Identifying Sources of Organic Matter to Benthic Organisms in the Beaufort and Chukchi Outer Continental Shelves

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Final Report
OCS Study BOEM 2019-030
May 2019

BOEM Bureau of Ocean Energy Management

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This study was funded by the U.S. Department of the Interior, Bureau of Ocean Energy Management (BOEM) Alaska OCS Region (Cooperative Agreement M16AC00005) and the University of Alaska Fairbanks. This report, OCS Study BOEM 2019-030, is available through the Coastal Marine Institute and select federal depository libraries. Electronic access is available at *http://www.boem.gov/Alaska-Scientific-Publications*.

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Abstract

Benthic invertebrate communities are an essential ecosystem component in Arctic food webs in terms of energy transfer to higher trophic levels and mineralization. Currently, the proportional contributions of different sources of organic matter (marine, terrestrial, or microbial production) that sustain benthic organisms in the Arctic are unclear. This project provided a better understanding of the organic matter sources consumed by benthic organisms using a state-of-the-art essential amino acid (EAA) "fingerprinting" approach. Unlike non-essential amino acids, the term "essential" means that they only originate from the organisms that synthesized them (e.g., photosynthetic or microbial organisms) and cannot be synthesized by consumers. The EAAs have specific stable carbon isotope fingerprints, depending on the producer type, and they differ between marine, terrestrial, and microbial producers. The EAA fingerprints are incorporated into and conserved within consumers, creating a pattern or "stable isotope fingerprint," which can be statistically compared with the EAA fingerprints of the primary producers. "Fingerprints" allow the separation of microbial and terrestrial carbon sources from marine production, filling a gap identified in previous benthic food web work involving systems in the Arctic. This is a particularly powerful tool to quantify the proportional contribution by microbial, terrestrial plant, and marine primary producers to consumers.

We found that EAA fingerprints in the soft tissues of clams from the Arctic marine environment were reflected in the signatures preserved in the shells of these organisms. This important methodological finding will allow future application of the approach to analyses of archeological and geological clam samples. We compared results from our analyses of primary producers (endmembers in terrestrial plants and phytoplankton) with literature values and found that most endmembers of the same category had very similar EAA fingerprints. This suggests that EAA isotope fingerprints of primary producers are taxon-specific and driven by broad and deep phylogenetic differences in EAA synthesis rather than environmental and geographic differences.

Archived benthic invertebrates were analyzed from three regions, Hanna Shoal, Chukchi Sea, and the Beaufort Sea. In the Hanna Shoal and Chukchi samples, which came from a relatively uniform water depth, we found that phytoplankton and terrestrial derived EAAs made the greatest proportional contributions to benthic invertebrates. This finding supports observations of highly productive phytoplankton blooms in the region. The Hanna Shoal bivalve model estimated that bacteria made up the next highest proportion of bivalve EAAs, which may reflect the reworking and ecological availability of more refractory organic matter. There appeared to be some differences between the two bivalve species analyzed from the Hanna Shoal. Phytoplankton was estimated to contribute a higher proportion of EAAs, and terrestrial organic matter and bacteria less, to *Astarte* spp. than found with *Macoma* spp. These differences are likely due to their different feeding modes.

In contrast to the Chukchi region and Hanna Shoal, the results from the Beaufort region indicated a greater contribution of EAAs from sources other than phytoplankton and terrestrial organic matter. Most notably, there appeared to be a greater contribution of bacterial and

macroalgal sources of EAAs in the invertebrates. Additionally, water depth influenced the source of the proportional contributions, with more bacterial-derived EAAs at greater depth. A future direction would be to apply compound-specific amino acid and fatty acid analyses on the same sample. This would allow the determination of the proportional contribution of marine photosynthetic sources as a whole (vs. terrestrial and bacterial sources), and the determination of proportional contributions of ice algae and phytoplankton based on the fatty acids data.

Introduction

Background

Arctic shelf systems are often considered benthic-dominated systems because much of the primary production from phytoplankton and sea ice (i.e., ice algal production) goes ungrazed by zooplankton and sinks to the seafloor (Grebmeier et al. 2007b, a). Tight pelagic-benthic coupling results in rich invertebrate communities dominating the benthic marine ecosystems in the Arctic (Piepenburg 2005; Dunton et al. 2006). Benthic invertebrates are important in remineralization processes and as prey for higher trophic levels (Seymour et al. 2014a, b).

The nature of the Arctic benthic communities is likely to be affected by changes in the climate, environment, and human use of the Arctic system. For example, changes in the organic matter sources supporting the complex benthic food webs could alter energy flow through the system (Iken et al. 2005, 2010; McTigue and Dunton 2014, 2017; Divine et al. 2015; Kędra et al. 2015). The Chukchi Sea, in particular, experiences dramatic sea-ice dynamics and, possibly, fluctuations in the overall quantity of marine primary production. Benthic food webs in the Chukchi Sea are also sensitive to hydrographic conditions (Iken et al. 2010; Feder et al. 2011; Tu et al. 2015), which increases the probability that climate warming will have an impact. Currently, the proportional contributions of organic matter sources (phytoplankton, microphytobenthos, ice algae, terrestrial sources, and microbial production) that sustain these benthic organisms through food web links are unclear (McTigue and Dunton 2014, 2017).

Depending on the specificity of the analysis, fatty acids and their stable isotope composition can be used to distinguish sources of organic matter (Graham et al. 2014; Wang et al. 2015; Wang et al. 2016). To date, Arctic food web research has largely employed bulk stable isotope analysis of the total organic carbon and nitrogen in tissue samples (Bentzen et al. 2007, 2014; Feder et al. 2011; Savory et al. 2014; Seymour et al. 2014a, b). This project used a novel approach, "stable isotope fingerprinting," to identify and quantify links between organic matter sources and benthic organisms in the Arctic marine environment. Stable isotope fingerprinting provides greater organic matter source specificity and differentiation by examining the isotopic signatures of individual essential amino acids (EAAs) originating from the food sources consumed by an organism (Larsen et al. 2009, 2013). Unlike non-essential amino acids, EAAs cannot be synthesized by consumers; rather, EAAs are synthesized by photosynthetic or microbial organisms and transferred to consumers. The EAAs have specific stable isotope values depending on whether the producer is marine photosynthetic (EAAs derived from dissolved organic carbon), terrestrial photosynthetic (EAAs derived from atmospheric carbon dioxide), or microbial (Figure 1). Isotope values are conserved within a consumer, so the stable carbon isotopic values of the eight EAAs (phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, and lysine) can be individually measured. The relative isotopic difference between these values creates a "stable isotope fingerprint," which can be used to identify the source and calculate source-specific proportional contributions of EAAs to a consumer organism (Larsen et al. 2009, 2013).

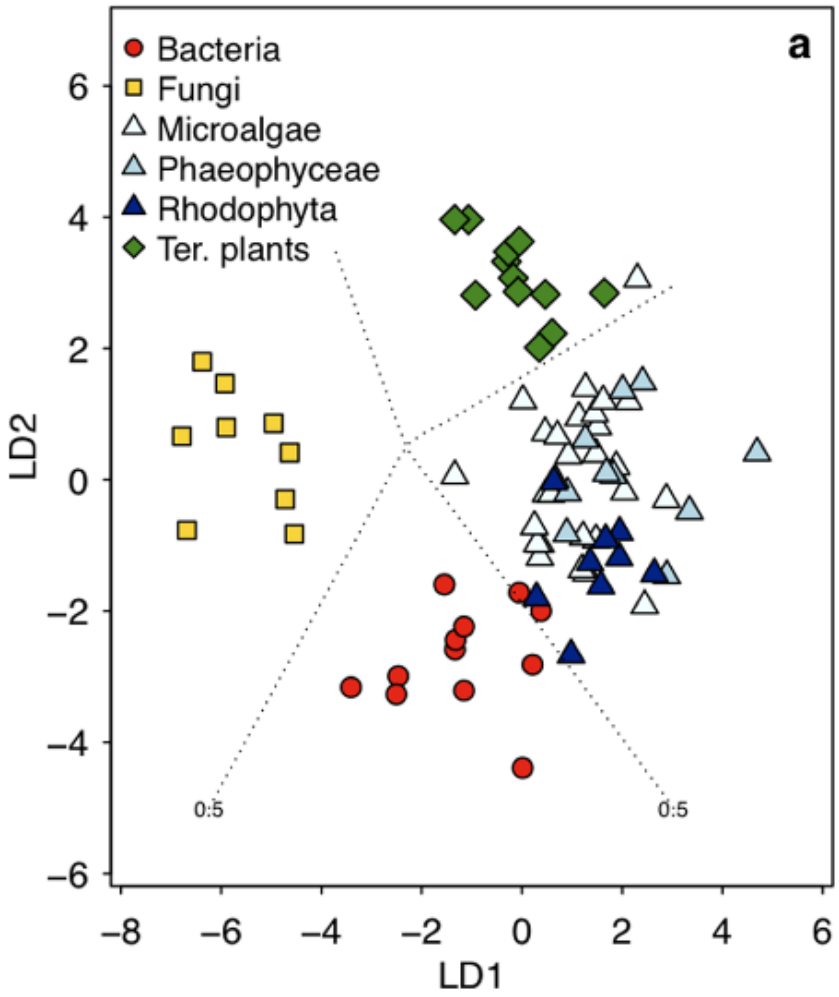

Figure 1: Stable carbon isotope amino acid "fingerprints" (linear discriminant function analysis) of organic matter sources (from Larsen et al. 2013).

Quantitatively determining links between producers and benthic consumers helps define the extent and strength of pelagic-benthic coupling in the Beaufort and Chukchi Seas. Further, the data can inform modeling projections based on changes in proportions of source inputs into the marine environment and provide a baseline for comparative studies and monitoring activities. Understanding the key organic matter sources and links that support the rich benthos of the Beaufort and Chukchi Seas contributes significantly to our knowledge of ecosystem function and resilience.

Objectives

The primary research goal for the project was to quantify proportional contributions of different organic matter sources consumed by benthic invertebrates on the Outer Continental Shelf (OCS) of the Beaufort and Chukchi Seas. Sources of organic matter in the Beaufort and Chukchi Seas include marine phytoplankton and sea ice algal, microphytobenthic, terrestrial, and microbial production. For this project, we used benthic invertebrate samples from existing archives

containing representatives of all benthic biomass-dominating taxa (crustaceans, mollusks, and echinoderms) in the region. We analyzed a subset of samples drawn from more than 5,000 specimens collected across the Beaufort and Chukchi shelf systems, which allowed us to cost-effectively estimate the proportional contribution of organic matter sources to a range of benthic invertebrates with different feeding modes and lifestyles.

- Objective 1: Measure the stable carbon isotope compositions of EAAs from archived benthic organisms that contribute substantially to benthic biomass and have a variety of feeding types and mobility in the Beaufort Sea OCS.
- Objective 2: Perform EAA fingerprinting of archived benthic samples from the Chukchi Sea OCS.
- Objective 3: Quantify the proportional contribution of marine photosynthetic, terrestrial photosynthetic, and microbial-derived EAAs in benthic organisms from the Beaufort and Chukchi Seas.

Hypotheses

There is significant terrestrial matter imported into the Beaufort Sea region through major river systems, coastal erosion, and possibly melting nearshore ice. Bulk stable isotope studies suggest that both terrestrial and microbial carbon sources may be important to benthic food webs in the region (Dunton et al. 2006; Divine et al. 2015).

- Hypothesis 1: Essential amino acids from microbial-reworking of organic matter from terrestrial and microbial sources provide the dominant proportional contribution to benthic organisms in the Beaufort Sea.
- Photosynthetic sympagic, microphytobenthic, and pelagic sources of organic matter have been proposed as sources of organic matter to benthic communities in the Chukchi Sea (Iken et al. 2010; McTigue and Dunton 2014, 2017). The marine photosynthetic contribution could be particularly significant in the Hanna Shoal region (northeast Chukchi shelf), where McTigue and Dunton (2014) recently documented unprecedented high chlorophyll *a* concentrations in surface sediment samples.
- Hypothesis 2: Marine, photosynthetically-derived organic matter (sympagic, microphytobenthic, or pelagic) provides the dominant proportional contribution of EAAs to benthic organisms in the Chukchi Sea.

Methods

Study Area/Sample Collection

Samples were drawn from a large archive (>5,000 samples) of benthic marine invertebrates taken from the Chukchi and Beaufort Seas (Figure 2) during the BOEM-funded 2014 Transboundary cruise in the Arctic Beaufort Sea (Figure 3) and the AMBON (Figure 4) and COMIDA-Hanna Shoal (Figure 5) projects in the Chukchi Sea. The AMBON cruise report (Iken 2015) includes detailed oceanographic information related to the project study area. For the Hanna Shoal samples, we analyzed two bivalve genera (*Astarte* spp. and *Macoma* spp.) collected in 2012 and 2013 as part of the Hanna Shoal Ecosystem Study, an extension of the COMIDA CAB program. Sampling stations were oriented around 72 °N, 162 °W in the Hanna Shoal region (Figure 5 and Table 1). Samples were collected using a van Veen grab, then sorted, identified, and dried at 60°C onboard.

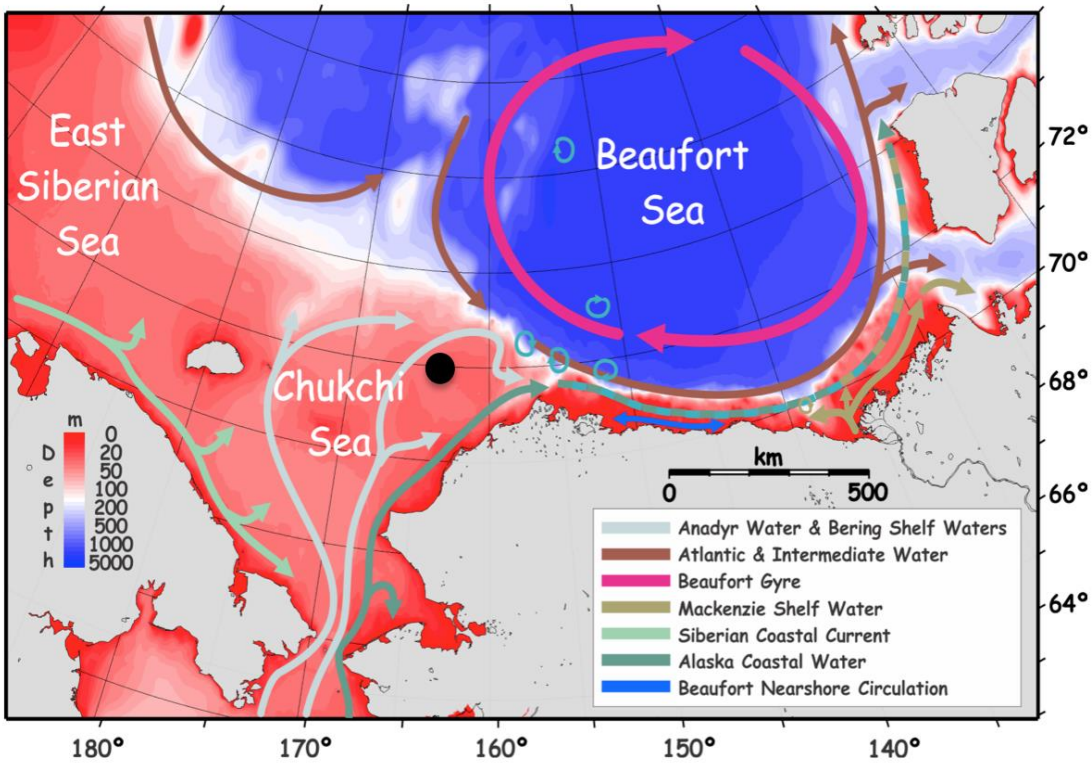

Figure 2: Locations of the Beaufort and Chukchi Seas and their major oceanographic influences (courtesy of Seth Danielson). The black circle represents the approximate position of the Katie's Floeberg feature as described by Barrett and Stringer 1978.

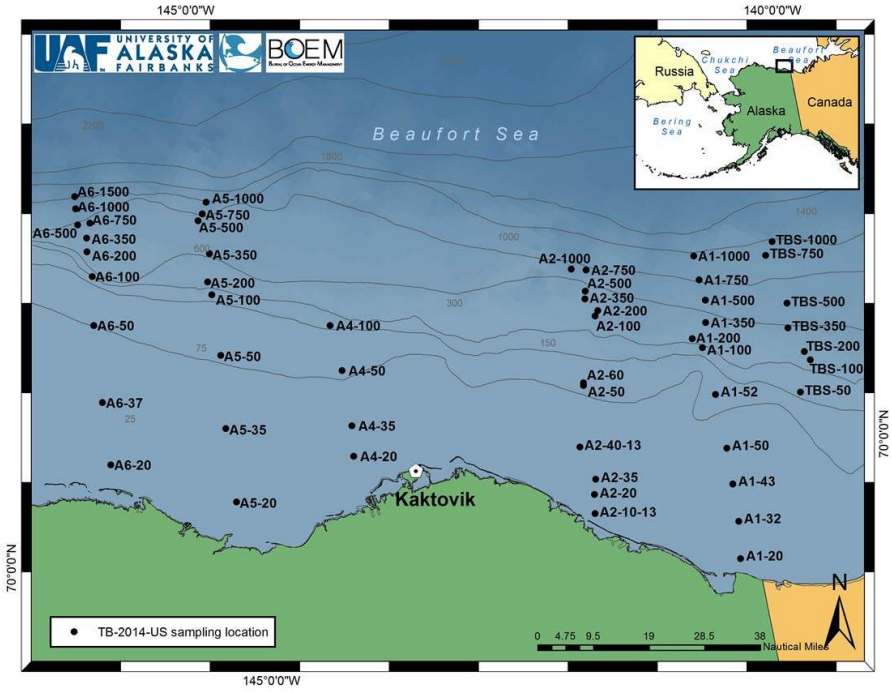

Figure 3: Beaufort Sea region where archived benthic invertebrates were collected (Transboundary project 2014).

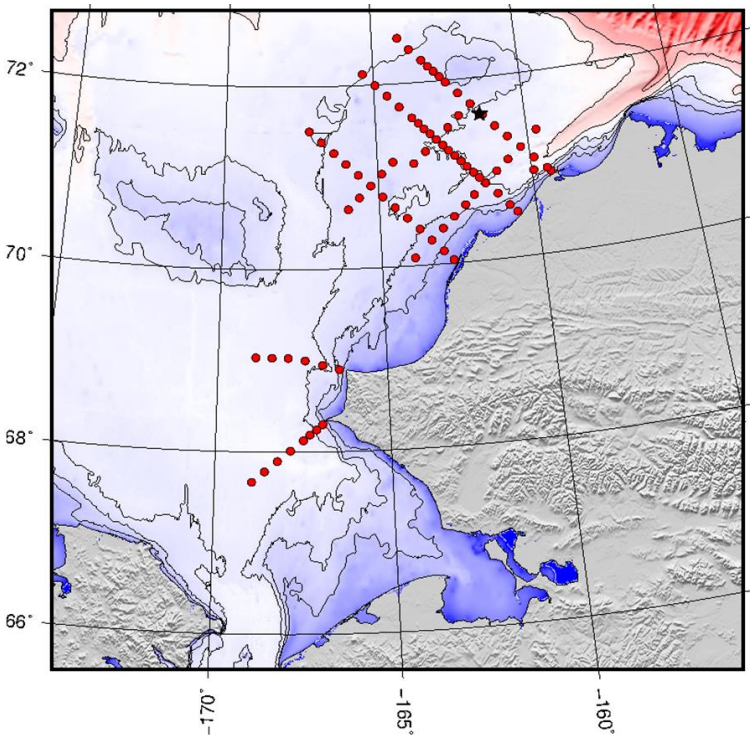

Figure 4: Chukchi Sea region where archived benthic invertebrates were collected (AMBON project 2015).

Table 1: Longitudes and latitudes of stations with samples available from the COMIDA-Hanna Shoal archives (from Kenneth Dunton).

Station	lat	long	Station	lat	long	Station	lat	long
E1-10	70.315	-147.732	H28	72.4006	-159.3462	H109	71.5	-159.512
E3-7	70.325	-147.649	H32	71.777	-159.007	H112	72.7937	-164.8982
CB05DSE-2	70.026	-145.259	H34	71.9874	-160.4038	H1	71.6513	-162.6365
DS4	70.025	-145.253	HS3	71.943	-162.6993	H3	71.8699	-162.0476
W3-9	70.376	-147.794	HS3	71.943	-162.6993	H3	71.8699	-162.0476
DS-11	70.322	-147.579	H112	72.7937	-164.8982	H4	72.5449	-162.2542
W3-9	70.376	-147.794	H112	72.7937	-164.8982	H10	72.303	-164.2588
H1	71.6513	-162.6365	UTX8	71.7255	-163.4562	H21	72.5213	-164.738
H3	71.8699	-162.0476	UTX8	71.7255	-163.4562	H21	72.5213	-164.738
H5	72.088	-161.7187	CBL11	72.1033	-165.4556	H24	71.6273	-164.7991
H6	72.1603	-163.5761	BARC10	71.62	-157.9305	H30	72.7425	-163.6716
H10	72.303	-164.2588	H4	72.5449	-162.2542	H37	71.553	-160.687
H10	72.303	-164.2588	H30	72.7425	-163.6716	H37	71.553	-160.687
H19	71.7144	-161.5679	H30	72.7425	-163.6716	H38	71.611	-159.36
H19	71.7144	-161.5679	H4	72.5449	-162.2542	CBL15	71.7274	-160.7183
H24	71.6273	-164.7991	H9	72.2189	-160.873	H17	71.9913	-163.3834
H6	72.1603	-163.5761	H9	72.2189	-160.873	H32	71.777	-159.007
H6	72.1603	-163.5761	H32	71.777	-159.007	UTX8	71.7255	-163.4562
H9	72.2189	-160.873	H33	71.8228	-159.7722	UTX8	71.7255	-163.4562
H17	71.9913	-163.3834	H33	71.8228	-159.7722	CBL11	72.1033	-165.4556
H17	71.9913	-163.3834	HS3	71.943	-162.6993	H17	71.9913	-163.3834
H28	72.4006	-159.3462	HS3	71.943	-162.6993			

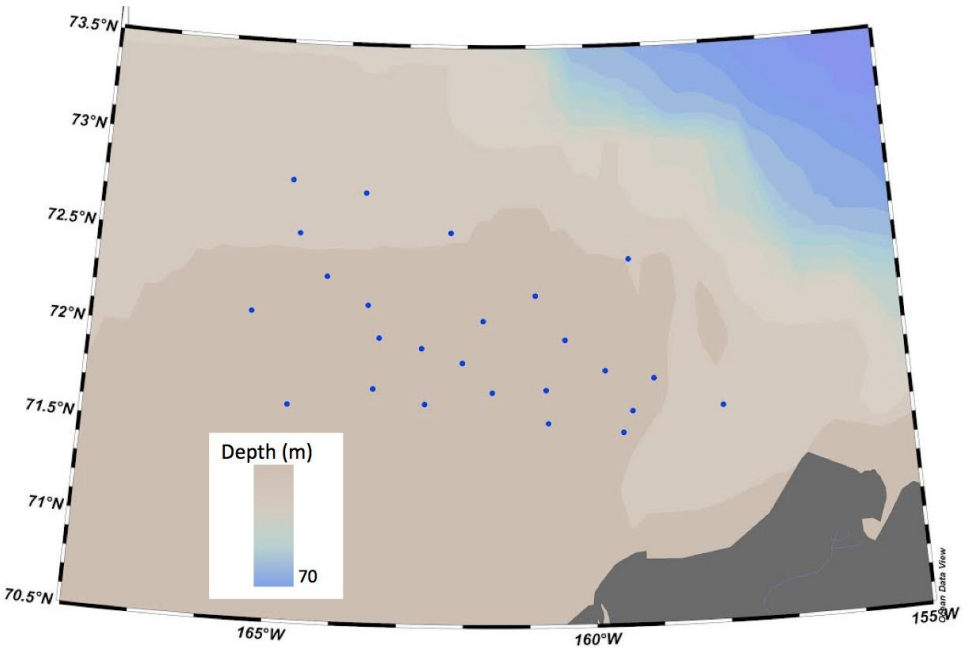

Figure 5: Hanna Shoal region where archived benthic invertebrates were collected (COMIDA-Hanna Shoal).

We also wished to examine the relationship between shell and muscle EAA stable carbon isotope fingerprints, which could allow this approach to be applied to ancient shells to investigate long-term changes. For this purpose, we selected 13 *Macoma calcarea* samples (with both shell and muscle materials present) to maximize the available geographic and isotopic range based on bulk carbon isotope analyses. These archived samples had been collected with van Veen grabs as part of the 2015 AMBON research cruise in the Chukchi Sea.

A suite of potential Arctic primary producers (endmembers) was compiled to compare with published EAA stable carbon isotope fingerprints (Larsen et al. 2009, 2013; McMahon et al. 2016). SCUBA divers collected five red algae species (*Coccotylus truncatus, Dilsea sp., Rhodomela sp., Odonthalia dentata,* and *Phycodrys sp*.) and two kelp species (*Laminaria saccharina* and *Alaria esculenta*) from 5–10 m water depth along the Beaufort Sea coast. Five terrestrial plant species (*Eriophorum angustifolium, Salix herbacea, Rhododendron groenlandicum, Alnus sp*., and *Betula pendula*) were collected from the University of Alaska Fairbanks campus. Terrestrial samples and macroalgae were dried and pressed in a herbarium press. Four Arctic diatom species (*Skeletonema marinoii, Coscinodiscus sp., Porosira glacialis,* and *Chaetoceros furallatus*) were cultured for use in the study.

The cultured diatoms were isolated from water samples or germinated from spore-containing sediment samples from the Barents Sea or the coast of northern Norway. Species were identified by a combination of morphological and molecular methods. Stock cultures were held in a climate-controlled room at the Norwegian College of Fishery Science, University of Tromsø, at 5±0.5°C and 50 µmol m^{-2} s^{-1} scalar irradiance with a photoperiod of 14:10 hour (light:dark) in Guillard's f/2×50 marine water enrichment solution. Cultures were grown semi-continuously in 100 L Plexiglas cylinders by diluting the cultures with fresh, nutrient-replete culture medium once they reached the 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). All cultures were aerated with compressed air to avoid sedimentation and CO_2-limitation. Culture samples were collected by concentrating cells onto a plankton net (mesh size 5–20 µm) before centrifuging at 3500 rpm for five minutes in a cooled centrifuge (4°C). The resulting wet pellets were transferred into 50 ml Falcon tubes and stored at -80°C.

Bulk Stable Isotope Analysis

The elemental composition of shell, muscle, and endmember samples was determined by bulk stable isotope analysis before proceeding with compound-specific isotope analysis. Endmember samples were lyophilized for approximately 48 hours 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 remaining powdered samples were 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 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, which also resulted 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 hours, 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 minutes, and lyophilized. The resulting organic matter was weighed to approximately 0.5 mg into tin capsules for bulk carbon and nitrogen stable isotope analysis. Due to small size, some shell samples were excluded from the bulk analysis to ensure 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 at the University of Alaska Fairbanks (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 before flushing with N_2 gas to remove oxygen. The samples were then hydrolyzed on a heating block at 110°C for 20 hours. 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 was 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 minutes. 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 minutes. 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 (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 minutes. 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} 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 minutes, then 10°C min^{-1} to 245°C and held for 10 minutes, and finally 5°C min^{-1} to 290°C and held for 5 minutes. 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 δ^{13}Cvalue was corrected by subtracting the difference between the δ^{13}C value of norleucine (the internal standard) from the same analysis (injection) and the average δ^{13}C value of norleucine across all project analyses. In accordance with published protocols (O'Brien et al. 2002), amino acid δ^{13}C values were corrected for the carbon added as a result of derivatization. The $\delta^{13}C_{EAA}$ values were mean-centered (normalized) by subtracting each value from the average of the $\delta^{13}C_{EAA}$ values for that sample (Larsen et al. 2009). This allowed direct comparison of $\delta^{13}C_{EAA}$ patterns ("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. Mixing models were generated (using R package in *simmr*; Parnell et al. 2013) to estimate proportional contributions of primary producers to bivalve EAAs. Phytoplankton endmember data generated in this study were used as mixing model inputs because our data were more taxonomically constrained (i.e., all diatoms) than the literature phytoplankton data. We also used terrestrial plant, red algae, and brown algae endmember data generated in this study. Published data values (Larsen et al. 2009, 2013) were used for bacterial endmembers because Arctic marine bacterial samples cultured on amino acid-free media were unavailable to us. To compare bivalve shell and muscle $\delta^{13}C_{EAA}$ fingerprints, the mixing model was performed separately for the two tissue types. The $\delta^{13}C_{EAA}$ 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.

We used polynomial contrasts to evaluate whether the $\delta^{13}C_{EAA}$ patterns of the endmembers generated in this study were distinct and to assess how they compared with previously published values of the same endmember categories (Larsen et al. 2009, 2013;

McMahon et al. 2016, 2018). These tests are critical as separation of endmember signatures is a necessary condition for further analysis. Mixed models were used with dataset and EAA identity as fixed factors and species by EAA interaction as the random factor. Significant interactions between dataset and EAA identity were considered evidence that the datasets followed statistically different patterns. We also performed a linear discriminant analysis (LDA) of the endmember datasets (Larsen et al. 2009, 2013; McMahon et al. 2016, 2018) to test whether the endmember categories were classified differently, regardless of any statistical differences detected between published and generated endmember data.

Results

The EAA fingerprinting data generated in this study were compared to a previously published database of amino acid fingerprints from primary production sources (Larsen et al. 2009, 2013) to identify amino acid sources for the tested benthic organisms. Eleven amino acids were resolved successfully for all samples: alanine (Ala), glycine (Gly), threonine (Thr), serine (Ser), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), aspartic acid (Asp), glutamic acid (Glu), and phenylalanine (Phe). Of these, we focused on the EAA (Thr, Val, Leu, Ile, Phe) because they are not synthesized by animals and must be derived from sources of primary production without significant fractionation.

Endmember Results

To validate the use of the Arctic diatom species endmembers as a model input for making estimates of diet proportions of Arctic bivalves, we tested how model results would change with differing endmember values. Three mixing model runs (sensitivity tests) were conducted using different datasets (Table 2). Dataset 1 included only published (literature) data (Larsen et al. 2009, 2013; McMahon et al. 2016, 2018). Dataset 2 included values generated from this study for Arctic diatoms (used as the phytoplankton –diatom endmember), Arctic red algae, Arctic brown algae and Arctic terrestrial matter (Rowe et al. in press) and published bacterial endmember values (Larsen et al. 2009, 2013; McMahon et al. 2016, 2018). Dataset 3 pooled both published and newly generated values for endmember inputs. The Hanna Shoal bivalve EAA $\delta^{13}C$ values were used as inputs for the mixing model sensitivity tests to estimate the proportional contributions of the endmembers to the diets of these bivalves. The outputs of the three different models were then compared to examine the effects of endmember source differences.

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 (Table 3). Based also on the LDA results (Figure 6), which showed that our Arctic diatoms (phytoplankton) were more distinct than the taxonomically diverse data set present by Larsen et al. (2009, 2013), we were justified in using Dataset 2 (our cultured Arctic phytoplankton data and published bacteria endmember data) in the mixing models presented here. Phytoplankton was estimated to make up the largest proportional contribution of EAA in our test series of *Macoma* sp.

Table 2: Datasets used for mixing model endmember inputs. $\delta^{13}C_{EAA}$ values have been centered to the mean values of all five EAAs 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

Table 3: Mixing model results (dietary proportions) based on EAA fingerprints for endmember-source dataset sensitivity tests. 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

The LDA confidence ellipses for terrestrial plants, bacteria, red algae, brown algae, and Arctic phytoplankton did not overlap with those of any other endmembers, indicating that these endmembers were distinct from each other (Figure 6) and satisfying the condition that sources used for stable isotope diet reconstructions should have significantly different values. With this condition met, we proceeded with using these endmember data to generate mixing models. However, the confidence ellipses of brown algae and phytoplankton using literature values did overlap, suggesting that these endmember sources may need further investigation or combining (Figure 6). Furthermore, the dataset by EAA interaction of the polynomial contrasts was

significant ($p<0.0001$) for all parameters investigated except brown algae. This finding indicated that there were subtle differences in how values changed between EAAs by dataset for each endmember category, despite the similarity of general fingerprints of the same endmember categories from different datasets.

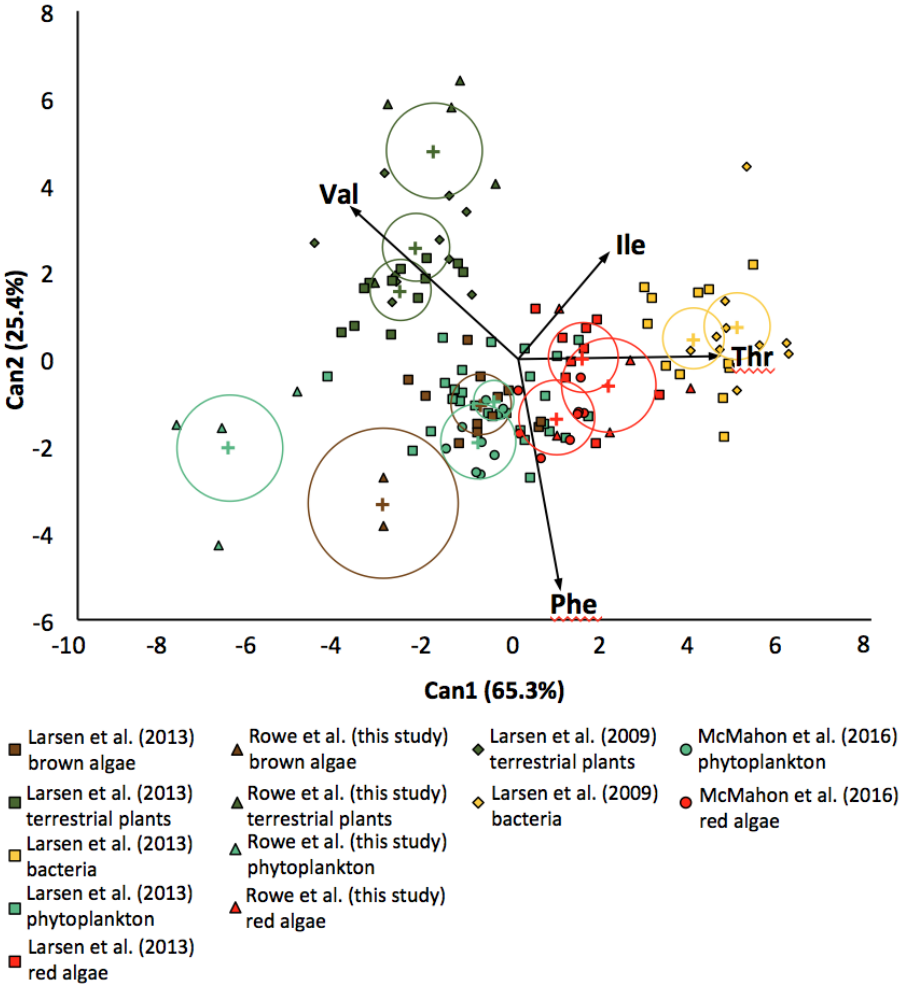

Figure 6: Linear discriminant analysis based on the mean-centered $\delta^{13}C_{EAA}$ values (Thr, Val, Leu, Ile, Phe) of all endmembers (McMahon et al. 2006, 2016, Larsen et al. 2009, 2013). The crosses indicate the mean value plus the standard ellipses for each primary production source of the same color (redrawn from Rowe et al., in press).

Hanna Shoal Region

Estimated dietary proportions of Hanna Shoal *Astarte* spp. and *Macoma* spp. samples from the mixing model are presented in Figures 7 and 8. The highest source contributions were from phytoplankton and bacteria for both genera, though the absolute proportions varied. Phytoplankton was estimated to contribute a higher proportion to EAAs of *Astarte* spp. than to *Macoma* spp. There were slightly higher contributions of bacteria and terrestrial plants to *Macoma* spp.

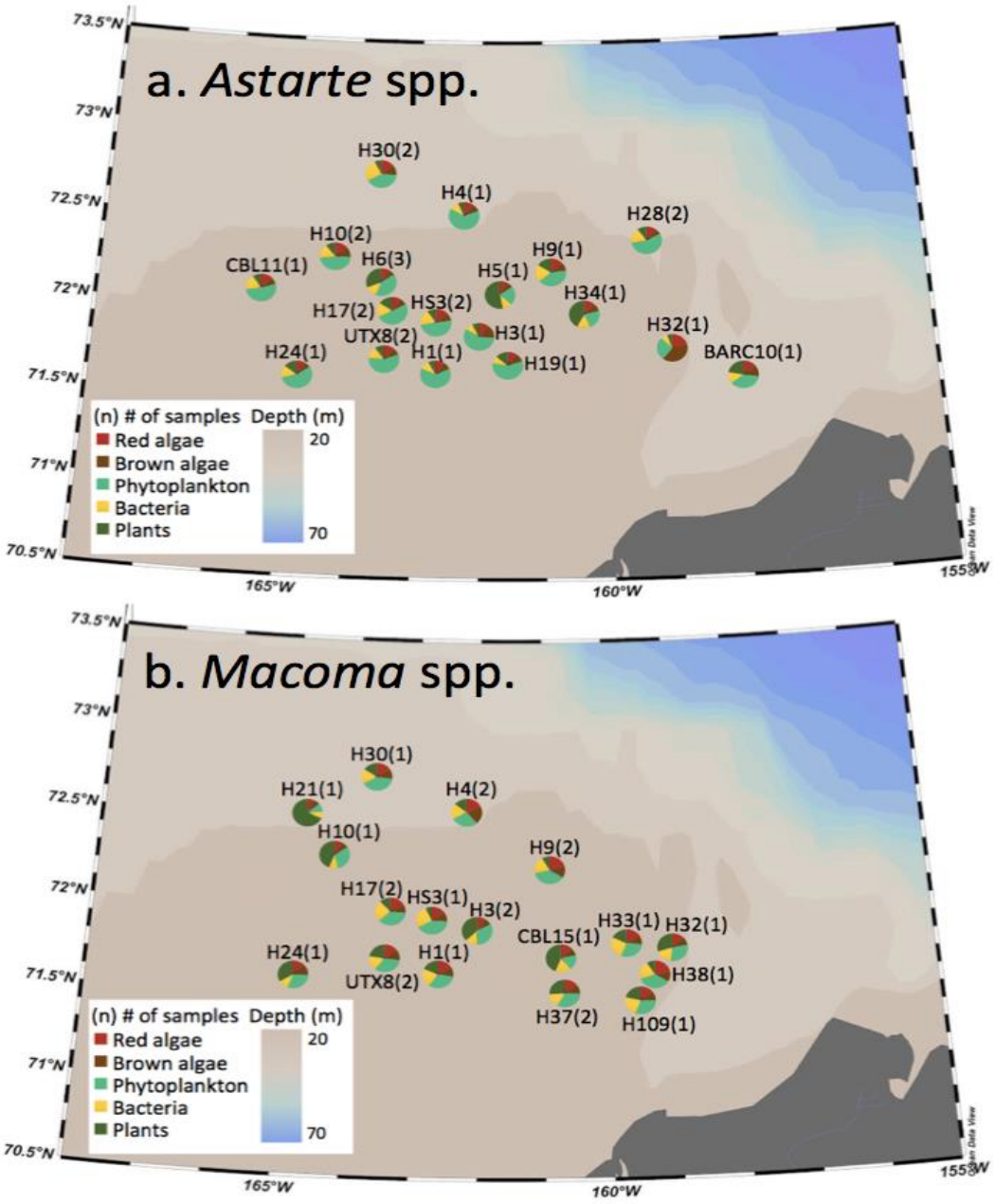

Figure 7: *Astarte* spp. (a) and *Macoma* spp. (b) sampling locations and estimated dietary contributions in Hanna Shoal individuals (*simmr* solo runs) (redrawn from Rowe et al., in press).

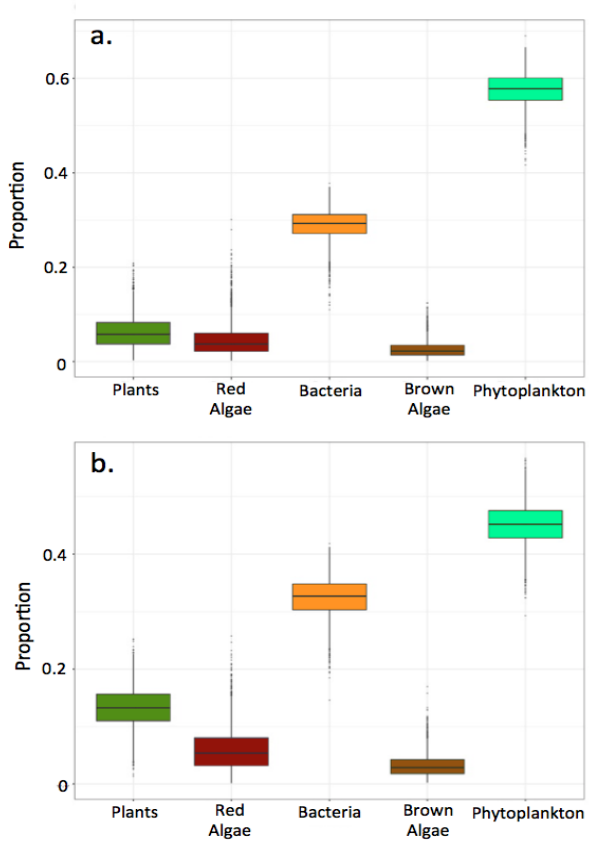

Figure 8: Proportional contributions of diet sources to *Astarte* spp. (a) and *Macoma* spp. (b) from Hanna Shoal as modeled by *simmr* (redrawn from Rowe et al., in press).

Chukchi Region

Estimated dietary proportions in the Chukchi Sea samples are presented in Figures 9–18. The proportional contributions of the different EAA sources varied according to species and by location. *Buccinum* sp. *Alcyonidium* sp. *Anonyx* sp. and *Macoma* sp. all showed high contributions from a terrestrial source at most locations sampled. In this regard, the findings were similar to the results from the Hannah Shoal, which indicated a large contribution of terrestrially derived EAAs at some stations. *Macoma* spp. was common to both Hannah Shoal and Chukchi Sea samples. For both regions, *Macoma* spp. samples reflected high contributions of terrestrial-derived EAAs, which could have originated from coastal inputs. Essential amino acids from phytoplankton and bacteria made major contributions to *Argis* sp. *Eualus* sp. *Chionoecetes* sp. and *Maldanidae* sp. No relationships for amino acid sources vs. water depth were defined due to the relatively uniform depth gradient in the sample area.

An unexpected result came from the analyses of echinoderms (e.g., *Ophiocten sericeum*) from Hanna Shoal and Chukchi Sea samples; echinoderms from both regions had a very limited number of amino acids. To our knowledge, ours was the first analyses of this kind for echinoderms, so we only speculate that this finding reflects an unknown taxon-specific biochemical issue.

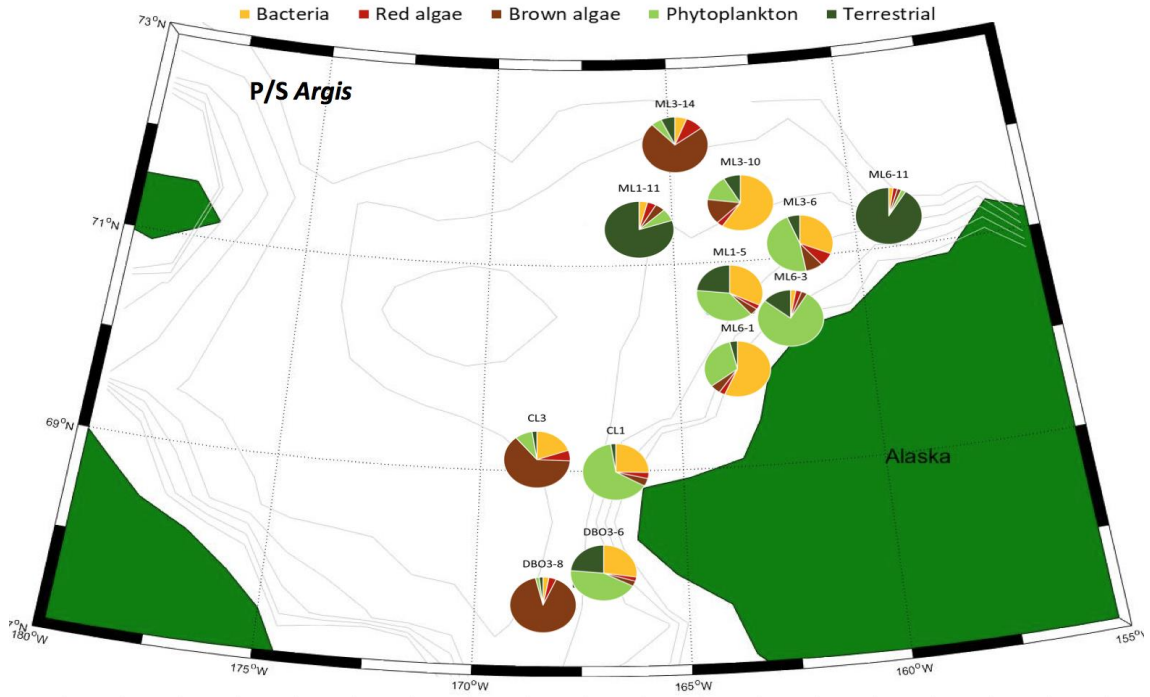

Figure 9: *Anonyx sp.* (amphipod) locations and estimated dietary contributions in the Chukchi Sea individuals (*simmr* solo runs).

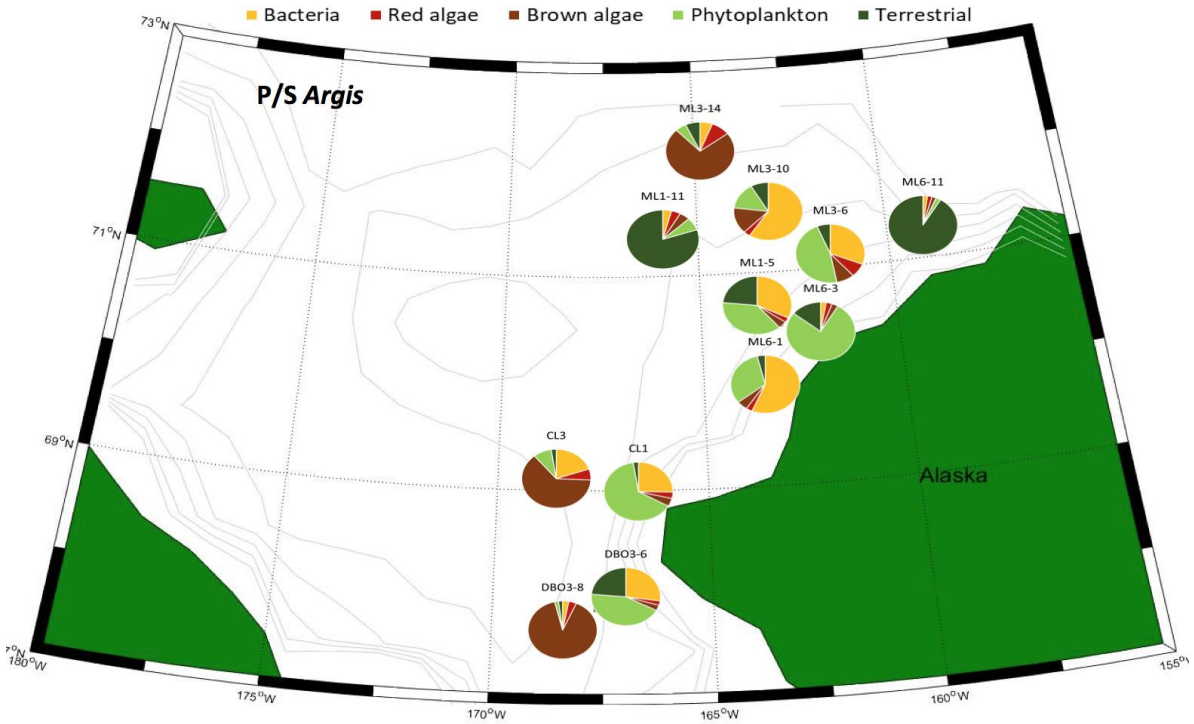

Figure 10: *Argis sp.* (shrimp) locations and estimated dietary contributions in the Chukchi Sea individuals (*simmr* solo runs).

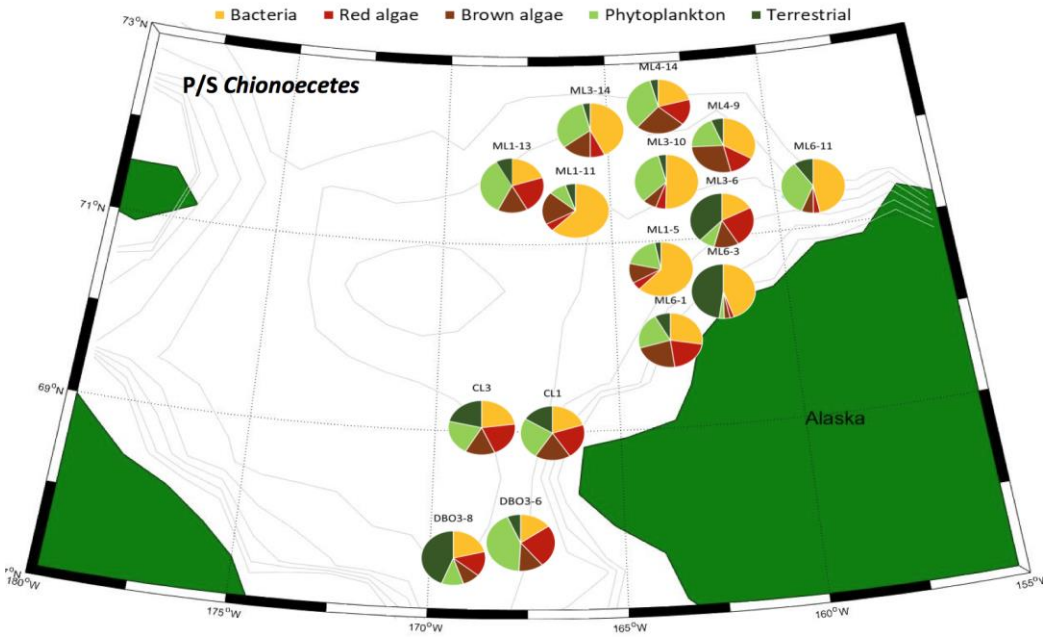

Figure 11: *Buccinum sp.* (sea snail) locations and estimated dietary contributions in the Chukchi Sea individuals (*simmr* solo runs).

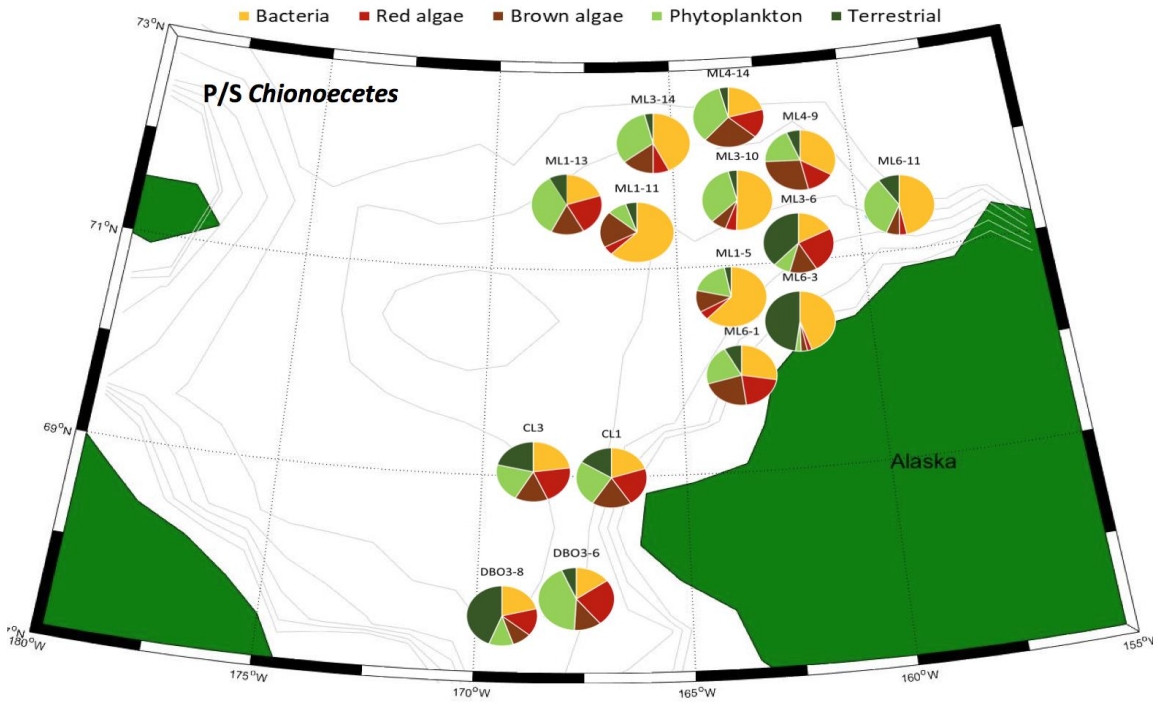

Figure 12: *Chionoecetes sp.* (snow crab) locations and estimated dietary contributions in the Chukchi Sea individuals (*simmr* solo runs).

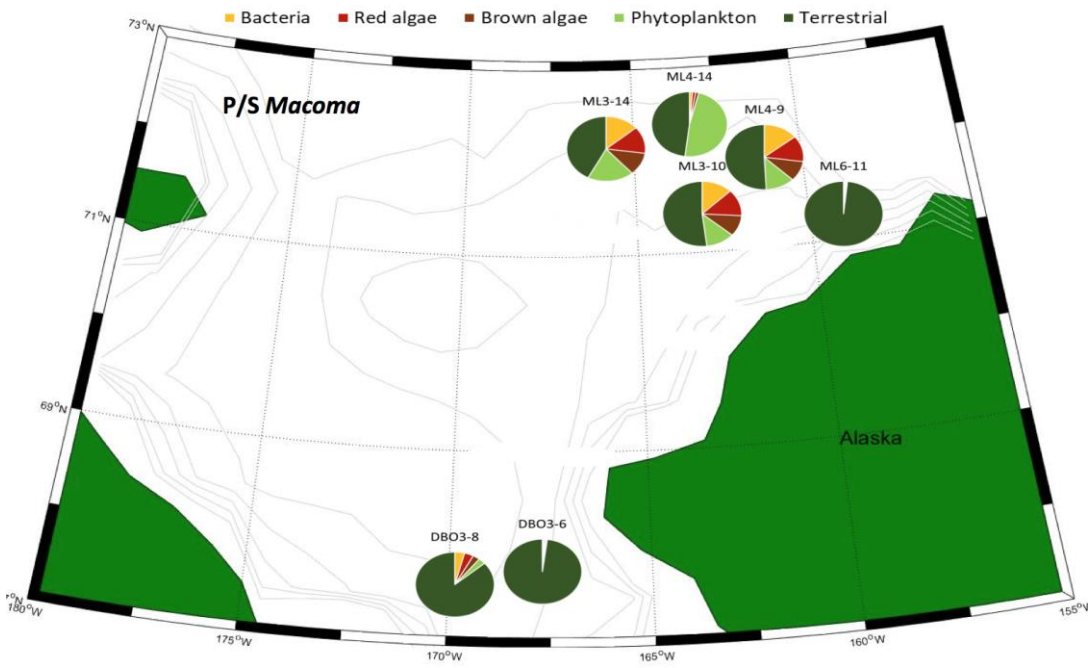

Figure 13: *Eualus sp.* (shrimp) locations and estimated dietary contributions in the Chukchi Sea individuals (*simmr* solo runs).

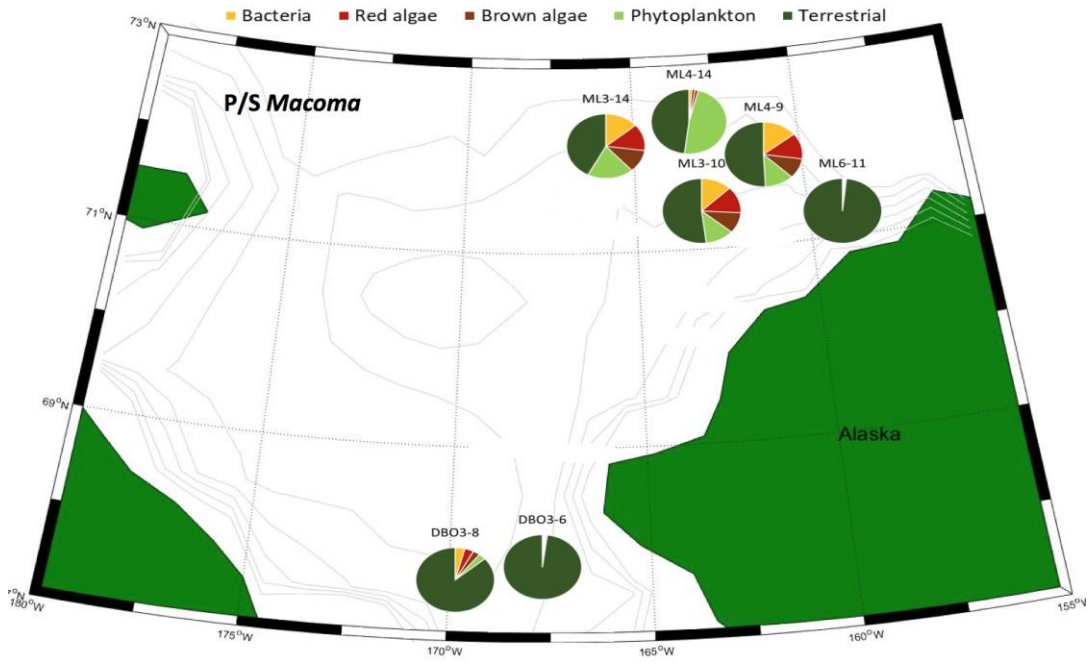

Figure 14: *Macoma sp.* (clam) locations and estimated dietary contributions in the Chukchi Sea individuals (*simmr* solo runs).

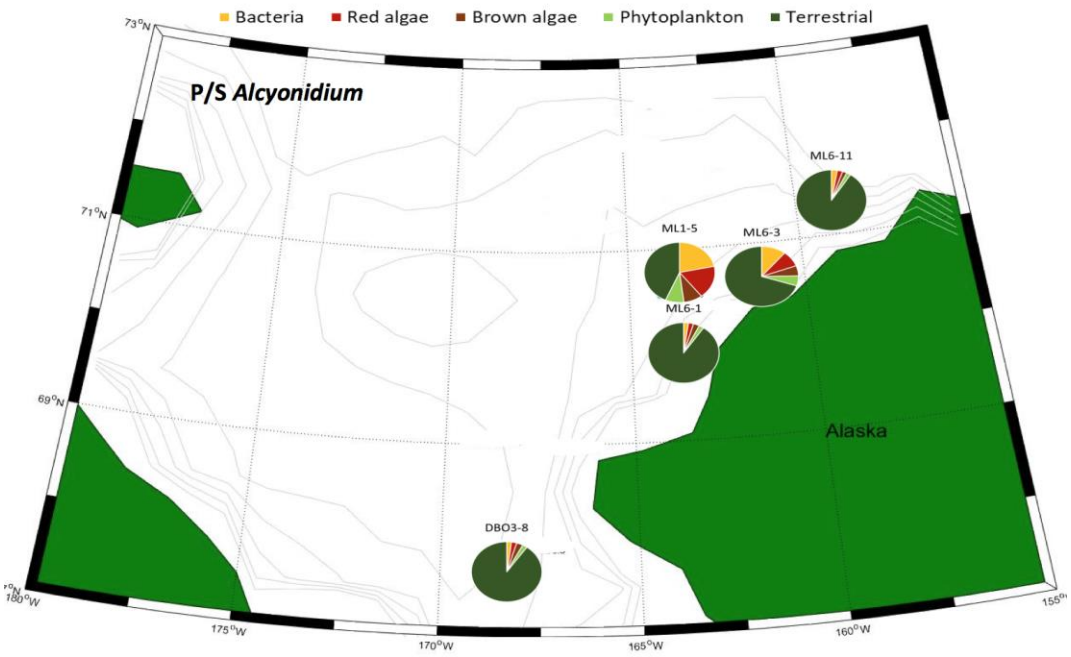

Figure 15: *Alcyonidium sp.* (bryozoan) locations and estimated dietary contributions in the Chukchi Sea individuals (*simmr* solo runs).

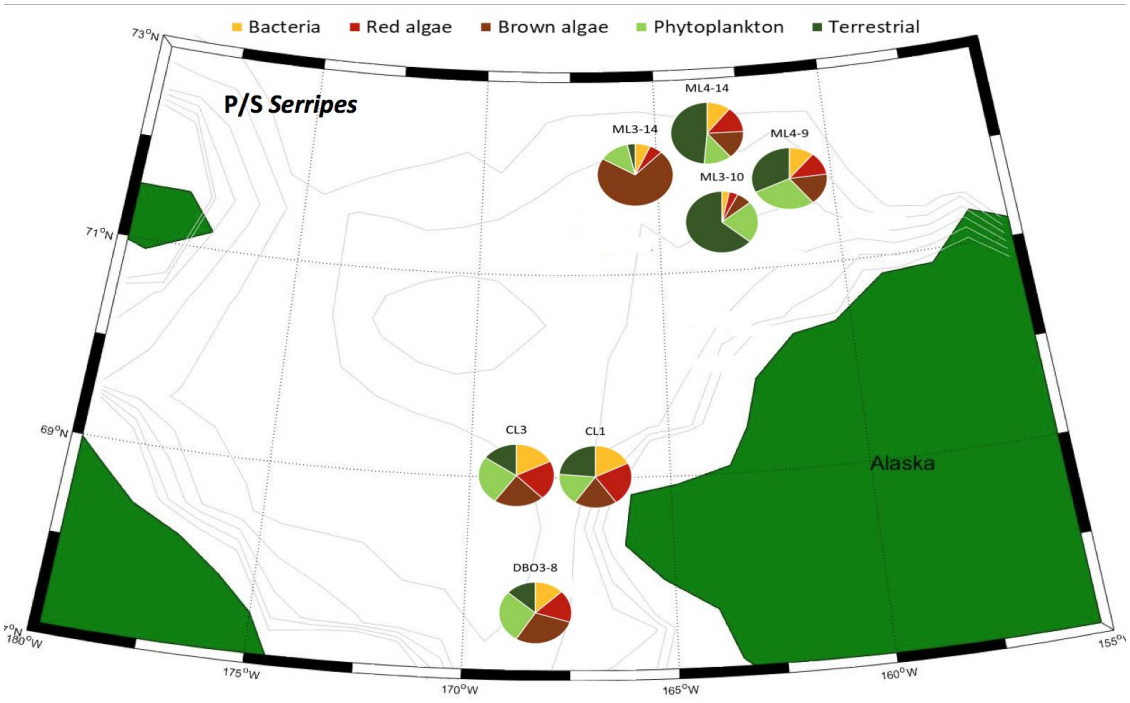

Figure 16: *Serripes sp.* (clam) locations and estimated dietary contributions in the Chukchi Sea individuals (*simmr* solo runs).

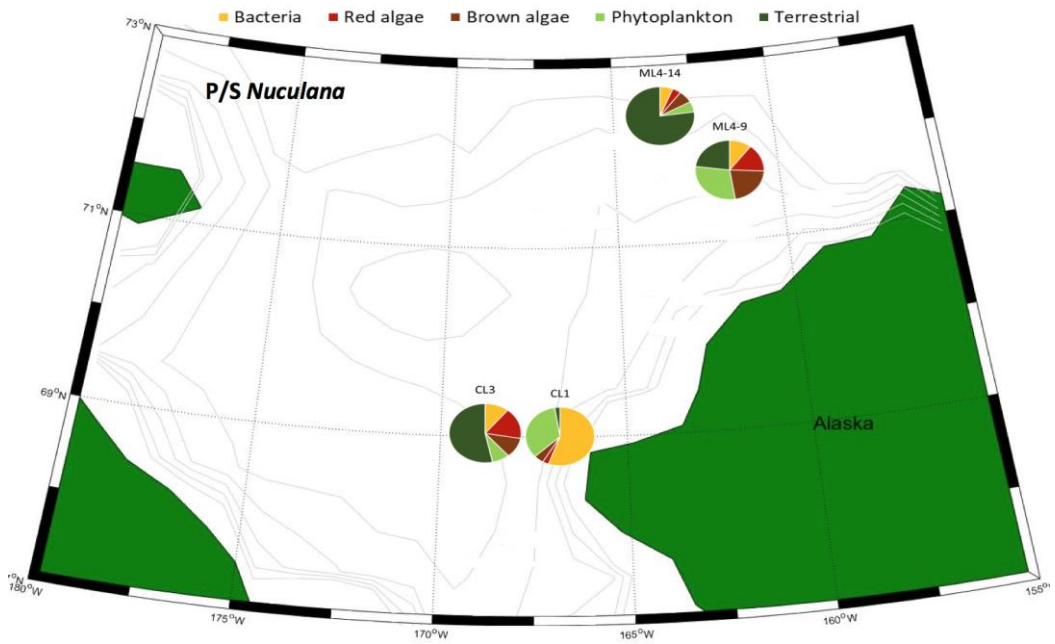

Figure 17: *Nuculana sp.* (clam) locations and estimated dietary contributions in the Chukchi Sea individuals (*simmr* solo runs).

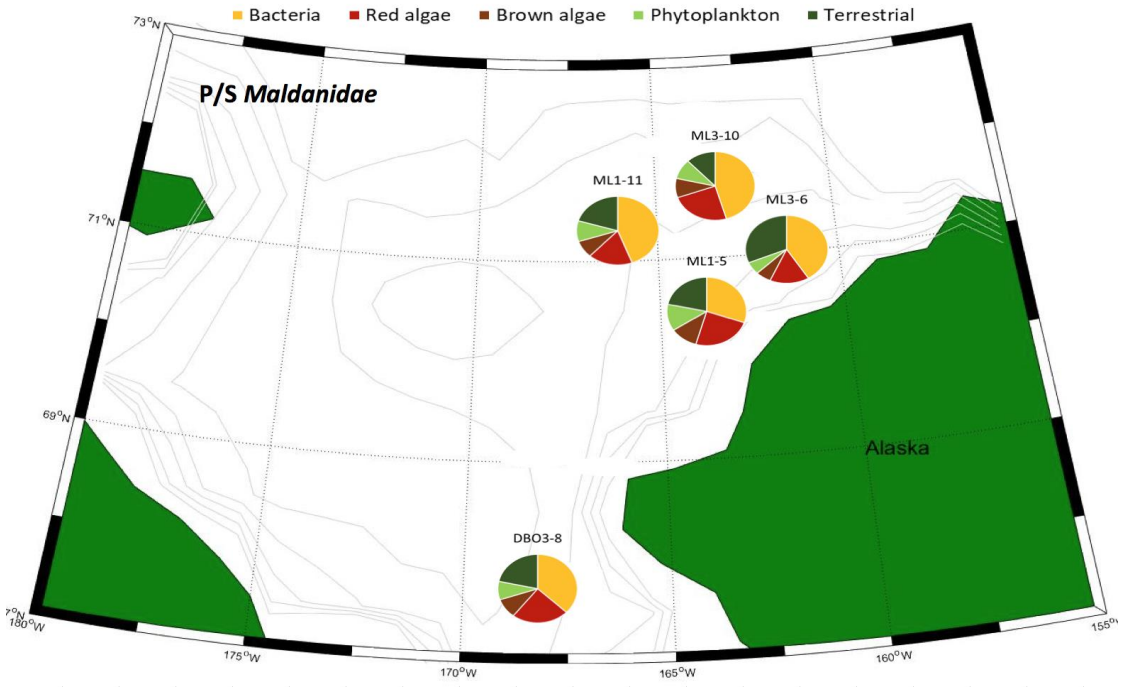

Figure 18: *Maldanidae sp.* (worm) locations and estimated dietary contributions in the Chukchi Sea individuals (*simmr* solo runs).

Beaufort Region

Estimated dietary proportions in the Beaufort Sea samples are presented in Figures 19–22. The source contributions varied by species, and bacterial EAA contributions were higher in many of the Beaufort Sea samples compared with those from the Hanna Shoal and Chukchi Sea regions. Samples from deeper locations had a greater contribution of bacterial EAAs.

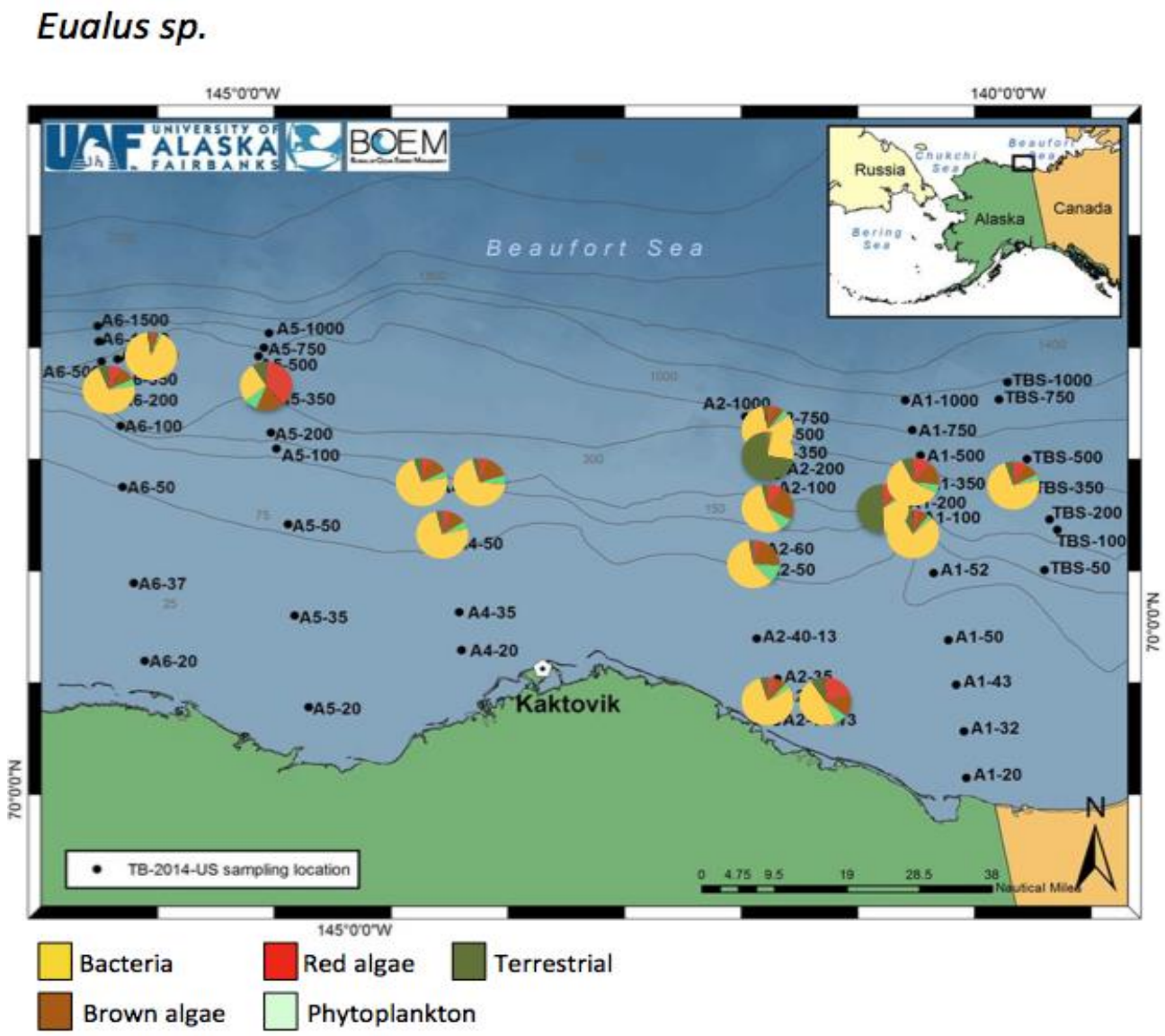

Figure 19: *Eualus* sp. (shrimp) locations and estimated dietary contributions in the Beaufort Sea individuals (*simmr* solo runs).

Astarte spp (no label = A. crenata, Am = A. montagui, Ab = A. borealis).

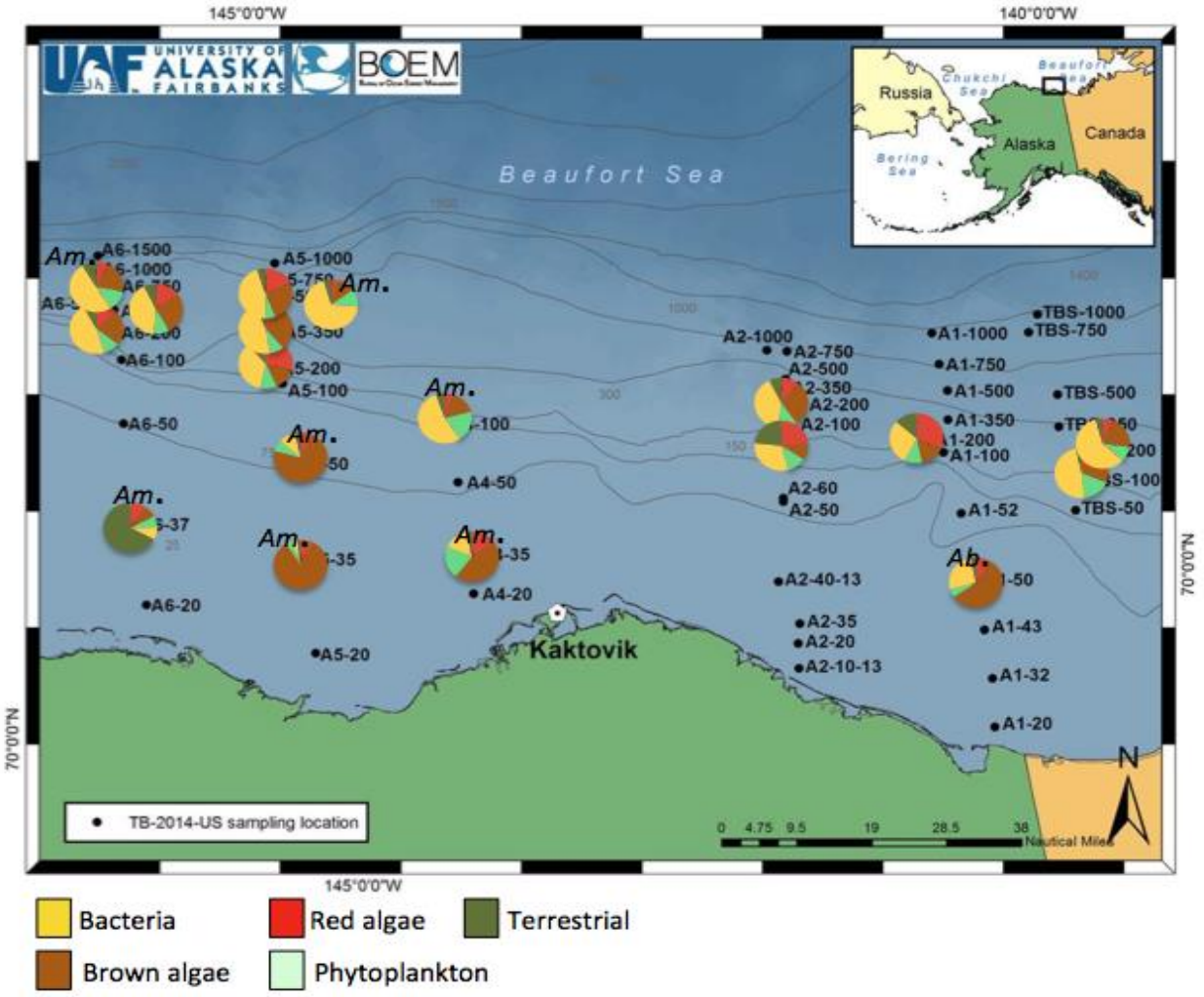

Figure 20: *Astarte* spp. (clam) locations and estimated dietary contributions in the Beaufort Sea individuals (*simmr* solo runs).

Sabinea

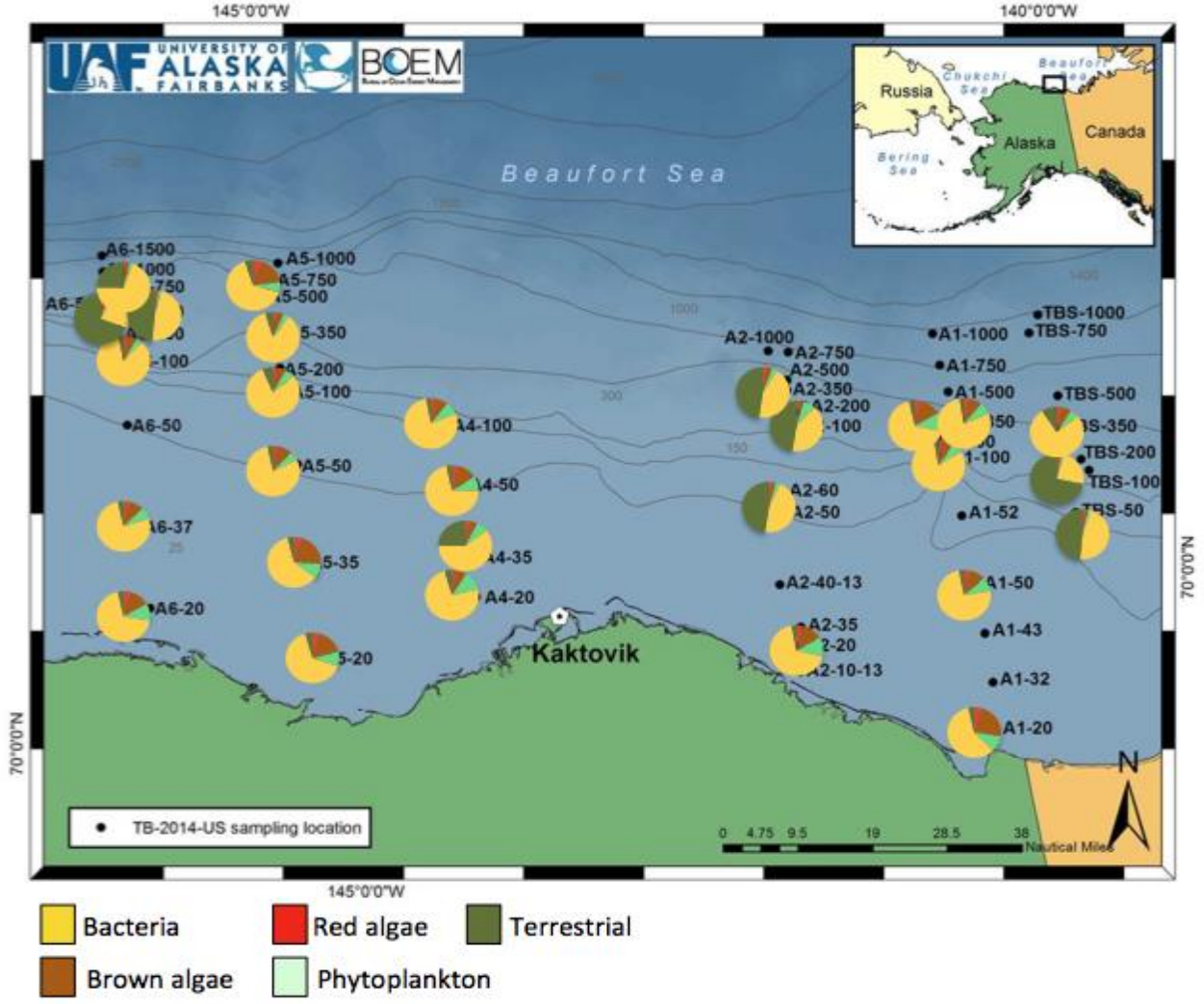

Figure 21: *Sabinea* sp. (shrimp) locations and estimated dietary contributions in the Beaufort Sea individuals (*simmr* solo runs).

Bathyarca glacialis

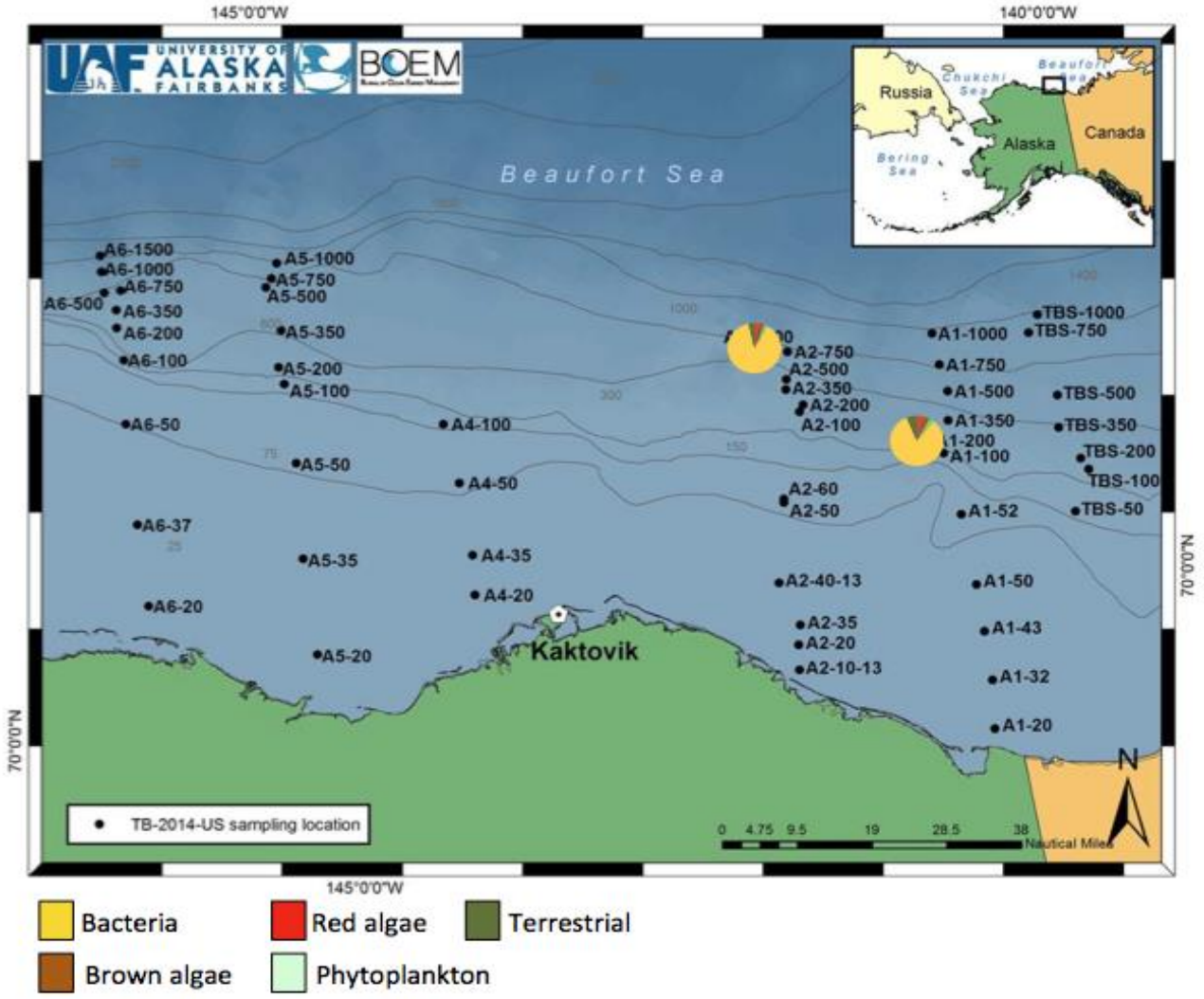

Figure 22: *Bathyarca* sp. (bivalve) locations and estimated dietary contributions in the Beaufort Sea individuals (*simmr* solo runs).

Leucine to Isoleucine Index

Beaufort Sea samples represented a range of sampling water depths, so it was possible to examine the isoleucine to leucine index (ratio of one to the other, Larsen et al. 2009, 2013) as a marker of bacterial contribution (i.e., a higher ratio indicates a higher relative contribution of bacterially derived amino acids). The isoleucine to leucine index for both *Sabinea septemcarinata* and *Astarte* spp. showed significant increases along an exponential-linear relationship with the water depth (Figure 23 and 24), though both organisms did not cover the complete depth range. No linear relationship was evident for *Eualus* sp. vs. depth, and the index was highly variable within and between depth brackets above ~ 400 m water depth for this organism (Figure 23). *Astarte* spp. had the lowest isoleucine to leucine values, at water depths of <50 m.

We found that the isoleucine to leucine index values of our phytoplankton endmembers were the lowest of all the sources we tested, while the bacteria values were the highest, which is consistent with the previous findings of Larsen et al. (2009, 2013). This finding indicates that the isoleucine to leucine index is useful as an indicator of the contribution of both phytoplankton and bacteria when one source is low and the other is high. For example, the index showed increasing bacterial contributions with depth in *Astarte* spp. This was consistent with the mixing model results for *Astarte* spp., which indicated that bacterial contributions of EAAs increased from ~10% at the shallowest depths sampled (~25 m) to ~50% at the deeper depths (200 m – 500 m). The increase in bacterial contributions with depth correlated with a decrease in phytoplankton contributions. In the shallowest sampling locations, phytoplankton contributed up to 50% in *Astarte* spp. but dropped to ~25% beyond 100 m (Figure 25).

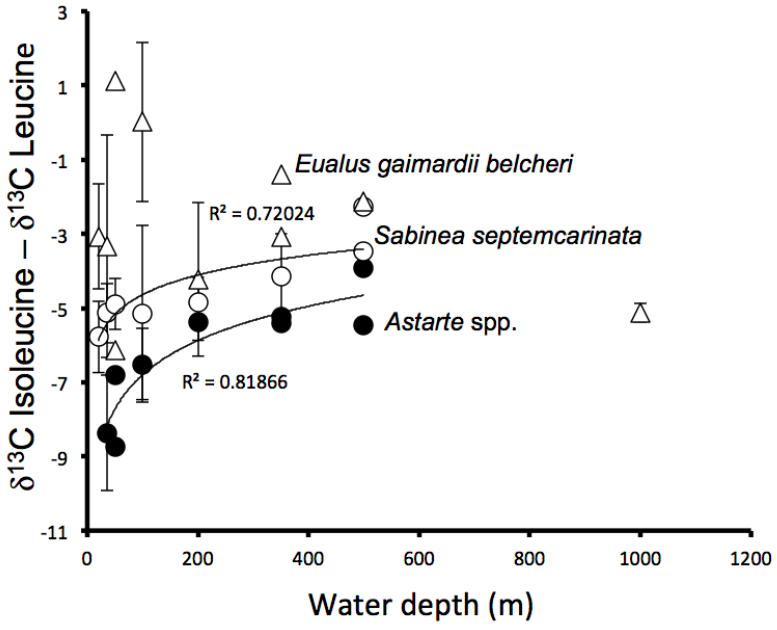

Figure 23: Leucine to isoleucine indexes of inverts from the Beaufort Sea vs. station depth (±1 SD).

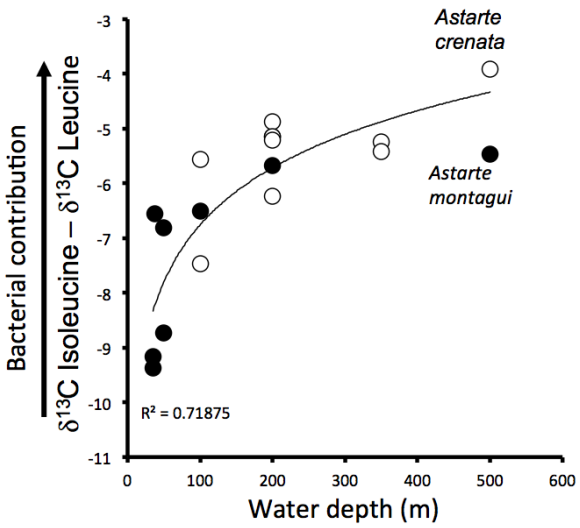

Figure 24: Leucine to isoleucine indexes of *Astarte* spp. from the Beaufort Sea vs. station depth.

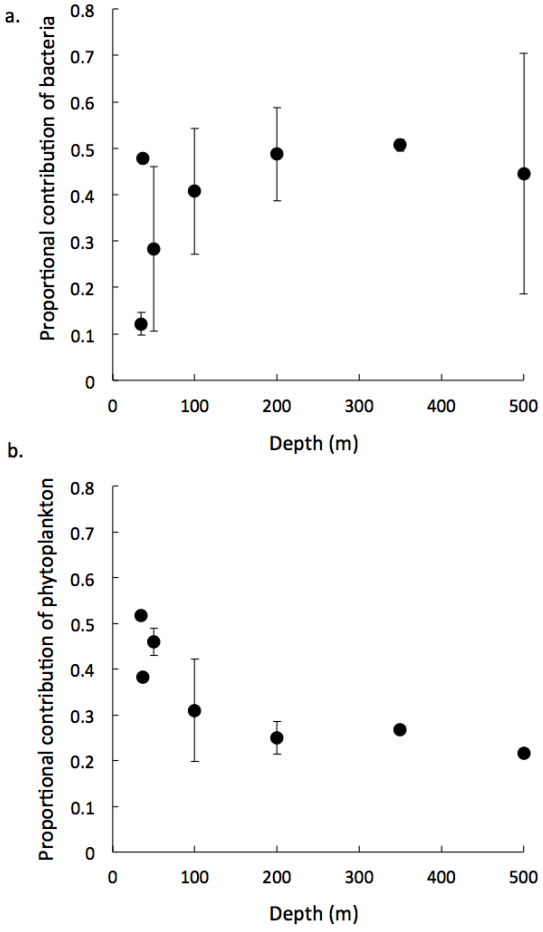

Figure 25: Proportional contributions of bacterial (a) and phytoplankton (b) EAAs vs. water depth for *Astarte* spp. from the Beaufort Sea (error bar ± 1 SD).

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 organic shell materials was 3.9 (±0.2).

The relationship between shell and muscle $\delta^{13}C_{EAA}$ values was tightly correlated and close to a 1:1 line ($y = 0.85x$, $R^2 = 0.91$), suggesting similar values of EAA across tissues and similar patterns in the EAA fingerprints (Figure 26a). 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 26b). The model estimated phytoplankton and bacteria as the two highest-ranking EAA sources to *Macoma calcarea* muscle (Table 4). When using shell values from the same individuals, the model also estimated phytoplankton as the highest dietary proportion, but the subsequent rankings differed with terrestrial plants ranked second, and bacteria ranked last (Table 4).

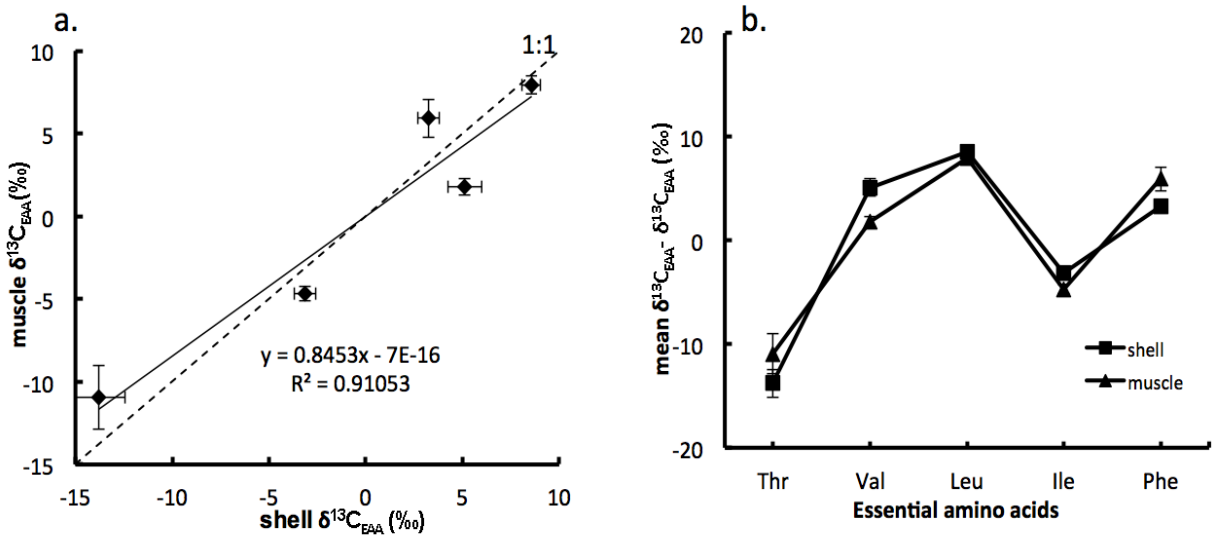

Figure 26: Shell and muscle centered average amino acid $\delta^{13}C$ values for *Macoma calcarea* plotted against each other (a) and on the same axis by amino acid (b) (error bar ±1 SD).

Table 4: Mixing model dietary proportion estimates based on EAA fingerprints for *Macoma calcarea* muscle and shell samples. Values are expressed in percent (%).

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

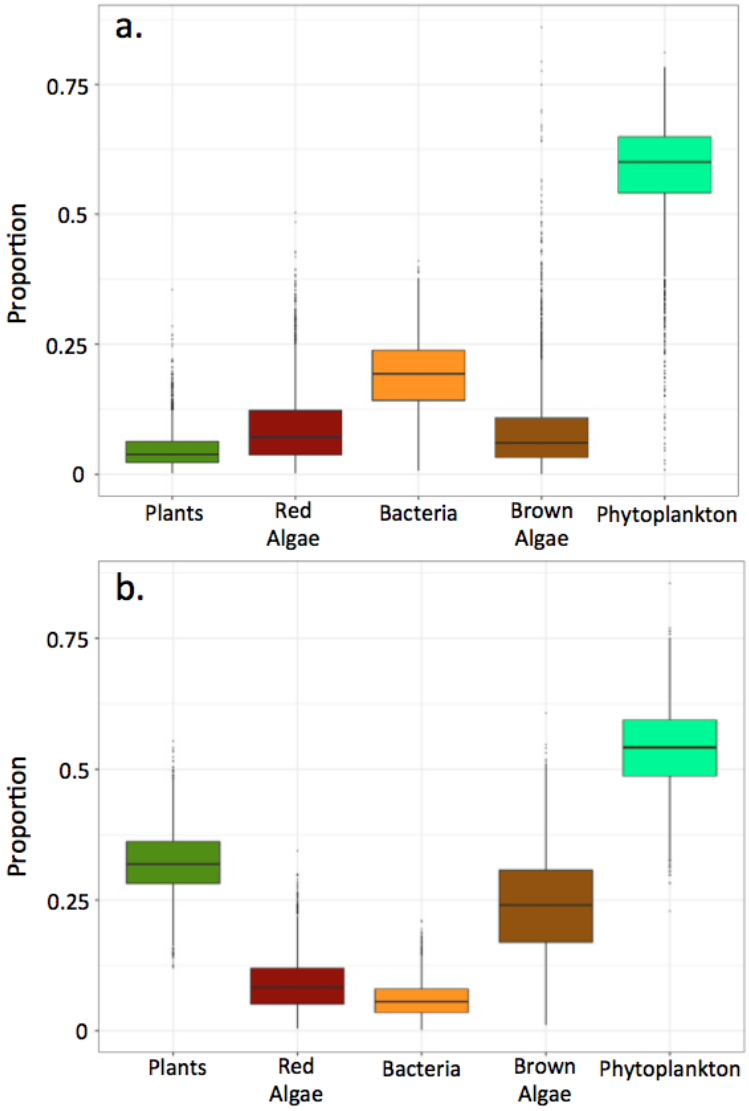

Figure 27: Proportional contributions of diet sources to *Macoma calcarea* muscle (a) and shell (b) as modeled by *simmr*.

Discussion

Endmember Results

In comparisons between our endmembers and literature values, most endmembers of the same category had very similar EAA isotope fingerprints, despite some statistical differences. These 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 (McMahon et al. 2016) were remarkably similar. This finding was consistent with previously published conclusions (Larsen et al. 2009, 2013) that EAA isotope fingerprints of primary producers are taxon-specific, driven by broad and deep phylogenetic differences in amino acid synthesis rather than environmental and geographic differences. However, there were slight differences in fingerprints between datasets, most notably for phytoplankton. The differences in phytoplankton values from different datasets 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 EAA isotope fingerprints regardless of location, "phytoplankton," as characterized here, is a metabolically and taxonomically diverse functional group and species compositions can change dramatically with location, environmental conditions, and time.

Similarly, bacteria are very diverse in EAA synthesis, and these pathways are not yet well understood. Categorizing primary production sources with common broad groupings like "phytoplankton" and "bacteria" is useful for simplicity but could mask 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. However, diatoms are taxonomically much closer to brown algae, which were classified as a separate category in Larsen et al. (2013). The phytoplankton cultures used in this study were from specific species of diatoms. Therefore, we suggest that continued efforts need to be made to determine endmember values by increasing phylogenetically consistent representation, especially concerning phytoplankton and bacteria.

Hanna Shoal and Chukchi Sea Regions

Our mixing models showed that phytoplankton is the most important contributor of EAAs to Hanna Shoal bivalves. This finding was consistent with our Hypothesis 2 and observations of highly productive phytoplankton blooms in the region (Arrigo et al. 2014; Arrigo and 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 species (McMahon et al. 2006; Dunton et al. 2017). The Hanna Shoal bivalve model estimated that bacteria made up the next highest proportion of bivalve EAAs, which may reflect the reworking and ecological availability of more refractory organic matter.

Surprisingly, 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. Sediment-laden ice sheets, transported by the Beaufort Gyre into the western Beaufort Sea and Chukchi Sea, may be one pathway for the introduction of terrestrial organic matter to this region (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 and Stringer 2006). As the ice melts, sediments containing terrestrial organic matter could collect around Hanna Shoal. However, this mechanism would require that tidewater glaciers entrain large amounts 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 entrains significant amounts of sediment and terrestrial organic matter and is advected out of shallow waters in the Beaufort and Chukchi Seas (Eicken et al. 2005). The terrestrial organic matter is released to the Hanna Shoal benthos when the ice melts. Another 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 source differences between the two bivalve species we investigated. Phytoplankton was estimated to contribute a higher proportion of EAAs to *Astarte* spp. than to *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 different feeding modes. As suspension feeders, *Astarte* spp. filter particles from the water column. In contrast, *Macoma* spp. are surface deposit feeders and 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 consume more bacteria in deposited carbon.

The mixing models showed that phytoplankton and terrestrial derived EAAs were, in most cases and at most locations, the dominant contributors of EAAs to Chukchi Sea invertebrates. As in the Hanna Shoal region, this finding is also supported by observations of highly productive phytoplankton blooms and terrestrial organic material inputs in the region (Arrigo et al. 2014; Arrigo and 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, as has been suggested for various arctic bivalve species (McMahon et al. 2006; Dunton et al. 2017). Marine photosynthetic sources could include ice algae, open-ocean phytoplankton, and the microphytobenthos. The mixing models using amino acid fingerprinting are not able to distinguish the contribution of ice algae vs. open-ocean phytoplankton; however, we have previously used compound-specific carbon isotope analyses of fatty acids to do so. Applying compound-specific amino acid and fatty acid analyses on the same sample would allow determination of the proportional contribution of marine photosynthetic sources as a whole (based on the amino acids) vs. other sources (e.g., terrestrial and bacterial) and the proportional contribution of ice algae vs. phytoplankton (based on the fatty acids).

Beaufort Region

In contrast to the Chukchi Sea and Hanna Shoal samples, the Beaufort Sea samples indicated a greater contribution of EAAs from sources other than phytoplankton and terrestrial organic matter. The Beaufort benthic food web may be particularly sensitive to environmental changes (Divine et al. 2015). Low redundancy and high trophic separation make the ecosystem less effective at responding to changes in environmental conditions (Divine et al. 2015). Compared to the Chukchi Sea, the Beaufort Sea is more nutrient-poor, the pelagic-benthic coupling is weaker, and the ice algae contribution is an order of magnitude smaller (Dunton et al. 2005, 2006). Consequently, benthic invertebrate communities in the western Beaufort rely on terrestrial organic matter inputs and the advection of allochthonous carbon from the Chukchi Sea (Divine et al. 2015). These sources of organic matter are more refractory than fresh primary production and are likely to shift in abundance due to changing hydrographic patterns and increased freshwater inputs brought upon by climate change (Divine et al. 2015). Energy flow through the system could be altered as the proportions of different organic matter sources delivered to benthic food webs in the Arctic change (Iken et al. 2010; McTigue and Dunton 2014; Divine et al. 2015; Kedra et al. 2015; McTigue et al. 2015).

Consistent with Hypothesis 1, there appeared to be a greater contribution of bacterial and macroalgal sources of EAAs in the invertebrates from the Beaufort region. Water depth also appeared to be an influence on the source of the proportional contributions, with an increased contribution of bacterial-derived EAAs observed at greater water depth. This finding was evident in both the isoleucine to leucine index, used as a proxy for bacterial contributions (Larsen et al. 2009, 2013), and the mixing model results from the *Astarte* spp. samples. The mixing model results also showed a decrease in the contribution of phytoplankton with an increase in water depth.

Terrestrial organic matter has typically been considered an inferior carbon source for marine consumers (Schell 1983), but results from bulk stable isotope analyses of benthic ecosystems in the Beaufort Sea indicate that terrestrial carbon may be utilized in marine benthic food webs in significant amounts, possibly after microbial processing (Dunton et al. 2006; Garneau et al. 2009; Divine et al. 2015). Permafrost melting and increased river discharge influence the delivery of terrestrial organic matter into the marine system, as in the area of the Mackenzie River Delta in the eastern Beaufort Sea (McClelland et al. 2014) and many smaller river systems in the western Beaufort (Dunton et al. 2006).

We are not certain why we did not observe larger terrestrial signal in the samples collected in the Beaufort Sea, where there is extensive landfast ice and large volumes of sediment deposition. However, the large contributions of bacterial-derived amino acids to individuals sampled from the region could be fueled by terrestrial organic matter. The depth gradient in the Beaufort may add distance and time between input and consumption, allowing time for bacteria to act on the terrestrial organic matter. In theory, break down of terrestrial organic matter should result in the conversion of organic matter from terrestrial form to bacterial form. We are unable to distinguish autotrophic vs. heterotrophic bacterial production using this

method. The mixing model results provide estimates of the proportional contribution of the different sources of EAAs to benthic organisms and are not a proxy for source materials being deposited on the sediment.

We did not find obvious spatial trends in the proportional contribution of terrestrial sources. This was surprising, as we had expected that the influences of the Mackenzie River or coastal erosion might be evident at stations closest to them, or along water currents carrying these potential sources of terrestrial organic matter. Instead, terrestrial organic matter was estimated to contribute a relatively small proportion to most samples in the Beaufort, regardless of location. We hesitate to jump to the conclusion that terrestrial sources are unimportant to the Beaufort benthos. For instance, it has previously been shown that terrestrial organic matter is heavily utilized by fish in Beaufort lagoons (Dunton et al. 2006). Our results may indicate that terrestrial organic matter is reworked by bacteria before it can be consumed by benthic invertebrates (Garneau et al. 2009).

Shell vs. Muscle Comparison

The EAA isotope fingerprints of *Macoma calcarea* shell and muscle were very similar. The mixing model results of using the EAA isotope fingerprints from these two tissue types both identified phytoplankton as the highest contributor to the bivalves' EAAs. This similarity indicates that shells can be used to estimate the proportional contributions of the dominant source of EAAs to the diets of bivalves when soft tissues are not available for analyses. However, the model results for the other dietary sources differed in estimated proportion and the relative ranking of their importance between the two tissue types; possibly 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, and potentially irregular growth bands (Moss et al. 2018), we homogenized whole shells to yield enough sample for analysis. This resulted in time-averaging the lifespan of the bivalves while muscle tissue would likely have a shorter temporal window, which 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 identify the factors causing differences between EAAs 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 EAAs we have observed or from the results of feeding studies, 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 changes in the proportional contribution of phytoplankton over time. This 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.

Conclusions

We found that the EAA isotope fingerprinting method can be used to distinguish sources of Arctic primary producers to two invertebrate species. However, this method is limited in its resolution and cannot separate taxonomically similar endmembers. 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 composed largely of diatoms. This problem might be resolved by completing stable carbon isotope analyses of EAAs and fatty acids on the same samples. Stable carbon isotope analyses of fatty acids, coupled with fatty acid profiling, has been successfully used at high-latitude marine locations to determine the proportional contribution of ice algal-derived fatty acids. Based on prior fatty acid stable carbon isotope data, the proportional contribution of ice-derived particulate organic matter to Bering Sea bivalve species *Macoma calcarea* and *Nuculana radiata* has been estimated to be as high as 47% (Oxtoby et al. 2016), suggesting that it may be a highly important food source to bivalves in the Chukchi Sea as well.

Results from this study indicate that phytoplankton and terrestrial sources were the most important sources of EAAs to the invertebrate samples from Hanna Shoal and the Chukchi Sea. Bacterial and macro-algal sources made high contributions to invertebrates in the Beaufort Sea, and bacterial sources seemed to make a higher proportional contribution to species with increasing water depth. Bacteria were also the second most important source to the invertebrates investigated from Hanna Shoal and the Chukchi Sea, so it is clear that bacteria are certainly an important contributor of EAAs throughout the entire region. Mixing model estimates of individual invertebrate samples showed that terrestrial organic matter contributed a substantial dietary proportion to some individuals in the Hanna Shoal and the Chukchi Sea, particularly in *Macoma* spp.

Paired shell and muscle samples from *Macoma calcarea* had similar EAA carbon stable isotope fingerprints, with differences in some amino acids that may reflect the different time frames recorded in the two tissue types. Mixing models run with the muscle and shell values revealed phytoplankton to be the highest contributing source of EAAs to both 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.

Acknowledgments

This study was funded through the Coastal Marine Institute/BOEM with cost-share funding from the University of Alaska Fairbanks. We thank Kenneth Dunton (Texas A&M) for contributing samples from Hanna Shoal, Martina Uradnikova and Renate Døving Osvik (UiT Norway's Arctic University) for supplying us with phytoplankton cultures, and Arny Blanchard (UAF) for assistance with statistics. We also thank Ann-Christine Zinkann and Kyungcheol Choy (UAF) for preparing and analyzing samples from the Chukchi Sea and Beaufort Sea regions, respectively, and Kate Wedemeyer and Warren Horowitz (BOEM) for valuable input.

Study Products

Audrey Rowe, Arny Blanchard, Katrin Iken, Diane O'Brien, Martina Uradnikova, Renate Døving Osvik, Matthew Wooller (In Press). Estimates of primary production sources to Arctic bivalves using amino acid stable carbon isotope fingerprinting. Stable Isotopes in Environmental and Health Studies.

Ann-Christine Zinkann, Katrin Iken, Diane O'Brien, Matthew Wooller (2019). Digging Deep: Depth distribution and utilization of carbon sources in the Chukchi Sea sediments. Oral presentation, Benthic Ecology Conference, Newfoundland, Canada.

Ann-Christine Zinkann, Katrin Iken, Diane, O'Brien, Matthew Wooller (2019). Digging Deep: Depth distribution and utilization of carbon sources in the Chukchi Sea sediments. Oral presentation, Alaska Marine Science Symposium, Anchorage, Alaska.

Matthew Wooller, Katrin Iken, Audrey Rowe, Arny Blanchard, Diane O'Brien (2019). Identifying sources of organic matter to benthic organisms in the Beaufort. Poster presentation, Alaska Marine Science Symposium, Anchorage, Alaska.

Matthew Wooller, Katrin Iken, Audrey Rowe, Arny Blanchard, Diane O'Brien (2019). Identifying sources of organic matter to benthic organisms in the Beaufort. Oral presentation, CMI Annual Research Review, Anchorage, Alaska.

Audrey Rowe, Arny Blanchard, Katrin Iken, Diane O'Brien, Matthew Wooller (2018). Developing stable isotope fingerprinting of bivalve shells to detect long-term changes in organic matter sources into the Arctic marine ecosystem. Poster presentation, Alaska Marine Science Symposium, Anchorage, Alaska.

Matthew Wooller, Katrin Iken, Audrey Rowe, Arny Blanchard, Diane O'Brien (2018). Identifying sources of organic matter to benthic organisms in the Beaufort. Oral presentation, CMI Annual Research Review, Anchorage, Alaska.

Audrey Rowe, Arny Blanchard, Katrin Iken, Diane O'Brien, Martina Uradnikova, Renate Døving Osvik, Matthew Wooller (2018). Stable carbon isotope amino acid fingerprinting of shells from two Arctic clam genera to track primary production sources. Poster presentation, ISOECOL, Viña Del Mar, Chile.

Audrey Rowe (2018). Estimates of primary production sources to Arctic bivalves using amino acid stable carbon isotope fingerprinting. MSc Thesis, College of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Fairbanks, Alaska.

Matthew Wooller, Arny Blanchard, Ann Christine Zinkann, Kyungcheol Choy, Katrin Iken, Diane O'Brien, Audrey Rowe (2018). Determining primary production sources to benthic organisms in the Arctic using stable isotope fingerprinting. Oral presentation, Alaska Marine Science Symposium, Anchorage, Alaska.

Ann-Christine Zinkann, Katrin Iken, Diane O'Brien, Matthew Wooller (2018). Identifying the sources of amino acids to benthic invertebrates across the Chukchi Sea shelf using compound-specific stable isotope analyses. Poster presentation, Alaska Marine Science Symposium, Anchorage, Alaska.

Audrey Rowe, Katrin Iken, Diane O'Brien, Matthew Wooller (2017). Analyzing shifts in the base of the Arctic marine food web using amino acid stable isotope fingerprinting. Oral presentation, Water and Environmental Research Center, University Seminar Series, Fairbanks, Alaska.

Ann-Christine Zinkann, Katrin Iken, Diane O'Brien, Matthew Wooller (2018). Contribution of microbially-derived carbon to benthic invertebrates across the Chukchi Sea shelf using amino acid specific stable isotope analyses. Oral presentation, Ocean Science Meeting, Portland, Oregon.

References

Arrigo KR, Perovich DK, Pickart RS, et al (2014) Phytoplankton blooms beneath the sea ice in the Chukchi Sea. Deep Res Part II Top Stud Oceanogr. doi: 10.1016/j.dsr2.2014.03.018

Arrigo KR, van Dijken GL (2015) Continued increases in Arctic Ocean primary production. Prog Oceanogr. doi: 10.1016/j.pocean.2015.05.002

Babb DG, Galley RJ, Asplin MG, et al (2013) Multiyear sea ice export through the Bering Strait during winter 2011-2012. J Geophys Res Ocean. doi: 10.1002/jgrc.20383

Barrett SA, Stringer WJ (2006) Growth mechanisms of "Katie's Floeberg." Arct Alp Res. doi: 10.2307/1550744

Barrett SA, Stringer WJ (1978) Growth Mechanisms of "Katie's Floeberg." Arct Alp Res. doi: 10.1080/00040851.1978.12004015.

Bentzen TW, Follman EH, Amstrup SC, et al (2007) Variation in winter diet of southern Beaufort Sea polar bears inferred from stable isotope analysis. Can J Zool. doi: 10.1139/Z07-036

Bentzen TW, Shideler RT, O'Hara TM (2014) Use of stable isotope analysis to identify food-conditioned grizzly bears on Alaska's North Slope. Ursus. doi: 10.2192/ursus-d-13-00002.1

Budge SM, Wooller MJ, Springer AM, et al (2008) Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. Oecologia. doi: 10.1007/s00442-008-1053-7

Divine LM, Iken K, Bluhm BA (2015) Regional benthic food web structure on the Alaska Beaufort Sea shelf. Mar Ecol Prog Ser. doi: 10.3354/meps11340

Dunton KH, Grebmeier JM, Trefry JH (2017) Hanna Shoal: An integrative study of a High Arctic marine ecosystem in the Chukchi Sea. Deep Sea Res Part II Top Stud Oceanogr. doi: 10.1016/j.dsr2.2017.09.001

Dunton KH, Weingartner T, Carmack EC (2006) The nearshore western Beaufort Sea ecosystem: Circulation and importance of terrestrial carbon in arctic coastal food webs. Prog Oceanogr. doi: 10.1016/j.pocean.2006.09.011

Eicken H, Gradinger R, Gaylord A, et al (2005) Sediment transport by sea ice in the Chukchi and Beaufort Seas: Increasing importance due to changing ice conditions? Deep Res Part II Top Stud Oceanogr. doi: 10.1016/j.dsr2.2005.10.006

Feder HM, Iken K, Blanchard AL, et al (2011) Benthic food web structure in the southeastern Chukchi Sea: An assessment using $\delta^{13}C$ and $\delta^{15}N$ analyses. Polar Biol. doi: 10.1007/s00300-010-0906-9

Feder HM, Naidu AS, Jewett SC, et al (1994) The northeastern Chukchi Sea: Benthos-environmental interactions. Mar Ecol Prog Ser. doi: 10.3354/meps111171

Garneau MÈ, Vincent WF, Terrado R, Lovejoy C (2009) Importance of particle-associated bacterial heterotrophy in a coastal Arctic ecosystem. J Mar Syst. doi: 10.1016/j.jmarsys.2008.09.002

Graham C, Oxtoby L, Wang SW, et al (2014) Sourcing fatty acids to juvenile polar cod (*Boreogadus saida*) in the Beaufort Sea using compound-specific stable carbon isotope analyses. Polar Biol. doi: 10.1007/s00300-014-1470-5

Grebmeier J, Feder H, McRoy C (2007a) Pelagic-benthic coupling on the shelf of the northern Bering and Chukchi Seas. II. Benthic community structure. Mar Ecol Prog Ser. doi: 10.3354/meps051253

Grebmeier J, McRoy C, Feder H (2007b) Pelagic-benthic coupling on the shelf of the northern Bering and Chukchi Seas. I. Food supply source and benthic bio-mass. Mar Ecol Prog Ser. doi: 10.3354/meps048057

Iken K (2015) Arctic Marine Biodiversity Observing Network (AMBON) 2015 cruise report. http://ambon-us.org/wp-content/uploads/2015/09/AMBON-2015-cruise-report.pdf

Iken K, Bluhm B, Dunton K (2010) Benthic food-web structure under differing water mass properties in the southern Chukchi Sea. Deep Res Part II Top Stud Oceanogr. doi: 10.1016/j.dsr2.2009.08.007

Iken K, Bluhm BA, Gradinger R (2005) Food web structure in the high Arctic Canada Basin: Evidence from $\delta^{13}C$ and $\delta^{15}N$ analysis. Polar Biol. doi: 10.1007/s00300-004-0669-2

Kędra M, Moritz C, Choy ES, et al (2015) Status and trends in the structure of Arctic benthic food webs. Polar Res. doi: 10.3402/polar.v34.23775

Larsen T, Lee Taylor D, Leigh MB, O'Brien DM (2009) Stable isotope fingerprinting: A novel method for identifying plant, fungal, or bacterial origins of amino acids. Ecology. doi: 10.1890/08-1695.1

Larsen T, Ventura M, Andersen N, et al (2013) Tracing carbon sources through aquatic and terrestrial food webs using amino acid stable isotope fingerprinting. PLoS One. doi: 10.1371/journal.pone.0073441

Macdonald TA, Burd BJ, Macdonald VI, et al (2010) Taxonomic and Feeding Guild Classification for the Marine Benthic Macroinvertebrates of the Strait of Georgia, British Columbia Canadian Technical Report of Fisheries and Aquatic Sciences 2874. Can Tech Rep Fish Aquat Sci. doi: 10.1002/prot.20320

McClelland JW, Townsend-Small A, Holmes RM, et al (2014) River export of nutrients and organic matter from the North Slope of Alaska to the Beaufort Sea. Water Resour Res. doi: 10.1002/2013WR014722

McMahon KW, Ambrose WG, Johnson BJ, et al (2006) Benthic community response to ice algae and phytoplankton in Ny Ålesund, Svalbard. Mar Ecol Prog Ser. doi: 10.3354/meps310001

McMahon KW, Thorrold SR, Houghton LA, Berumen ML (2016) Tracing carbon flow through coral reef food webs using a compound-specific stable isotope approach. Oecologia. doi: 10.1007/s00442-015-3475-3

McMahon KW, Williams B, Guilderson TP, et al (2018) Calibrating amino acid $\delta^{13}C$ and $\delta^{15}N$ offsets between polyp and protein skeleton to develop proteinaceous deep-sea corals as paleoceanographic archives. Geochim Cosmochim Acta. doi: 10.1016/j.gca.2017.09.048

McTigue ND, Dunton KH (2017) Trophodynamics of the Hanna Shoal Ecosystem (Chukchi Sea, Alaska): Connecting multiple end-members to a rich food web. Deep Res Part II Top Stud Oceanogr. doi: 10.1016/j.dsr2.2017.08.010

McTigue ND, Dunton KH (2014) Trophodynamics and organic matter assimilation pathways in the northeast Chukchi Sea, Alaska. Deep Res Part II Top Stud Oceanogr. doi: 10.1016/j.dsr2.2013.07.016

Misarti N, Gier E, Finney B, et al (2017) Compound-specific amino acid $\delta^{15}N$ values in archaeological shell: Assessing diagenetic integrity and potential for isotopic baseline reconstruction. Rapid Commun Mass Spectrom. doi: 10.1002/rcm.7963

Moss DK, Surge D, Khaitov V (2018) Lifespan and growth of *Astarte borealis* (Bivalvia) from Kandalaksha Gulf, White Sea, Russia. Polar Biol. doi: 10.1007/s00300-018-2290-9

O'Brien DM, Fogel ML, Boggs CL (2002) Renewable and nonrenewable resources: Amino acid turnover and allocation to reproduction in Lepidoptera. Proc Natl Acad Sci. doi: 10.1073/pnas.072346699

Oxtoby LE, Budge SM, Iken K, et al (2016) Feeding ecologies of key bivalve and polychaete species in the Bering Sea as elucidated by fatty acid and compound-specific stable isotope analyses. Mar Ecol Prog Ser. doi: 10.3354/meps11863

Parnell AC, Phillips DL, Bearhop S, et al (2013) Bayesian stable isotope mixing models. Environmetrics. doi: 10.1002/env.2221

Piepenburg D (2005) Recent research on Arctic benthos: Common notions need to be revised. Polar Biol. doi: 10.1007/s00300-005-0013-5

Rowe A, Blanchard A, Iken K, O'Brien D, Uradnikova M, Osvik RD, Wooller MJ (in press). Estimates of primary production sources to Arctic bivalves using amino acid stable carbon isotope fingerprinting. Stable Isotopes in Environmental and Health Studies.

Savory, GA, Hunter, CM, O'Brien DM (2014) Anthropogenic food use and diet overlap between red foxes (*Vulpes vulpes*). Can J Zool. doi: 10.1139/cjz-2013-0283

Schell DM (1983) Carbon-13 and carbon-14 abundances in Alaskan aquatic organisms: Delayed production from peat in arctic food webs. Science. doi: 10.1126/science.219.4588.106

Seymour J, Horstmann-Dehn L, Wooller MJ (2014a) Proportion of higher-trophic-level prey in the diet of Pacific walruses (*Odobenus rosmarus divergens*). Polar Biol. doi: 10.1007/s00300-014-1492-z

Seymour J, Horstmann-Dehn L, Wooller MJ (2014b) Inter-annual variability in the proportional contribution of higher trophic levels to the diet of Pacific walruses. Polar Biol. doi: 10.1007/s00300-014-1460-7

Tu KL, Blanchard AL, Iken K, Horstmann-Dehn L (2015) Small-scale spatial variability in benthic food webs in the northeastern Chukchi Sea. Mar Ecol Prog Ser. doi: 10.3354/meps11216

Wang SW, Budge SM, Iken K, et al (2015) Importance of sympagic production to Bering Sea zooplankton as revealed from fatty acid-carbon stable isotope analyses. Mar Ecol Prog Ser. doi: 10.3354/meps11076

Wang SW, Springer AM, Budge SM, et al (2016) Carbon sources and trophic relationships of ice seals during recent environmental shifts in the Bering Sea. Ecol Appl. doi: 10.1890/14.2421/suppinfo

Young JK, Black BA, Clarke JT, et al (2017) Abundance, biomass and caloric content of Chukchi Sea bivalves and association with Pacific walrus (*Odobenus rosmarus divergens*) relative density and distribution in the northeastern Chukchi Sea. Deep Res Part II Top Stud Oceanogr. doi: 10.1016/j.dsr2.2017.04.017

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