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

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ABSTRACT: Bivalves influence both the ecology and the economy of coastal regions. By filter-feeding on particles in the water column, these organisms reduce turbidity and link benthic and pelagic production. In addition, production and sales of harvested bivalves are a source of income in coastal areas like the Eastern Shore of Virginia (USA). Phytoplankton are known to be a main food source to many bivalves; however, ocean-side lagoons off the coast of Virginia support extensive aquaculture of *Mercenaria mercenaria* (hard clams) in waters with relatively low chlorophyll concentrations. 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 the C, N, and H isotopic ratios of aquaculture clams and a variety of primary producers in a Virginia coastal lagoon over an annual cycle and conducted a Bayesian mixing model analysis to identify current energy sources for clams. By adding a third isotopic ratio (hydrogen), we were able to improve precision over a 2-isotope model based on C and N isotopes. Our analysis reveals that field-cultured clams in Virginia coastal lagoons are significantly supported by microalgae (23 to 44 %) but gain most of their energy from macroalgae (55 to 66 %), and only a small fraction from macrophytes (0 to 14 %). While macroalgae are often an indicator of coastal eutrophication, these algae can be an important food source to bivalves when abundant in low nitrogen, oligotrophic systems. Our results also indicate hydrogen stable isotopes are useful in concert with other isotopes for tracing sources in coastal food webs.

KEY WORDS: Clams · *Mercenaria mercenaria* · Stable Isotope Analysis in R · 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 USA, the dominant environmental influences on its growth are not well understood (Henry & Nixon 2008). Many studies conclude that food supply controls the growth of bivalves (Peterson 1982, 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 food, aquaculture clams are often held in field grow-out pens to reach market size where they use *in situ* food sources and experience fluctuating environmental conditions. Clams respond positively to short-term increases in algal production, but eutrophication resulting in reduced sediment oxygen or harmful algal blooms is detrimental to the growth of clams (Weiss et al. 2002, Carmichael et al. 2004, 2012).

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The low chlorophyll waters of coastal lagoons in Virginia (USA) support aquaculture of hard clams that are grown under nets to protect them from predators (Murray & Kirkley 2005). 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, detrital particles from the degradation of vascular plants and macroalgal detritus, as well as microorganisms, small metazoans, and resuspended sediment (Bianchi 2006). Resuspended sediment from wind-driven turbulence can result in high turbidity in the shallow lagoons of Virginia (Lawson et al. 2007) with organic material making up less than 25% of the total suspended solids in the water (Fig. 1).

Although coastal bivalve growth and reproduction are often linked to annual cycles of phytoplankton production (e.g. Ansell et al. 1980, Boon et al. 1998), aquaculture clams in Virginia lagoons may be relying on alternative 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 (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m498p187_supp.pdf) supports the view that phytoplankton and benthic microalgae are the dominant food sources for most studied populations of bivalve suspension feeders. However, planktonic algal communities vary in nutritional quality (e.g. protein and lipid composition), and standard measures of water quality such as chlorophyll or total organic content do not consistently reflect relative food value for growth performance (Beukema & Cadee 1991, Grant 1996, Hawkins et al. 1998). O'Donnell et al. (2003) linked diet shifts in Virginia clams over long time scales with past changes in primary production due to changes in sea level. In the present and in the future, rising sea level (Erwin et al. 2006), increases in nitrogen loading from agricultural activities (Henry & 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 quantity and quality of their food supply (Havens et al. 2001, Carr et al. 2012, Kirwan 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).

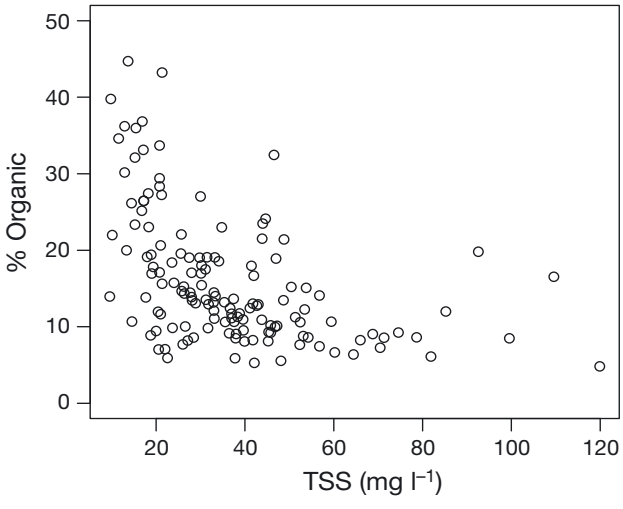

Fig. 1. Relationship between organic matter and total suspended solids (TSS) in water samples for 2005 to 2008 from 6 sites along a transect that is near the sampling site for stable isotopes near Cobb Island, Virginia. Measurements are from the Virginia Coast Reserve Long-Term Ecological Research program (VCR LTER) (www.vcrlter.virginia.edu)

Clams will also respond 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), as well as habitat degradation due to hypoxia, changes in predation, and sedimentation (Whetstone & Eversole 1981, Norkko et al. 2006, Henry & Cerrato 2007, Carmichael et al. 2012). As clams feed, they reduce suspended particles which results in increased light penetration in the water column, couple benthic and pelagic production, and help 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 organic matter supporting clam diets is critical. Nutrition and growth studies are complicated not only by 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 potential food sources is a way to assess if clams are selectively using a specific source of production.

Stable isotopes can be used to quantify proportional source contributions to consumers in coastal ecosystems (Peterson 1999). Carbon isotopic ratios in primary producers vary in relation to photosynthetic pathways that distinguish C3 and C4 autotrophs. Nitrogen isotopic ratios become enriched in successive levels of food webs, but their interpretation is complicated by variation in $\delta^{15}N$ among sources due to kinetic fractionation by processes such as nitrification and denitrification, requiring additional information. Since mixing model discrimination among sources is dependent on the number of sources, differences in isotopic composition among sources, and the number of distinct isotopes, adding a third isotope may help in source resolution (Peterson et al. 1985, Fry 2006). Here, we use the relative composition of the isotopes ^{13}C and ^{15}N along with ^{2}H (deuterium) in primary producers as one way to help resolve among many potential sources. Large differences in hydrogen isotope ratios between different macroalgae and vascular plant (i.e. seagrass, marsh grass) sources may allow for discrimination beyond what is possible with the stable isotopes of carbon and nitrogen. Specifically, hydrogen isotope ratios in primary producers are the result of fractionation during photosynthesis, biophysical fractionations especially differential evaporative losses of hydrogen isotopes in stomal water for emergent plants, and various biochemical fractionations including depletion of deuterium during lipid synthesis (DeNiro & Epstein 1981, Roden & Ehleringer 1999, Sessions et al. 1999). The net result of these processes can lead to substantial differences in the hydrogen isotope ratios of primary producers (Doucett et al. 2007).

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 there are 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 and conditions in the Virginia lagoons (McGlathery et al. 2007). We were interested in the possible significance for clams of other sources, particularly the abundant invasive *Gracilaria vermiculophylla* (Thomsen et al. 2006), as well as various macroalgae that tend to foul clam grow-out nets (Fig. 2). To test these hypotheses, we used a Bayesian mixing model approach (Moore & Semmens 2008) to explicitly incorporate uncertainty in potential sources. We determine that macroalgae along with microalgae are significant in supporting clams.

Fig. 2. *Ulva lactuca* and *Agardhiella subulata*. Macroalgae fouling antipredator nets over hard clam aquaculture pens in Cobb Island Bay, Virginia

MATERIALS AND 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 massive system-wide die-off of seagrass in the 1930s lead to a simultaneous collapse of the scallop fishery, but since 1999, high water quality and re-seeding have resulted in successful restoration of over 4000 acres of seagrass beds (Orth et al. 2006). In recent decades, clam aquaculture has developed into an extensive industry in Virginia (Murray & Kirkley 2005). Aquaculturalists obtain leases from the state of Virginia to use subtidal bottom ground in lagoons for shellfish beds (www.mrc.virginia.gov). We sampled aquaculture clams *Mercenaria mercenaria* from a leased aquaculture bed near Cobb Island, Virginia (37.307376° N, 75.780602° W). The study site is located 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 season, current, wind, and storm conditions. Winters are mild in the VCR and summers are hot with water temperatures in excess of 30°C. Salinities are >30 ppt except after strong precipitation events. Particulate organic matter usually ranges from 2 to 10 mg l^{-1} and total

suspended solids (TSS) range from 10 to 80 mg l^{-1} (Fig. 3a). Only a small portion of the total sediment load is organic and this portion is inversely related to sediment load (Fig. 1). Chlorophyll concentrations are low with no obvious seasonality (Fig. 3b). In a 2 yr study in a similar nearby lagoon, Hog Island Bay, water column chlorophyll *a* never exceeded 12 µg 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 at the study 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, leaves, and needles 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 carbon and nitrogen isotope ratio analysis and on nylon-based filters for hydrogen isotope 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 Nitex screen and then with silica. Trays were exposed to light for 12 to 24 h 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).

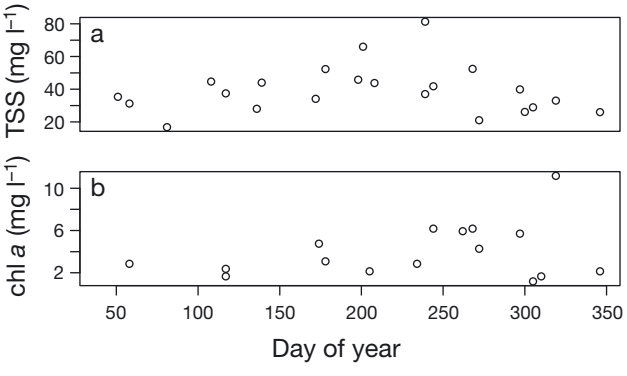

Fig. 3. Seasonal patterns of (a) total suspended solids (TSS) and (b) chlorophyll *a* (chl *a*) for Little Cobb Island measured by the Virginia Coast Reserve Long-Term Ecological Research program (www.vcrlter.virginia.edu). Data are measurements made from 2004 to 2008 by day of year (DOY)

Individual clam samples for isotopic analysis comprised muscle tissue aggregated from 3 animals 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 to reduce the effects of short-term spatial and temporal variation that influence other tissues with shorter turnover time (Yokoyama et al. 2005). We compared the isotopic composition of whole biomass tissue including adductor muscle to just adductor muscle tissue. A few samples of clams from natural populations were also collected from nearby seagrass beds and other lagoons in the VCR for comparison of their isotopic composition with aquaculture clams. Samples from hatchery facilities for H_2O, juvenile clams, and algal feed were also collected to measure C, N, and H isotope ratios for model parameterization of environmental water usage (Solomon et al. 2009) and species-specific trophic fractionation (Post 2002). Samples were collected 6 times during 2010 to 2011 to capture seasonal variability in isotopic ratios based on turnover time in bivalve tissue (Riera & Richard 1997). We analyzed isotopic ratio values derived from clam samples (see below) for seasonal variability using 1-way analysis of variance (ANOVA). We tested for differences in isotopic ratios between whole tissue and adductor muscle samples, as well as between aquaculture versus wild clams, with a *t*-test.

Sample collections of clams and sources were made in November 2010, and February, April, June, July, and September 2011. We typically collected 4 to 5 replicate samples at each sampling time for clams (12 to 15 clams with 3 animals pooled to make a single sample) and available source materials. In the case of clams, as an example, we had a total of 27 replicate samples that were analyzed for 3 stable isotopes, providing 81 observations that constituted the basis for our stable isotope mixing model analysis. Some sources were not available during certain seasons (e.g. some macroalgal taxa during winter). Other sources, like phytoplankton, were sampled indirectly using cultures (see above) or only at the outset of the study (terrestrial vegetation), and in

these cases replication was limited. Overall, replicates for source sample isotope composition were as follows: phytoplankton 4, benthic microalgae 16, *Ulva* 17, *Gracilaria* 16, *Agardhiella* 7, *Codium* 8, *Spartina* 17, *Zostera* 22, terrestrial vegetation 5.

Organic matter samples were rinsed with deionized water to remove salts, dried to constant weight at 60°C for at least 48 h, and powdered with mortar and pestle. Samples were not rinsed with acid to avoid alterations in the isotopic ratios (Mateo et al. 2008). Aliquots of powdered samples were weighed 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 hydrogen isotope ratio analysis, sample values were calibrated to local water vapor according to Wassenaar & Hobson (2003) and compared to a suite of normalization reference standards including algae (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). Values from this analysis represent the non-exchangeable organic hydrogen of samples. Isotope ratios are reported here in the standard del (‰) notation relative to international standards (C: Pee Dee Belemnite, N: atmospheric N_2, H: Vienna Standard Mean Ocean Water) expressed as $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ such that:

$$\delta X = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}}\right] \quad (1)$$

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 clams were evaluated using the Bayesian mixing model Stable Isotope Analysis in R (SIAR) described by Parnell & Jackson (2011). The computer code, user manual and details are available at http://cran.r-project.org/web/packages/siar and its application is further presented in the Supplement at www.int-res.com/articles/suppl/m498p187_supp.pdf. Bayesian models like SIAR expand on the analysis possible with simple mixing models by including many sources of uncertainty as well incorporating evidence from the observed data to interpret the likelihood of mathematically feasible solutions (Moore & Semmens 2008). 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 1×10^6 iterations with the first 400 000 discarded. Model equations for the 3 isotopes used in this analysis are as follows:

$$\delta^{13}C_{\text{clam}} = \sum_{k=1}^{K}\left[\phi_k\left(\delta^{13}C_k + \Delta_C\right)\right] + \varepsilon_C \quad (2)$$

$$\delta^{15}N_{\text{clam}} = \sum_{k=1}^{K}\left[\phi_k\left(\delta^{15}N_k + \Delta_N\right)\right] + \varepsilon_N \quad (3)$$

$$\delta^{2}H_{\text{clam corrected}} = \sum_{k=1}^{K}\left[\phi_k\left(\delta^{2}H_k\right)\right] + \varepsilon_H \quad (4)$$

where k is a source, K is the total number of sources, ϕ_k is the proportional contribution of source k, Δ_X is trophic fractionation for isotope X, and ε_X is residual error for isotope X. Each of the parameters Δ_X, ε_X, and source isotopic ratios are considered to be 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 and Δ_N. Trophic fractionation parameters were directly measured as Δ_C = 1.05‰ ± 0.75 (mean ± SD) and Δ_N = 3.24‰ ± 0.83 by comparing the isotopic ratios of hatchery-raised juvenile clams and their exclusive algal food source. We assumed there was no trophic fractionation for hydrogen based on Solomon et al. (2009). Proportional contributions and residual error were fitted by the posterior model distributions in the model. We included the uncertainty associated with Δ_C and Δ_N in the analysis.

To account for the environmental water contribution to clam tissue (as opposed to food) we directly measured $\delta^{2}H$ of hatchery clams, food source, and water (following Solomon et al. 2009). The resulting estimate of the environmental water contribution to clams (denoted as ω) was ω = 0.15 ± 0.09, which is within the range of values reviewed in Solomon et al. (2009). $\delta^{2}H_{\text{water}}$ at the study site was −9.99‰ ± 0.87. We used these mean values of ω and $\delta^{2}H_{\text{water}}$ to correct all measured clam isotope values. This correction accounts for the contribution of environmental water to the hydrogen of organic matter and is denoted as $\delta^{2}H_{\text{clam corrected}}$ in Eq. (4) above. We explored the consequences of using this correction by calculating maximum and minimum estimates of the correction and running the model analysis with these corrected values. The effects of uncertainty in the correction were small (see the Supplement).

Mixing models have fixed solutions if the number of sources is $n + 1$ relative to the number of isotopes

used (n) (Fry 2006). In our study, we measured the isotopic ratios of 3 elements, but we identified 9 potential sources of production available to clams. Hence, there were many more sources than isotopes. Since terrestrial vegetation had a negligible contribution in an initial 9-source model, is not abundant near our study site, and had $\delta^{15}N$ that was 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 grouping by performing 1-way ANOVAs on source values for each isotope and compared means with post-hoc Tukey's Honestly Significant Difference (HSD) test. These tests incorporated the data from sources across all replicate and seasonal samples. While some sources appeared clustered, grouping the data into categories like macroalgae (a combination of *Codium*, *Agardhiella*, *Gracilaria*, and *Ulva*) would have been inconsistent among isotopes (see below). Therefore, we used the model without pre-model grouping and combined the posterior distributions of related (e.g. all species of macroalgae) sources, as suggested by 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. In order to retain accuracy without excluding data, we modeled all feasible combinations of 2 to 8 sources. 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 3 elements (C, N, and H) to evaluate the utility of including hydrogen isotope ratio data. Since resolution of source contributions to clams 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 periods were averaged for each source prior to mixing model analysis. Thus, variability included in source contributions presented below encompasses a seasonal component.

Model output from the SIAR analysis produced residual sums of squares based on the means and the maximum likelihood of the model (see the Supplement). We compared models using the Bayesian Information Criterion (BIC), which is also known as the Schwartz Bayesian Information Criterion (SIC or SBC) (Burnham & Anderson 2002). The criterion is calculated as:

$$\mathrm{BIC} = -2\ln(L) + K \times \ln(n) \quad (5)$$

where L is the maximum 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 BIC from the minimum BIC (Δ_i) was greater than 10, which excluded models for which the normalized model likelihood was less than 5%. Two-isotope models were compared to 3-isotope models by visual analysis of source contribution posterior distributions.

RESULTS

Source and consumer isotopic ratios

The various primary producers which constitute potential organic matter sources for clams had overlapping isotope ratios; however, there were distinctions among taxa for some elements and in some cases among the 3 major groups of autotrophs (microalgae, macroalgae, and higher plants, i.e. macrophytes) (Fig. 4). Carbon isotopic ratios had relatively low variability within a taxa (error bars in Fig. 4). In the case of $\delta^{13}C$, higher plants, *Zostera* and *Spartina*, had the highest (most positive) values and phytoplankton had the lowest (most negative) values. The HSD test identified the following 5 groups: (1) *Zostera*; (2) *Spartina* and *Codium*; (3) *Codium*, *Gracilaria*, and *Agardhiella*; (4) *Agardhiella*, benthic microalgae (BMA), and *Ulva*; and (5) phytoplankton. While the carbon isotopic ratios of the macrophytes and the microalgae were different, the macroalgal species isotopic values were intermediate and overlapping. Nitrogen isotopic ratios had a small range and relatively low variability for most sources (Fig. 4). The HSD test identified 2 distinct groups: (1) the 4 macroalgal species *Codium*, *Agardhiella*, *Gracilaria*, and *Ulva*; and (2) *Zostera*, BMA, phytoplankton, and *Spartina*. $\delta^{2}H$ values had a much larger range than C or N isotopic ratios, with significant differences between several macroalgal species. The HSD test

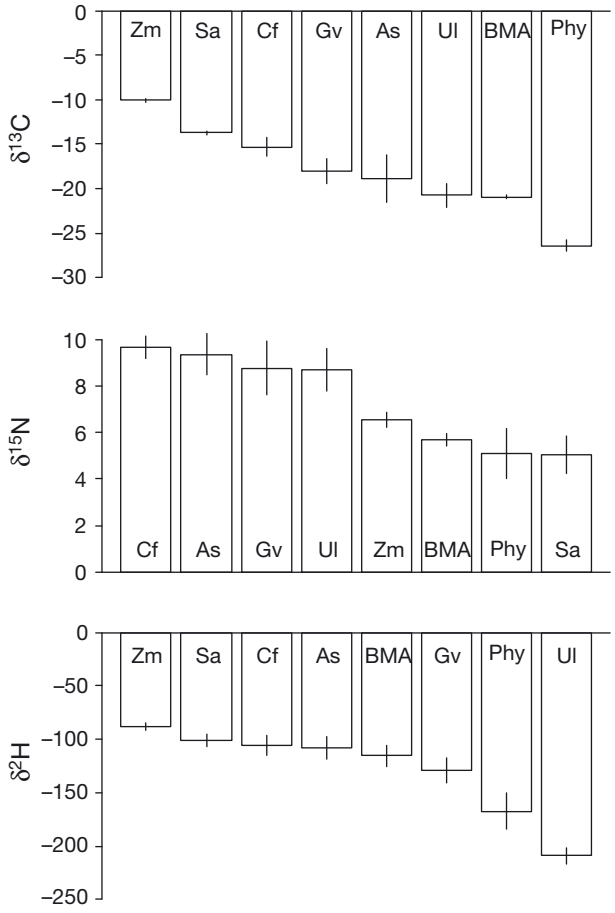

Fig. 4. Means of $\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$ for sources ranked from highest (most positive) to lowest (most negative). Sources are abbreviated as As = *Agardhiella subulata*, Cf = *Codium fragile*, Gv = *Gracilaria vermicuphylla*, Ul = *Ulva lactuca*, BMA = benthic microalgae, Phy = phytoplankton, Sa = *Spartina alterniflora*, Zm = *Zostera marina*. Error bars are 95% confidence intervals

identified 4 groups: (1) *Zostera*, *Spartina*, *Codium*, *Agardhiella*, and BMA; (2) BMA and *Gracilaria*; (3) phytoplankton; and (4) *Ulva*.

Clams had little variation in isotopic ratios throughout the study (Table 1). Temporal variation for C and N isotopes was low except in June when clams had lower $\delta^{13}C$ and higher $\delta^{15}N$. No other sampling period had significantly different C or N isotopic ratios. There was no difference in $\delta^{2}H$ among sampling times (Table 1). Given the low seasonal variation of the isotope ratios, samples were pooled for mixing model analysis. Whole tissue $\delta^{13}C$ of clams was significantly lower relative to muscle tissue, but this difference was relatively small (~1‰). Carbon and nitrogen isotopic ratios of aquaculture clams were not significantly different than those of wild clams found in adjacent seagrass beds and other lagoons. There was a small but significant difference in hydrogen isotope ratios (*t*-test: $p < 0.05$) with a mean difference of about 8‰ (Table 1). Overall, the 2 groups had similar isotopic composition; however, the comparisons were based on limited observations of wild clams and also included some wild clams where only whole animal tissue was measured.

Clam isotopic ratios averaged across the 6 sampling times fell within the polygon described by similarly averaged, source isotope ratios (Fig. 5). Clam tissue isotope ratios were similar to seston but the latter had a more negative $\delta^{13}C$ value (Fig. 5). Seston isotope ratios were closest to but also different from both phytoplankton and benthic microalgae isotope ratios (Fig. 5), indicating a mixed composition of suspended material. When comparing with potential organic sources, clams were closest to the isotope values of macroalgae and benthic microalgae (Fig. 5). *Ulva* and phytoplankton were the only sources with lower $\delta^{2}H$ than clam tissue, suggesting one or both of these resources was used to balance the more positive $\delta^{2}H$ of other sources.

Source contributions

Based on model selection criteria, the models with fewer than all 8 sources had a higher likelihood (Table 2). The 10 models that were ranked highest all had support as the differences in the model selection statistics were relatively small especially for the 3-

Table 1. *Mercenaria mercenaria*. Seasonal variability of the isotope ratios for C, N, and H in aquaculture clams as well as isotope ratios for a sample of wild clams pooled from different times, where n is the number of observations and SD is standard deviation

Clam type	Date	n	$\delta^{13}C$ Mean ± SD	$\delta^{15}N$ Mean ± SD	$\delta^{2}H$ Mean ± SD
Aquaculture	Nov 2010	4	−18.8 ± 0.1	11.6 ± 0.3	−135.4 ± 1.2
Aquaculture	Feb 2011	4	−18.6 ± 0.1	11.8 ± 0.2	−137.5 ± 4.9
Aquaculture	Apr 2011	5	−18.9 ± 0.1	11.3 ± 0.2	−136.9 ± 2.1
Aquaculture	Jun 2011	5	−19.6 ± 0.3	12.6 ± 0.2	−141.3 ± 3.6
Aquaculture	Jul 2011	5	−19.0 ± 0.0[a]	11.6 ± 0.2	−142.8 ± 2.8
Aquaculture	Sep 2011	5	−18.8 ± 0.1	11.3 ± 0.2	−139.3 ± 7.0
Wild	Various	7	−18.2 ± 1.9	11.8 ± 1.7	−132.2 ± 2.7

[a]SD for this sample was 0.015

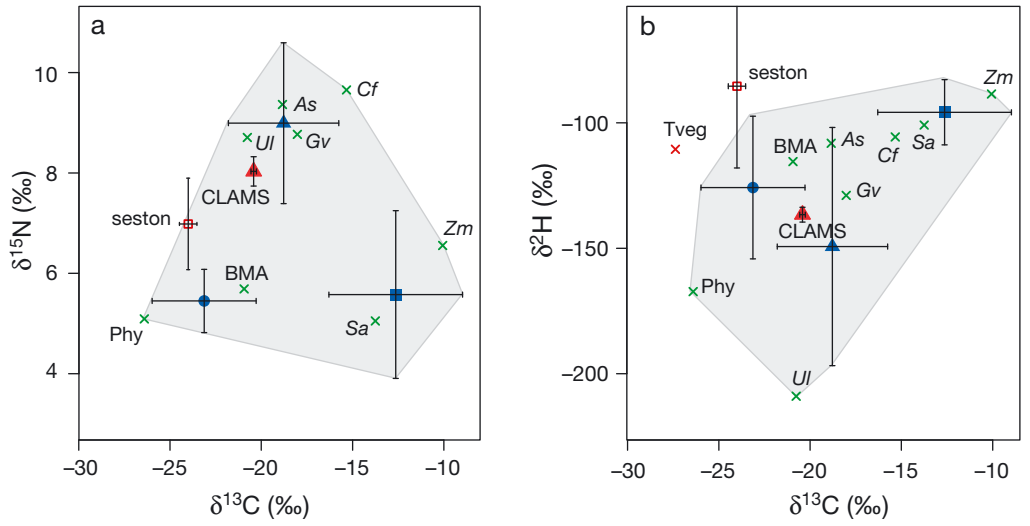

Fig. 5. Isotopic ratios ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$) of primary producer sources, seston, and clam tissue from an aquaculture site in Cobb Island Bay, Virginia. (a) $\delta^{13}C$ and $\delta^{15}N$, (b) $\delta^{13}C$ and $\delta^{2}H$. Sources indicated by a green × were considered individual sources and grouped after modeling, as microalgae, macroalgae and macrophytes. Grouped values are indicated by blue symbols: (●) microalgae, (■) macrophytes, (▲) macroalgae. Error bars are standard deviations. The light gray shape is the mixing polygon created by the sources. Clam isotope values 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 (a) because Tveg was highly depleted in N. Including this point would make the other sources less distinct

Table 2. Statistics and source contributions for the top 10 ranked 3-isotope and 2-isotope Bayesian mixing models. The 95% credible intervals for source contributions by taxa are given as percentages for each model. The last column is the source contributions for means of the post-model groupings as percentages, where the post-model groupings are in the order macroalgae, microalgae, macrophytes. Column headings are: number of parameters (*K*), Bayesian Information Criterion (BIC), difference of model *i* BIC from minimum BIC (Δ_i), and source taxa where the abbreviations are Phyto = phytoplankton, BMA = benthic microalgae, *Agardh.* = *Agardhiella*, *Gracil.* = *Gracilaria*

K	BIC	Δ_i	Phyto	BMA	*Codium*	*Agardh.*	*Ulva*	*Gracil.*	*Spartina*	*Zostera*	Grouped sources
(a) Three-isotope models											
5	−2.17	0.00	15–34	1–24	18–40	8–29	7–19				62/38/0
4	−2.13	0.04	27–40		26–47	6–31	4–15				66/34/0
5	−1.37	0.80	26–40		19–43	8–32	4–16			0–8	63/34/4
5	−1.24	0.93	25–39		19–44	7–32	5–16		0–8		64/32/4
3	−1.20	0.97	37–45		35–53			2–24			58/42/0
2	−1.18	0.99	41–48		51–58						55/45/0
4	−1.15	1.03	14–30			37–55	10–23		5–22		63/23/14
4	−0.83	1.34	19–35			33–52	9–22			6–20	59/28/14
3	−0.72	1.45	34–44		47–56		1–14				60/40/0
4	−0.46	1.71	20–40	1–27	33–52		5–19				56/44/0
(b) Two-isotope models											
3	−57.35	0.00	33–44		31–53	4–33					58/42/0
3	−57.02	0.33	31–44		38–55		1–27				60/40/0
2	−56.79	0.56	40–48		51–59						55/45/0
4	−56.09	1.26	27–40		26–47	1–29	0–23				66/34/0
3	−55.81	1.54	36–45		35–56			0–25			58/42/0
4	−55.42	1.93	28–42		28–50		1–25	0–22			62/38/0
4	−55.12	2.23	31–42		23–47	3–29		0–20			61/39/0
4	−54.81	2.54	14–34	0–21	8–36	3–27	2–25	0–19		0–6	63/35/2
5	−53.82	3.53	25–39		18–44	1–26	0–21	0–20			67/33/0
4	−52.92	4.43	32–45		25–50	4–35				0–6	58/39/3

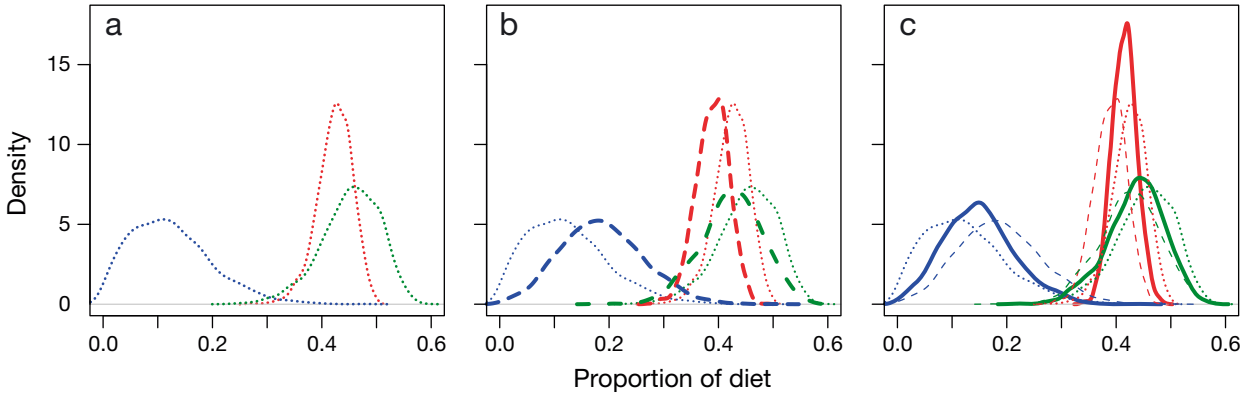

Fig. 6. Probability distributions of source contributions for a representative 3-source model including different isotopes where: (a) distributions (dotted lines) are for a 1-isotope model using only $\delta^{13}C$; (b) distributions are for the 1-isotope model (dotted lines) and a 2-isotope model (dashed lines) including $\delta^{13}C$ and $\delta^{15}N$ data; and (c) distributions are for the 1-isotope (dotted line), 2-isotope (dashed line), and a 3-isotope model (solid line) including $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ data. Sources are: phytoplankton = red, *Codium fragile* = green, *Agardhiella subulata* = blue. Note that distributions for the 3-isotope model are more distinct and have higher maximum probability densities

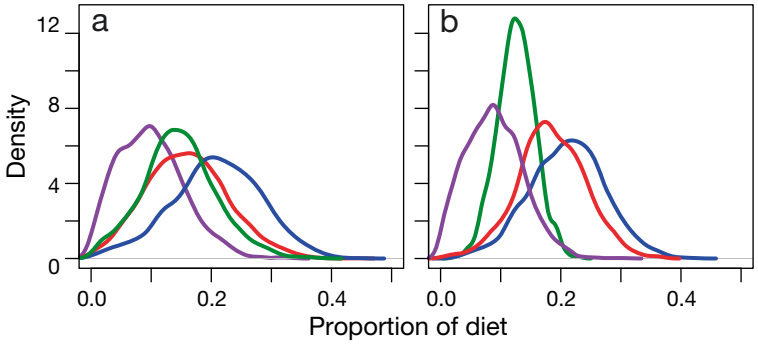

Fig. 7. Probability distributions of source contributions for 4 types of macroalgae from a representative model: purple = *Gracilaria vermiculophylla*, green = *Ulva lactuca*, red = *Agardhiella subulata*, blue = *Codium fragile*. (a) Distributions are from a 2-isotope model that included $\delta^{13}C$ and $\delta^{15}N$. (b) Distributions are from a 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

isotope model (differences in BIC <2, Table 2). The overall best combination of sources for a 3-isotope model was benthic microalgae, phytoplankton, *Ulva*, *Agardhiella*, and *Codium*. The highest ranked source combinations for the 2-isotope model (C and N) were phytoplankton, *Agardhiella*, and *Codium*. Few of the highest ranked models included either *Spartina* or *Zostera*. Although the 2- and 3-isotope models had similar mean values for proportional source contributions, 3-isotope models had more precise posterior distributions (Fig. 6) and allowed for higher resolution of individual source contributions (Fig. 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 macrophytes. Phytoplankton and benthic microalgae were grouped as microalgae, all 4 types of macroalgae were considered a group, and *Spartina* and *Zostera* were grouped as macrophytes (Fig. 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 3), which indicated that the individual models could not easily distinguish between certain sources. Although the general distribution patterns were similar, the 95% credibility intervals with post-model grouping overlap less than pre-model grouping (Fig. 8). Model output with post-model grouping improved both accuracy and precision. Post-model groupings for the top ranked models based on BIC all had a similar pattern. The ranges for these contributions were 55 to 66% macroalgae, 23 to 45% microalgae, and 0 to 14% macrophytes (Table 2, see the Supplement).

DISCUSSION

The isotope mixing models are limited in discriminating among the 8 possible sources supporting hard clams at the Virginia coastal site we studied. Models

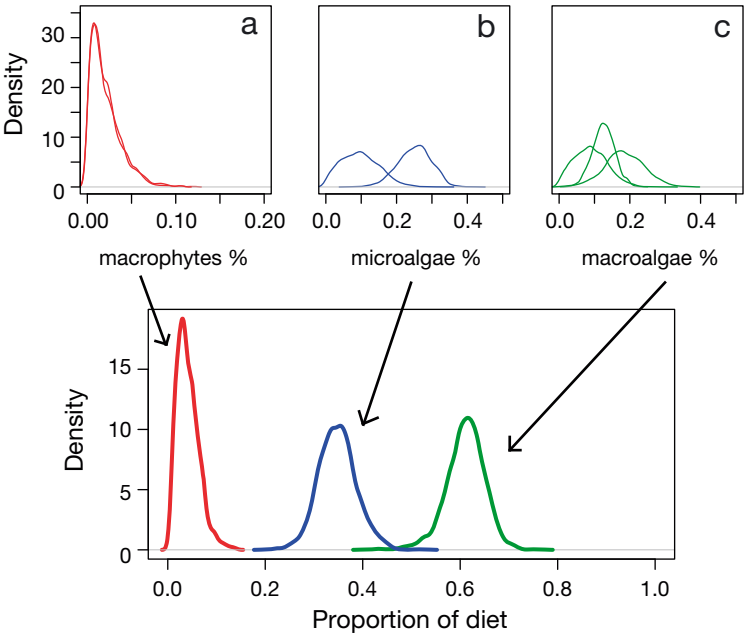

Fig. 8. Probability distributions of post-model grouping of sources as macrophytes (red), microalgae (blue), and macroalgae (green). Small panels (a,b,c) are posterior distributions of individual sources from each of the 3 groups which were summed to form (d) post-model group distributions

Table 3. Correlation coefficients of posterior distributions from paired simulated values of dietary proportions. 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

identifying various mixtures of macroalgae along with benthic microalgae and phytoplankton had nearly equal evidence of fitting the data (Table 2). However, the top ranked models were highly consistent in identifying a combination of macroalgae and microalgae in supporting aquaculture clams. Macroalgae provided greater than 50% of the support in all the top models and there was little or no contribution from macrophytes.

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 a high uncertainty in the models. 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. 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 BIC, clams likely feed only on certain food sources.

Based on post-model groupings, macroalgae are a significant food source and, along with microalgae, support the production of clams. The nutritional value of the macroalgae *Agardhiella* and *Codium* adds support to their potentially large contributions (C:N approximately 12 to 14). Based on mathematically feasible solutions from mixing polygons (Fig. 5), if phytoplankton are not included, then both *Ulva* and benthic microalgae must be included to balance more positive source isotope values. Since the BIC prioritizes fewer model parameters (sources in this case), models that include just phytoplankton were ranked higher than those that include both benthic microalgae and *Ulva*. However, the low concentrations of chlorophyll *a* in these waters lend some support for inclusion of benthic microalgae and *Ulva* rather than phytoplankton as primary sources.

Hydrogen isotopes improved 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 nitro-

gen data improved the accuracy of the model but not the precision; probability distributions of source contributions shifted when $\delta^{15}N$ was added (Fig. 6b). In nearly all cases, credibility intervals were smaller when hydrogen isotopic ratios were included in the model (Fig. 6c). Second, hydrogen isotope ratios potentially 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 overlapped considerably and had similar means when only carbon and nitrogen isotopes were used (Fig. 7a), we determined more precise proportions when hydrogen isotopes were included (Fig. 7b). The contribution of *Gracilaria* remained 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 possible way to improve model performance with the number of possible sources in this system would be to include $\delta^{34}S$ as an additional measurement. Sulfur isotopes are distinct between producers that derive sulfides with low isotopic ratios of $\delta^{34}S$ from reduced anoxic sediments and those that derive sulfates with relatively high $\delta^{34}S$ from seawater (Knoff et al. 2001, O'Donnell et al. 2003, Fry 2006). However, both spatial variability in sulfur isotopic ratios of primary producers (Deegan & Garritt 1997, Stribling et al. 1998), as well as 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 & Lajtha 2007). Hence, adding a fourth isotope would have also resulted in additional complexity.

Implications of source contributions to clams

Anti-predator nets that cover clam aquaculture operations acquire a dense fouling of macroalgae (Luckenbach 2009, Fig. 2). This fouling can have negative impacts on cultured bivalves by causing variability in dissolved oxygen concentrations and by reducing water flow with consequent decreases in food availability. Fouling organisms that are filter feeders can also compete with aquaculture clams, and eventual decomposition of fouling organisms may reduce oxygen supply (Fernandez et al. 1999, Carmichael et al. 2012). However, the accumulation of macroalgae on nets may also 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).

Clam use of fouling macroalgal material might suggest a difference in the sources supporting wild and aquaculture clams. We made a limited comparison of these 2 groups and found they had similar isotopic composition, which suggests they may rely on the same food sources. This possibility requires further analysis for at least 2 reasons. First, clam netting supports substantial epibiotic growth when compared to areas occupied by wild clams (e.g. tidal flats) without netting, as documented by Powers et al. (2007) in North Carolina lagoons. Second, in New England estuaries, there are significant differences in isotopic composition of fauna between areas dominated by seagrass versus macroalgae over relatively short distances of <10 m (Olsen et al. 2013). Hence, our results may apply only or mainly to clams within aquaculture cages where nets promote a localized food source for clams. Further research is warranted to evaluate differences and similarities in resource use between aquaculture and wild clams. For the aquaculture clams that were the focus of our study, all models indicated that macroalgae were significant in supporting the production of this provisioning ecosystem service.

Patchy occurrence of macroalgal species suggests that there is a variable composition of algae at any one time. Stable isotope analysis is further complicated by seasonal variation in isotopic composition, as was measured by Dethier et al. (2013) for macroalgae and for *Zostera marina* in a northwest Pacific estuary. These authors illustrate how consumer diet analysis can be compromised by this variability. We measured relatively low isotopic variability in the sources, partly by focusing on a single site. Further, 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 clams. We suggest that although much of the production in Virginia coastal lagoons is seasonal (McGlathery et al. 2001), a persistent pool of dissolved or particulate detrital material is likely important in supporting aquaculture populations. Post-model grouping indicated the significant reliance of clams on macroalgae sources despite the temporal variation in macroalgal abundances. For the entire Virginia Coastal Reserve,

there is considerable spatial variation among and within the coastal lagoons in types of primary producers as well as production rates (Giordano et al. 2012). We only studied one location and hence our results, especially given the variability documented in other systems (e.g. Dethier et al. 2013), do not address the potential effects of the larger scale spatial variability in sources supporting clam production.

Based on prior studies, macroalgae are rarely the dominant energy source for bivalves (see the Supplement). Macroalgal support of food webs has been identified as characteristic of nutrient-enriched estuaries (Olsen et al. 2011); however, Virginia lagoons that support aquaculture have very low nitrogen inputs and are not eutrophic (McGlathery et al. 2001). Nevertheless, these lagoons support abundant production of potentially nutritious macroalgae from both native and invasive populations (Thomsen et al. 2006, McGlathery et al. 2007). Macroalgal contributions through detrital food chains have been demonstrated in some systems (Riera 1998, Kharlamenko et al. 2001), and grazing on macroalgae is an important control in New England estuaries (Fox et al. 2012). Hence, macroalgae contribute to food webs across gradients of eutrophication, but the relative contribution and the consumers affected likely vary (Olsen et al. 2011, Fox et al. 2012). When specifically considering bivalves, high abundances of macroalgae can have negative effects. For example, invasive macroalgae like *Caulerpa taxifolia* are associated with decline in the abundance and condition of bivalves (Wright et al. 2007, Gribben et al. 2009) due to reduced oxygen levels created by proliferation of these algae. The balance of positive and negative impacts of macroalgae on bivalves likely varies substantially among systems, based on the biomass and types of macroalgae present.

Macrophyte detritus (from *Spartina* spp.) has been linked to bivalve production previously (e.g. Newell & Langdon 1986, Duggins et al. 1989, O'Donnell et al. 2003). The population of clams we analyzed is located proximal to marshes and seagrass beds. Material from these areas is likely significant in the detrital portion of seston, but these macrophytes were not identified as contributing significant support to clams based on mixing model analysis.

Since they are filter feeders, clams only feed on macroalgae and macrophytes as fine particles or in 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 to 3‰ depletion in $\delta^{13}C$ 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 macrophyte detritus in the water column could, therefore, account for a larger contribution to hard clam basal resources if the detritus is enriched with ^{15}N or ^{13}C relative to the living plants we sampled. Less is known about changes in hydrogen isotopic ratios during decomposition, and the limited studies are contradictory (Estep & Hoering 1981, Macko et al. 1983, Fenton & Ritz 1988). The hydrogen isotope results suggest macrophytes are not an important resource for clams. Furthermore, decomposing macroalgae is abundant locally, and their detrital particles could be similarly enriched (in ^{15}N and ^{13}C) during microbial degradation. The observations that macroalgal sources are abundant in this system, degrade into particulates easily, and are a potentially nutritious substrate (Tyler et al. 2001, Thomsen et al. 2006) support the isotope model evidence for a significant contribution to clams.

Since hard clams control feeding processes by reducing clearance rates when there are high levels of suspended particulate matter (Bricelj et al. 1984, Kraeuter & Castagna 2001), eutrophication of Virginia coastal lagoons could 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 hypoxia, 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). Although bivalve populations declined historically in the Virginia Coast Reserve through loss of oyster reefs and scallop populations, restoration and conservation of oysters as well as clam aquaculture have at least partially replaced this lost ecosystem service. Ecosystem managers wishing to sustain successful hard clam aquaculture should consider the importance of macroalgae to this fishery. With adequate food supply and habitat, clams and other bivalves provide benefits to humans 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 aquaculture clams in a low chlorophyll Virginia coastal lagoon use macro- and microalgae as primary food sources. This finding differs from many coastal systems, where phytoplankton are the dominant food source for bivalves. While additional study is needed, our results imply that macroalgal resources likely provide significant support to consumers in other coastal systems with low phytoplankton and persistent macroalgae. Hydrogen isotopes extended the ability to discriminate among sources and improved the precision of mixing models. This analysis supports 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 8 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 macroalgae to clams.

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