ESTIMATES OF PRIMARY PRODUCTION SOURCES TO ARCTIC BIVALVES USING AMINO ACID STABLE CARBON ISOTOPE FINGERPRINTING

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Abstract

Benthic invertebrates are a crucial trophic link in Arctic marine food webs. However, estimates of the contribution of primary production sources sustaining these organisms are not well characterized. Potential sources could include sinking particulate organic matter from sea ice algae and phytoplankton, terrestrial organic matter eroded from the coastal environment, macroalgal material, or microbial organic matter. Proportions of these sources could also be significantly altered in the future as a result of environmental change. We measured the stable carbon isotope values of essential amino acids in muscle tissue from two common bivalve genera (*Macoma* spp. and *Astarte* spp.) collected in Hanna Shoal in the northeastern Chukchi Sea, considered an Arctic benthic hotspot. We used stable isotope mixing models in R (*simmr*) to compare the stable carbon isotope amino acid fingerprints of the bivalves to a suite of amino acid source endmembers, including marine phytoplankton, brown and red macroalgae, bacteria, and terrestrial plants, to estimate the proportional contributions of primary production sources to the bivalve species from Hanna Shoal. The models revealed relatively high contributions of essential amino acids from phytoplankton and bacteria averaged across both species in the region as a whole. We also examined whether stable carbon isotope fingerprints could be measured from essential amino acids preserved in bivalve shells, which could then allow proportional contributions of food sources to be estimated from ancient bivalve shells, allowing source estimates to be extended back in time. To investigate this, we measured the stable carbon isotope values of essential amino acids in a suite of paired modern bivalve shells and muscle from *Macoma calcarea* from the Chukchi Sea. These analyses revealed a correspondence between the fingerprints and mixing model estimates of the dominant primary production source of essential amino acids derived from analyses of these two tissue types. Our findings indicate that stable carbon isotope amino acid fingerprinting of marine bivalves can be used to examine dominant organic matter sources in the Arctic marine benthos in recent years as well as in deeper time.

Key words: Compound-specific stable isotope analysis, amino acid fingerprinting, benthos, bivalves, Hanna Shoal, Chukchi Sea

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Introduction

Zooplankton consume less than half of water column primary production in the Arctic, leaving the majority of the remaining organic matter available to the benthos (Campbell et al. 2009). As a result of the available production, benthic invertebrates dominate the base of the food web in the Arctic and sustain large populations of diving mammals and birds (Schonberg et al. 2014). However, the rapidly changing environmental conditions at high latitudes (Piepenburg 2005, Dunton et al. 2006), which include changes in phytoplankton growth (e.g., Blais et al. 2017, Neukermans et al. 2018), sea ice extent and thickness (e.g., Lavoie et al. 2010, Labe et al. 2018), coastal erosion (e.g., Farquharson et al. 2018) and river discharge (e.g.,Carmack et al. 2016) may significantly influence the proportions of different organic matter sources entering the marine environment of the Arctic. For example, the thinning and receding of annual sea ice of the Arctic shelf systems and consequent increase in light availability has led to a noticeable increase in phytoplankton production, a trend that will likely continue as long as adequate nutrient concentrations are available (Arrigo & Van Dijken 2011, Arrigo et al. 2014). However, warmer water temperatures and increased advection of Pacific water masses are also promoting faster development and higher biomass of zooplankton in the Chukchi (Matsuno et al. 2011, Woodgate et al. 2012). This trend of increased biomass from meso-zooplankton can lead to higher grazing pressure on phytoplankton, undermining the strong pelagic-benthic coupling that currently sustains bivalves in the Chukchi (Grebmeier 2012a). Declining sea ice is also decreasing the abundance of ice algae, an important early spring food source to the benthos (McMahon et al. 2006, Gradinger 2009).

In addition to phytoplankton, terrestrial organic matter may also be increasing in the Arctic. Approximately 10% of global river runoff flows into the Arctic Ocean, resulting in substantial terrestrial organic matter inputs (Macdonald 2000). Climate warming is further increasing terrestrial inputs to the Arctic due to increases in river discharge and permafrost thaw (Peterson et al. 2006, Lantuit et al. 2012). Recent increases in coastal erosion due to warming, storm activity, and sea ice retreat may be contributing additional terrestrial carbon to the Arctic shelf systems (Dunton et al. 2006, Serreze et al. 2007). These may heighten the importance of terrestrial organic matter to the carbon cycle in the Arctic. However, terrestrial organic matter tends to be highly refractory and less bioavailable to benthic invertebrates than marine primary

production sources (Dunton et al. 2006, Harris et al. 2018). The predicted directions of changes in availability of these primary production sources could thus threaten benthic invertebrates in the Arctic. Some of these benthic invertebrates are crucial links to higher trophic levels, being the primary food source of threatened species such as Pacific walrus (*Odobenus rosmarus*) and spectacled eider (*Somateria fischeri*) (Grebmeier 2012a, Young et al. 2017). Consequentially, a decline in sources of bioavailable sinking organic matter may pose an additional threat to these species.

Arctic "hotspots" of productivity are of particular interest due to their high ecological importance to a variety of marine trophic levels. Hanna Shoal is one such feature in the northeastern Chukchi Sea, with high benthic biomass due to the abundant sources of primary productivity supporting high secondary production (Dunton et al. 2017). Autochthonous sources of production in Hanna Shoal include ice algae, phytoplankton, and microalgae present on sediment (i.e., microphytobenthos) (Dunton et al. 2017). Hanna Shoal also receives high inflow of organic matter-rich water masses from the North Pacific, contributing to its productivity (Dunton et al. 2017). Moreover, grazing pressure from zooplankton is low, allowing much of the primary production to sink to the benthos, promoting strong pelagic-benthic coupling (Dunton et al. 2017). These high levels of primary production support high densities of calorie-rich bivalves in Hanna Shoal, making the region an important summer feeding ground for Pacific walrus (Young et al. 2017). Changes to primary production due to climate warming could induce bottom-up effects that alter the structure of the ecosystem, reducing food availability to consumers and placing further pressure on species such as Pacific walrus that are already influenced by retreating sea-ice habitats (Young et al. 2017). There is, thus, a pressing need to better characterize benthic food web structure in these systems in order to improve predictions regarding changes in primary production sources.

Trophic studies of marine benthic communities in the Pacific Arctic have often used "bulk" stable carbon and nitrogen isotope values (expressed as $\delta^{13}C$ and $\delta^{15}N$) to assess feeding ecology, trophic structure, and organic matter sources (Iken et al. 2010, Feder et al. 2011, Divine et al. 2015, Tu et al. 2015). Bulk refers to stable carbon and/or nitrogen isotope analyses of total organic matter of primary producers and consumers. Although bulk isotope analyses provide a cost-effective and relatively quick method of examining food webs, there are some shortcomings, including limitations concerning source specificity, differential routing to tissues,

and uncertainties about trophic fractionation (Gannes et al. 1997, Phillips et al. 2014). To increase source specificity in studies of consumer diets, additional isotopic techniques have been developed, including compound specific stable isotope analyses of fatty acids (Budge et al. 2008, Wang et al. 2014, 2015, 2016, Oxtoby et al. 2016, 2017, Schollmeier et al. 2018) and essential amino acid stable carbon isotope fingerprinting (Larsen et al. 2009, 2013, McMahon et al. 2016).

Although essential amino acid stable carbon isotope fingerprinting has not yet been applied in the marine environments of the Arctic, this approach can distinguish between bacterial, marine, and terrestrial primary producers. This approach relies on the stable carbon isotope values of essential amino acids having consistent and unique patterns within primary producer clades due to differing biosynthetic pathways (Larsen et al. 2009, 2013). Essential amino acids cannot be synthesized by animals, so patterns of stable carbon isotopic values of essential amino acids, or "fingerprints," from primary producers are conserved in the amino acids of consumers (Larsen et al. 2009, 2013). These fingerprints, analyzed from soft tissues such as muscle from consumers can then subsequently be used to estimate the proportional contributions of primary producers to essential amino acids of consumers (Larsen et al. 2009, 2013). Essential amino acid stable carbon isotope fingerprinting could, therefore, provide a new and quantitative approach to determine primary production sources sustaining benthic invertebrates in the Arctic.

Bivalve shells as well as soft tissues contain amino acids (Ellis et al. 2014) and could potentially be used to conduct amino acid stable carbon isotope fingerprinting (Misarti et al. 2017). Shell precipitation is guided by an organic matrix, which becomes tightly bound to the calcium carbonate structure, resulting in a small fraction of organic material being entrained in the shell (Wheeler 1992). This organic fraction can be preserved for thousands of years without significant alteration (O'Donnell et al. 2007). Because bivalve shells preserve much more readily than soft tissue, allowing them to remain in the fossil and archeological record (O'Donnell et al. 2007), their amino acid stable carbon isotope fingerprints could potentially be analyzed and used to reconstruct long-term changes in primary production sources to bivalves in the Arctic. Previous research has indicated that although some amino acids in fossil bivalve shells tend to become slightly enriched in ^{13}C over time, the overall pattern of $\delta^{13}C$ values of different amino acids relative to each other largely remain the same (O'Donnell et al. 2007). Fossil shells could, therefore, be good candidates for essential amino acid stable carbon isotope fingerprinting, which

uses the relative patterns of amino acid $\delta^{13}C$ values rather than their absolute values to gain information about carbon source proportions (Larsen et al. 2009). However, little research has been done on stable carbon isotope analyses of amino acids in shells (e.g., Ellis et al. 2014), and no attempts to date have compared amino acid stable carbon isotope fingerprints from bivalve shells and soft tissues. If a relationship between the two tissue types can be established, then fossil shells could be used to infer the primary production sources in ancient clams and compare them to their modern counterparts (McMahon et al. 2018).

In this study, we aimed to generate a set of essential amino acid stable carbon isotope fingerprints for a suite of primary production sources (endmembers) from the Arctic, including macroalgae, phytoplankton, and terrestrial plants, to supplement previously published endmember data (Larsen et al. 2009, 2013, McMahon et al. 2016). We then aimed to apply essential amino acid stable carbon isotope fingerprinting and Bayesian mixing models (Larsen et al. 2009, 2013, Parnell et al. 2013) to analyses of two common bivalve genera (*Astarte* spp. and *Macoma* spp.) from Hanna Shoal to estimate the proportional contribution of different primary producers to bivalves in this region. We hypothesized that essential amino acid stable carbon isotope fingerprints of bivalves from Hanna Shoal will reflect diets high in contributions from microalgae. This would support their proposed reliance on marine algal "food banks" (Dunton et al. 2017), as well as observations of large phytoplankton blooms in the region (Arrigo et al. 2014, Arrigo & van Dijken 2015). Finally, we aimed to use paired samples of muscle and shell from a modern Arctic clam species, *Macoma calcarea* to examine and compare the amino acid stable carbon isotope fingerprints and mixing model results between the two tissue types. We hypothesized that shell and muscle essential amino acids will have similar stable carbon isotope fingerprints.

Methods:

Sample collection

We compiled a suite of potential primary producers (endmembers) from the Arctic to complement the endmembers that have previously been analyzed for their essential amino acid stable carbon isotope fingerprints and published (Larsen et al. 2009, 2013, McMahon et al.

2016). We obtained four Arctic diatom species (*Skeletonema marinoii*, *Coscinodiscus sp.*, *Porosira glacialis*, and *Chaetoceros furallatus*) that were either isolated from water samples or germinated from spore-containing sediment samples collected in the Barents Sea or along the coast of northern Norway. Species were identified by a combination of morphological and molecular methods. Stock cultures for inoculation were kept in a climate-controlled room at 5±0.5°C and 50 µmol m^{-2} s^{-1} scalar irradiance and a photoperiod of 14:10 h (light:dark) in Guillard's f/2×50 marine water enrichment solution at the Norwegian College of Fishery Science, University of Tromsø. Cultures were grown semi-continuously in 100 l Plexiglas cylinders by diluting the cultures with fresh nutrient-replete culture medium once they reached late exponential phase. The culture medium was prepared from filtered (0.22 µm), pasteurized, local seawater (Tromsø Sound, 25 m depth) by adding silicate (final concentration 12.3 µM) and a commercial, amino acid-free, nutrient mixture (SubstralTM, 0.25 ml l-1; The Scotts Company [Nordics] A/S, Denmark). This nutrient mixture was chosen due to the need for adding economically feasible amounts of nutrients to large culture volumes. All cultures were aerated with compressed air to avoid sedimentation and CO_2-limitation. Culture samples were collected by first concentrating cells onto a plankton net (mesh size 5-20 µm) before centrifuging at 3500 rpm for 5 min in a cooled centrifuge (4°C). The obtained wet pellets were transferred into 50 ml Falcon tubes and stored at -80°C.

We also obtained five red algae species (*Coccotylus truncatus*, *Dilsea* sp., *Rhodomela* sp., *Odonthalia dentata*, and *Phycodrys* sp.) and two kelp species (*Laminaria saccharina* and *Alaria esculenta*) using hand-collection by SCUBA divers from between 5-10 m depth along the Beaufort Sea coast. Five terrestrial plant species were also collected from the University of Alaska Fairbanks (UAF) campus (*Eriophorum angustifolium*, *Salix herbacea*, *Rhododendron groenlandicum*, *Alnus sp.*, and *Betula pendula*). Terrestrial samples and macroalgae were dried and pressed in a herbarium press.

Samples of two bivalve genera (*Astarte* spp. and *Macoma* spp.) were collected during research cruises aboard the USCGC Healy in August 2012 and 2013 as part of the Hanna Shoal Ecosystem Study, an extension of the COMIDA CAB program (Grebmeier 2012b, Cooper 2013). Sampling stations were oriented around 72 °N, 162 °W (Dunton 2016) (Figure 1). Samples were collected using a van Veen grab, then sorted, identified, and dried at 60°C onboard (Grebmeier 2012b, Cooper 2013). A total of n=26 *Astarte* spp. and n=24 *Macoma* spp. samples,

with 1-3 individuals per station, were selected for compound-specific stable carbon isotope analysis of amino acids.

To examine the relationship between shell and muscle amino acid stable carbon isotope fingerprints, a total of 13 *Macoma calcarea* samples with both shell and muscle materials present were chosen to maximize available geographic and isotopic range based on bulk carbon isotope analyses. These samples were collected with Van Veen grabs as part of the 2015 AMBON research cruise in the Chukchi Sea (Iken 2015).

Bulk stable isotope analysis

Endmember samples were lyophilized for approximately 48 h and weighed to 0.2-0.5 mg into tin capsules for bulk stable carbon (and nitrogen) isotope analysis. The foot of each bivalve specimen was removed, rinsed with deionized water, lyophilized, and powdered using a Wig-L-Bug® grinding mill. The samples were then weighed to approximately 0.5 mg into tin capsules for bulk stable carbon (and nitrogen) isotope analysis. The powdered samples were then lipid-extracted by repeatedly soaking in 2:1 chloroform methanol, decanting, and adding fresh solution until the supernatant was clear (approximately three times per sample). The samples were then lyophilized and weighed to approximately 0.5 mg into tin capsules for bulk stable carbon (and nitrogen) isotope analysis.

Whole shells were thoroughly scrubbed and rinsed with deionized water to remove potential surface contaminants, resulting also in the removal of the periostracum. Shells were then powdered using a Wig-L-Bug® grinding mill. The powder was demineralized by soaking in 6N HCl for ~24 h, decanting, and adding fresh HCl until bubbling ceased. Samples were then rinsed in deionized water to remove HCl until the pH was neutral, centrifuged at 5000 rpm for 10 min, and lyophilized. The resulting organic matter was weighed to approximately 0.5 mg into tin capsules for bulk carbon (and nitrogen) stable isotope analysis. Some shell organic samples were excluded from bulk analysis due to small size so that there would be adequate material left for amino acid stable carbon isotope analysis (described below).

Bulk carbon and nitrogen samples were analyzed using continuous-flow isotope ratio mass spectrometry on a Thermo Scientific Flash 2000 elemental analyzer interfaced via a Thermo Scientific Conflo IV to a Thermo Scientific DeltaV^{Plus} Isotope Ratio Mass Spectrometer

(IRMS). Stable isotope ratios are reported in delta (δ) notation as $((R_{sample}/ R_{standard}) -1) \times 1000‰$, where R is the ratio of heavy to light isotope. The standard for carbon was Vienna Pee Dee Belemnite (VPDB) and the standard for nitrogen was air. Analytical error from multiple (n = 22) analyses of an internal laboratory standard (peptone) was ≤0.3‰. Analyses were performed at the Alaska Stable Isotope Facility (ASIF) at UAF.

Compound specific stable carbon isotope analyses of amino acids

Lipid-extracted and lyophilized endmembers and bivalve muscle samples were weighed to approximately 2.5-3.0 mg into 13 x 100 mm Pyrex VWR culture tubes with PFTE lined screw caps. Some shell samples did not yield sufficient organic matter to weigh out to this sample weight. For these specimens, all of the organic matter remaining after demineralization was used for amino acid stable isotope analysis, and samples were concentrated down accordingly in the final step of amino acid derivatization. One ml of 6N HCl was added to each sample, and each was flushed with N_2 gas to remove oxygen. The samples were then hydrolyzed on a heating block at 110°C for 20 h. After hydrolysis, samples were passed through a 0.2 µm Millex-GP filter into new dram vials. Next, 25 µl of 0.1 mM norleucine were added to each sample as an internal standard. Samples were dried on an N-evaporator in a 60°C water bath. To form amino acid isopropyl esters, 2 ml of freshly prepared 2-propanol acidified with acetyl chloride was added to each sample, and samples were heated to 110°C for 60 min. Samples were then dried on an N-evaporator in a 60°C water bath and washed and evaporated twice with dichloromethane (DCM). To acetylate the samples, 0.5 ml of DCM and 0.5 ml of trifluoroacetic anhydride were added, and samples were heated to 100°C for 10 min. Samples were then dried on an N-evaporator at room temperature and washed and evaporated twice with DCM. Finally, 250 µl of DCM was added to each sample to transfer them to GC vials. A pure 12-amino acid standard of equal concentrations of alanine (Ala), glycine (Gly), threonine (Thr), serine (Ser), valine (Val), leucine (Leu), isoleucine (Ile), norleucine (Nle), proline (Pro), aspartic acid (Asp), glutamic acid (Glu), and phenylalanine (Phe) was prepared concurrently with each batch of samples using the same methods described above to account for fractionation during preparation (e.g., O'Brien et al. 2002). Derivatized samples were injected using an auto-sampler (Thermo-Scientific TriPlus RSH) into an Agilent Single Taper Ultra Inert Liner (#5190-2293) held at 280°C for 2 min. The

compounds were separated on a Thermo TraceGOLD TG-200MS GC column (60 m x 0.32 mm x 0.25 um) installed on an Agilent 6890N gas chromatograph (GC) interfaced with a Thermo Scientific DeltaV^{Plus} Isotope IRMS via a GC-III combustion (C) interface. The oven temperature of the GC started at 50°C and heated at 15°C min^{-1} to 140°C, followed by 3°C min^{-1} to 152°C and held for 4 min, then 10°C min^{-1} to 245°C and held for 10 min, and finally 5°C min^{-1} to 290°C and held for 5 min. Each sample was run in triplicate and the average reproducibility (1 standard deviation) across all amino acids from samples was ≤1‰. Average reproducibility from all amino acids from the pure standards was ≤0.6‰. Average reproducibility for the internal standard (norleucine) from all analyses was ≤0.7‰.

Data analysis

Each amino acid specific isotope analysis was corrected by subtracting the difference between the run-specific and average isotopic value of norleucine (the internal standard) to each sample's isotopic value. Amino acid $\delta^{13}C$ values were also corrected for the carbon added as a result of derivatization in accordance with previously published protocols (O'Brien et al. 2002). Essential amino acid $\delta^{13}C$ values were also mean-centered (normalized) by subtracting each value from the average of the essential amino acid $\delta^{13}C$ values for that sample (Larsen et al. 2009). This allowed direct comparison of essential amino acid $\delta^{13}C$ patterns (i.e., fingerprints) among samples (Larsen et al. 2013).

Statistical analyses were performed in Microsoft Excel 2011 version 14.7.0 and R version 3.4.0 with RStudio interface version 1.0.143 (R Development Core Team 2011). To estimate proportional contributions of primary producers to bivalve amino acids, mixing models were generated using the R package *simmr* (Parnell et al. 2013). We chose to use the phytoplankton endmember data we generated in this study as part of the mixing model inputs, rather than previously published phytoplankton values, because they were more taxonomically constrained than the literature phytoplankton data (i.e., all diatoms). We also used terrestrial plant, red algae, and brown algae endmember data generated in this study. We used published values from Larsen et al. 2009 and 2013 for bacterial endmembers because Arctic marine bacterial samples cultured on amino acid-free media were unavailable to us. For the Hanna Shoal bivalves, the mixing models were run with samples separated by genus (*Astarte* and *Macoma*). To compare bivalve

shell and muscle essential amino acid fingerprints, the mixing model was run separately for the two tissue types. The essential amino acid carbon stable isotope values for bivalve shells and muscle were also compared with paired t-tests to test the hypothesis that shell and tissue endmember signatures were not different.

To examine how essential amino acid $\delta^{13}C$ patterns of the endmembers generated in this study compared with previously published values of the same endmember categories (Larsen et al. 2009, 2013, McMahon et al. 2016), we used polynomial contrasts to compare the patterns of endmembers of the same category from various datasets. These tests are critical in that endmember source signatures must not overlap, and separation of endmembers is a necessary condition for further analysis (Phillips et al. 2014). Mixed models were used with dataset and essential amino acid identity as fixed factors and species by essential amino acid interaction as the random factor. Significant interactions between dataset and essential amino acid identity were considered as evidence that the datasets followed statistically different patterns, rather than simply offsets in mean values. We also performed a linear discriminant analysis (LDA) of the endmember datasets (from this study and previously published data: Larsen et al. 2009, 2013, McMahon et al. 2016) to test whether the endmember categories were significantly different from each other regardless of any statistical differences detected between published and our own endmember data.

In order to validate the use of the Arctic diatom species endmember as a model input for making estimates of diet proportions of Arctic bivalves, we tested how model results would change with differing endmember values. To do this, three mixing model runs (sensitivity tests) were conducted using different datasets: dataset 1 refers to pooled literature values for endmembers (Larsen et al. 2009, 2013, McMahon et al. 2016); dataset 2 refers to Arctic diatom values newly generated in this study, with the addition of literature values for bacteria due to a lack of availability of isolated Arctic bacteria cultures for this study; and dataset 3 refers to using mean values based on pooled literature and our newly generated values (Supplementary Table 1). The Hanna Shoal bivalve essential amino acid $\delta^{13}C$ values were used as inputs for the mixing model sensitivity tests to estimate the proportional contributions of the endmembers (phytoplankton, red algae, brown algae, bacteria, and terrestrial plants) to the diets of these bivalves. The outputs of the three different models were then compared to examine the effects of endmember source differences.

Results

Eleven amino acids were resolved successfully for all samples: Ala, Gly, Thr, Ser, Val, Leu, Ile, Pro, Asp, Glu, and Phe. Of these, we focused on the essential amino acids (Thr, Val, Leu, Ile, Phe) because they are not synthesized by animals and, therefore, must be derived from sources of primary production without significant fractionation.

Mixing model results for bivalves from Hanna Shoal

Estimated dietary proportions of *Astarte* spp. and *Macoma* spp. from the mixing model are presented in Table 1. The highest source contributions were from phytoplankton and bacteria for both genera, though the absolute proportions varied (Figure 2). Phytoplankton was estimated to contribute a higher proportion to essential amino acids of *Astarte* spp. than to *Macoma* spp. (Table 1). This was balanced by slightly higher contributions of bacteria and terrestrial plants in *Macoma* spp. (Table 1).

Shell vs. muscle comparison

Before lipid extraction, the bulk $\delta^{13}C$ values of individual shell samples of *Macoma calcarea* were significantly different from their corresponding muscle samples (paired 2-sample t-test, $p<0.002$). After lipid extraction of the muscle, the bulk $\delta^{13}C$ values of the two tissue types were not significantly different (paired 2-sample t-test, $p=0.479$). The mean C:N value of shell organic materials was 3.9 (±0.2).

The relationship between shell and muscle essential amino acid $\delta^{13}C$ values was tightly correlated and close to a 1:1 line ($y = 0.85x$, $R^2 = 0.91$), suggesting overall similar patterns in the essential amino acid fingerprints (Figure 3a). However, $\delta^{13}C$ values of Thr, Val, and Ile were significantly different across tissue types (paired 2-sample t-tests, all $p<0.003$) (Figure 3b).

When using *Macoma calcarea* muscle values, the model estimated phytoplankton and bacteria as the two highest-ranking essential amino acid sources (Table 2, Figure 4a). When using *Macoma calcarea* shell values from the same individuals, the model likewise estimated phytoplankton as the highest dietary proportion, but the subsequent rankings differed (Table 2,

Figure 4b). The model estimated that the second ranking source was terrestrial plants, followed by brown algae, and bacteria were the lowest ranking source (Table 2, Figure 4).

Endmembers

The LDA confidence ellipses for terrestrial plants, bacteria, red algae, Arctic brown algae, and Arctic phytoplankton did not overlap with those of any other endmembers, indicating that these endmembers were distinct from each other (Figure 5). This satisfies the condition that sources used for stable isotope diet reconstructions should have significantly different values (Phillips et al. 2014). This finding allowed us to proceed with using these endmember data to generate mixing models. However, the confidence ellipses of brown algae and phytoplankton from literature values did overlap, suggesting that these endmember sources may need further investigation or combining (Figure 5). Furthermore, the dataset by essential amino acid interaction of the polynomial contrasts was significant ($p<0.0001$) for all parameters investigated except brown algae, indicating that although the general fingerprints of the same endmember categories from different datasets were the same, there were subtle differences in how values changed between essential amino acids by dataset for each endmember category.

We report *simmr* mixing model results for Hanna Shoal bivalves from three sensitivity tests based on three different datasets of endmember amino acid carbon isotope values. Phytoplankton was estimated to make up the largest proportional contribution of essential amino acids in all three of the mixing model tests (Supplementary Table 2). However, the mixing model outputs differed in their determination of subsequent rankings of endmember contributions (Supplementary Table 2). Overall, these sensitivity tests confirmed that the phytoplankton values were driving most of the differences in the estimates of proportional contributions from different sources between the models (see Supplementary Materials for more details).

Discussion

Our mixing models showed that phytoplankton is the most important contributor of essential amino acids to Chukchi Sea bivalves. This finding supports observations of highly productive phytoplankton blooms in the region (Arrigo et al. 2014, Arrigo & van Dijken 2015).

Additionally, the phytoplankton endmembers in the models encompass ice algae, which are largely composed of diatoms (Budge et al. 2008). Therefore, these results may also reflect a contribution of ice algae as a food source for bivalves, as has been suggested for various arctic bivalve species (McMahon et al. 2006, Dunton et al. 2017).

The Hanna Shoal bivalve model estimated that bacteria made up a higher proportion of bivalve essential amino acids than terrestrial plants. Given that bacteria break down and rework more refractory organic matter, this ranking seems ecologically sensible. Nevertheless, the model estimated larger diet contributions of terrestrial organic matter to Hanna Shoal bivalves than brown algae or red algae, particularly to *Macoma* spp. This is difficult to explain geographically, as Hanna Shoal is far offshore. One potential mechanism for the availability of terrestrial organic matter to bivalves in this region may be from sediment-laden ice sheets transported by the Beaufort Gyre into the western Beaufort Sea and Chukchi Sea (Babb et al. 2013). During their westward transport, these large ice sheets can get trapped behind Katie's Floeberg, a shallow-water feature on top of Hanna Shoal (Barrett & Stringer 1978). As the ice melts, sediments that could contain terrestrial organic matter could subsequently collect around Hanna Shoal. However, this mechanism would rely on the tidewater glaciers entraining a sufficient quantity of terrestrial organic matter for transfer to the Hanna Shoal. A more plausible explanation is that Hanna Shoal is located downstream of sediment-laden ice that is advected out of shallow waters in the Beaufort and Chukchi Sea, entraining significant amounts of sediment and terrestrial organic matter in the region (Eicken et al. 2005), which possibly delivers terrestrial organic matter to the Hanna Shoal region and is subsequently released to the benthos when the ice melts. Another possible scenario is the advection of river-derived organic matter with currents running adjacent to Hanna Shoal (Feder et al. 1994).

There also appeared to be some differences between the two bivalve species we investigated. Phytoplankton was estimated to contribute a higher proportion of essential amino acids to *Astarte* spp. than *Macoma* spp., while terrestrial organic matter and bacteria were estimated to contribute less to *Astarte* spp. The differences in endmember contributions are likely due to their differing feeding modes. As suspension feeders, *Astarte* spp. filter particles mostly from the water column (Macdonald et al. 2010). In contrast, *Macoma* spp. are surface deposit feeders, which extract organic matter from ingested surface sediments (Macdonald et al. 2010).

This may allow *Macoma* spp. to take advantage of a wider range of deposited organic matter sources (Young et al. 2017), and therefore, consume more bacteria in deposited carbon.

The essential amino acid isotope fingerprints (patterns) of *Macoma calcarea* shell and muscle were very similar. The mixing model results of using the essential amino acid isotope fingerprints from these two tissue types both also identified phytoplankton as the highest contributor to the bivalves' essential amino acids. This similarity indicates that shells can be used to estimate the proportional contributions of the dominant source of essential amino acids to the diets of bivalves when soft tissues are not available for analyses. However, the model estimates of the other potential dietary sources differed not only in estimated proportion, but also in the relative ranking of their importance between the two tissue types. This may be because the formation of soft tissues and shell organics represent different time frames within the lifetime of a bivalve (Misarti et al. 2017). Due to the minute organic matter fraction in each shell as well as potentially irregular growth bands (Moss et al. 2018), we homogenized whole shells in order to yield enough sample for analysis. This resulted in time averaging over the entire lifespan of the bivalves that likely did not reflect the same temporal window as muscle samples. This may explain some of the offset between shell and muscle isotopic values. Future research efforts could be dedicated to a long-term controlled feeding study of bivalve species to better identify the factors causing differences between essential amino acids in shells and muscle.

After the relationship between shell and muscle fingerprints are refined, either by applying a correction based on the differences for certain essential amino acids we have observed or from the results of future controlled feeding studies of bivalves, future research directions could include analyses of archaeological bivalve remains (i.e., from archeological middens) or death assemblages to establish a pre-industrial baseline. Patterns of amino acid $\delta^{15}N$ values of modern bivalve shells are consistent with archaeological shell samples of the same taxa (Misarti et al. 2017), suggesting that amino acid $\delta^{13}C$ patterns are likely consistent as well. The similarities in the estimates of the proportional contributions of phytoplankton to bivalves using either shell or muscle samples indicate that archived *Macoma* spp. shells could be used to investigate possible changes in the proportional contribution of phytoplankton over time. This in itself would be a valuable parameter to examine given predictions for phytoplankton biomass to increase in the future and the likelihood that it has changed in the past. Identifying how organic

matter pathways have changed both in recent years and over longer (millennial) timescales will yield a better understanding of how current changes are altering the Arctic ecosystem.

Here we showed that the essential amino acid fingerprinting method can be used to distinguish sources of Arctic primary producers to two bivalve species. However, this method is limited in its resolution when separating taxonomically similar endmembers is desired. For example, the importance of ice algae to the diets of the bivalves in this study remains obscured by the inability to distinguish them from pelagic phytoplankton, as both are largely composed of diatoms (Budge et al. 2008). A future solution may be the combination of stable carbon isotope analyses of essential amino acids and of fatty acids from the same samples. Stable carbon isotope analyses of fatty acids coupled with fatty acid profiling have been successfully used at high-latitude marine locations to determine the proportional contribution of ice algal-derived fatty acids (Budge et al. 2008, Wang et al. 2014, 2015, 2016, Oxtoby et al. 2016). For example, the proportional contribution of ice-derived particulate organic matter, based on prior fatty acid stable carbon isotope data, to some bivalve species (*Macoma calcarea* and *Nuculana radiata*) in the Bering Sea was up to 47% (Oxtoby et al. 2016), suggesting that it may be a highly important food source to bivalves in the Chukchi Sea as well.

In comparisons between our endmembers and literature values, most endmembers of the same category had very similar essential amino acid isotope fingerprints, despite some statistical differences. These similar patterns were apparent even over a wide geographic spread. For example, the fingerprints of Arctic red algae analyzed in this study and tropical red algae from McMahon et al. (2016) were remarkably similar. This finding supports the conclusions of Larsen et al. (2009, 2013) that essential amino acid isotope fingerprints of primary producers are taxon-specific, driven by broad phylogenetic differences in amino acid synthesis, rather than driven by environmental differences. However, there were slight differences in fingerprints between different datasets, most notably for phytoplankton. The differences in phytoplankton values from different datasets also had some effects on our mixing model results. While our results suggest that phylogenetically close endmember groups such as red algae, brown algae, or terrestrial plants produce consistent essential amino acid fingerprints regardless of location, "phytoplankton" as characterized here is a metabolically and taxonomically diverse functional group that changes dramatically with location, environmental conditions, and time. Similarly, bacteria are very diverse in essential amino acid synthesis, and these pathways are not yet well

understood (Price et al. 2018). Categorizing primary production sources with common broad groupings like "phytoplankton" and "bacteria" is useful for their simplicity, but could lead to the pitfall of masking differences in levels of diversity within these groups. For example, the Larsen et al. (2013) microalgae category (equivalent to our phytoplankton category) contained samples of both cyanobacteria and diatoms. This is despite diatoms being taxonomically much closer to brown algae (Ruggiero et al. 2015), which was classified as its own separate category in Larsen et al. (2013). The phytoplankton cultures we used were from specific species of diatoms only. Therefore, we suggest that continued efforts need to be made to determine endmember values by increasing phylogenetically consistent representation, especially concerning phytoplankton and bacteria.

Conclusions

Results from this study indicate that phytoplankton are the most important source of essential amino acids to the two species of bivalves investigated in this study. Bacteria were the second most important source to the bivalves investigated from Hanna Shoal. Mixing model estimates of individual bivalve samples showed that terrestrial organic matter contributed a substantial dietary proportion to some individuals in the Hanna Shoal, particularly in *Macoma* spp. Paired shell and muscle samples from *Macoma calcarea* had similar amino acid carbon stable isotope fingerprints, with differences in some amino acids that may have been due to different time frames recorded in the two tissue types. Mixing models run with these muscle and shell values revealed phytoplankton to be the highest contributing source of essential amino acids to the essential amino acids in shell and muscle samples of *Macoma calcarea.* Overall, our results indicate that amino acid fingerprinting shows considerable potential for tracking changes in essential amino acid sources in the Arctic marine environment.

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Tables

Table 1. Mixing model dietary proportion estimates based on essential amino acid fingerprints for *Astarte* spp. and *Macoma* spp. from Hanna Shoal using dataset 2 endmember inputs. Values are expressed in percent plus or minus the standard deviation.

Endmember	***Astarte* spp.**	***Macoma* spp.**
Red algae	5±3	6±4
Brown algae	3±2	3±2
Phytoplankton	58±3	45±4
Bacteria	29±3	33±3
Terrestrial plants	6±3	13±3

Table 2. Mixing model dietary proportion estimates based on essential amino acid fingerprints for *Macoma calcarea* shell and muscle samples. Dataset 2 endmember inputs were used. Values are expressed in percent (%).

Endmember	**shell**	**muscle**
Red algae	7±4	9±7
Brown algae	19±8	9±9
Phytoplankton	43±6	59±10
Bacteria	5±3	19±7
Terrestrial plants	26±5	5±4

Figures

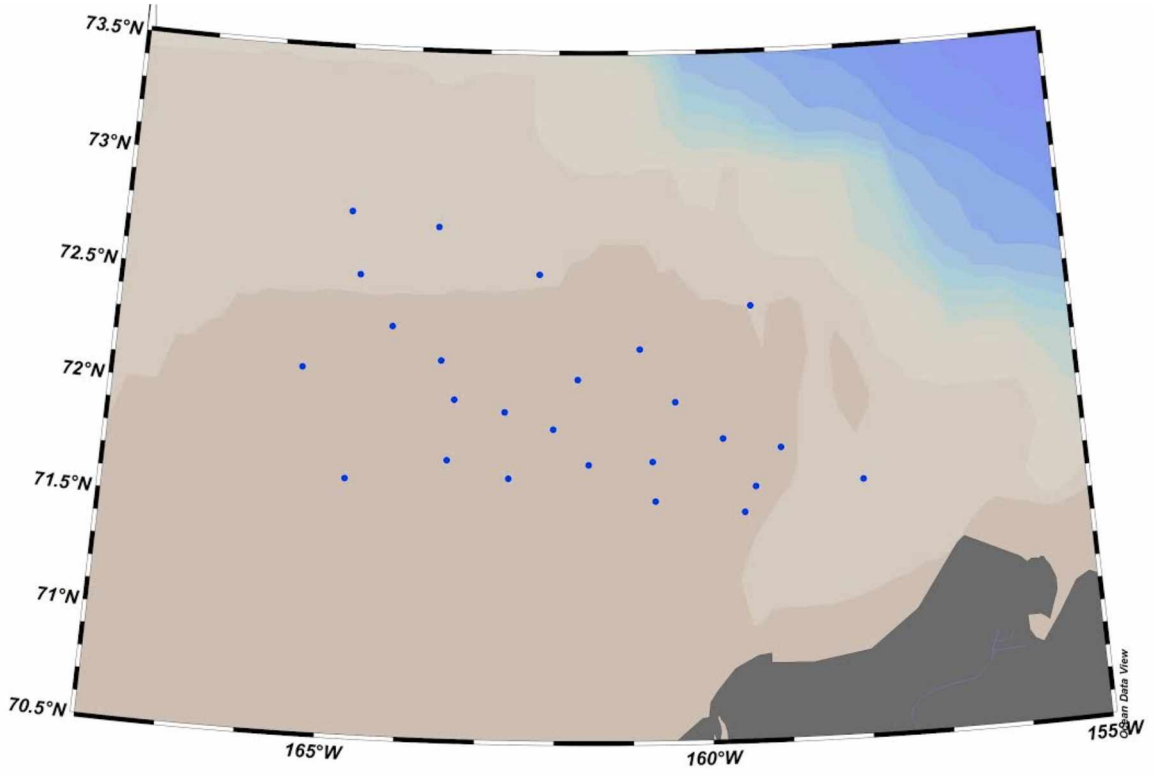

Figure 1: *Astarte* spp. (a) and *Macoma* spp. (b) sampling locations in Hanna Shoal.

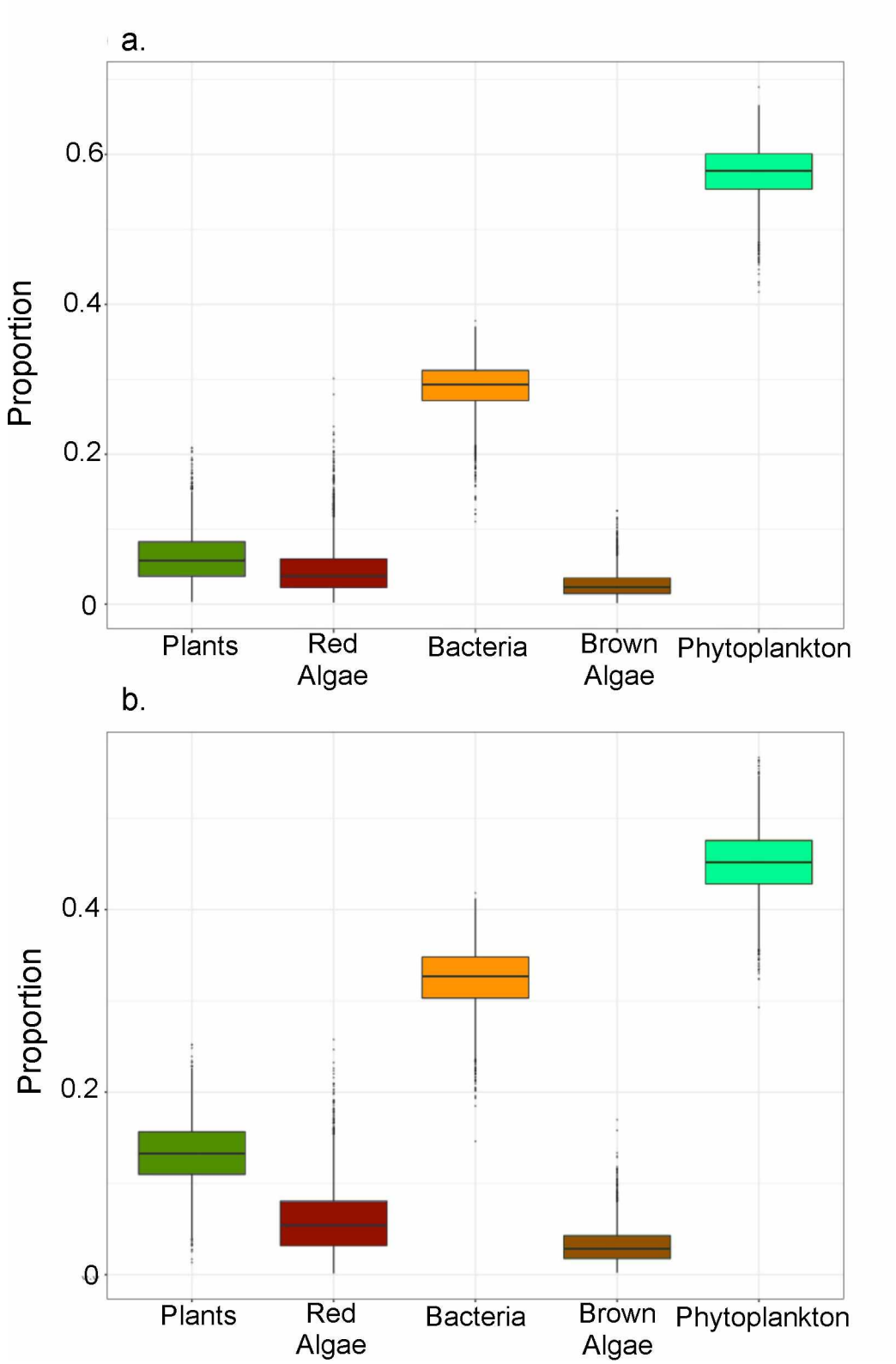

Figure 2: Proportional contributions of diet sources to *Astarte* spp. (a) and *Macoma* spp. (b) as modeled by *simmr*.

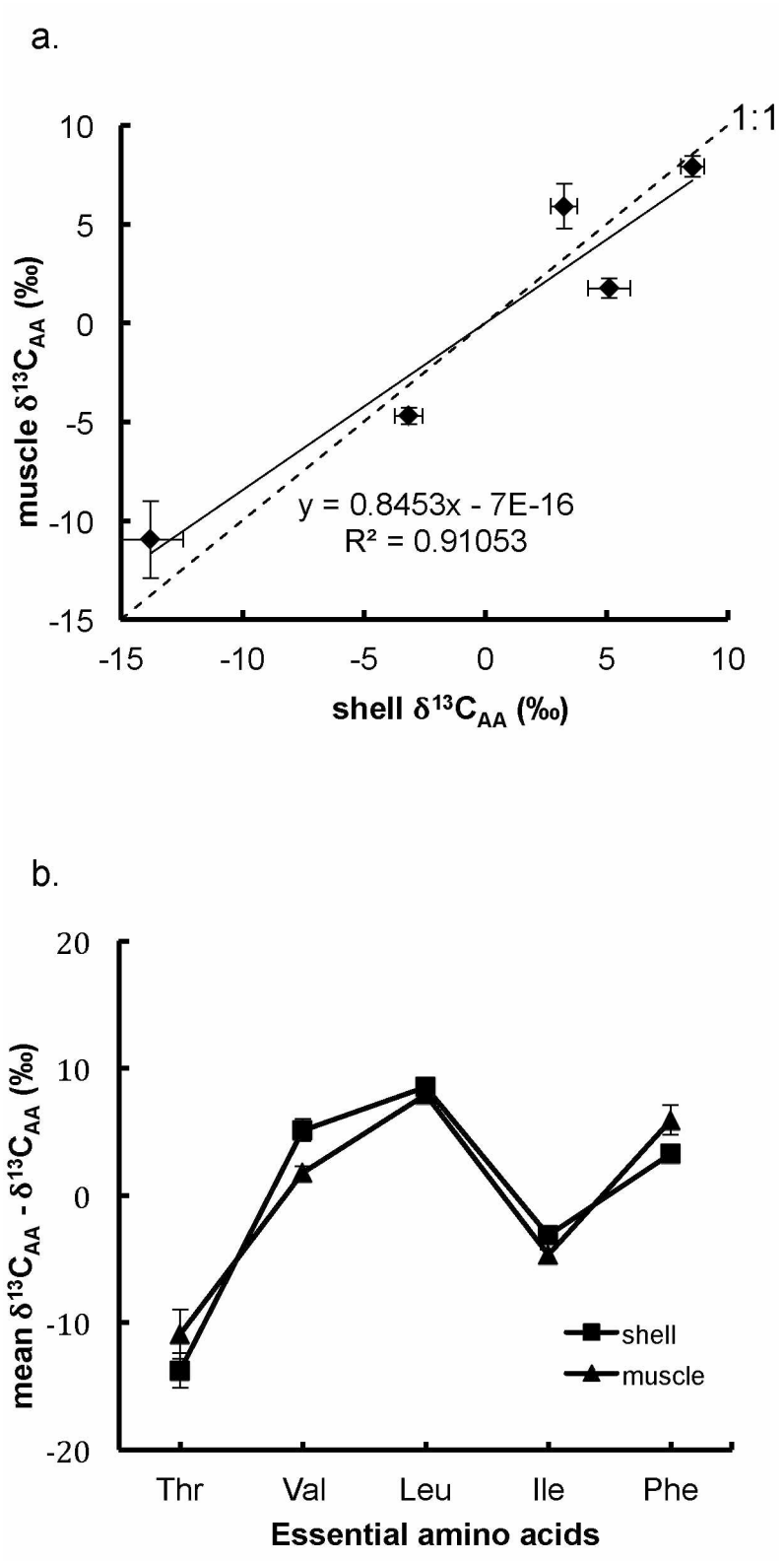

Figure 3: Shell and muscle centered average amino acid $\delta^{13}C$ values for *Macoma calcarea* plotted against each other (a) and on the same axis by amino acid (b).

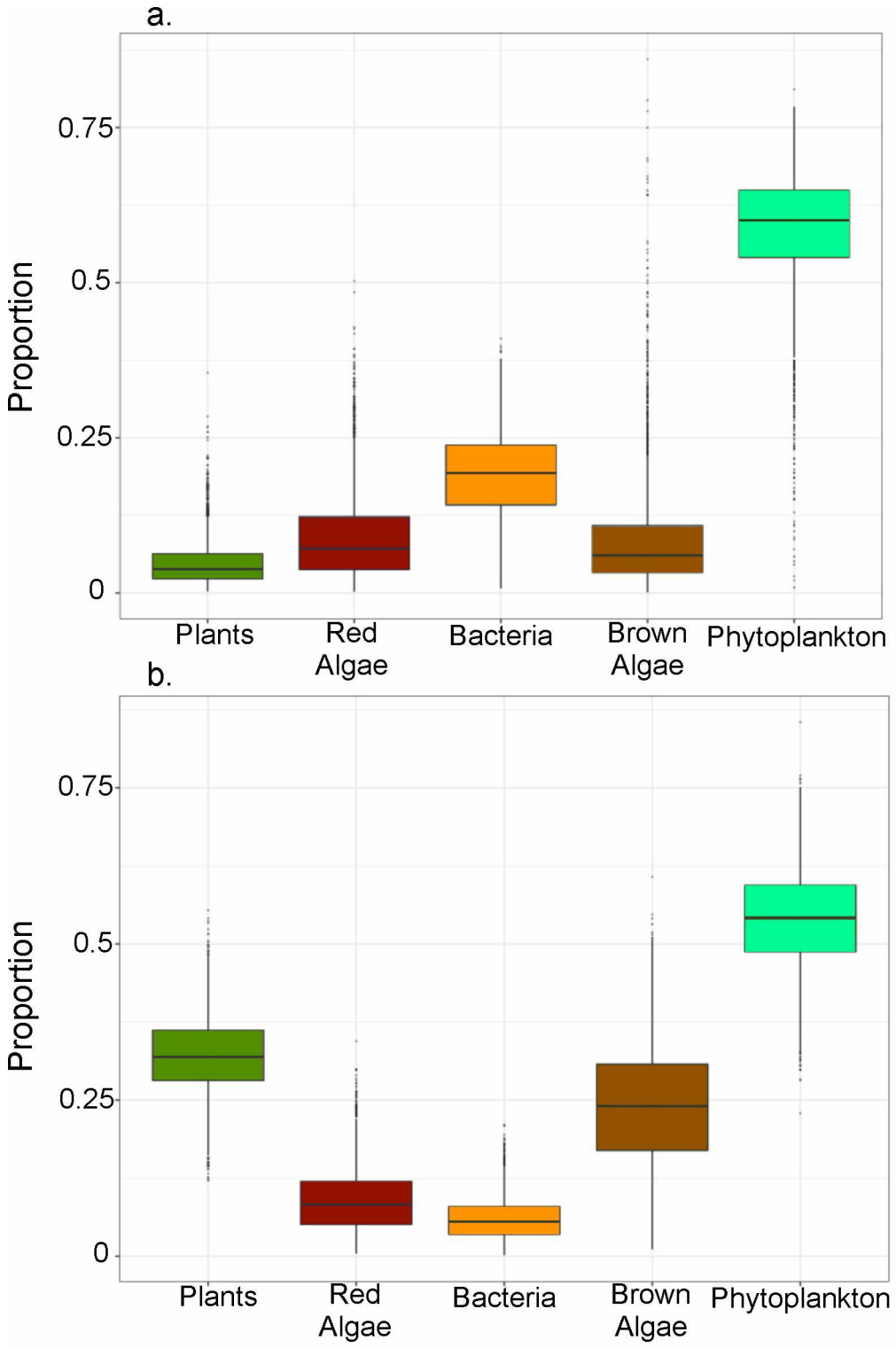

Figure 4: Proportional contributions of diet sources to *Macoma calcarea* muscle (a) and shell (b) as modeled by *simmr*.

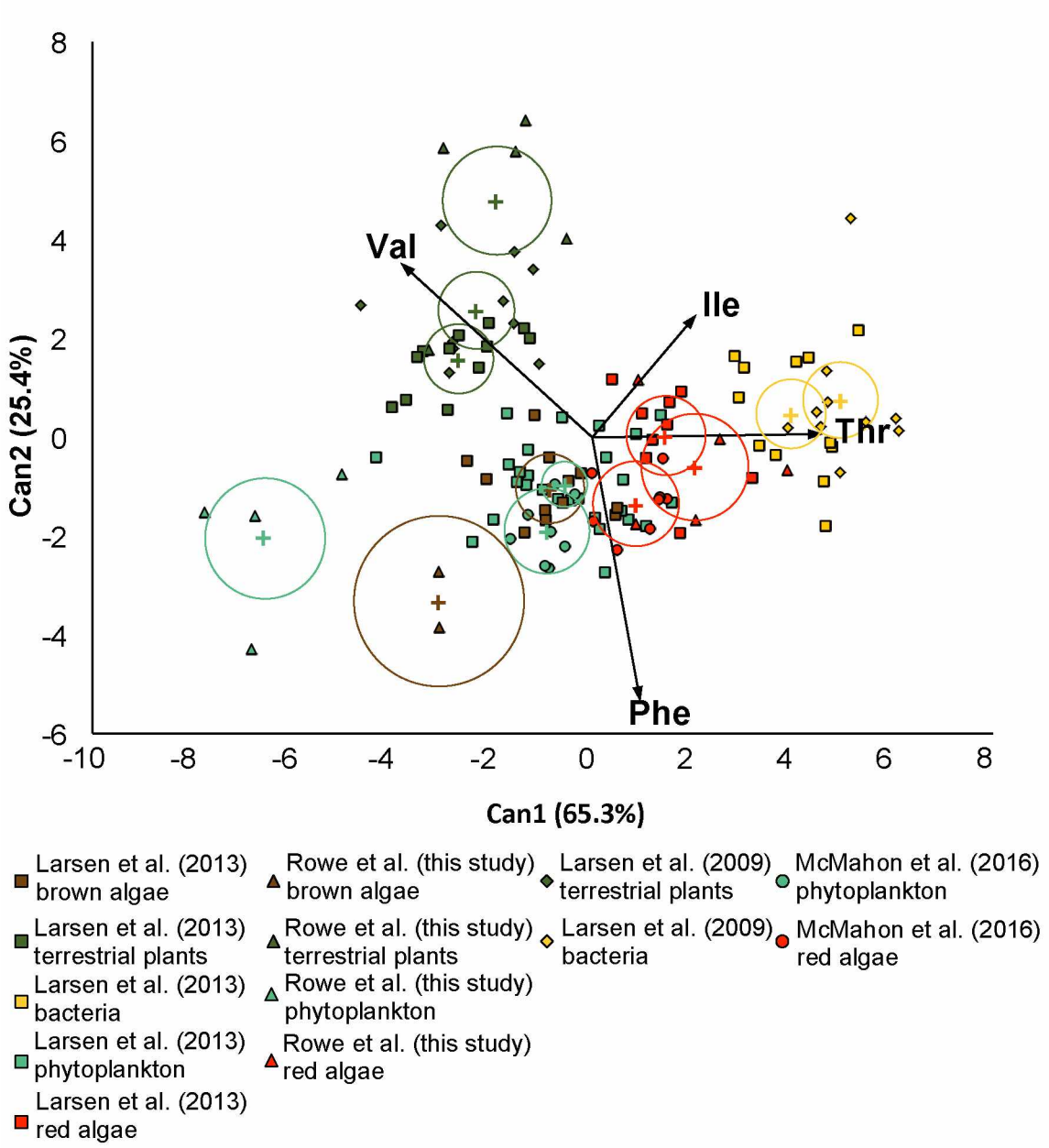

Figure 5: Linear discriminant analysis based on the mean-centered $\delta^{13}C_{EAA}$ values (Thr, Val, Leu, Ile, Phe) of all endmembers. The crosses indicate the mean value plus the standard ellipses for each primary production source of the same color.

Supplementary documents

Supplementary Table 1. Mixing model inputs of endmember datasets. Dataset 1 used only literature data (Larsen et al. 2009, 2013, McMahon et al. 2016) as endmember inputs. Dataset 2 used only Arctic-specific endmembers newly generated in this study and bacteria values from Larsen et al. (2009, 2013). $\delta^{13}C$ amino acid values have been centered to the mean values of all five essential amino acids per data set, and are expressed in per mil (‰).

Dataset 1

Endmember	Thr	Val	Leu	Ile	Phe
Red algae	-8.9±2.2	2.0±1.5	3.9±0.9	-0.7±1.0	3.7±0.7
Brown algae	-13.3±3.7	2.6±1.1	6.5±1.2	0.2±1.6	4.0±1.4
Phytoplankton	-10.7±2.0	2.7±1.0	5.7±1.2	-1.9±1.5	4.1±1.0
Bacteria	-4.8±3.4	1.5±1.5	0.2±1.0	-0.1±1.2	3.1±1.6
Terrestrial plants	-13.9±2.2	5.1±0.8	7.9±1.1	-0.1±0.9	0.9±1.3

Dataset 2

Endmember	Thr	Val	Leu	Ile	Phe
Red algae	-9.8±2.7	2.1±0.4	3.2±1.5	0.2±1.1	4.3±1.7
Brown algae	-15.8±1.0	2.3±0.3	8.9±0.2	-1.3±1.4	5.9±0.8
Phytoplankton	-15.0±3.0	4.8±0.8	11.6±1.4	-5.6±1.3	4.2±1.7
Bacteria	-4.8±3.4	1.5±1.5	0.2±1.1	-0.1±1.2	3.1±1.6
Terrestrial plants	-10.7±3.3	6.5±1.3	6.7±1.1	-0.7±2.6	-1.8±1.7

Dataset 3

Endmember	Thr	Val	Leu	Ile	Phe
Red algae	-9.1±2.3	2.0±1.3	3.7±1.1	-0.5±1.0	3.8±1.0
Brown algae	-13.6±3.5	2.5±1.0	6.8±1.4	0.0±1.7	4.3±1.5
Phytoplankton	-11.1±2.6	2.9±1.2	6.3±2.4	-2.3±2.1	4.2±1.1
Bacteria	-4.8±3.4	1.5±1.5	0.2±1.1	-0.1±1.2	3.1±1.6
Terrestrial plants	-13.3±2.6	5.4±1.0	7.7±1.2	-0.2±1.3	0.4±1.7

Supplementary Table 2. Mixing model dietary proportion results based on essential amino acid fingerprints for endmember source dataset sensitivity tests. All Hanna Shoal bivalve data were used as inputs for the mixing model. Dataset 1 used only literature data (Larsen et al. 2009, 2013, McMahon et al. 2016) as endmember inputs. Dataset 2 used only Arctic-specific endmembers newly generated in this study and bacteria values from Larsen et al. (2009, 2013). Dataset 3 pooled both literature and newly generated values for endmember inputs. Values are expressed in percent plus or minus the standard deviation.

Endmember	Dataset 1	Dataset 2	Dataset 3
Red algae	2±2	3±2	5±3
Brown algae	3±2	2±1	7±4
Phytoplankton	48±5	54±2	46±7
Bacteria	1±1	33±2	3±2
Terrestrial plants	46±4	8±3	39±4

Supplementary Table 3: Mixing model dietary proportion results of Leu tests. Leu was removed from the model, and the two most contrasting datasets of the sensitivity tests (Table 1) were run again. Values are expressed in percent (%).

Endmember	Dataset 1	Dataset 2
Red algae	3±2	3±2
Brown algae	2±2	2±1
Phytoplankton	60±9	65±3
Bacteria	3±2	26±3
Plants	32±7	5±2

Supplementary Table 4: Mixing model dietary proportion results of phytoplankton sensitivity tests. The phytoplankton model inputs of datasets 1 and 2 were switched to test the sensitivity of the model to phytoplankton input values. Values are expressed in percent (%).

Endmember	Dataset 1	Dataset 2
Red algae	3±2	2±1
Brown algae	2±1	28±4
Phytoplankton	57±2	34±5
Bacteria	35±2	1±1
Plants	3±2	35±2

Supplementary text:

Sensitivity tests:

Phytoplankton was estimated to make up the largest proportional contribution (46 to 54%) of essential amino acid carbon in all three of the mixing model tests (Supplementary Table 2). However, the mixing model outputs differed in their determination of subsequent rankings of endmember contributions. Terrestrial plants were calculated to contribute a mean of approximately 46% and 39% of the bivalve diets in dataset 1 and dataset 3, respectively (Supplementary Table 2). This proportion was only 8% when using dataset 2. Bacteria were calculated to contribute mean values of 1-3% of the bivalve diets in the model dataset 1 and dataset 3, and 33% in dataset 2 (Supplementary Table 2). Red algae and brown algae had consistently low mean proportions in all three models, with red algae ranging from approximately 2-5% and brown algae ranging from 1-7% (Supplementary Table 2).
A visual inspection of endmember amino acid patterns led us to suspect that the phytoplankton values in dataset 2 were largely responsible for driving the differences between the model iterations. To further probe this idea, we removed Leu (the amino acid that differed most dramatically between phytoplankton from dataset 2 and the other datasets) from the model and ran the models again with datasets 1 and 2. This resulted in an increase in phytoplankton by approximately 12% in dataset 1 and 10% in dataset 2 (Supplementary Table 3). The second-ranking endmembers in each set decreased by approximately the same amount, resulting in no overall changes in rankings of endmember proportions due to the deletion of Leu. We replaced the phytoplankton value of dataset 1 with the phytoplankton value of dataset 2 and reran the model. The resulting estimates were very similar to the original dataset 2 results: the proportional contributions of phytoplankton and bacteria were approximately 57% and 35%, respectively (Supplementary Table 4). Finally, we also replaced the phytoplankton value of dataset 2 with the phytoplankton value of dataset 1 and reran the model (Supplementary Table 4). This resulted in average estimates of 34% phytoplankton, 28% brown algae, and 35% terrestrial plants. Overall, these sensitivity tests confirmed that the phytoplankton values were driving most of the differences in the estimates of proportional contributions from different sources between the models.