids are a substantial component of plant biomass. However, the highest lipid contents we found reported in the literature for the most deuterium-depleted algae in our dataset were only 6.4% and 9% for *Ulva* and *Enteromorpha* respectively

(Wahbeh 1997), which is likely not high enough to explain the additional 30‰ depletion observed in these algae.

Variability in ε for benthic microalgae across our systems could be related to differences in community composition of the organisms we sampled. Although inspection suggested algae dominated our samples, other microbes were present. Some benthic bacteria may rely on sources of hydrogen that vary in δ^2H based on different methanotrophic pathways (Deines et al. 2009). Additionally, although uptake is not considered a fractionating process for hydrogen, variable availability in hydrogen ions modified by boundary layer conditions could differ between types of autotrophs.

In terrestrial plants, enriched δ^2H is used as empirical evidence for identifying Crassulacean acid metabolism (CAM) because C3, C4, and CAM photosynthetic pathways in land plants each have distinct combinations of $\delta^{13}C$ and δ^2H signatures (Ziegler et al. 1976; Sternberg and DeNiro 1983; Sternberg et al. 1984). We do not find this predictable separation of carbon and hydrogen signatures in our data (Fig. 3): both carbon and hydrogen signatures are highly variable. These patterns are consistent with the high variability previously observed in δ^2H of cellulose nitrate of brown and green algae (Sternberg et al. 1986).

Terrestrial succulent plants use CAM as an adaption to water stress—an adaptation not expected in the aquatic environment. However, CAM has been inferred in aquatic species by observations of substantial diel hydrogen ion changes (5–290 mmol H^+ kg^{-1}) (Keeley 1998), which indicates the operation of acid metabolism. 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. In the absence of aquatic acid metabolism, some aquatic primary producers use bicarbonate.

Bicarbonate uptake in aquatic plants and algae is widely observed (Table 2), however genera that use CO_2 are not more or less enriched than those that use HCO_3^- (Fig. 3). While the combination of δ^2H and $\delta^{13}C$ is useful in identifying three distinct photosynthetic pathways in terrestrial plants, this combination did not seem to be associated with the variability in carbon metabolism of aquatic primary producers.

The high variability we found for ε in macroalgae may be a promising area to investigate for a more complete understanding of drivers of δ^2H signatures. *Chara,* a completely submergent macroalgae that grows in deep 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 *Agardhiella* and *Codium* were also enriched in deuterium relative to more simply structured *Ulva*, which grows as sheets only two cells thick. The balance of photosynthate produced from water and from stored reserves may be the driving influence of δ^2H variability between these aquatic primary producers.

Conclusions

We found large and consistent differences in hydrogen fractionation between groups of aquatic primary producers across freshwater and marine ecosystems: relative depletion in microalgae, high variability in macroalgae, and relative enrichment in both terrestrial vegetation and aquatic macrophytes. These patterns can be used to partition energy sources in many aquatic ecosystems with a high degree of certainty. Hydrogen isotopes may be less useful in distinguishing sources in aquatic food webs where both macrophytes and terrestrial vegetation are important, or when many species of marine macroalgae are considered as a single source.

Variability within groups observed here supports the idea of DeNiro and Epstein (1981) that fractionation is under biological control but modified by environmental factors. Food web studies using hydrogen isotopes must account for variability in fractionation, and causes of the large variability of ε in macroalgae require further investigation.

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Figure 1.1 Conceptual diagram of fractionation processes affecting net ε 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, e.g. evapotranspiration is an enrichment process of known magnitude and heterotrophic carbon metabolism is an enrichment process of variable magnitude. (*evapotranspiration*: Roden and Ehleringer 1999; Cuntz et al. 2007; Roden et al. 2000; Barbour et al. 2004; *diffusion:* Roden and Ehleringer 1999; Yakir et al. 1989; Flanagan et al. 1991; Shu et al. 2008; *photosynthesis*: Solomon et al. 2011; Yakir and DeNiro 1990; Luo and Sternberg 1991; but see Luo et al. 1991; *heterotrophic carbon metabolism*: Yakir, 1992; Sessions 2006; Luo and Sternberg 1992; *biochemical synthesis*: Yakir, 1992; Sternberg et al. 1986; Yakir and DeNiro 1990; Sessions et al 1999).

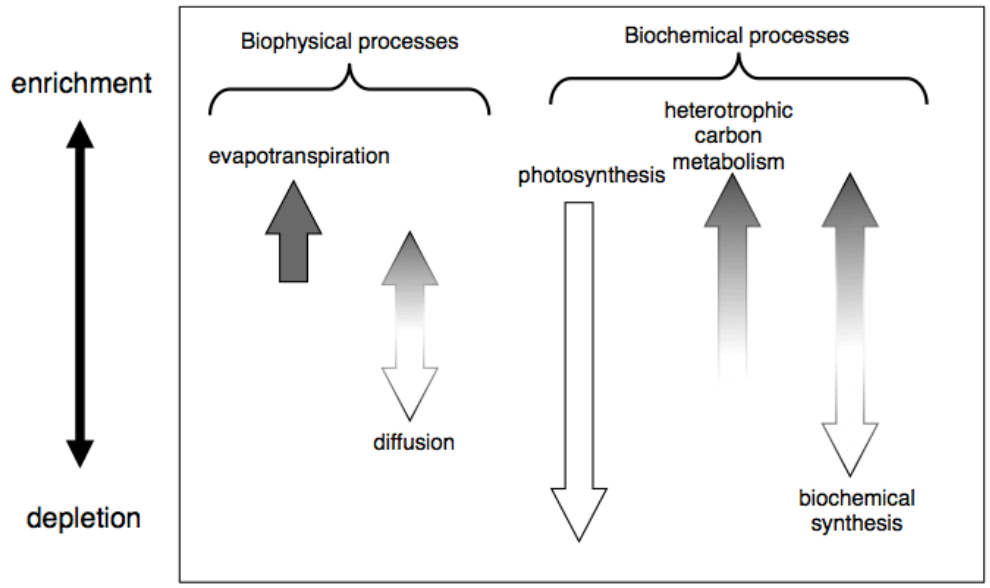

Figure 1.2 Mean values of fractionation (ε) between water δ^2H and organic matter δ^2H for 5 categories of primary producers from 3 different watershed types. $\varepsilon = \Delta\delta^2H = \delta^2H_{water} - \delta^2H_{om}$. The upper left panel summarizes data from all 3 systems. Categories are abbreviated as follows: MA = macroalgae; MP = macrophytes; BMA = benthic microalgae; PHY = phytoplankton; TV = terrestrial vegetation. Error bars show standard error; 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.

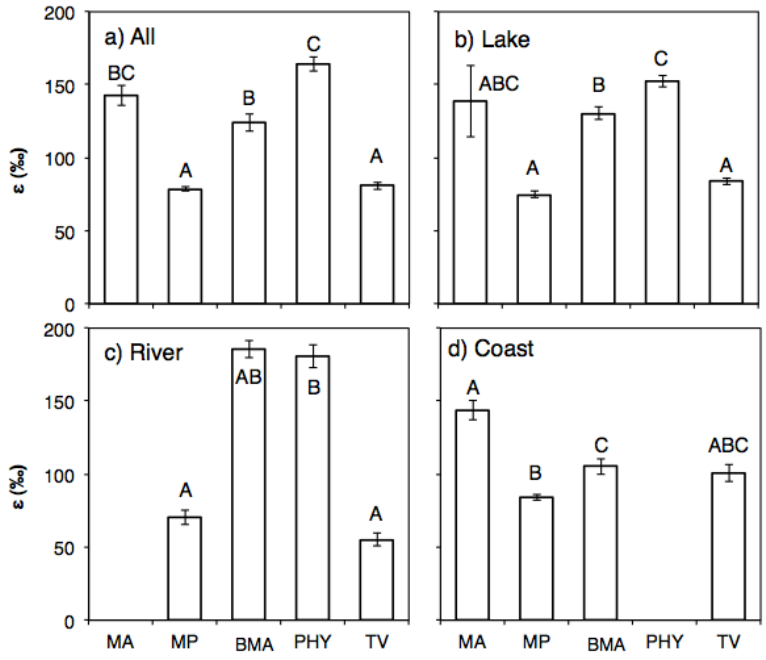

Figure 1.3 Hydrogen fractionation (ε_H) and $\delta^{13}C$ of aquatic plant genera. Circles are macroalgae species; triangles are macrophyte species. Coloring indicates evidence from literature (Table 2) of main carbon sources: gray filled in symbols are species that can use both HCO_3^- and CO_2, dark filled in symbols are species that are obligate CO_2 users (CAM), and open symbols are species that are assumed to use HCO_3^-.

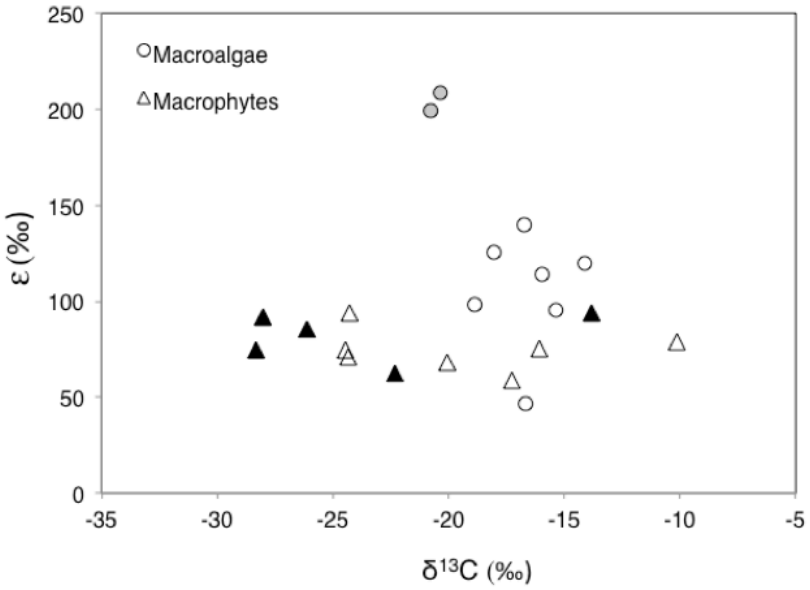

Table 1.1 Mean hydrogen fractionation values between water and organic matter grouped by primary producer types considered in this study.

	Description	ε ± s.d (‰)	n	System
Macroalgae	*Agardhiella*	97.93 ± 14	8	Coast
	Chara	46.38 ± 12	6	Lake
	Codium fragile	95.41 ± 13	8	Coast
	Ectocarpus	140.05	1	Coast
	Enteromorpha	208.38	1	Coast
	Gracilaria vermiculophylla	125.38 ± 15.2	14	Coast
	Scytosiphon	119.42 ± 9	2	Coast
	Ulva lactuca	199.04 ± 16	18	Coast
	filamentous algae	204.26 ± 28	7	Lake
	All macroalgae	**142.73 ± 55**	**64**	
Macrophytes	*Brasenia schreberi*	68.34 ± 20	6	Lake
	Isoetes	85.63 ± 6	3	Lake
	Myroiphyllum fawelli	95.21	1	Lake
	Najas	58.98	1	Lake
	Nuphar variegata	74.30 ± 9	6	Lake
	Nymphea odorata	70.95 ± 7	6	Lake
	Pontederia cordata	74.36 ± 4	2	Lake
	Potamogeton pusillus	73.10 ± 8	8	Lake
	Sparganium angustifolium	89.58 ± 7	5	Lake
	Spartina alterniflora	93.81 ± 12	13	Coast
	*Trapa natans***	62 ± 7	5	River
	Vallisneria americana	62.65 ±3	4	River
	Zostera marina	78.63 ± 9	22	Coast
	All macrophytes	**78.31 ± 14**	**82**	
Benthic microalgae	Benthic microalgae (rock scrapings)	185.61 ± 12	4	River
	Benthic microalgae (tile scrapings)	130.35 ± 12	9	Lake
	Benthic microalgae (vertical migration)	105.3 ±22	16	Coast
	All benthic microalgae	**124.15 ± 32**	**29**	
Phytoplankton	Blue green algae	207 ± 17	5	River
	Diatoms	161.22 ± 3	5	River
	net tows	152 ± 18	10	Lake
	regrowth cultures*	153.75 ± 1	4	Lake
	All phytoplankton	**164.36 ± 24**	**24**	
Terrestrial vegetation	Broadleaf/deciduous	107.90 ± 11	3	Coast
	Evergreen	89.85 ± 2	2	Coast
	Broadleaf/deciduous	74.81 ± 7	20	Lake
	Evergreen	90.21 ± 7	12	Lake

Broadleaf/deciduous	51.75 ± 9	7	River
Evergreen	79.91	1	River
Spaghnum	77.89 ± 10	4	Lake
Chamaedaphne	113.40 ± 7	4	Lake
All terrestrial vegetation	**80.97 ± 18**	**49**	

*from Solomon et al. 2011

**from Caraco et al. 2010

Table 1.2 Evidence of bicarbonate usage and acid metabolism by macrophytes and macroalgae species in this study.

	Genera	Source	Carbon source	evidence
Macrophytes	*Pontederia cordata*	Pagano and Titus 2007	CO_2	pH drift technique
	Isoetes	Keeley and Bowes 1982; Keeley 1998; Boston et al. 1989	CO_2	anatomy, observed pH changes
	Myriophyllum fawelli	Hough and Wetzel 1977; Allen and Spence; Boston et al. 1989	HCO_3^-	anatomy
	Potamogeton crispus	Hough and Wetzel 1977; Sand-Jensen 1982; Sand-Jensen et al. 1992; Allen and Spence 1981	HCO_3^-	anatomy
	Sparganium angustifolium	Sand-Jensen et al. 1982; Sand-Jensen et al 1992	CO_2	gas measurements
	Spartina alterniflora	Hwang and Morris 1992	CO_2	isotopic tracer
	Valisneria americana	Keeley 1998	CO_2	anatomy
	Zostera marina	Boston et al. 1989; Hellblom et al 2001	HCO_3^-	proton pump, CA
Macroalgae	*Chara*	Ray et al. 2003; Allen and Spence 1981; Lucas et al. 1983	HCO_3^-	proton pump, internal CA, external CA, anion exchange
	Cladophera	Raven 1982; Kremer 1981	HCO_3^-	enzyme activity
	Codium fragile	Maberly et al. 1990; Kremer 1981	HCO_3^-	enzyme activity
	Enteromorpha flexuosa	Maberly et al. 1990; Jolliffee and Tregunna 1970	CO_2 and HCO_3^-	pH drift technique
	Fucus vesiculosus	Maberly et al. 1990; Kremer 1981	HCO_3^-	enzyme activity
	Gracilaria vermiculophyla	Andria et al. 1999; Bidwell and McLachlan 1985; Zou et al. 2004	HCO_3^-	internal CA, external CA
	Polysiphonia	Maberly et al. 1990	HCO_3^-	pH drift technique
	Ulva lactuca	Maberly et al. 1990; Bidwell and McLachlan 1985; Jolliffee and Tregunna 1970; Cornwall et al. 2012	CO_2 and HCO_3^-	modeled and observed pH changes

Table 1.S1 Supplemental Material

Hydrogen fractionation calculated from literature data where both organic matter and environmental water isotope signatures were reported. Asterisk (*) indicates studies that reported using benchtop equilibration and high temperature pyrolysis in isotopic measurements.

	Reference	Autotroph	δ^2H organic matter	δ^2H water	ε
Macroalgae	Fenton and Ritz 1988	*Acrocarpia paniculata*	-88	6	94
	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	102
	Estep and Dabrowski 1980	*Chondrus crispus*	-103	2	105
	Finlay et al. 2010*	*Cladophora*	-195	-48	146
	Finlay et al. 2010*	*Cladophora*	-214	-48	166
	Finlay et al. 2010*	*Cladophora*	-229	-46	183
	Finlay et al. 2010*	*Cladophora*	-242	-46	195
	Smith and Epstein 1970	*Corallina chilense*	-47	-9	38
	Fenton and Ritz 1988	*Eklonia radiata*	-39	6	45
	Estep and Dabrowski 1980	*Enteromorpha clathrata*	-174	0	174
	Smith and Epstein 1970	*Enteromorpha marginata*	-72	-12	60
	Doucett et al. 2007*	Filamentous algae	-240	-104	136
	Doucett et al. 2007*	Filamentous algae	-292	-107	185
	Doucett et al. 2007*	Filamentous algae	-277	-81	197
	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	128
	Smith and Epstein 1970	*Gigartina cristata*	-88	-9	79
	Fenton and Ritz 1988	*Gigartina sp.*	-41	6	47
	Smith and Epstein 1970	*Grateloupia setchellii*	-94	-9	85
	Schlieg and Vogel 1970	Green seaweed	-57	0	57
	Schlieg and Vogel 1970	Green seaweed	-103	0	103
	Fenton and Ritz 1988	*Heterozostera tasmanica*	-83	3	86
	Fenton and Ritz 1988	*Hormosira banksii*	-31	3	34
	Finlay et al. 2010*	*Lemanea*	-136	-48	88
	Finlay et al. 2010*	*Lemanea*	-138	-46	92
	Smith and Epstein 1970	*Macrocystis pyrifera*	-70	-9	61
	Schlieg and Vogel 1970	Mosslike alga	-166	0	166
	Finlay et al. 2010*	*Nostoc*	-184	-48	135

	Finlay et al. 2010*	*Nostoc*	-196	-47	149
	Finlay et al. 2010*	*Nostoc*	-218	-48	170
	Finlay et al. 2010*	*Nostoc*	-226	-46	180
	Estep and Dabrowski 1980	*Ulva lacutca*	-166	2	168
	Estep and Dabrowski 1980	*Ulva lacutca*	-180	12	192
	Fenton and Ritz 1988	*Ulva spathulata*	-107	3	110
	Fenton and Ritz 1988	*Ulva taeniata*	-70	6	76
	total				**117 ± 49**
Macrophytes	Smith and Epstein 1970	*Frankenia grandifolia*	-61	-12	49
	Smith and Epstein 1970	*Phyllospadix torreyi*	-17	-9	8
	Smith and Epstein 1970	*Salicornia bigelovii*	-82	-12	70
	Smith and Epstein 1970	*Zostera marina*	-76	-10	66
	total				**48 ± 28**
Benthic algae	Solomon et al. 2011*	Benthic algae	-173	-52	121
	Solomon et al. 2011*	Benthic algae	-174	-44	130
	Solomon et al. 2011*	Benthic algae	-180	-44	136
	Solomon et al. 2011*	Benthic algae	-189	-40	149
	Cole et al. 2011*	Benthic algae	-180	-43	137
	Cole et al. 2011*	Benthic algae	-186	-40	146
	Finlay et al. 2010*	Diatoms	-146	-48	97
	Doucett et al. 2007*	Diatoms	-214	-103	111
	Doucett et al. 2007*	Diatoms	-231	-104	127
	Finlay et al. 2010*	Diatoms	-185	-46	140
	Finlay et al. 2010*	Diatoms	-190	-48	142
	Doucett et al. 2007*	Diatoms	-251	-81	171
	total				**134 ±19**
Phytoplankton	Cole et al. 2011*	Phytoplankton	-198	-43	155
	Cole et al. 2011*	Phytoplankton	-195	-40	155
	total				**155**

CHAPTER 2

Macroalgal support of cultured hard clams in a low nitrogen coastal lagoon

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Abstract

Bivalves play significant roles in both the ecology and the economy of coastal regions. By filter-feeding on particles in the water column, these organisms reduce turbidity and connect benthic and pelagic production. In addition to the indirect benefits of improved water quality, production and sales of harvested livestock are major sources of income in coastal areas like the Eastern Shore of Virginia (USA). Phytoplankton are known to be a main food source to bivalves; however, low chlorophyll waters off the Virginia coast support extensive aquaculture of *Mercenaria mercenaria* (hard clams). The ultimate energy sources supporting these clams are uncertain but significant because seagrass restoration, sea level rise, and climate change will potentially change the quality and quantity of primary production available to these populations. We measured ^{13}C, ^{15}N, and ^{2}H isotopic composition of aquaculture clams and a variety of primary producers in a Virginia coastal lagoon over a seasonal cycle and conducted a Bayesian mixing model analysis to identify current energy sources of clams. By using a third isotope, ^{2}H, we were able to improve precision over a 2-isotope model, as well as partially resolve source contributions from individual species of macroalgae. Our analysis reveals that clams are significantly supported by microalgae (23–44%) but gain most of their energy from macroalgae (55–66%), and only a small fraction from macrophytes (0–14%). While macroalgae are often an indicator of coastal eutrophication, these highly nutritious algae can be an important food source to bivalves when abundant in low nitrogen, oligotrophic systems.

Keywords: Clams, *Mercenaria mercenaria*, SIAR, hydrogen isotopes, macroalgae, aquaculture

Introduction

Although *Mercenaria mercenaria* (hard clam) is one of the most abundantly harvested and cultured species along the Atlantic coast of the US, the dominant environmental influences on its growth are not well understood (Henry and Nixon 2008). Many studies conclude that food supply controls the growth of bivalves (Dame 1996, Bayne 1998, Weiss et al. 2002, Carmichael et al. 2004); so successful field culture of clams depends on sufficient production and supply of appropriate food. While feedstock in artificial aquaria can be controlled to provide highly nutritious material, aquacultured clams are often held in field grow-out pens to reach market size where they are subject to *in situ* food sources and fluctuations in environmental conditions. Clams may respond positively to short-term changes in food supply from increased nitrogen loads that stimulate algal production (Weiss et al. 2002, Carmichael et al. 2004), but excess N-inputs can result in reduced sediment oxygen or harmful algal blooms that are detrimental to clam growth (Carmichael et al. 2012).

Low chlorophyll waters in the coastal lagoons of Virginia (USA) support dense populations of hard clams in aquaculture beds. In these lagoons between the barrier islands and the mainland, hard clams are extensively cultured (Murray and Kirkley 2005). In field grow-out pens, clams meet their energetic requirements by feeding on the mixture of naturally occurring organic material in the water column. This seston comprises a variety of material including microalgae, cellulose-rich detrital particles from the degradation of vascular plants, and macroalgal detritus, as well as microorganisms and small metazoans, and resuspended sediment. Resuspended sediment from wind-driven turbulence can result in high turbidity (Lawson et al. 2007) in shallow lagoons; at our study location organic material usually makes up less than 25% of the total suspended solid load in the water (Figure 1).

Although traditional ideas regarding feeding strategies and food availability of coastal bivalves have linked their growth and reproductive activities to annual cycles of phytoplankton production (e.g. Boon et al. 1998, Ansell et al. 1980), aquacultured clams in Virginia coastal bays may be relying on sources of primary production that are more prevalent than phytoplankton (mean chlorophyll *a* < 5 μg L^{-1}) such as benthic algae, macroalgae, seagrasses, and marsh grasses. A literature review of studies that evaluated marine bivalve diets using stable isotopes (Table 1) supports the view that phytoplankton and benthic microalgae are the dominant food sources for these and most bivalve suspension feeders. However, planktonic algal communities vary widely in nutritional quality, and standard measures of water quality such as chlorophyll or total organic content do not consistently reflect relative food value to correlate to growth performance (Beukema & Cadee 1991, Grant 1996, Hawkins et al. 1998). O'Donnell et al. (2003) linked diet shifts in Virginia clams with changes in primary production due to rising sea level from historical reliance on benthic algae and terrestrial detritus to modern use of phytoplankton and *Spartina alterniflora*. Rising sea level (Erwin et al. 2006), increases in nitrogen loading from agricultural activities (Henry and Cerrato 2007, Giordano et al. 2011, Carmichael et al. 2012), and large-scale seagrass restoration (Orth et al. 2012) will potentially affect Virginia clam populations by altering the quality and quantity of the food supply (Havens et al. 2001, Kirwan et al. 2012, Carr et al. 2012). These large-scale changes could impact the success of aquaculture operations as well as the ecosystem services clams provide (Lonsdale et al. 2009).

Clams will also have direct physiological responses to changes in environmental parameters like temperature (Joyner-Matos et al. 2009), pH (Waldbusser et al. 2010, Talmage & Gobler 2011) and turbidity (Ellis et al. 2002, Wall et al. 2011) in addition to indirect responses to

habitat degradation from hypoxia, increased predation, and other factors (Whetstone & Eversole 1981, Norkko et al. 2006, Henry and Cerrato 2007, Carmichael et al. 2012). As clams feed, they increase light penetration in the water column, couple benthic and pelagic production, and can maintain high water quality by promoting dominance of large nutritious algae (Lonsdale et al. 2009). Since both the production and the ecological significance of bivalves depend on their feeding behavior (Hawkins et al. 1998, Lonsdale et al. 2009), knowing the sources of support to clam diets is critical. Nutrition and growth studies are complicated by not only the variability in food quality and quantity in natural systems, but also by the complex feeding physiology of clams that includes both pre- and post-ingestive selection of food items (Kraeuter & Castagna 2001). Uncertainty also arises from inconsistencies between laboratory results and *in situ* studies on feeding in bivalves (Grizzle et al. 2008). Therefore, indirect determination of the major energy source contributions that sustain clam aquaculture populations is complex and may not be reliable. Comparing clam tissue isotopic ratios to isotopic ratios of seston and other primary producers is a way to assess if clams are selectively utilizing a specific source of production in this system.

Stable isotopes can be used to quantify proportional source contributions to consumer diets in coastal ecosystems (Peterson 1999, Michener & Kaufman 2007). Carbon isotopic ratios vary in primary producers in a predictable way based on photosynthetic pathways that distinguish between C3 and C4 autotrophs. Nitrogen isotope signatures become enriched in successive levels of food webs but their interpretation is complicated by variation in $\delta^{15}N$ among sources, requiring additional information. Since mixing model discrimination among sources is dependent on the number of sources, differences in isotopic signature among sources, and the number of distinct isotopes, adding a third isotope may help in source resolution. Here we use

^{13}C and ^{15}N along with a third isotope ^{2}H as one way to help resolve among many potential sources. Large differences in hydrogen isotope ratios between different macroalgae and vascular plants (i.e. seagrass, marsh grass) sources may allow for discrimination in models beyond what is possible just with ^{13}C and ^{15}N.

The purpose of this study was to identify the ultimate sources of organic matter supporting hard clam aquaculture in a Virginia coastal lagoon through stable isotope analysis. Since we had many potential organic matter sources, we hypothesized that adding a third isotope, ^{2}H, would reduce ambiguity in mixing model results. We also hypothesized that benthic microalgae would be a key resource based on prior studies (Table 1). We were interested in the possible significance for clams of other sources, particularly the abundant invasive *Gracilaria vermiculophylla* (Thomsen et al. 2006) and other macroalgae that tend to foul clam grow-out pens (Figure 2). By using a 3-isotope approach in a Bayesian framework, we determine the potential utility of incorporating hydrogen isotopes into coastal food web analysis.

Methods

Site description

The Virginia Coast Reserve (www.vcrlter.virginia.edu) comprises fringing marshes and shallow lagoons within the barrier islands system of the Eastern Shore of Virginia (Barnes & Truitt 1998). Historically the lagoons were the location of a productive and lucrative scallop fishery facilitated by extensive beds of the habitat-forming seagrass *Zostera marina*. A system-wide state change in the 1930s resulted in loss of the seagrass beds and a simultaneous collapse of the scallop fishery, but high water quality and efforts to re-seed seagrass since 1999 have resulted in successful restoration of over 4,000 acres of seagrass beds (Orth et al. 2006). In recent

decades, clam aquaculture has developed into an extensive industry (Murray & Kirkley 2005). Private citizens can obtain leases from the state of Virginia to use subtidal bottom ground in lagoons for shellfish aquaculture (www.mrc.virginia.gov). We sampled aquaculture clams, *Mercenaria mercenaria* from a leased aquaculture bed near Cobb Island, VA (37.307376 N, -75.780602 E). The study site is located in the Virginia Coast Reserve near a long-term water quality monitoring site.

Based on data from the Virginia Coast Reserve Long Term Ecological Research (VCR LTER) program (McGlathery et al. 2008), the water quality conditions at this site vary as a function of seasons, currents, wind, and storm conditions. Winters are mild in the VCR and summers are hot with water temperatures in excess of 30 °C, and salinities are > 30 ppt except after strong precipitation events. Water quality data from 2004–2008 at the nearest LTER monitoring station indicate that total suspended solids range from near 0 mg L^{-1} to over 100 mg L^{-1} during storm events (Figure 1a). Particulate organic matter usually ranges from 2–10 mg L^{-1} and TSS ranges from 10–80 mg L^{-1} (Figure 3a). Chlorophyll concentrations are low with no obvious seasonality (Figure 3b). Only a small portion of the total sediment load is organic and the portion that is organic decreases with increased sediment load (Figure 3). In a 2-yr study in a nearby similar lagoon, Hog Island Bay, water column chlorophyll a never exceeded 12 mg L^{-1} (McGlathery et al. 2001).

Sample collection and isotopic analysis

Our study was designed to evaluate the isotopic signatures from all potential sources of organic matter supporting the clams in this location. Macroalgae (*Gracilaria vermicuphylla, Ulva lactuca, Codium fragile,* and *Agardhiella subulata*) and macrophytes (*Zostera marina* and

Spartina alterniflora) (all hereafter referred to by genus only) were collected as grab samples. Macroalgae were collected directly from fouling on anti-predator nets over growing clams, seagrass blades were collected from the water column, blades of marsh grass were collected from the closest marsh, and blades of terrestrial vegetation such as wax myrtle (*Myrica cerifera*) and Virginia pine (*Pinus virginiana*) were sampled from the mainland shoreline. Seston was collected by filtering water at the study site on pre-combusted GF/F filters for ^{13}C and ^{15}N analysis and on nylon-based filters for ^{2}H analysis. Benthic diatoms were collected using a modified vertical migration technique (Riera & Richard 1996): diatoms were sampled from the top layer of sediment with a putty knife and gently spread to a depth of approximately 1 cm in shallow trays, covered with a 64 μm mesh Nytex screen and covered with silica. Trays were left to incubate in light for 12–24 hours and phototactic diatoms were harvested after they migrated vertically into the silica layer. Harvested material was suspended in filtered seawater and then processed as seston samples. Phytoplankton were sampled from incubated laboratory cultures of native planktonic assemblages grown in filtered site water as per Caraco et al. (2010).

Clam samples comprised muscle tissue aggregated from three individuals collected from grow-out pens. Market-sized clams were sacrificed in a drying oven before dissection and only adductor muscle tissue was extracted from the whole soft tissue biomass for analysis. Muscle tissue was selected to evaluate diet without the effects of short-term spatial and temporal variation that influences other tissues with shorter turnover time (Yokoyama et al. 2006). We compared the isotopic composition of whole biomass tissue including adductor muscle to just adductor muscle tissue. Clams from natural populations were also collected from seagrass beds in nearby bays for comparison. Samples of environmental water for $^{2}H_2O$, juvenile clams, and hatchery algal feed were also collected for model parameterization of environmental water usage

(Solomon et al. 2009) and species-specific trophic fractionation (Post 2002). Samples were collected 6 times from 2010–2011 to capture seasonal variability in isotopic ratios based on turnover time in bivalve tissue (Riera & Richard 1997). Sample collections were made in Nov 2010, and February, April, June, July, and September of 2011.

Organic matter samples were rinsed with deionized water to remove salts, dried to constant weight at 60 ºC for at least 48 hours, and powdered with mortar and pestle. No acidification was applied to the samples to avoid alterations in the isotopic ratios (Mateo et al. 2008). Aliquots of powdered samples were weighted into tin (^{13}C, ^{15}N) or silver (^{2}H) capsules for analysis. All isotopic analyses were performed at the Colorado Plateau Stable Isotope Laboratory. For ^{2}H analysis, sample values were calibrated to local water vapor according to Wassenaar & Hobson (2003) and compared to a suite of normalization reference standards including powdered caribou hoof and powdered kudo horn (Doucett et al. 2007). 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). Isotope ratios are reported here in the standard del (‰) notation relative to international standards (H: Vienna Standard Mean Ocean Water, C: Peedee Belemnite, N: atmospheric N_2), expressed as $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ such that $\delta X = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 10^3$, where X is ^{13}C, ^{15}N, or ^{2}H and R is $^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{2}H/^{1}H$.

Mixing model analysis

Proportional source contributions to clam diets were evaluated using the freely available Bayesian mixing model Stable Isotope Analysis in R (SIAR) (Parnell et al. 2010) adjusted for dietary water contributions for ^{2}H as in Solomon et al. (2009). Bayesian models like SIAR expand on the analysis possible with strictly linear models by incorporating many sources of

uncertainty as well incorporating evidence from the observed data to interpret the likelihood of mathematically feasible solutions. Posterior distributions of source contributions in model output adjust the mathematically possible solutions for the likelihood of observed consumer isotopic ratios. The model was run with uninformative Dirichlet distributed priors for 1 x 10^6 iterations with the first 400,000 discarded. Model equations for the three isotopes used in this analysis are as follows:

$$\delta^{13}C_{clam} = \sum_{k=1}^{K}[\phi_k(\delta^{13}C_k + \Delta_C)] + \varepsilon_C$$

$$\delta^{15}N_{clam} = \sum_{k=1}^{K}[\phi_k(\delta^{15}N_k + \Delta_N)] + \varepsilon_N$$

$$\delta^{2}H_{clam} = \omega * \delta^{2}H_{water} + (1-\omega) * \sum_{k=1}^{K}[\phi_k(\delta^{2}H_k)] + \varepsilon_H$$

where k is the number of sources, ϕ_k is the proportional contribution of source k, Δ_x is trophic fractionation for isotope X, ω is dietary water contribution of hydrogen to organic matter, and ε_X is residual error for isotope X. Each of the parameters Δ_X, ω, ε_X, and source isotopic ratios are normally distributed. Proportional contributions, ϕ_X, have a Dirichlet distribution where all sources are treated independently but must sum to 1 (Gelman et al. 2003). Model input fitting parameters measured for this study were source isotopic ratios, Δ_C, Δ_N, ω, and δ^2H_2O. Trophic fractionation parameters were determined as Δ_C = 1.05‰ ± 0.75 standard deviation (sd) and Δ_N = 3.24‰ ± 0.83 sd by comparing the isotopic ratios of hatchery raised juvenile clams and their exclusive algal food source. Environmental water contribution to clam tissue (as opposed to food) was calculated as ω = 0.15 ± 0.09 sd using hatchery clams, food source, and water for δ^2H of consumer, water, and food respectively (Solomon et al. 2009). δ^2H_2O at the study site was

measured as δ^2H = –9.99‰ ± 0.87 sd. Proportional contributions and residual error were fitted by the posterior model distributions in the model.

Mixing model results have determined solutions if the number of sources is n + 1 relative to the number of isotopes used (n) (Fry 2006), however, as in this case, there are often many more sources than isotopes measured. The addition of hydrogen isotope data only means that our mixing solution can be determined for 4 sources, but we identified 9 potential sources of production available to these consumers. Since terrestrial vegetation had the lowest contribution in an initial 9-source model, is not abundant near our study site, and had $\delta^{15}N$ much more depleted than either clams or any other source (1.14‰), we excluded it from the analysis. One common way to reduce the number of sources is to form groups of either functionally related or isotopically similar sources before the analysis. This is referred to as *a priori* grouping (Phillips et al. 2005). To avoid confusion with Bayesian terminology of prior and posterior distributions, we will refer to this as pre-model grouping. One problem with pre-model grouping is that the resulting model is often less able to distinguish source contributions since the grouped source combines the variability associated with multiple individual sources (Phillips et al. 2005). We tested our source data for potential pre-model groups by performing one-way ANOVAs on sources for each isotope and compared means with post-hoc Tukey's HSD test. While some sources appeared clustered, these groupings were inconsistent across isotopes and had unclear separations (Figure 4). Therefore, we used the model without pre-model grouping and combined the posterior distributions of related (e.g. all species of macroalgae) sources as per Phillips et al. (2005) to draw conclusions.

Model accuracy declines when the number of sources increases relative to the number of isotopes used (Parnell et al. 2010). However, excluding potential sources could lead to inaccurate

conclusions since clams could potentially be feeding on any combination of the sources we sampled. Therefore, in order to retain accuracy without excluding data, we modeled all feasible combinations of 2–8 sources where clams were contained in the mixing polygon created by sources (Figure 5). Additional source combinations that included pre-model grouping of related sources (e.g. macroalgae or macrophytes) were included in the analysis for comparison. In aggregate we considered 166 possible source combinations. All source combinations were evaluated using isotopic ratios of just carbon and nitrogen as well as with all three isotopes to evaluate the utility of including hydrogen data.

Model output was evaluated using Schwarz Bayesian Criteria (SBC), calculated as SBC $= -2\ln(L) + K \times ln(n)$, where L is the likelihood of the model, K is the number of parameters included, and n is the sample size (Rust et al. 1995). We discarded models where the difference of model SBC from the minimum SBC (Δ_i) was greater than 10, which excluded models for which the normalized model likelihood (*w)* was less than 5%. 2-isotope models were compared to 3-isotope models by visual analysis of source contribution posterior distributions.

Results

Consumer and source isotopic ratios

Clam isotopic ratios were closest to those of macroalgae and benthic microalgae (Figure 5). Microalgae were the most depleted in ^{13}C and macrophtyes were the most enriched. Carbon signatures of macroalgal species were overlapping and variable. Benthic microalgae were well constrained but did not differ significantly with *Ulva* or *Gracilaria* (Figure 4). Phytoplankton were the most depleted in ^{13}C. *Spartina* and *Zostera* were the most enriched, and *Spartina* did not differ significantly from the most enriched macroalga, *Codium*. Nitrogen isotopic ratios were

well constrained for most sources but only macroalgae had distinctly different nitrogen composition relative to the other sources (Figure 4). They were the only group of sources more enriched in ^{15}N than clams. $\delta^{2}H$ values had a much larger range, with significant differences between several macroalgal species. *Ulva* and phytoplankton were the only sources more depleted in ^{2}H than clam tissue. Hydrogen isotopic ratios were clustered for some macroalgae, all higher plants, and benthic microalgae. Macroalgal signatures, however, were significantly different from each other except for *Codium* and *Agardhiella*. These two macroalgae were not significantly different than benthic microalgae, *Spartina*, or *Zostera* (Figure 4).

Clams had little variation in isotopic ratios throughout the study and there were no significant differences between $\delta^{2}H$ of clams from different sampling periods ($p > 0.05$). Temporal variation for C and N isotopes was low: in June, clams were depleted in ^{13}C and enriched in ^{15}N, but no other sampling had significantly different isotopic ratios. Therefore, samples were pooled into one group for mixing model analysis. Whole tissue $\delta^{13}C$ was significantly depleted relative to muscle tissue (~1‰) but this difference was consistent seasonally and relatively small. Carbon and hydrogen ratios of aquaculture clams were significantly different ($p < 0.01$) than those of natural clams found in adjacent seagrass beds. However, these differences were small (~1‰ depleted in ^{13}C, ~8‰ enriched in ^{2}H), and much lower than the differences observed among sources potentially supporting clams.

Clam tissue isotope ratios did differ substantially from those of seston (Figure 5). Seston isotope ratios were closest to but also different from both phytoplankton and benthic microalgae isotope ratios (Figure 5) indicating a mixed composition of suspended material. There was some seasonal variation in source isotopic ratios but few consistent patterns. Since spatial and temporal resolution of source contributions could be complicated by a lag between temporal changes in

source ratios and when particulates from the source are available for consumption, data from different sampling period were grouped for each source prior to mixing model analysis. Variability included in source contributions therefore includes seasonal variability.

Source contributions

Based on model selection criteria the models with fewer than all 8 sources have a higher likelihood (Table 2). The overall best combination of sources to include with a 3-isotope model includes benthic microalgae, phytoplankton, *Ulva*, *Agardhiella*, and *Codium*. The highest ranked source combinations for the 2-isotope model includes phytoplankton, *Agardhiella*, and *Codium*. Few of the highest ranked models include either *Spartina* or *Zostera*. Although the 2- and 3-isotope models have similar mean values for proportional source contributions, 3-isotope models have more precise posterior distributions (Figure 6) and allow for higher resolution of individual source contributions (Figure 7).

Phillips et al. (2005) suggest several methods for grouping sources when the number of sources exceeds isotopes as is the case in this study. We evaluated pre-model and post-model grouping of sources as macroalgae, microalgae, and macrophtyes. Phytoplankton and benthic microalgae were grouped as microalgae, all four types of macroalgae were considered a group, and *Spartina* and *Zostera* were considered macrophtyes (Figure 8). Post-model grouping allows for greater resolution of source contributions since the uncertainty associated with individual sources is smaller than when distinct sources are grouped together (Phillips et al. 2005). Post-model groupings also help resolve the problem of correlated posterior distributions (Table S2), which indicated that the individual models could not easily distinguish between certain sources. Although the general pattern of distributions are similar, the 95% credibility intervals with post-

model grouping overlap less than they do with pre-model grouping. Model output with post-model grouping improves in both accuracy and precision. Post-model groupings for the top ranked models according to SBC all have a similar pattern: 55–66% contribution of macroalgae, 23–45% of microalgae, and 0–14% of macrophtyes (Table 2; Figure S1).

Discussion

Mixing model performance

SIAR performance decreases as the number of sources included in the analysis increases (Parnell et al. 2010) such that true values of simulated data fall outside of the 95% credibility intervals of the posterior distributions. Although we likely conserve model performance here by using 3 isotopes in our analysis, the large number of possible sources in this system leads to more uncertainty in our model output. Since least reliable model performance occurs with *a priori* grouping of sources (Parnell et al. 2010) we ran the model with all possible combinations of the 8 individual sources and evaluated model performance using a model selection criteria statistic for Bayesian models. Grouping sources also means losing ability to infer contributions from those individual sources that are combined (Phillips et al. 2005). However, a large number of sources increases model complexity, and may not lead to better estimates because any source included in the model will necessarily contribute. Since models with fewer than 8 sources were ranked highest by SBC, we are confident that clams selectively feed on certain food sources.

Based on post-model groupings, macroalgae are the dominant food source. The nutritional value of *Agardhiella* and *Codium* adds support to their potentially large contributions (C:N approximately 12–14). Based on mathematically feasible solutions from mixing polygons, if phytoplankton are not included, then both *Ulva* and benthic microalgae must be included.

Since the Schwarz Bayesian Criteria prioritizes fewer model parameters (sources in this case), models that include just phytoplankton are ranked higher than those that include both benthic microalgae and *Ulva*. However, the low concentrations of chlorophyll *a* in these waters lend support for inclusion of benthic microalgae and *Ulva* rather than phytoplankton.

Hydrogen isotopes improve model performance in 2 distinct ways. First, addition of the third isotope allows for improved precision in estimates of source contributions. In most circumstances, adding nitrogen data improves the accuracy of the model but not the precision; probability distributions of source contributions shift when $\delta^{15}N$ is added (Figure 6b). In nearly all cases, credibility intervals become smaller when hydrogen isotopic ratios are included in the model (Figure 6c). Secondly, hydrogen isotope ratios can allow for resolution of source distributions beyond the functionally significant groupings considered here. As a group, macroalgae contribute approximately 60% of the diet. While the posterior distributions of all macroalgae species overlap considerably and have similar means when only carbon and nitrogen isotopes are used (Figure 7a), we can determine more precise proportions when hydrogen is included (Figure 7b). The contribution of *Gracilaria* remains similar, but the model including hydrogen distinguishes between a more certain smaller contribution of *Ulva* as well as larger contributions of *Agardhiella* and *Codium*.

One way to improve model performance with the number of possible sources in this system would be to include $\delta^{34}S$ as an additional source of information. Sulfur isotopes are distinct between producers that derive sulfides depleted in ^{34}S from reduced anoxic sediments and sulfates in seawater enriched in ^{34}S (Knoff et al. 2001, O'Donnell et al. 2003, Fry 2006), however the assimilation of microbial biomass and detrital material may lead to uncertainty in

the interpretation of sulfur isotopic ratios in consumers in detritus-supported benthic food webs (Michener & Lathja 2007).

Implications of source contributions to clams

Field growout pens in aquaculture operations acquire a dense fouling of macroalgae on anti-predator nets (Figure 2). This fouling can have negative impacts on cultured bivalves by reducing water flow and consequently decreasing food availability. Fouling organisms that are filter feeders can also compete with the fishery, and eventual decomposition of these organisms may reduce oxygen supply (Fernandez et al. 1999, Carmichael et al. 2012). However, the accumulation of macroalgae on nets may provide a locally important nutritious food source for clams. In addition to detrital particles from macroalgae, clams may be able to incorporate dissolved organic material released by living macroalgae as a significant source of energy similar to other bivalves (Baines et al. 2007). Clams found in nearby seagrass beds had isotopic signatures similar to aquaculture clams and therefore mixing model analysis indicates that they may rely on similar food sources. However, both the density of clams and the abundance of macroalgae in natural seagrass beds are much lower than in aquaculture pens.

Patchy occurrence of macroalgal species suggests that there is a variable composition of algae in the system at any one time: the species most present or abundant in the area may not always reflect the quality or availability of detrital particles. Similar to the findings of Baeta et al. (2009), seasonal changes in environmental conditions in the lagoon we studied did not lead to significant differences in the stable isotope ratios of this consumer. Although much production in this system is seasonal (McGlathery et al. 2001), a persistent pool of detrital material could support aquaculture populations. Post-model grouping indicates the significant reliance on

macroalgae sources while accounting for temporal variation in macroalgal abundances. While we can identify that the organic matter from macroalgae supports clams, further investigation is needed to determine if this is a dissolved and/or detrital pool, and to determine the relative importance to clams of macroalgal abundance at the local (e.g. macroalgae on nets) and landscape (e.g. for entire lagoon areas) level.

Macroalgae are rarely observed as a dominant energy source for bivalves (Table 1). Macroalgal support of food webs has been identified as an early stage of coastal eutrophication (Olsen et al. 2011); however, Virginia lagoons that support aquaculture have very low nitrogen inputs (McGlathery et al. 2001). These bays support abundant production of potentially nutritious macroalgae from both native and invasive populations (Thomsen et al. 2006, McGlathery et al. 2007). Further, these macroalgae are especially abundant on anti-predator nets above aquaculture clams (Luckenbach 2009).

Usually nematodes (Riera & Hubas 2003), mud snails (Giannotti & McGlathery 2001), and polycheates (Lefebvre et al. 2009, Nordstrom et al. 2009) are the organisms that feed on macroalgae. For bivalves, macroalgal contributions to detrital food chains have been demonstrated in Marenne-Oléron Bay (Riera 1998) and in Possyet Bay, Russia (Kharlamenko et al. 2001) (Table 1). But in many systems macroalgae have negative effects on bivalves. For example, invasive macroalgae like *Caulerpa taxifolia* are associated with decline in the abundance and condition of bivalves (Gribben et al. 2009, Wright et al. 2007) due to reduced oxygen levels created by proliferation of these algae.

Macrophtye detritus (from *Spartina spp.*) has been linked to bivalve production previously (e.g. Newell & Langdon 1986, Duggins et al. 1989, O'Donnell 2003) but does not appear to be an appreciable source to clams in this study. The population of clams analyzed in

our study is adjacent to abundant macrophytes from nearby marshes and seagrass beds. Material from these areas is likely significant in the detrital portion of seston, but these macrophytes do not appear to make up a significant portion of their diet based on mixing model analysis of source isotopic signatures.

Since they are filter feeders, clams only feed on macroalgae and macrophtyes in particulate or dissolved form. Consequently, source material must necessarily undergo a physical transformation in order to be small enough to be ingested by clams. In the process of degradation, isotopic signatures may be affected. ^{15}N enrichment from preferential loss of ^{14}N during particulate N decomposition, as well as possible 2–3‰ depletion in carbon during decomposition (Macko et al. 1983, Fogel et al. 1989) could alter the isotopic ratios of source material before consumption by filter feeders. $\delta^{13}C$ analysis may also be complicated due to *in situ* ecosystem metabolism, the effects of ambient pH, and uptake of bicarbonate by phytoplankton (Oczkowski et al. 2010). Persistent macrophtye detritus in the water column could therefore account for a larger contribution to hard clam basal resources with enriched ^{15}N or ^{13}C ratios. Changes to hydrogen isotopic ratios during decomposition of *Acer rubrum* leaves in water were insignificant (C. Yang unpubl. data), however, the limited studies on changes in $\delta^{2}H$ during decomposition are contradictory (Estep & Hoerign 1980, Macko et al. 1983, Fenton & Ritz 1988). Thus, the hydrogen isotope results are important indicators that macrophytes are not an important resource to clams. Furthermore, decomposing macroalgae is abundant locally, and their detrital particles would be similarly enriched during microbial degradation. The observations that macroalgal sources are abundant in this system, degrade into particulates easily, and have low C:N ratios (Tyler et al. 2001, Thomsen et al. 2006) provides *a prioiri* evidence that macroalgae could contribute a large proportion to clam diets.

Since hard clams control feeding processes by reducing clearance rates when there are high levels of suspended particulate matter (Bricelj 1984, Kraeuker & Castagna 2001), eutrophication of Virginia coastal lagoons would affect aquaculture of this species. Under different conditions of food supply, clams may allocate resources to support different types of growth (Eversole et al. 2000) with a resultant change in biochemical composition and harvest quality. Under highly eutrophic conditions that result in hypoxic conditions, loss of grazing pressure by clams could change the planktonic community to favor production of low quality seston and harmful algae (Newell et al. 2009), which may cause a persistent reduction in productivity of the fishery. Although bivalve populations declined historically in the Virginia Coast Reserve through loss of oyster reefs and scallop populations, restoration and aquaculture has at least partially replaced this lost ecosystem service. Clams also act as a nutrient sink in this ecosystem since they are harvested for sale and consumption, thereby transferring nutrients out of the local environment that could otherwise contribute to habitat degradation. Consequently, ecosystem managers wishing to sustain successful hard clam aquaculture should consider the importance of macroalgae as a food supply to this fishery. With adequate food supply and habitat, clams and other bivalves provide benefits to human both as a food resource and by promoting better water quality. These benefits, however, may be lost if eutrophication causes habitat degradation and a shift away from the dominance of benthic primary production.

Conclusions

Using a 3-isotope Bayesian mixing model, we determined that aquacultured clams in a low chlorophyll Virginia coastal lagoon selectively rely on macroalgal detritus as their primary food source. This finding is significantly different than most coastal systems, where phytoplankton are

the dominant food source for bivalves. Hydrogen isotopes extended our ability to discriminate among sources and improved the precision of models. This analysis warrants broader application of hydrogen isotopes, in combination with carbon and nitrogen, to the analysis of coastal food webs. While a 3- isotope model could not fully distinguish among the eight possible sources of primary production that we considered, consistency among model analyses as well as among methods of grouping sources strongly support the importance of organic matter derived from macro- and micro-algae to clams.

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Figure 2.1 Relationship of organic matter and total suspended solids in water quality samples between 2005–2008 from 6 sites along a transect through the isotope sampling site near Cobb Island used in this study. Measurements are from VCR–LTER (www.vcrlter.virginia.edu).

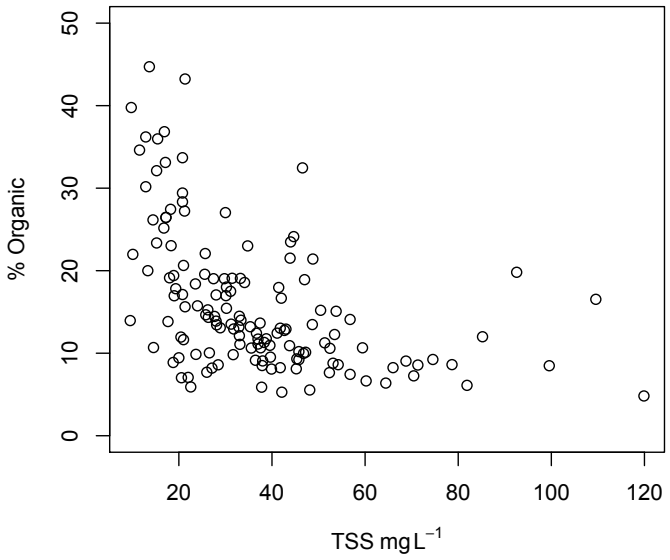

Figure 2.2 Macroalgae (*Ulva lactuca* and *Agardhiella subulata*) fouling on antipredator nets over hard clam aquaculture pens in Cobb Island Bay, Virginia.

Figure 2.3 Seasonal patterns of total suspended solids (TSS) and chlorophyll *a* for Little Cobb Island measured by Virginia Coast Reserve Long Term Ecological Research program (www.vcrlter.virginia.edu). Data show measurements made from 2004–2008 by day of year (DOY).

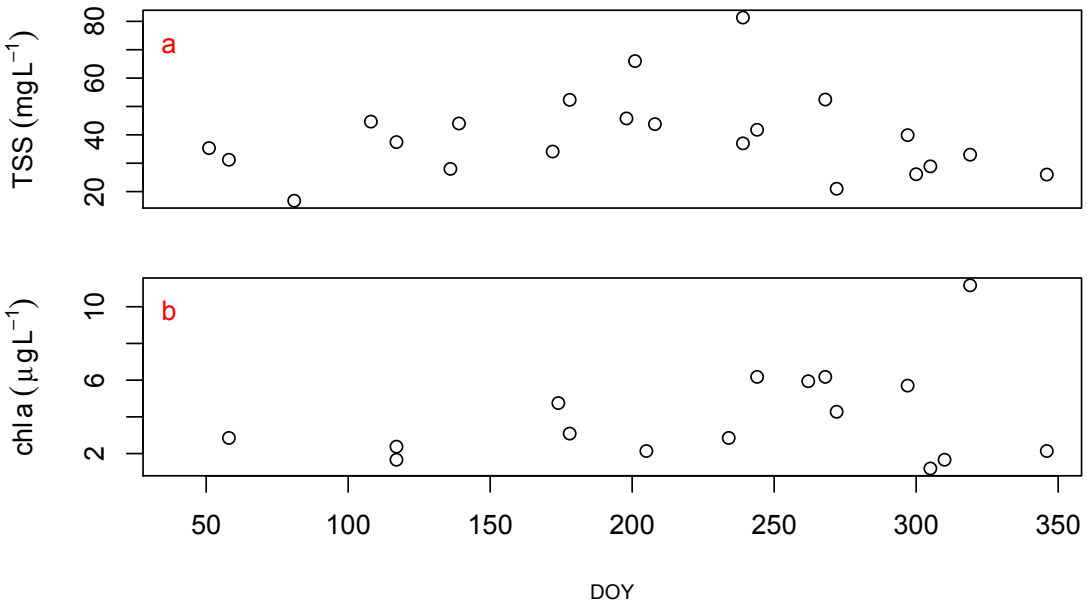

Figure 2.4 Significant differences among primary producer sources as determined by one-way analysis of variance of isotopic ratios. For each given isotope, source ratios that are connected by solid bars are not significantly different. Sources are ranked from most enriched to most depleted. Sources are abbreviated as *As* = *Agardhiella subulata*, *Cf* =*Codium fragile, Gv* = *Gracilaria vermicuphylla, Ul* = *Ulva lactuca,* BMA = benthic microalgae, Phy = phytoplankton, *Sa* = *Spartina alterniflora*, *Zm* = *Zostera marina*.

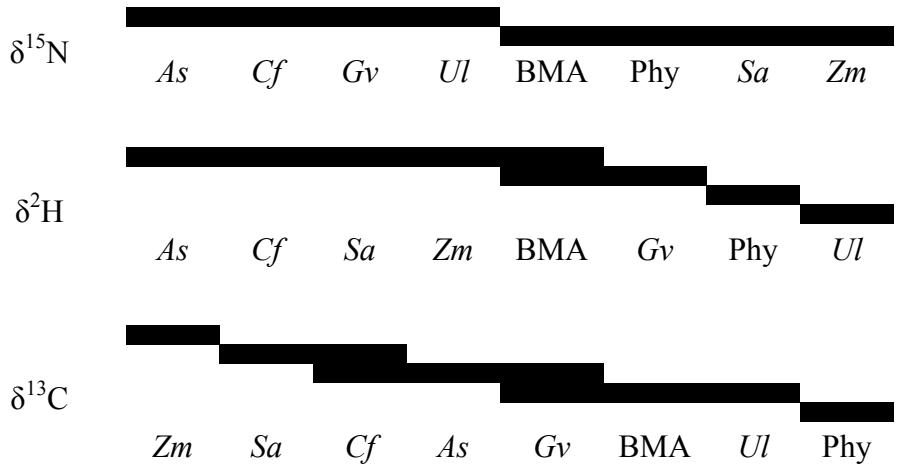

Figure 2.5 Isotopic ratios ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$) of primary producer sources, seston, and clam tissue from an aquaculture growout site in Cobb Island Bay, Va. Sources indicated by an X were considered individual sources and grouped after modeling, as microalgae, macroalgae and macrophtyes. Grouped values are indicated by blue symbols: circle = microalgae, square = macrophytes, triangle = macroalgae. Error bars show standard deviations. Light gray shape shows the mixing polygons created by the sources. Clam isotope values shown here are corrected for trophic fractionation ($\delta^{15}N$ and $\delta^{13}C$) and dietary water contributions, ω ($\delta^{2}H$). Seston and terrestrial vegetation (Tveg) were not included as sources but are shown for comparison. Sources are abbreviated as *As* = *Agardhiella subulata*, *Cf* =*Codium fragile, Gv* = *Gracilaria vermicuphylla, Ul* = *Ulva lactuca,* BMA = benthic microalgae, Phy = phytoplankton, *Sa* = *Spartina alterniflora*, *Zm* = *Zostera marina*. Tveg values are excluded from the C and N plot because Tveg was highly depleted in N. Including this point would make the other sources less distinct.

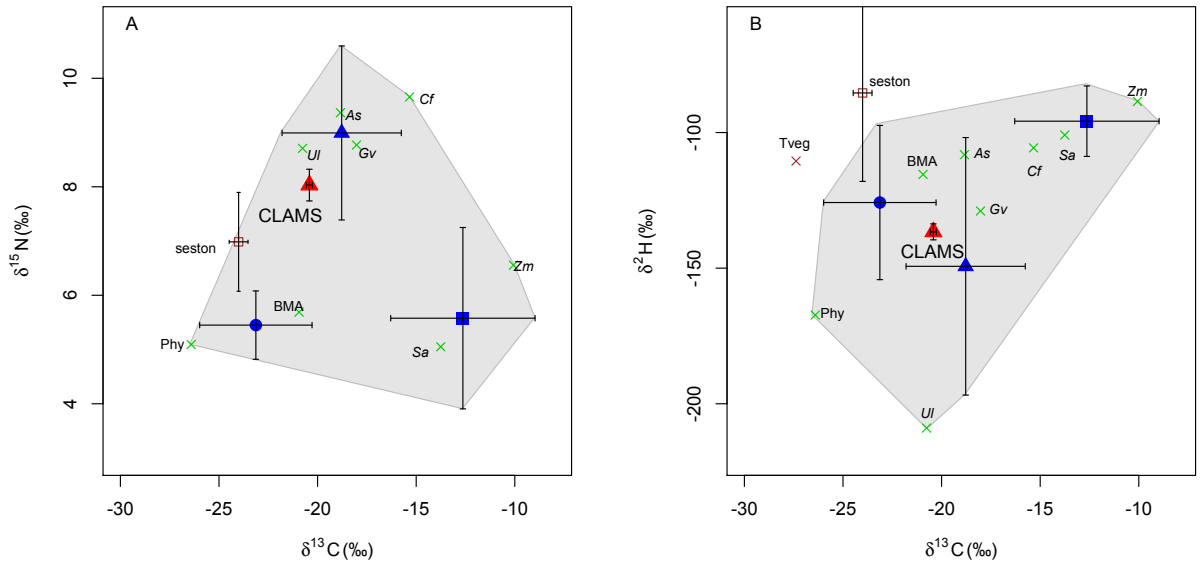

Figure 2.6 Probability distributions of source contributions for a representative 3-source model. Panel (a) shows distributions from a 1-isotope model using only $\delta^{13}C$. Panel (b) overlays distribution from 2-isotope model including $\delta^{15}N$ data. Panel (c) overlays distributions from 3-isotope model including $\delta^{2}H$ data. Phytoplankton = red, *Codium fragile* = green, *Agardhiella subulata* = blue

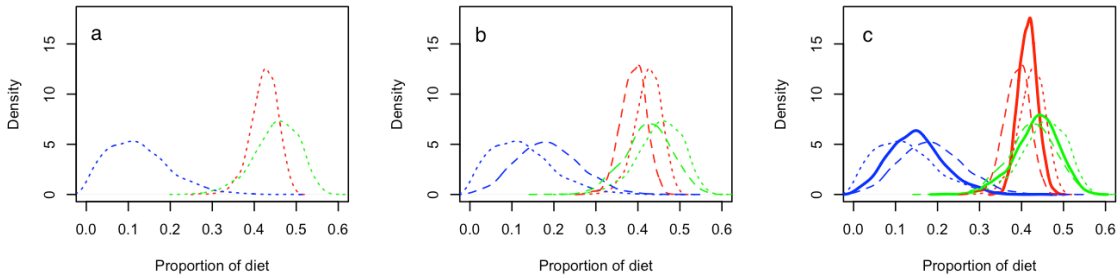

Figure 2.7 Probability distributions of source contributions for 4 types of macroalgae from a representative model: purple = *Gracilaria vermicuphylla*, green = *Ulva lactuca*, red = *Agardhiella subulata*, blue = *Codium fragile*. Panel (a) shows distributions from 2-isotope model that included $\delta^{13}C$ and $\delta^{15}N$. Panel (b) shows distributions from 3-isotope model including $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$. Distributions of source contributions in the 3-isotope model are more distinct and have higher maximum probability densities.

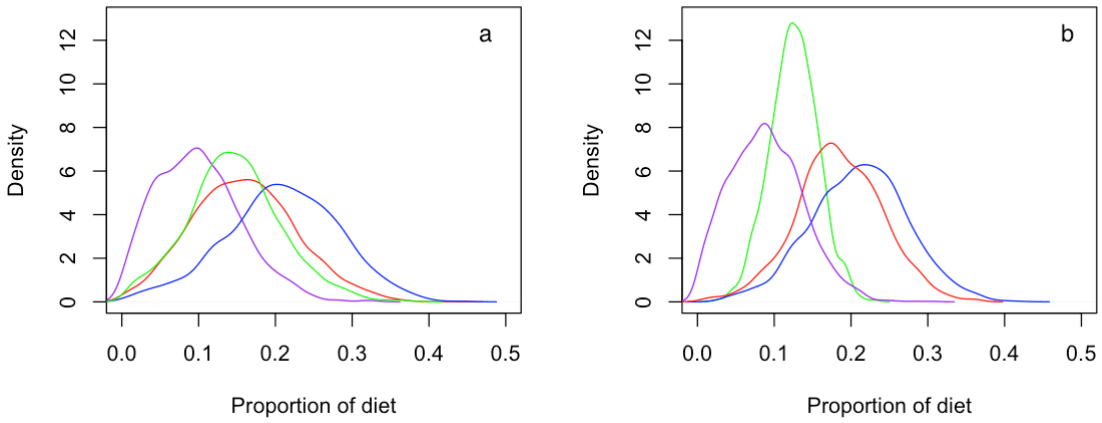

Figure 2.8 Probability distributions of post-model grouping of sources as macrophytes (red), microalgae (blue), and macroalgae (green). Small panels (a–c) show posterior distributions of individual sources from each of the 3 groups which were summed to form post-model group distributions (d).

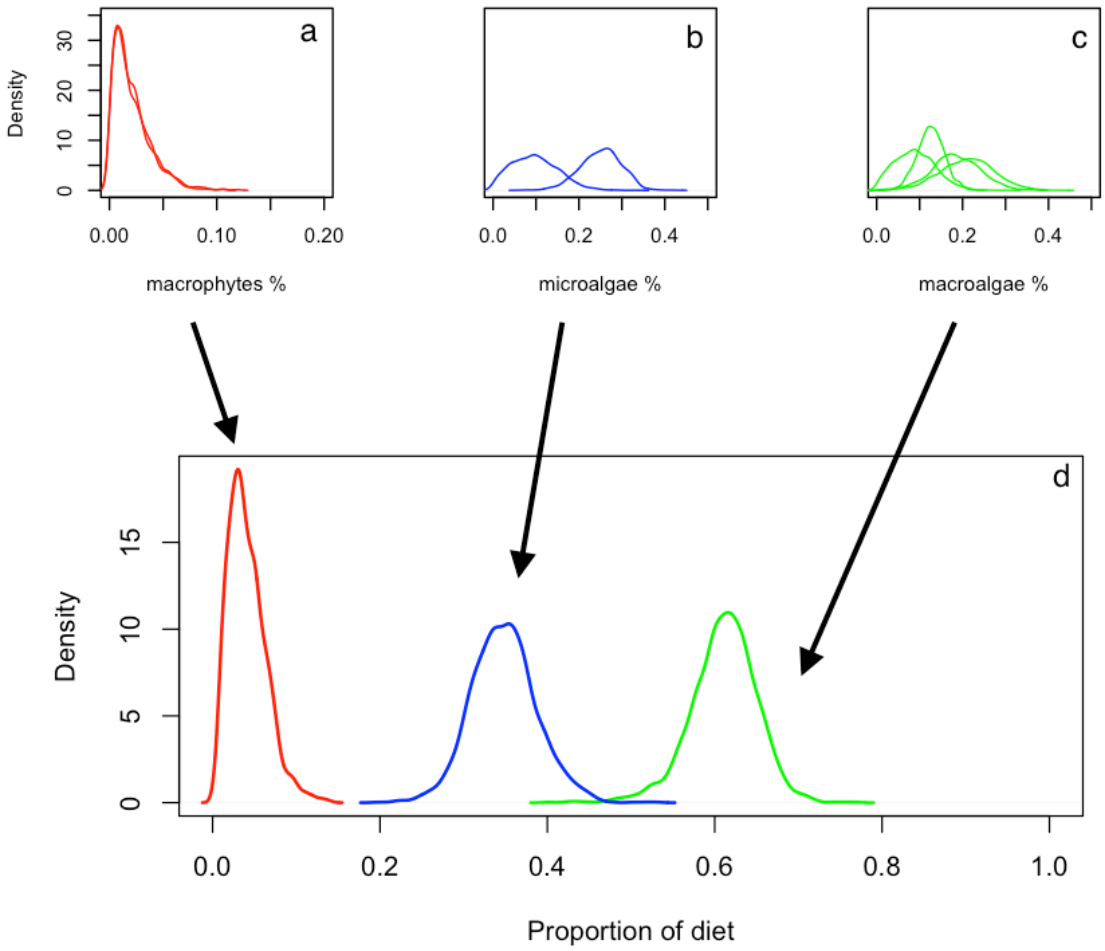

Table 2.1 Literature review of main food sources of bivalves as determined by stable isotope analysis. All sources considered in the given study are listed, main food sources (> 50% reliance) are indicated by *. Abbreviations for source material are: MPB = microphotobenthos, SPOM = suspended particulate organic matter, POM = particulate organic matter, SOM = sedimentary organic matter, BMA = benthic microalgae.

Citation	Isotopes used	Location	Bivalve species	Sources
Bode et al 2006	$\delta^{13}C$, $\delta^{15}N$	Galicia coast, NW Spain	*Mytilus galloprovincialis*	Chlorophyceae, Phaeophyceae, Rhodophyceae, Zosteraceae, phytoplankton*
Bouillon et al 2002	$\delta^{13}C$, $\delta^{15}N$	Coringa Wildlife Sanctuary (Andhra Pradesh, India)	*Tellina sp, Meretric meretrix, Pinctada radiata, Macoma sp, Crassostrea gigas, Mytilus galloprovincialis, Cerastroderma glaucum, Loripes lacteus, Abra ovata, Scrobicularia plana*	SPOM*, SOM, litter
Carlier et al 2007	$\delta^{13}C$, $\delta^{15}N$	Lapalme Lagoon, French Mediterranean coast	*Brachiodontes pharaonis, Cerastoderma glaucum, Chlamys varia, Crassostrea gigas, Modiolus adriaticus, Mytilus galloprovincialis, Paphia aurea, Tapes decussata, Liripes lacteus*	upland plants, salt marsh plants, seagrasses, macroaglae, seagrass epiphytes, POM*, SOM*
Carlier et al 2009	$\delta^{13}C$, $\delta^{15}N$	Salses-Leucate Lagoon (northwest Mediterranean)	*Anadora granosa, Anomalocardia squamosa, Barbatia pistachio, Gafrarium tumidum, Placamen berryi*	*Zostera noltii*, seagrass epiphytes, POM*, A*cetabularia, Ulva*, other macroalgae
Compton et al 2008	$\delta^{13}C$, $\delta^{15}N$	Dampier Flat, Roebuck Bay (NW Australia)		Mangrove leaves*, POM*, macroalgae, diatoms, phytoplankton*
Darnaude et al 2004a	$\delta^{13}C$, $\delta^{15}N$	Gulf of Lions (NW Mediterranean)	*not specified*	river POM, seawater POM*, surface sediment POM and SPOM
Darnaude et al 2004b	$\delta^{13}C$, $\delta^{15}N$	mouth of Rhone River delta (France)	*not specified*	terrestrial POM, seawater POM*
Decottignies et al 2007	$\delta^{13}C$, $\delta^{15}N$	Bourgneuf Bay, France	*Crassostrea gigas*	C3 angiosperm detritus, macroalgae-C4 plant detritus, marine phytoplankton*, benthic diatoms*
Dubois et al 2007	$\delta^{13}C$, $\delta^{15}N$	Bay of Veys, northern French coast	*Crassostrea gigas, Mytilus edulis*	POM*, TOM (detritus and freshwater microalgae), *Ulva**, MPB*

Fukumori et al 2008	$\delta^{13}C$	Uwa Sea, Japan	*Pinctada fucata martensii*	phytoplankton*, benthic microalgae, attached microalgae, POM
Gao et al 2006	$\delta^{13}C$, $\delta^{15}N$	Kau Sai Bay (eastern Hong Kong)	*Perna viridis*	POM*, fish feed, fish faeces
Herman et al 2000	$\delta^{13}C$, $\delta^{15}N$	Westerschelde estuary (Belgium)	*Cerastoderma edule, Ensis sp, Macoma balthica, Mya arenaria, Mytilus edulis, Scrobicularia plana*	pelagic algae*, benthic algae
Kanaya et al 2007	$\delta^{13}C$, $\delta^{15}N$	Gamo Lagoon	*Macoma contabulata, Nuttallia olivacea, Ruditapes philippinarum, Mya arenaria*	riverine POM, *Gracilaria,* marine POM*, benthic and epiphytic diatoms*, *Enteromorpha*
Kanaya et al 2008	$\delta^{13}C$, $\delta^{15}N$	Gamo and Idoura Lagoons (Japan)	*Laternula marilina, Ruditapes philippinarum and Crassostrea gigas*	marine POM*, lagoon POM, *Phragmites* leaves, *Pinus* leaves, Rhodophyta, benthic diatoms*, riverine POM
Kang et al 1999	$\delta^{13}C$, $\delta^{15}N$	Marennes-Oleron Bay, France	*Cerastoderma edule*	MPB*, *Enteromorpha, Fucus, Phorphyra, Ulva, Zostera noltii*, POM*
Kang et al 2006	$\delta^{13}C$, $\delta^{15}N$	Kwangyang Bay (Korea)	*Laternula marilina, Moerella rutila*	marsh plants, river POM, bay POM*, offshore POM*, MPB*, SOM, macroalgae, *Zostera*
Kharlamenko et al 2008	$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$	Vostok Bay (Sea of Japan)	*Mactra chinensis, Pandora pulchella, Felaniella usta, Megangulus zyonoensis*	SPOM, benthic microalgae*, SOM
Leal et al 2008	$\delta^{13}C$, $\delta^{15}N$	Baie des Veys, Lingreville area (Normandy, France) - oyster culture sites	*Crassostrea gigas*	marine SPOM, terrestrial POM, MPB, detrital OM from superficial sediment, *Ulva, phytoplankton**
Leduc et al 2006	$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$		*Austrovenus stutchburyi, Diloma subrostrata*	seston, MPB*, *Zostera capricorni*, Ulva*/Polysiphonia, Gracilaria*
Lefebvre et al 2009	$\delta^{13}C$, $\delta^{15}N$	Lingreville sur-mer (Normandy, France)	*Crossostrea gigas, Mytilus edulis, Cerastoderma edule*	marine POM*, MPB, *Ulva,* riverine POM
Machas et al 2003	$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$	Ria Formosa, Portugal	*Mytilus galloprovincialis, Tapes decussales*	phytoplankton*, MPB*, Ulvales, Bostrychia, Seagrasses, *Spartina, Sarcocornia,* POM
Nordstrom et al 2009	$\delta^{13}C$, $\delta^{15}N$	Åland Islands, northern Baltic Sea	*Macoma balthica*	*Cladophora, Fucus*, drift algae, epiphytes, vascular plants, phytoplankton*, SOM*
Page and Lastra 2003	$\delta^{13}C$, $\delta^{15}N$	Ría de Arosa (NW Spain)	*Cerastroderma edule, Tapes decussatus, Mytilus galloprovincialis*	suspended POM*, *Fucus, Ulva*, BMA*

Petersen et al 1986	$\delta^{13}C$, $\delta^{34}S$	Great Sippewissett salt marsh (Cape Cod)	*Crassostrea gigas, Mytilus edulis, Mercenaria mercenaria*	Phytoplankton*, *Spartina**, upland plants, *Fundulus, Cyprinodon*
Riera and Richard 1996	$\delta^{13}C$	Marennes-Oleron Bay (Atlantic coast, France)	*Crassostrea gigas*	POM, DIC from water, BMA*, macroalgae, terrestrial leaves
Riera et al 1999	$\delta^{13}C$, $\delta^{15}N$	Aiguillon Bay, France	*Macoma balthica, Scrobicularia plana, Mytilus edulis*	*Fucus, Spartina*, benthic diatoms*, phytoplantkon*, SOM, POM
Sara 2007	$\delta^{13}C$, $\delta^{15}N$	western Mediterranean sandy bottomed pond	*Cerastoderma glaucum*	POM, SOM, macroalgae, heterotrophic detritus, biodeposits*, seagrass*
Sauriau and Kang 2000	$\delta^{13}C$, $\delta^{15}N$	Marennes-Oleron Bay (Atlantic coast, France)	*Cerastoderma edule*	SPOM, MPB*, macroalgae, seagrass
Schaal et al 2008	$\delta^{13}C$, $\delta^{15}N$	Arcachon Bay (France)	*Chlamys varia, Tapes decussatus, Mytilus edulis, Crassostrea gigas*	Macroalgae, *Zostera* spp, sedimented OM*, marine POM
Yokoyama and Ishihi 2003	$\delta^{13}C$, $\delta^{15}N$	Gokasho Bay (central Japan)	*Theora lubrica*	BMA*, *Theora lubrica*, SOM, POM
Yokoyama et al 2005	$\delta^{13}C$, $\delta^{15}N$	Ariake Sound	*Mactra veneriformis, Ruditapes philippinarum*	estuarine POM*, riverine POM, sewage POM, SOM, terrestrial plant material, BMA, seaweed
Yokoyama et al 2009	$\delta^{13}C$, $\delta^{15}N$	Ariake Sound, Kyushu (southern Japan)	*Scapharca spp, Modiolus sp, Musculista sp, Atrina sp, Limaria sp,Anomia sp, Mactra sp, Raetellops sp, Solen sp, Ruditapes sp*	riverine POM, reeds, BMA*, macroalgae, coastal phytoplankton*

Table 2.2 Bayesian criteria selection for top 10 ranked source combinations for 3-isotope and 2-isotope models. Source contributions given are means of post-model groupings. Probability distributions of given models are shown in Figure 5. Calculations shown in table are: number of parameters (K), residual sum of squares (RSS), Schwarz Bayesian Criterion (SBC), difference of model *i* SBC from minimum SBC (Δ_i), and normalized relative likelihood (*w*). Expanded table without post-model grouping can be found in supplementary information.

3-isotope models	K	RSS	SBC	Δ_i	*w*	Macroalgae	Microalgae	Macrophytes
	5	4.10	-2.17	0.00	0.164	62%	38%	0%
	4	5.01	-2.13	0.04	0.161	66%	34%	0%
	5	4.36	-1.37	0.80	0.110	63%	34%	4%
	5	4.41	-1.24	0.93	0.103	64%	32%	4%
	3	6.56	-1.20	0.97	0.101	58%	42%	0%
	2	8.00	-1.18	0.99	0.100	55%	45%	0%
	4	5.41	-1.15	1.03	0.098	63%	23%	14%
	4	5.54	-0.83	1.34	0.084	59%	28%	14%
	3	6.80	-0.72	1.45	0.079	60%	40%	0%
	4	5.70	-0.46	1.71	0.070	56%	44%	0%
2-isotope models								
	3	0.09	-57.35	0.00	0.163	58%	42%	0%
	3	0.09	-57.02	0.33	0.138	60%	40%	0%
	2	0.11	-56.79	0.56	0.123	55%	45%	0%
	4	0.08	-56.09	1.26	0.087	66%	34%	0%
	3	0.10	-55.81	1.54	0.076	58%	42%	0%
	4	0.08	-55.42	1.93	0.062	62%	38%	0%
	4	0.09	-55.12	2.23	0.054	61%	39%	0%
	4	0.09	-54.81	2.54	0.046	63%	35%	2%
	5	0.08	-53.82	3.53	0.028	67%	33%	0%
	4	0.10	-52.92	4.43	0.018	58%	39%	3%

Electronic Supplementary Information

Figure 2.S1 Post-model groupings of source contributions from macrophytes (red), microalgae (blue), and macroalgae (green) from 10 highest ranked source combinations according to Schwarz Bayesian Criterion model selection. Panel (a) shows distributions from 3-isotope models, panel (b) shows distributions from 2-isotope models. Note the consistent relative contributions for macrophytes, microalgae, and macroalgae.

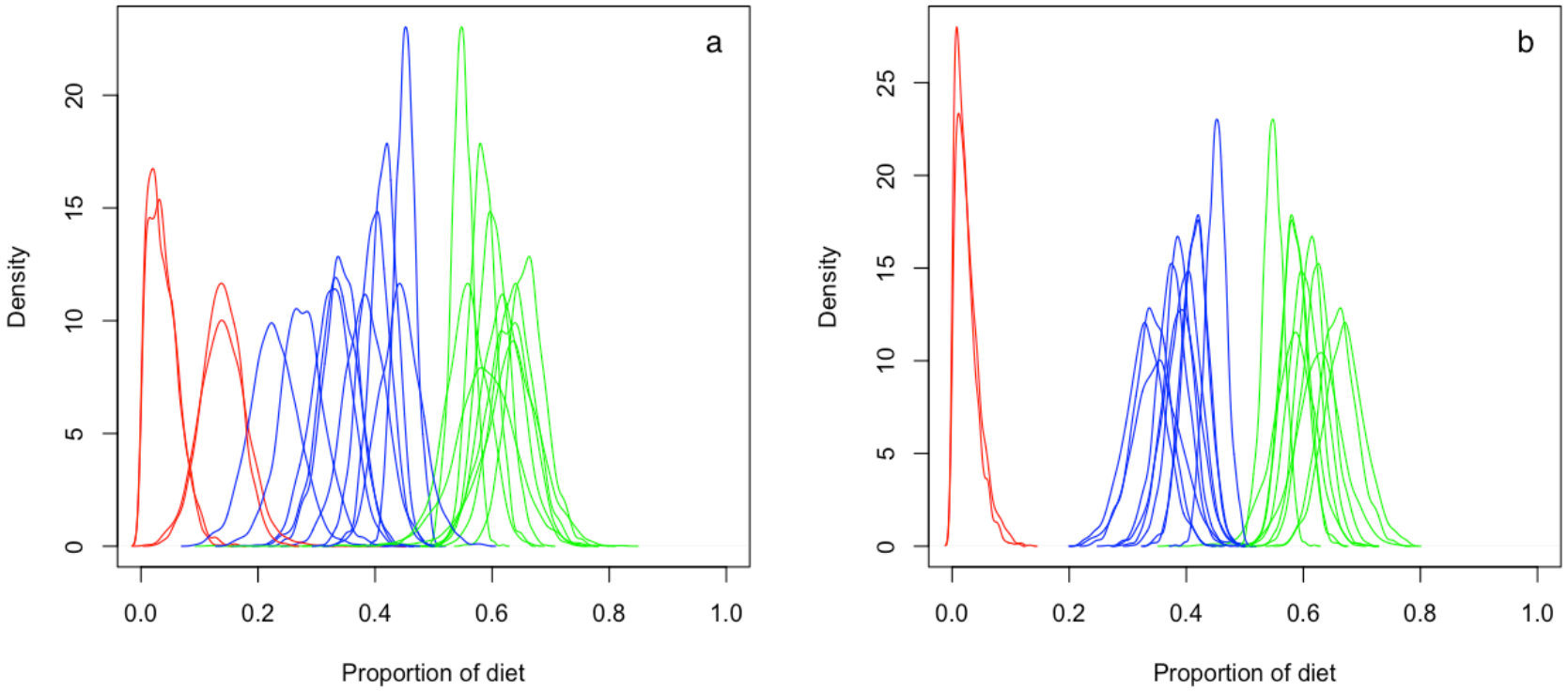

Table 2.S1. Bayesian criteria selection for top 10 ranked source combinations for 3-isotope and 3-isotope models. Source contributions given are means of post-model groupings. Probability distributions of given models are shown in Figure 5. Calculations shown in table are: number of parameters (K), residual sum of squares (RSS), Schwarz Bayesian Criterion (SBC), difference of model *i* SBC from minimum SBC (Δ_i), and normalized relative likelihood (*w*).

						Microalgae		Macroalgae				Macrophtyes	
3-isotope models	RSS	K	SBC	Δ	*w*	Phytoplankton	BMA	*Codium*	*Agardh*	*Ulva*	*Gracilaria*	*Spartina*	*Zostera*
	4.10	5	-2.17	0.00	0.164	15−34%	1−24%	18−40%	8−29%	7−19%			
	5.01	4	-2.13	0.04	0.161	27−40%		26−47%	6−31%	4−15%			
	4.36	5	-1.37	0.80	0.110	26−40%		19−43%	8−32%	4−16%			0−8%
	4.41	5	-1.24	0.93	0.103	25−39%		19−44%	7−32%	5−16%		0−8%	
	6.56	3	-1.20	0.97	0.101	37−45%		35−53%			2−24%		
	8.00	2	-1.18	0.99	0.100	41−48%		51−58%					
	5.41	4	-1.15	1.03	0.098	14−30%			37−55%	10−23%		5−22%	
	5.54	4	-0.83	1.34	0.084	19−35%			33−52%	9−22%			6−20%
	6.80	3	-0.72	1.45	0.079	34−44%		47−56%		1−14%			
	5.70	4	-0.46	1.71	0.070	20−40%	1−27%	33−52%		5−19%			
2-isotope models													
	0.09	3	-57.35	0.00	0.163	33−44%		31−53%	4−33%				
	0.09	3	-57.02	0.33	0.138	31−44%		38−55%		1−27%			
	0.11	2	-56.79	0.56	0.123	40−48%		51−59%					
	0.08	4	-56.09	1.26	0.087	27−40%		26−47%	1−29%	0−23%			
	0.10	3	-55.81	1.54	0.076	36−45%		35−56%			0−25%		
	0.08	4	-55.42	1.93	0.062	28−42%		28−50%		1−25%	0−22%		
	0.09	4	-55.12	2.23	0.054	31−42%		23−47%	3−29%		0−20%		
	0.09	4	-54.81	2.54	0.046	14−34%	0%−21%	8%−36%	3−27%	2−25%	0−19%	0−6%	
	0.08	5	-53.82	3.53	0.028	25−39%		18−44%	1−26%	0−21%	0−20%		
	0.10	4	-52.92	4.43	0.018	32−45%		25−50%	4−35%				0−6%

Table 2.S2. Correlation coefficients of posterior distributions from paired simulated values of dietary proportions drawn by each MCMC iteration. High absolute values of correlations indicate that the model cannot easily differentiate between sources. Sources are abbreviated as *As* = *Agardhiella subulata*, *Cf* =*Codium fragile, Gv* = *Gracilaria vermicuphylla, Ul* = *Ulva lactuca,* BMA = benthic microalgae, Phy = phytoplankton, *Sa* = *Spartina alterniflora*, *Zm* = *Zostera marina.*

	As	*Cf*	*Gv*	*Ul*	*Sa*	*Zm*	Phy
BMA	-0.08	-0.33	-0.06	0.43	-0.16	-0.15	-0.71
As		-0.61	-0.19	0.26	0.10	0.10	-0.36
Cf			-0.35	-0.36	-0.21	-0.29	0.54
Gv				-0.30	-0.04	0	-0.04
Ul					0.12	0.03	-0.73
Sa						-0.15	-0.04
Zm							0.11