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Sulfur isotopes ($\delta^{34}S$) in Arctic marine mammals: indicators of benthic vs. pelagic foraging

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ABSTRACT: Consumer tissue stable carbon isotope compositions ($\delta^{13}C$) are well established indicators of benthic and pelagic foraging in marine ecosystems. Stable sulfur isotope compositions ($\delta^{34}S$) are also potentially useful in this regard but have not been widely utilized outside of estuaries and salt marsh ecosystems. To test the ability of $\delta^{13}C$ and $\delta^{34}S$ to reflect benthic and pelagic foraging, we analyzed the stable carbon, nitrogen ($\delta^{15}N$), and sulfur isotope compositions of bone collagen from walrus (an obligate benthic feeder) and ringed seal (a mixed benthic/pelagic feeder) sampled from across the North American Arctic. Both had relatively low $\delta^{34}S$ values compared to those typically observed in marine consumers. These data suggest an important role for benthic microalgae in coastal marine food webs in the Arctic. At all of the 10 locations where both taxa could be sampled, walrus had lower $\delta^{34}S$ values than ringed seal, suggesting that this measurement is a useful indicator of benthic and pelagic foraging in nearshore Arctic environments. Contrary to expectations, there were no consistent differences in $\delta^{13}C$ between walrus and ringed seal at any of these sites, suggesting that this measurement may not always be best interpreted in light of benthic vs. pelagic foraging, particularly when comparisons are made across trophic levels. When the foraging ecology of a consumer is unknown, our data suggest that $\delta^{34}S$ may be a more sensitive indicator of the relative importance of benthic and pelagic prey in the diet than $\delta^{13}C$.

KEY WORDS: Sulfur isotopes · Carbon isotopes · Benthic · Pelagic · Arctic · Stable isotopes

1. INTRODUCTION

One of the most frequently cited principles governing the isotopic compositions of marine consumers is that animals foraging in benthic habitats tend to have higher tissue stable carbon isotope compositions ($\delta^{13}C$) than animals foraging in pelagic habitats (McMahon et al. 2013). These benthic–pelagic gradients tend to occur for a number of reasons related to nutrients and primary producers, rather than due to processes at the level of the consumers themselves. Benthic environments may see higher inputs of organic matter from macrophyte algae, which tend to have higher $\delta^{13}C$ values than pelagic phytoplankton. Macroalgae tend to be exposed to less water turbulence than pelagic phytoplankton, which causes the boundary layer (a stagnant layer of water) of cells to be thicker, and more prone to becoming progressively enriched in $^{13}CO_2$ (France 1995). Benthic microalgae and macroalgae may also rely on bicarbonate rather than CO_2 to a greater extent than pelagic phytoplankton (Raven et al. 2002); the $\delta^{13}C$ values of bicarbonate are 9‰ higher than those of CO_2 (Mook et al. 1974). These differences in primary producers are reflected in the tissues of higher trophic level consumers. In Alaska, for example, gray whales *Eschrichtius robustus*, which feed primarily on benthic mysids, were found to have $\delta^{13}C$ values

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*Corresponding author: paulszpak@trentu.ca that were approximately 4‰ higher than bowhead whales *Balaena mysticetus*, which feed primarily on pelagic zooplankton (Dehn et al. 2006). This pattern has been observed for other marine taxa (Hobson et al. 2002a, Tucker et al. 2013, Kopp et al. 2015) and these benthic–pelagic gradients have been invoked to interpret the foraging ecology of historic and ancient marine predators (Drago et al. 2009b, 2017, Skovrind et al. 2019).

Among marine mammals, walrus *Odobenus rosmarus* are among the most specialized benthic feeders, consuming primarily bivalves and other benthic invertebrates (Fay 1982). A limited range of benthic bivalves (*Mya* sp., *Serripes* sp., and *Hiatella* sp.) generally comprise >80% of the diet (Born et al. 2003) and walrus should therefore possess relatively high tissue $\delta^{13}C$ values in comparison to co-occurring pinnipeds and other pelagic consumers. In 3 studies of the $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen from Arctic marine mammals, this pattern was not observed, with walrus possessing very similar or lower $\delta^{13}C$ values than conspecific pinnipeds (ringed seal *Pusa hispida*, bearded seal *Erignathus barbatus*, and spotted seal *Phoca largha*) (Coltrain et al. 2004, Jaouen et al. 2016, Szpak et al. 2018). All of these other pinnipeds have diets with smaller contributions of benthic prey relative to walrus (Dehn et al. 2007). It is, therefore, surprising that walrus would not possess higher $\delta^{13}C$ values than these other pinnipeds. The expected pattern of higher $\delta^{13}C$ values in walrus relative to ringed seals has been observed in Barrow, Alaska (Dehn et al. 2007).

Sulfur isotopic compositions ($\delta^{34}S$) are a potentially useful marker for the importance of benthic productivity in marine ecosystems (Fry & Sherr 1989). There is little discrimination against ^{34}S associated with the uptake and assimilation of sulfur by producers (Canfield 2001). Like $\delta^{13}C$, $\delta^{34}S$ values vary minimally according to trophic level and therefore better reflect isotopic compositions at the base of the food web (Fry 1988, Nehlich 2015). For bone collagen specifically, Krajcarz et al. (2019) found no difference between the $\delta^{34}S$ values of bone collagen from predators (red fox *Vulpes vulpes*) and prey from a natural setting, suggesting that trophic level discrimination is negligible for ^{34}S, but additional studies are needed. Marine sulfate has a homogenous $\delta^{34}S$ value of approximately +20.3‰ globally and in the Arctic specifically (Rempillo et al. 2011). Marine consumers should therefore have $\delta^{34}S$ values close to +20‰, with values between +15 and +20‰ being commonly observed (Fry 1988, Thomas & Cahoon 1993). When sulfate is reduced to sulfide, the bacteria involved discriminate strongly against ^{34}S, with the resulting sulfide having a $\delta^{34}S$ value much lower (by 20 to 70‰) than the initial sulfate (Brunner & Bernasconi 2005). Sulfates produced from the reoxidation of these sulfides have $\delta^{34}S$ values that are lower than the overlying water column sulfates (Connolly et al. 2004). Consumers that inhabit benthic habitats where such processes occur tend to have lower $\delta^{34}S$ values than pelagic marine consumers (Thomas & Cahoon 1993). In estuarine and seagrass ecosystems, consumers may have $\delta^{34}S$ values <10‰ because of a significant contribution of organic matter derived from rooted plants utilizing sulfides or terrestrial organic matter with lower $\delta^{34}S$ values (Fry & Chumchal 2011). Accordingly, $\delta^{34}S$ values may decrease in consumers along an inshore–offshore gradient (Barros et al. 2010, Nelson et al. 2012). Relative to $\delta^{13}C$ and $\delta^{15}N$, $\delta^{34}S$ measurements are relatively rare, particularly for ancient organisms because bone collagen, the primary analytical substrate, contains only approximately 0.2 wt% S in mammals (Nehlich & Richards 2009). In this study, we sought to characterize the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of Arctic marine mammal bone collagen in an effort to assess if any of these isotope systems displayed systematic variation according to benthic vs. pelagic foraging by focusing on walrus (a benthic-feeding specialist) and ringed seal (a more pelagic-feeding taxon).

2. MATERIALS AND METHODS

2.1. Materials

Bones (n = 159) were sampled from 26 archaeological sites spanning from northwestern Alaska to northwestern Greenland. These 26 sites were grouped into 13 regions, since many are located in close proximity to one another (Fig. 1). The materials sampled are derived exclusively from Late Holocene deposits spanning the last 3000 yr, with the vast majority of the material dating to between 500 and 700 calibrated years BP (Table 1). Ringed seal was selected as the main comparator for walrus because this is the only taxon that is well represented at all sites across the Arctic. Additionally, ringed seals have a more pelagic diet, with Arctic cod *Boreogadus saida* representing the most important prey species in most cases (Born et al. 2004). A limited number of bearded seal and little auk *Alle alle* were also sampled, but since these species are relatively rare at most sites, they could not be included in the broad geographic comparisons. Ringed seal is represented

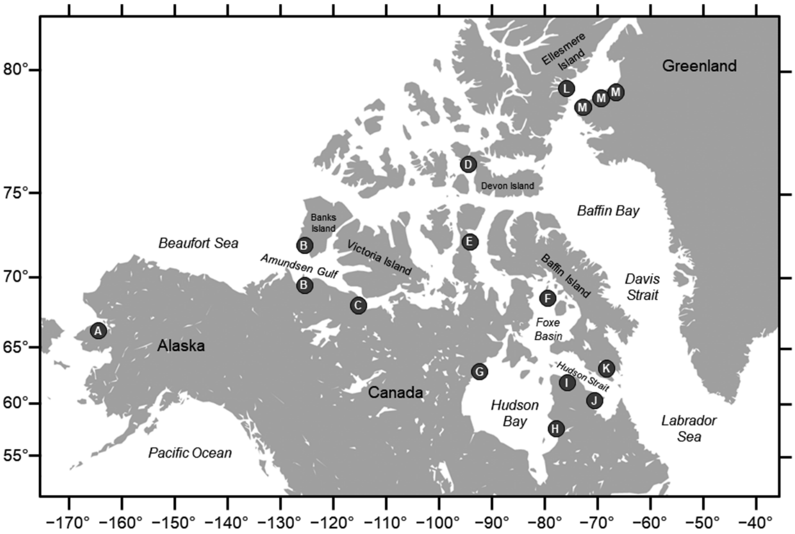

Fig. 1. Study area. Letters correspond to the 13 regions outlined in Table 1

in all 13 of the regions shown on Fig. 1, while walrus is represented in only 9 of these, since they have a more limited distribution (Table 1). The δ^{13}C and δ^{15}N values for ringed seals and a single walrus from some of the sites have been previously published and these 34 samples are indicated in Table S1 in Supplement 1 (www.int-res.com/articles/suppl/m653p205_supp1.xls). The δ^{13}C and δ^{15}N values were determined for all samples before they were selected for δ^{34}S analysis because the possibility exists that walrus may consume seals under some circumstances (Lowry & Fay 1984). We avoided any walrus samples that had high δ^{15}N values (>+15‰) to ensure that the walruses that were included had diets dominated by benthic bivalves and to avoid juvenile individuals; 4 walrus specimens were excluded based on this criterion. Based on epiphyseal fusion and size, the individuals that were sampled represented adults, a strategy meant to avoid the potential complications of the suckling effect on δ^{15}N and possibly on δ^{13}C values (Newsome et al. 2006).

Table 1. Summary of samples selected. Parenthetical letters beside the region indicate their position on Fig. 1

Site	Region	Approximate date (calibrated years BP)	Walrus	Ringed seal	Bearded seal	Little auk
KTZ-304	NW Alaska (A)	650–850[1]	2	2	1	
KTZ-087	NW Alaska (A)	350–550[1]	2	3		
KTZ-088	NW Alaska (A)	100–150[1]		3		
NkRi-3	Amundsen Gulf (B)	650–750[1]		3		
OjRl-3	Amundsen Gulf (B)	2150–2750[1]		3		
OkRn-1	Amundsen Gulf (B)	300–500[1]		3		
OlRr-1	Amundsen Gulf (B)	650–750[1]		1		
NaPi-2	Coronation Gulf (C)	550–650[2]		2		
NcPf-1	Coronation Gulf (C)	550–650[2]		3		
NcPf-12	Coronation Gulf (C)	550–650[2]		3		
RbJr-1	Devon Island (D)	500–650[1]	7	8		
PcJq-5	Somerset Island (E)	550–650[1]	2			
PeJr-1	Somerset Island (E)	550–650[1]	1	3		
NgFv-9	Foxe Basin (F)	500–600[1]	1	2		
KkJg-1	NW Hudson Bay (G)	325–500[1]	7	7		
IbGk-3	Eastern Hudson Bay (H)	0–650[1]		3		
KbFk-7	Nunavik (I)	1900–2100[1]	5	5		
KcFs-2	Nunavik (I)	450–1450[1]	1	3		
JfEl-4	Hudson Strait (J)	550–700[2]	4	3		
KkDo-1	SW Baffin Island (K)	650–150[1]	2	3		
SfFk-4	Ellesmere Island (L)	550–650[2]	8	9		
Iita	NW Greenland (M)	850–20[1]	5			5
Cape Grinnell	NW Greenland (M)	550–650[1]	4	4	1	
Qaqaitsuit	NW Greenland (M)	1300–1600[1]	2	5	1	

[1] Based on previously obtained radiocarbon dates, detailed in Table S12 in Supplement 2

[2] Based on new AMS radiocarbon dates reported in this paper (see Table S11)

2.2. Methods

2.2.1. Stable isotope analysis

The exterior surface of the bone was abraded using an NSK dental drill with a diamond-tipped cutting wheel. Chunks of bone weighing approximately 200 mg were cut away from the cleaned section using the dental drill and demineralized in 0.5 M HCl at 4°C for several days. The samples were then rinsed to neutrality with Type I water (resistivity >18.2 MΩ cm) and treated with 0.1 M NaOH for successive 20 min treatments until there was no color change in the solution. Samples were again rinsed with Type I water to neutrality and then 8 ml of 0.01 M HCl was added and the collagen was solubilized at 75°C for 48 h. The solution containing the collagen was filtered using a 5–8 µm filter to remove sediments and other insoluble residues, and then filtered using a 30 kDa molecular weight cut-off ultrafilter to remove low molecular weight compounds (Brown et al. 1988). The >30 kDa fraction was freeze-dried, and the collagen yield (an indicator of collagen preservation; Ambrose 1990) was calculated.

Carbon and nitrogen isotopic and elemental compositions were determined using an IsoPrime continuous flow isotope-ratio mass spectrometer (CF-IRMS) coupled to a Vario Micro elemental analyzer. Carbon and nitrogen isotopic compositions were calibrated relative to the Vienna PeeDee Belemnite (VPDB) and AIR scales, respectively, using a 2-point calibration anchored by 2 glutamic acid standards, USGS40 (accepted δ^{13}C = −26.39 ± 0.04‰, δ^{15}N = −4.52 ± 0.06‰) and USGS41 (accepted δ^{13}C = +37.63 ± 0.05‰, δ^{15}N = +47.57 ± 0.11‰). All of the samples were analyzed at least in duplicate. Accuracy and precision were monitored using the following isotopic reference materials: methionine (δ^{13}C = −28.61 ± 0.10‰, δ^{15}N = −5.04 ± 0.13‰), NIST-1577c (bovine liver; δ^{13}C = −17.52 ± 0.09‰, δ^{15}N = +8.15 ± 0.14‰), SRM-1 (caribou bone collagen; δ^{13}C = −19.40 ± 0.08‰, δ^{15}N = +1.82 ± 0.11‰), SRM-2 (walrus bone collagen; δ^{13}C = −14.72 ± 0.14‰, δ^{15}N = +15.59 ± 0.13‰), USGS42 (human hair; δ^{13}C = −21.09 ± 0.10‰, δ^{15}N = +8.05 ± 0.10‰), and USGS43 (human hair; δ^{13}C = −21.28 ± 0.10‰, δ^{15}N = +8.44 ± 0.10‰). Standard uncertainty was determined to be ±0.19‰ for δ^{13}C and ±0.24‰ for δ^{15}N (Szpak et al. 2017). Additional details on measurement calibration and analytical uncertainty are provided in Supplement 2 (see Tables S2, S3, & S4 at www.int-res.com/articles/suppl/m653p205_supp2.pdf).

Sulfur elemental and isotopic compositions were determined with a Europa ANCA-SL/20-20 elemental analyzer/isotope-ratio mass spectrometer. Sulfur isotopic compositions were calibrated relative to Vienna Canyon Diablo Troilite (VCDT), using IA-R0611 (Ba_2SO_4; δ^{34}S = +20.3‰) and IAEA-SO-5 (Ba_2SO_4; δ^{34}S = +0.5‰). Accuracy and precision were monitored using the following isotopic reference materials: IA-R068 (soy protein; δ^{34}S = +5.3 ± 0.2‰) and IA-R069 (tuna protein; δ^{34}S = +18.9 ± 0.2‰). A total of 62 samples were analyzed in duplicate. Standard uncertainty was determined to be ±0.4‰ for δ^{34}S (Szpak et al. 2017). Additional details on measurement calibration and analytical uncertainty are provided in Supplement 2 (Tables S5, S6, & S7).

2.2.2. Peptide mass fingerprinting

For confirmation of species identification on individual samples with uncertain morphological identifications (e.g. seal identified only as Phocidae or bearded seal with an unusually low δ^{15}N value), we carried out collagen peptide mass fingerprinting, also known as Zooarchaeology by Mass Spectrometry (ZooMS; Buckley et al. 2009). This involved resuspending approximately 1 mg lyophilized collagen for each sample with 50 mM ammonium bicarbonate (ABC) and enzymatically digesting with 0.4 µg sequencing grade trypsin (Promega) at 37°C overnight (~18 h). The peptide mixtures were then diluted and 1 µl was spotted onto a stainless steel Matrix Assisted Laser Desorption Ionization (MALDI) target plate and subsequently co-crystalized with 1 µl α-cyano hydroxycinnamic acid matrix. These were then analyzed using a Bruker Ultraflex II MALDI time-of-flight mass spectrometer with up to 2000 laser acquisitions, and the fingerprints compared with previously published marine mammal collagen species biomarkers (Buckley et al. 2014). Samples initially yielding poor spectra were further purified by C18 resin ZipTips (Varian), following the manufacturer's protocol, into 50% acetonitrile (ACN)/0.1% trifluoroacetic acid (TFA) and evaporated by centrifugal evaporator prior to being resuspended with 10 µl 0.1% TFA and then an aliquot subjected to the above procedure.

2.2.3. Data treatment

Statistical comparisons of isotopic compositions between species involved a 2-way analysis of variance (ANOVA) with species and regions as factors.

Only those regions with both ringed seal and walrus, each having at least 3 specimens, were included in this comparison. Comparisons of ringed seal and walrus within regions involved a Shapiro-Wilks test to assess normality, followed by either a Student's *t*-test (normally distributed data with equal variances), a Welch's *t*-test (normally distributed data with unequal variances), or a Mann-Whitney *U*-test (non-normally distributed data). Comparisons among multiple taxa in NW Greenland were made with a 1-way ANOVA followed by a post hoc Tukey's HSD test. Correlations between isotopic measurements were assessed using Pearson's correlation coefficient. Summary statistics present arithmetic means and standard deviations unless otherwise noted. Statistical tests were performed using PAST 3.20 (Hammer et al. 2001).

3. RESULTS

Overall, the δ^{34}S values of walrus bone collagen (+14.0 ± 1.0‰, n = 52) were lower than ringed seal bone collagen (+14.8 ± 0.8‰, n = 58); this difference was statistically significant (2-way ANOVA; $F = 44.84$, $p < 0.001$). In the 2-way ANOVA, a greater proportion of the variance was explained by species (partial $\eta^2 = 0.39$) than by region (partial $\eta^2 = 0.32$). In the 8 regions for which there were a sufficient number of walrus and ringed seal samples to make a comparison ($n \geq 3$ for both taxa), walrus had lower mean δ^{34}S values than ringed seal in every case (Fig. 2) and this difference was statistically significant for 4 of the 8 comparisons (Fig. 2; Table S8 in Supplement 2).

The δ^{13}C values of walrus (−14.38 ± 0.71‰, n = 52) and ringed seal (−14.00 ± 0.72‰, n = 58) were significantly different from one another (2-way ANOVA; $F = 11.23$, $p = 0.001$). In the 2-way ANOVA, a greater proportion of the variance was explained by region (partial $\eta^2 = 0.50$) than by species (partial $\eta^2 = 0.11$). At the level of individual regions, walrus had lower δ^{13}C values in 7 of the 8 comparisons (Northern Nunavik was the lone exception) (Fig. 3), but only 2 regions (Ellesmere Island and Devon Island) were characterized by sig-

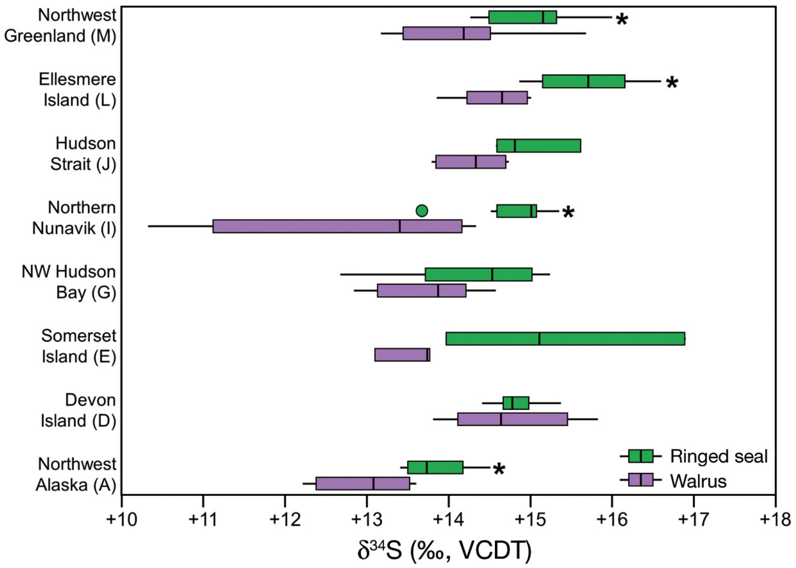

Fig. 2. Comparison of δ^{34}S values for walrus and ringed seal among the 8 regions for which a sufficient number of samples of both taxa were available. *Regions where δ^{34}S was significantly different between walrus and ringed seal (additional details are provided in the Supplement). Boxes represent the interquartile (Q1–Q3) range and vertical lines represent the medians. Whiskers represent maximum and minimum values, excluding outliers, which are presented as filled circles. VCDT: Vienna Canyon Diablo Trolite

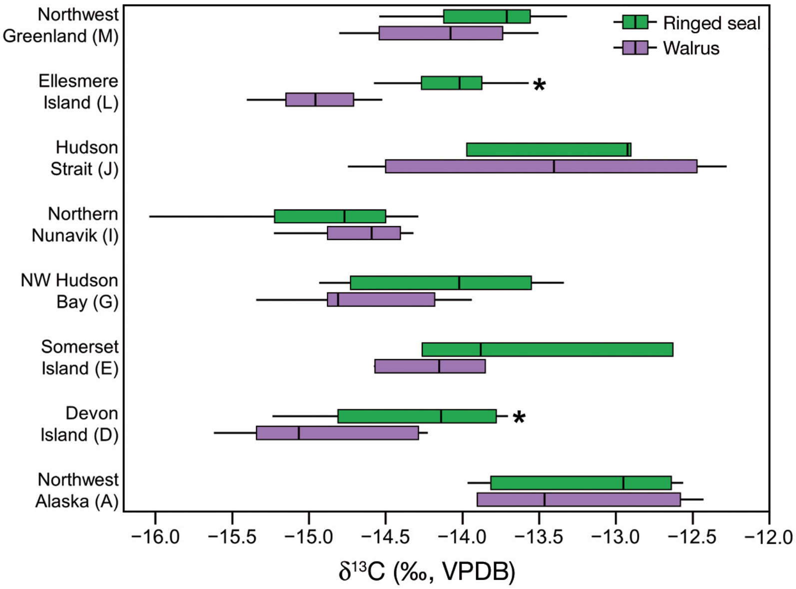

Fig. 3. Comparison of δ^{13}C values for walrus and ringed seal among the 8 regions for which a sufficient number of samples of both taxa were available. *Regions where δ^{13}C was significantly different between walrus and ringed seal (additional details are provided in the Supplement). VPDB: Vienna PeeDee Belemnite. Boxes and whiskers are defined as in Fig. 2

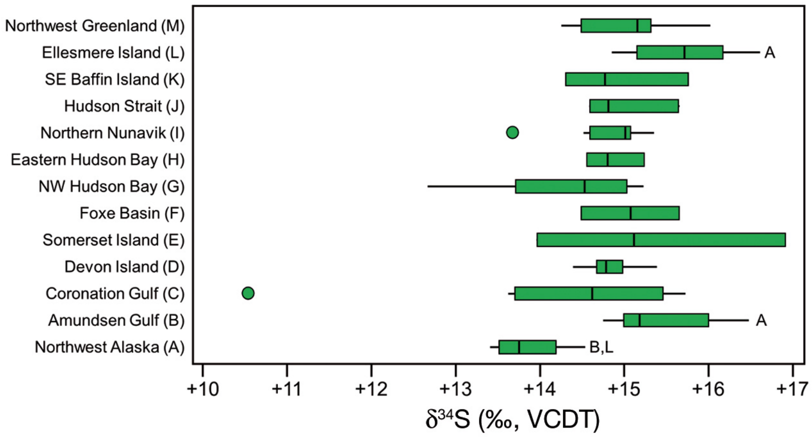

Fig. 4. Comparison of δ^{34}S values for ringed seal among all of the regions. Letters beside the boxplots indicate the region or regions from which that region's ringed seals possess significantly different δ^{34}S values. Boxes and whiskers are defined as in Fig. 2

nificant differences between the 2 taxa (Table S9 in Supplement 2).

For δ^{15}N, walrus (+11.81 ± 1.39‰, n = 52) had significantly lower values than ringed seal (+17.14 ± 1.38‰, n = 58) (2-way ANOVA; $F = 459.89$, $p < 0.001$). In the 2-way ANOVA, a greater proportion of the variance was explained by species (partial $\eta^2 = 0.83$) than by region (partial $\eta^2 = 0.38$). Walrus had significantly lower δ^{15}N values in all of the 8 regions for which this comparison could be made (Table S10 in Supplement 2). The isotopic and elemental compositions are presented in Table S1 for all of the samples analyzed in this study.

Among all of the regions, ringed seal δ^{34}S values were homogenous, with means for individual regions between +13.8‰ (NW Alaska) and +15.7‰ (Ellesmere Island) (Fig. 4). Ringed seals from NW Alaska were characterized by relatively low δ^{34}S values relative to the other sites, although they were only significantly lower than Amundsen Gulf (B) and Ellesmere Island (L) (Fig. 4).

Of the 4 taxa analyzed from NW Greenland (Fig. 5), walrus had the lowest δ^{34}S values, significantly lower than both ringed seal (p = 0.005) and little auk (p < 0.001). The little auks from NW Greenland had, on average, the highest δ^{34}S values (+15.9 ± 0.4‰, n = 5) of any of the taxa from any of the regions. In NW Greenland, the 2 bearded seals had δ^{34}S values that were intermediate between those of walrus and ringed seals. The δ^{13}C values of little auks (−17.44 ± 0.29‰, n = 5) were significantly lower than both ringed seal (−13.84 ± 0.38‰, n = 12; p < 0.001) and walrus (−14.15 ± 0.43‰, n = 13; p = 0.001). Walrus and ringed seal δ^{13}C values were not significantly different from one another (p = 0.23). The 2 bearded seals had the highest δ^{13}C values of any of the taxa sampled (Fig. 5).

4. DISCUSSION

The δ^{34}S values for walrus were quite low for marine consumers, with the mean for all samples being +14.0 ± 1.0‰ (range +10.3 to +15.8‰). Ringed seals also had relatively low δ^{34}S values across all sites (Fig. 4), with a mean of +14.9 ± 0.9‰ (range +10.5 to +16.9‰). Typically, marine consumers possess δ^{34}S values in the range of +15 to +20‰ (muscle, egg, scale, otolith, whole organism) approaching those of marine sulfate (+20.3‰) (Fry 1988, Thomas & Cahoon 1993, Godbout et al. 2010). The only published δ^{34}S values for Arctic marine mammals that we are aware of are for beluga whale (*Delphinapterus leucas*) muscle from Hudson Bay, which had δ^{34}S values consistently between +17 and +18‰ (Kelley 2014). Typical 'mar-

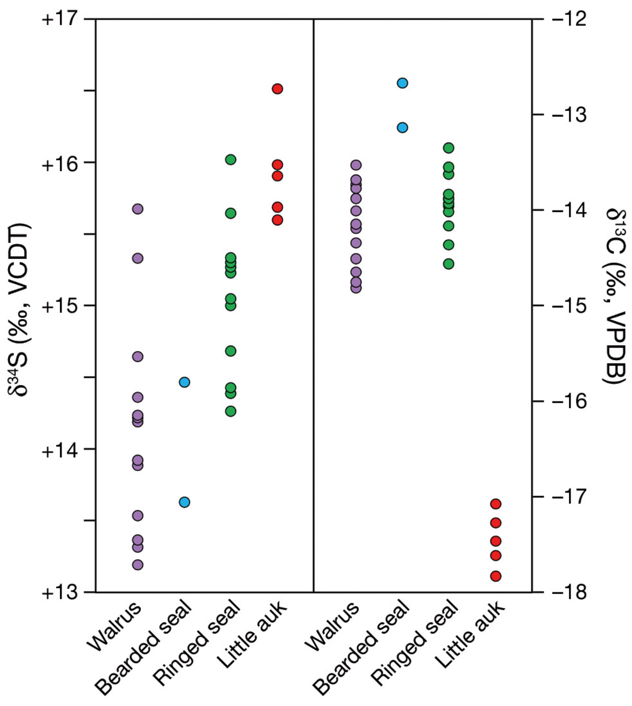

Fig. 5. Comparison of δ^{34}S and δ^{13}C values for all of the taxa sampled from NW Greenland (M)

ine′ δ^{34}S values were observed for little auks, a seabird that feeds predominantly on pelagic calanoid copepods (Hobson et al. 2002b) (Fig. 5). The low δ^{34}S values observed in Arctic marine mammals in this study therefore merit further discussion.

One reason that marine consumer δ^{34}S values may be relatively low is due to a high input of freshwater or terrestrial organic matter delivered by rivers or coastal erosion; these organic matter sources typically have lower δ^{34}S values than marine sulfates (Godbout et al. 2010, Fry & Chumchal 2011). In the North American Arctic, the input of terrestrial organic matter through erosion and freshwater organic matter through riverine discharge are relatively small compared to the European Arctic (Polyak et al. 2002, Macdonald et al. 2015). The Mackenzie River is an exception, depositing substantial amounts of organic matter into the Beaufort Sea (annual discharge of 307 km^3 freshwater); it is by far the largest source of freshwater in the region, delivering more sediment than all other North American Arctic rivers combined (McClelland et al. 2006). None of the sites from which samples were taken are located in close proximity to the Mackenzie River (the closest lies 500 km to the east). One site (KkJg-1) is located near Chesterfield Inlet (NW Hudson Bay), which has an annual discharge of 49 km^3 of water, but the walrus and ringed seal from this site do not possess especially low δ^{34}S values relative to the other regions (Fig. 2). Taking both ringed seal and walrus into account, the lowest δ^{34}S values occur in Kotzebue Sound (NW Alaska) where freshwater discharge comes from the Kobuk and Noatak Rivers and amounts to only 8.9 and 14.8 km^3 annually. Additionally, freshwater and terrestrial organic matter have lower δ^{13}C values than marine organic matter and this will be reflected in consumers at higher trophic levels (Simenstad & Wissmar 1985). The δ^{13}C values observed in this study, however, are not particularly low for bone collagen in marine consumers (Coltrain et al. 2004, Jaouen et al. 2016, Dyke et al. 2019, Skovrind et al. 2019). If high inputs of freshwater or terrestrial organic matter with low δ^{34}S values were responsible for the patterns observed in this study, we would expect there to be a positive correlation between consumer δ^{13}C and δ^{34}S values. This pattern was absent for all but 1 location and there was a negative, weak correlation between δ^{13}C and δ^{34}S for ringed seals at all other locations (Fig. 6). An alternative explanation for the overall low δ^{34}S values in the Arctic marine mammals analyzed in this study relates to variable reliance on productivity derived from benthic microalgae.

In the Arctic, the potential importance of benthic microalgae has generally been assumed to be negligible (Welch et al. 1992), or of limited importance beyond small spatial scales in shallow waters (Leu et al. 2015). The role of benthic microalgae in Arctic nearshore food webs has been overlooked for 2 reasons. First, it is difficult to differentiate organic matter on sediment surfaces that is derived from *in situ* production from organic matter originating in the pelagic or sympagic (ice-associated) environment that is delivered to the benthos without being grazed (Cahoon 1999). Secondly, even if these benthic microalgae can be isolated, they may possess isotopic compositions that are indistinguishable from other sources of production (Oxtoby et al. 2013). In a number of studies (Evrard et al. 2012, Oakes & Eyre 2014, Oxtoby et al. 2016), benthic microalgae have been observed to contradict the widely observed trend of benthic algae having higher δ^{13}C values than pelagic algae (France 1995). Nonetheless, there is mounting evidence that in shallow (<30 m depth) marine environments in the Arctic, benthic microalgae can make substantial contributions to food webs (Glud et al. 2009). These contributions may even exceed those of pelagic phytoplankton in oligotrophic areas (Glud et al. 2009). Coastal waters in the Arctic are relatively nutrient-poor (Smith & Sakshaug 1990) and it is under these oligotrophic conditions that the productivity of benthic microalgae should be high (Glud et al. 2009). The abundance of bivalves such as *Mya truncata*, *Hiatella arctica*, and *Serripes groenlandicus* (the primary prey of walrus) is highest at depths <30 m (Welch et al. 1992) where benthic microalgae tends to be most abundant (Cahoon 1999). It is at these depths that walrus spend most of their time foraging (Born et al. 2003). Ringed seals also forage primarily in waters <100 m deep, but unlike walrus, spend a much greater proportion of their time in the upper 50 m of water, rather than at or near the bottom (Born et al. 2004). These differences in foraging habitats between walrus and ringed seals are consistent with the observed variation in δ^{34}S between the 2 species if benthic microalgae are a more important source of production in the prey of walrus relative to ringed seals.

The relatively low δ^{34}S values observed in ringed seal and walrus in this study are consistent with the notion that benthic microalgae must make appreciable contributions to the primary production in shallow coastal food webs in the Arctic. The δ^{34}S values of marine sulfate, pelagic phytoplankton, and macroalgae are fairly homogenous, but the δ^{34}S values of benthic microalgae are highly variable depending on

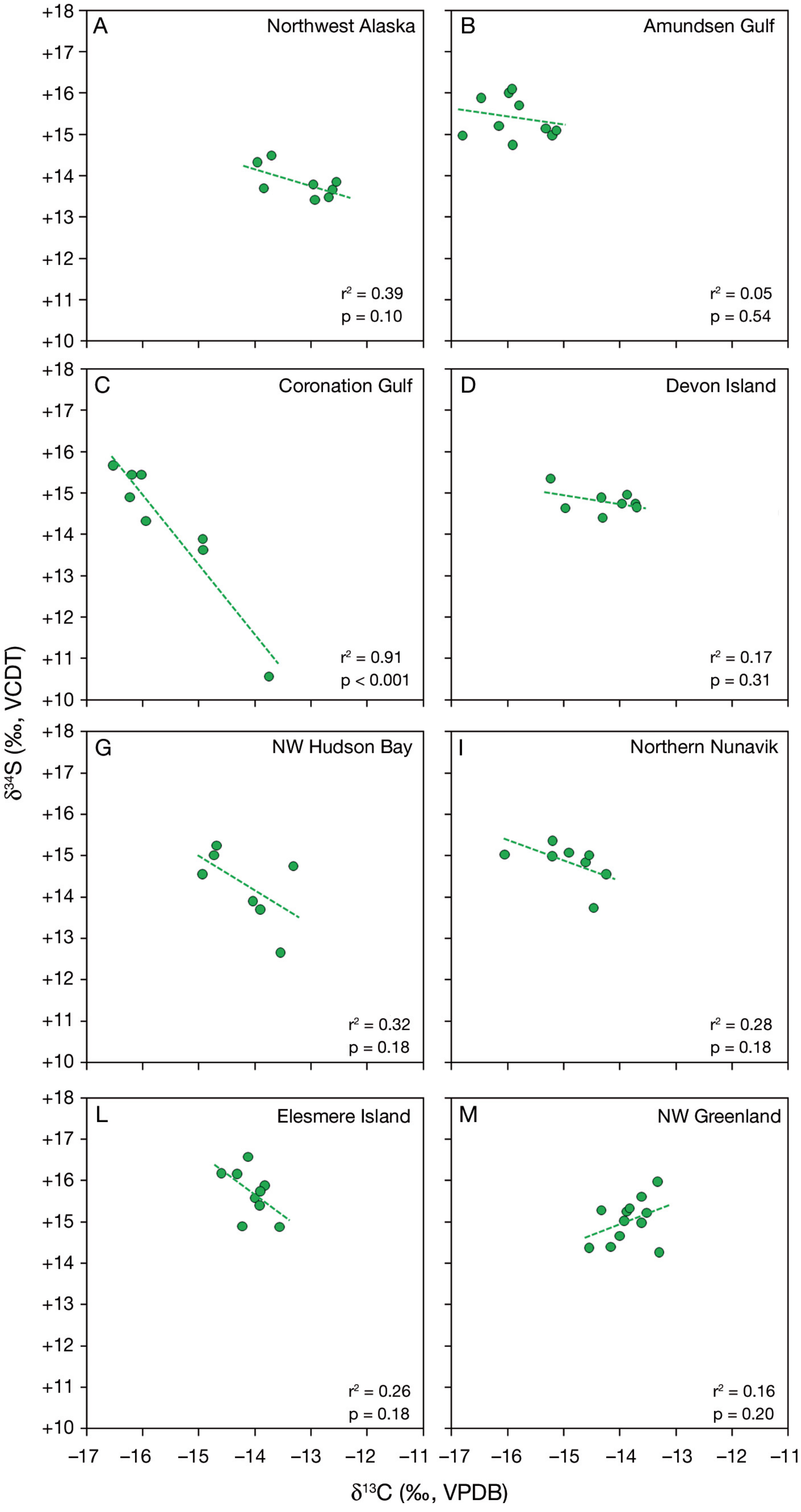

Fig. 6. Relationships between $\delta^{13}C$ and $\delta^{34}S$ values for ringed seals for each of the study regions. The letters in the top left of each panel correspond to the location of the sites in Fig. 1

the conditions in the surrounding sediments (Stribling & Cornwell 1997, Moncreiff & Sullivan 2001). Accordingly, without knowledge of the isotopic compositions of the appropriate end members (i.e. phytoplankton, benthic microalgae, macroalgae, sympagic algae), it is not possible to make an informed quantitative estimation of the relative proportion of organic matter derived from these different sources that reaches higher trophic levels beyond stating that it is not insignificant. While measuring the stable carbon isotope composition of benthic algae and phytoplankton may not provide these answers (Oxtoby et al. 2016), future studies should incorporate δ^{34}S measurements of nutrients, producers, and consumers in shallow Arctic ecosystems.

In addition to the overall low δ^{34}S values, walrus had lower δ^{34}S values than ringed seal. Given the isotopic compositions that have been recorded for benthic and pelagic feeding consumers, we expected that walrus would have significantly higher δ^{13}C values than ringed seal for each of the regions. Mean walrus δ^{13}C values were lower than ringed seal overall, and walrus δ^{13}C values were higher than those of ringed seal in only 1 of 8 regions; this difference was not statistically significant ($p = 0.33$) and was very small in magnitude (<0.3‰) (Fig. 3). Conversely, walrus had lower δ^{34}S values than ringed seal in all 8 of the regions, with significant differences in 4 of those (Fig. 2). Since consumer δ^{34}S values reflect the ultimate source of sulfur in the food web (Peterson & Fry 1987), these data suggest differences in the sulfur source of the prey consumed by walrus and ringed seal. Following the discussion above, we believe specifically that these data reflect the more specialized benthic foraging habits of walrus relative to ringed seals. The low δ^{34}S values of walrus suggest that bivalves, their primary prey, utilize food with low δ^{34}S values, most likely benthic microalgae that have assimilated sulfate that has undergone microbial sulfate reduction and is depleted of ^{34}S. This difference between ringed seal and walrus δ^{34}S is noteworthy because it suggests the potential of δ^{34}S for differentiating benthic vs. pelagic foraging in higher trophic level consumers where δ^{13}C may be equivocal or misleading. There were clear differences in δ^{13}C between the pelagic-feeding little auk and all other taxa in NW Greenland (Fig. 5), fitting with the expected pattern, but this was not true for walrus vs. ringed seal comparisons. This is an important point, because benthic–pelagic gradients are often invoked as an explanation for differences in foraging ecology, not only between disparate species (e.g. walrus and little auk), but also closely related species and even intraspecific variation according to sex, age, size, and reproductive status (Tucker et al. 2007, Drago et al. 2009a, Orr et al. 2012). The interpretation of consumer isotopic data in light of a detailed isotopic study of potential prey items would remove the uncertainty regarding the relative differences between benthic and pelagic prey, but this is not always feasible, especially for specimens sampled from historic, archaeological, and paleontological collections. The collection of isotopic data from many of the relevant prey taxa is not possible because they either do not preserve in ancient contexts (e.g. cephalopods, polychaetes, crustaceans) or obtaining isotopic measurements from tissues that do preserve (e.g. bivalve shells) is challenging (Schlacher & Connolly 2014). In the case of the Arctic sites that were sampled in this study, none of the relevant prey taxa were present (inclusive of Arctic cod and bivalves). Therefore, while our results are suggestive, we would emphasize that additional studies of this nature in well characterized modern ecosystems that include all of the potential prey taxa will be crucial to further exploring the potential of δ^{34}S as a marker of benthic vs. pelagic foraging in nearshore species.

An additional complication relates to differences in the routing of carbon and sulfur from dietary macronutrients to body tissues. For sulfur, this routing is straightforward, as bone collagen contains only methionine in very low abundance (approximately 2 residues/1000 for mammals). Methionine is an essential amino acid in vertebrates and therefore its sulfur isotopic composition should closely reflect the δ^{34}S of methionine in the foods consumed (Nehlich 2015). Since bone collagen contains no cysteine, a bulk δ^{34}S measurement of bone collagen can be understood as a de facto compound-specific δ^{34}S measurement of methionine, assuming the sample is not contaminated. The carbon in bone collagen, however, represents a mixture of essential and non-essential amino acids, and while this carbon is predominantly routed from dietary protein (Ambrose & Norr 1993, Jim et al. 2004), carbon in non-essential amino acids can also be routed from dietary lipids (Newsome et al. 2014). Within an organism, lipids have δ^{13}C values much lower than those of proteins (DeNiro & Epstein 1977), so variable proportions of proteins and lipids in the diets of consumers could represent an additional source of variation in bone collagen δ^{13}C, but not δ^{34}S. Therefore, variable dietary macronutrient contents could contribute to the unexpected pattern of variation in δ^{13}C between walrus and ringed seal in this study. Further δ^{13}C measurements of essential amino acids that better represent the isotopic composition of dietary protein would be useful for clarifying this issue.

The higher δ^{13}C values in walrus relative to ringed seal may be related to the different trophic levels at which these species feed, as evidenced by their very different δ^{15}N values. Ringed seal had tissue δ^{15}N values that were on average approximately 6‰ higher than those of walrus, equivalent to 1.5 to 2.0 trophic levels (Post 2002). It is possible that the small trophic-level enrichment for ^{13}C (Caut et al. 2009) has obscured any benthic vs. pelagic differences in prey δ^{13}C that would have otherwise been recorded in the tissues of walrus and ringed seal. If, as has been suggested, there is little or no change in δ^{34}S values across trophic levels (Nehlich 2015, Krajcarz et al. 2019), δ^{34}S may be more appropriate for making comparisons among taxa across trophic levels, but trophic enrichment for ^{34}S remains poorly characterized. Alternatively, it is possible that benthic and pelagic prey in these environments had δ^{13}C values that were too similar to be detectable isotopically in these consumers (Oxtoby et al. 2016) or that benthic microalgae had similar or possibly lower δ^{13}C values relative to other primary producers, as has been observed in other studies (Hobson et al. 2002b, Evrard et al. 2012, Oakes & Eyre 2014, Oxtoby et al. 2016). The data presented in this study suggest that δ^{34}S is a promising marker of benthic vs. pelagic foraging beyond just estuarine and salt marsh ecosystems.

5. CONCLUSION

Ringed seal and walrus from a variety of sites across the North American Arctic had relatively low δ^{34}S values compared to those typically observed in marine consumers. These data suggest an important role for benthic microalgae in coastal marine food webs in the Arctic. Benthic-feeding walrus had lower δ^{34}S values than more pelagic-feeding ringed seal, also supporting the notion that the low δ^{34}S values are driven by organic matter derived from the microphytobenthos. In contrast to expectations based on other studies, walrus had very similar and slightly lower δ^{13}C values than ringed seal, suggesting that this measurement may not always be best interpreted in light of benthic vs. pelagic foraging, particularly when comparisons are made across trophic levels. When the foraging ecology of a consumer is unknown, our data suggest that δ^{34}S may be a more sensitive indicator of the relative importance of benthic and pelagic prey in the diet than δ^{13}C.

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