

The following supplement accompanies the article

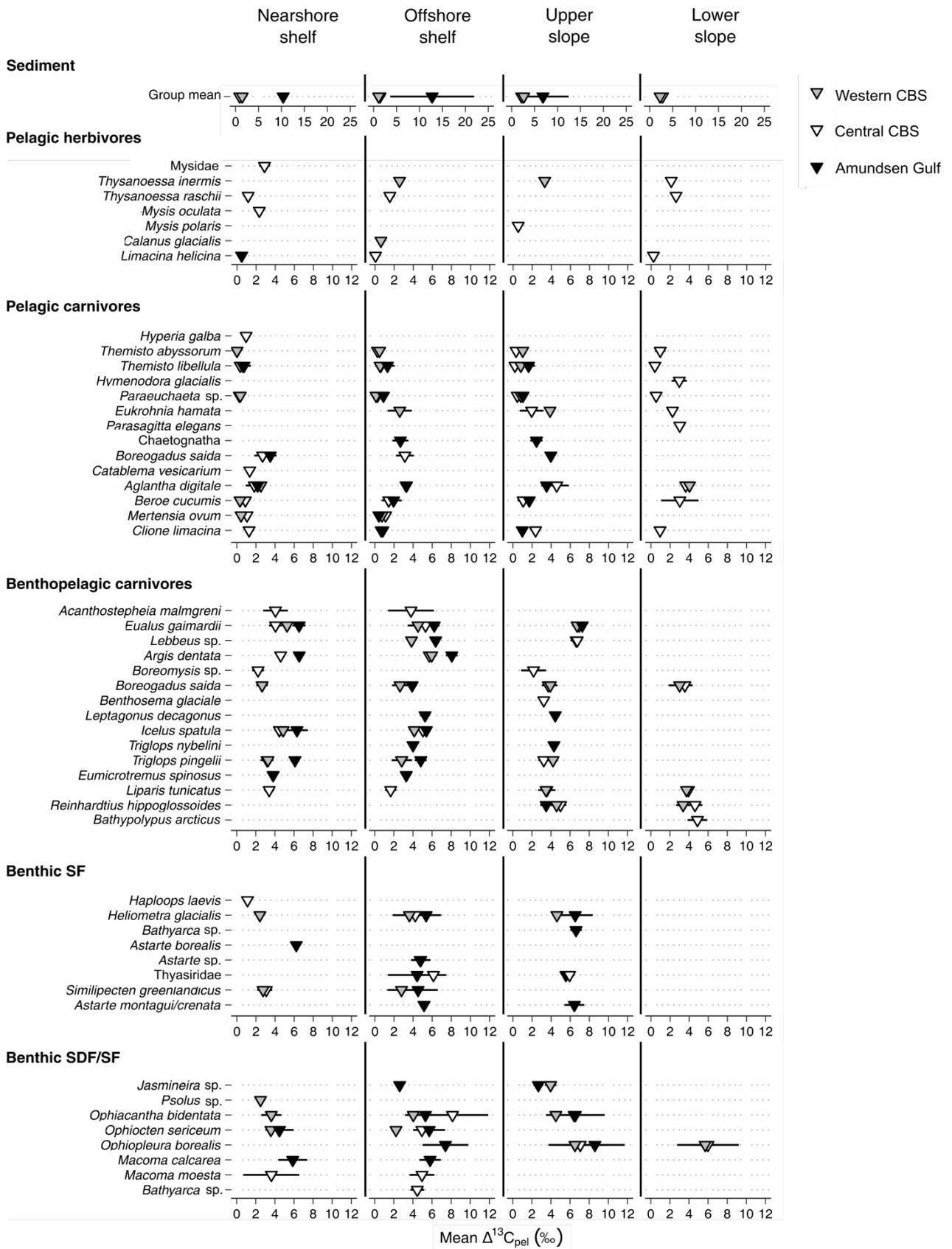
Benthic–pelagic trophic coupling in an Arctic marine food web along vertical water mass and organic matter gradients

**Ashley D. Stasko*, Bodil A. Bluhm, Christine Michel, Philippe Archambault,
Andrew Majewski, James D. Reist, Heidi Swanson, Michael Power**

*Corresponding author: ashley.stasko@dfo-mpo.gc.ca

Marine Ecology Progress Series 594: 1–19 (2018)

Supplement 2



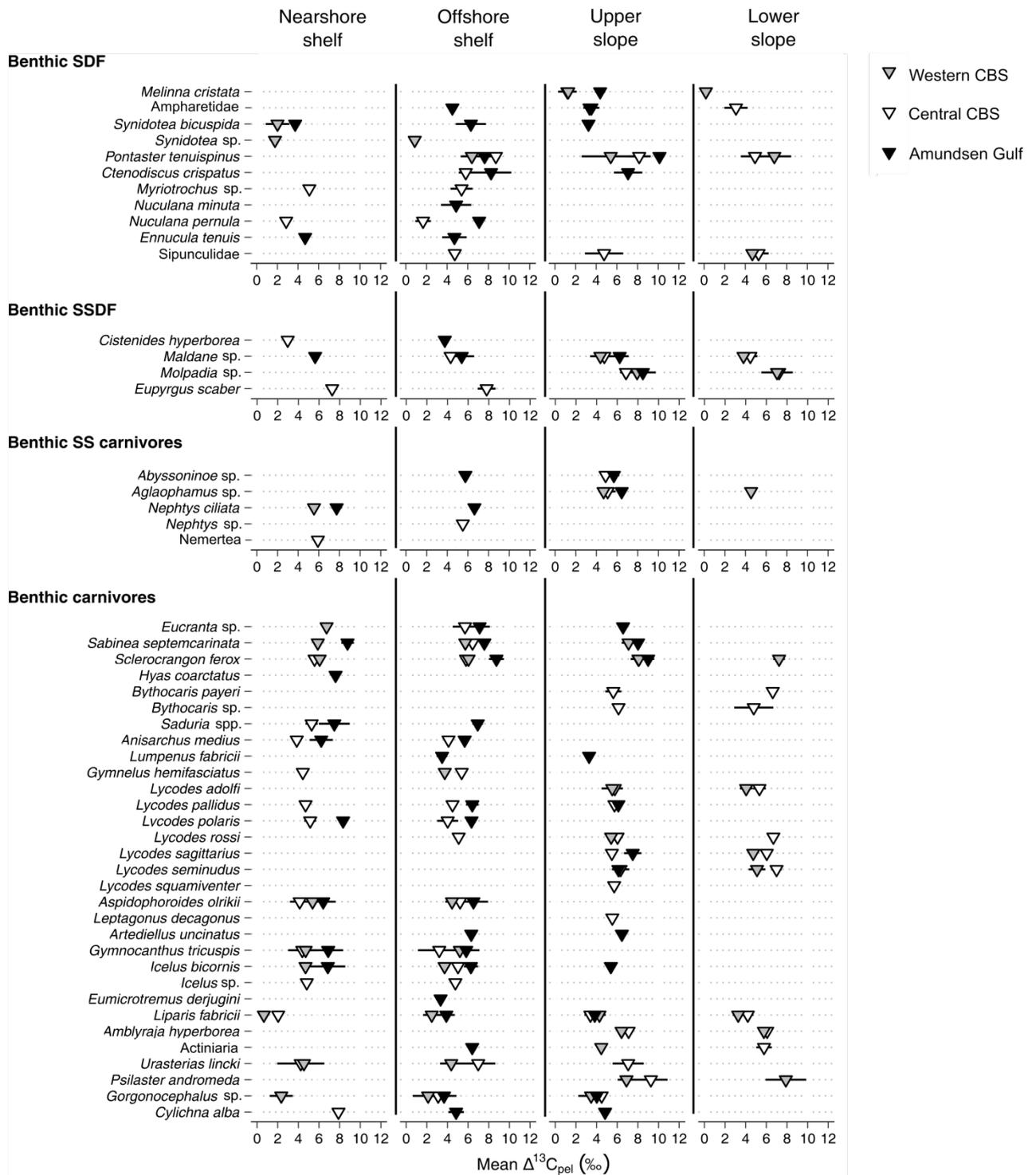


Fig. S2-1. Mean $\Delta^{13}\text{C}_{\text{pel}}$ (‰) for sediment and individual taxa across longitudinal regions, within each water mass assemblage. Whiskers for individual taxa represent ± 1 standard deviation. Note that sediment is presented on a wider axis than are taxa.

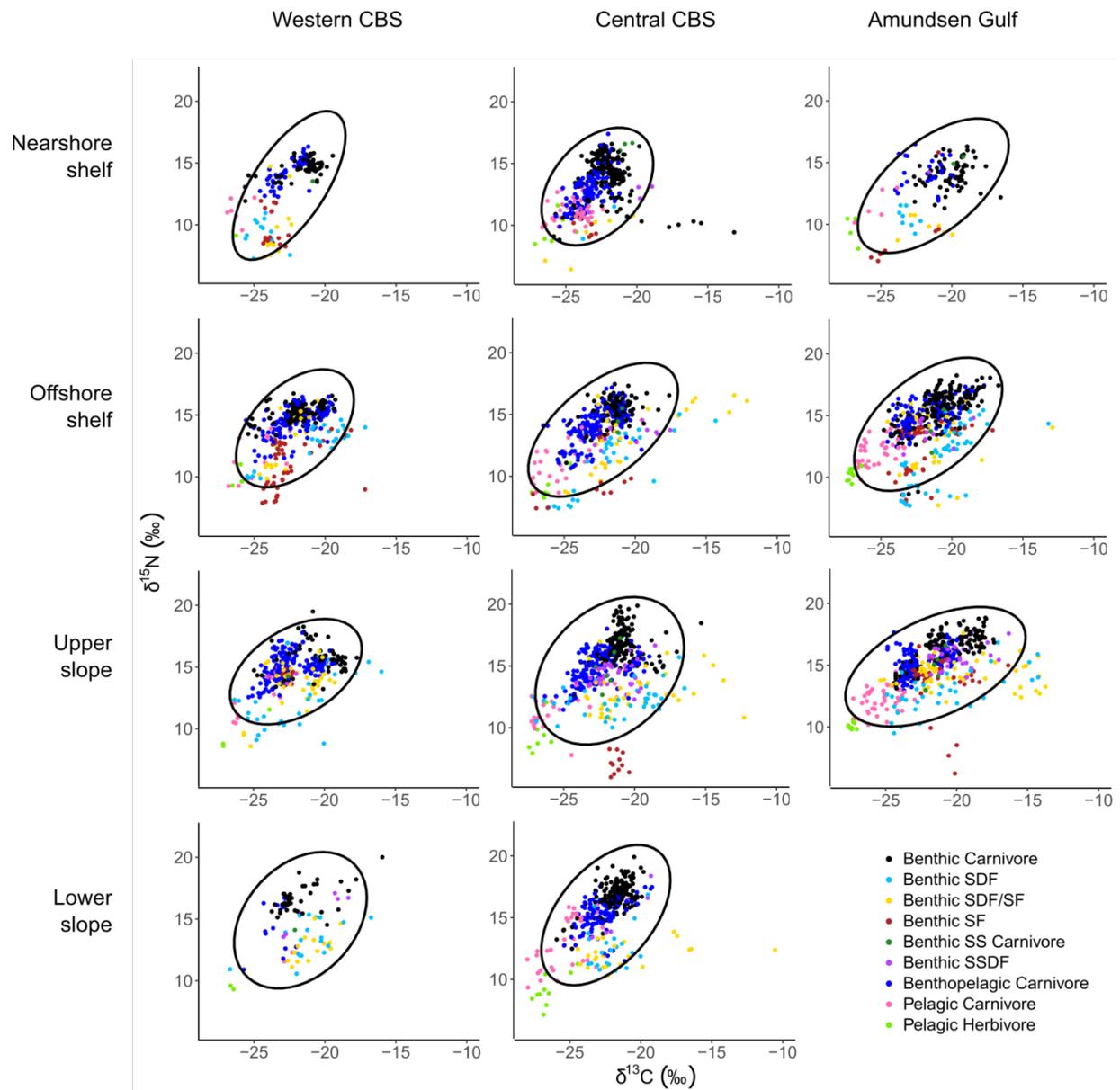


Fig. S2-2. Plots of unadjusted $\delta^{15}\text{N}$ against unadjusted $\delta^{13}\text{C}$ for all individuals included in the study, divided by longitudinal study region (Western CBS, Central CBS, and Amundsen Gulf) and by water mass assemblage (nearshore shelf, offshore shelf, upper slope, and lower slope). Individuals are coloured by functional group. Values of $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and C:N for each taxon averaged by the same community groups are published in Stasko et al. (2017).

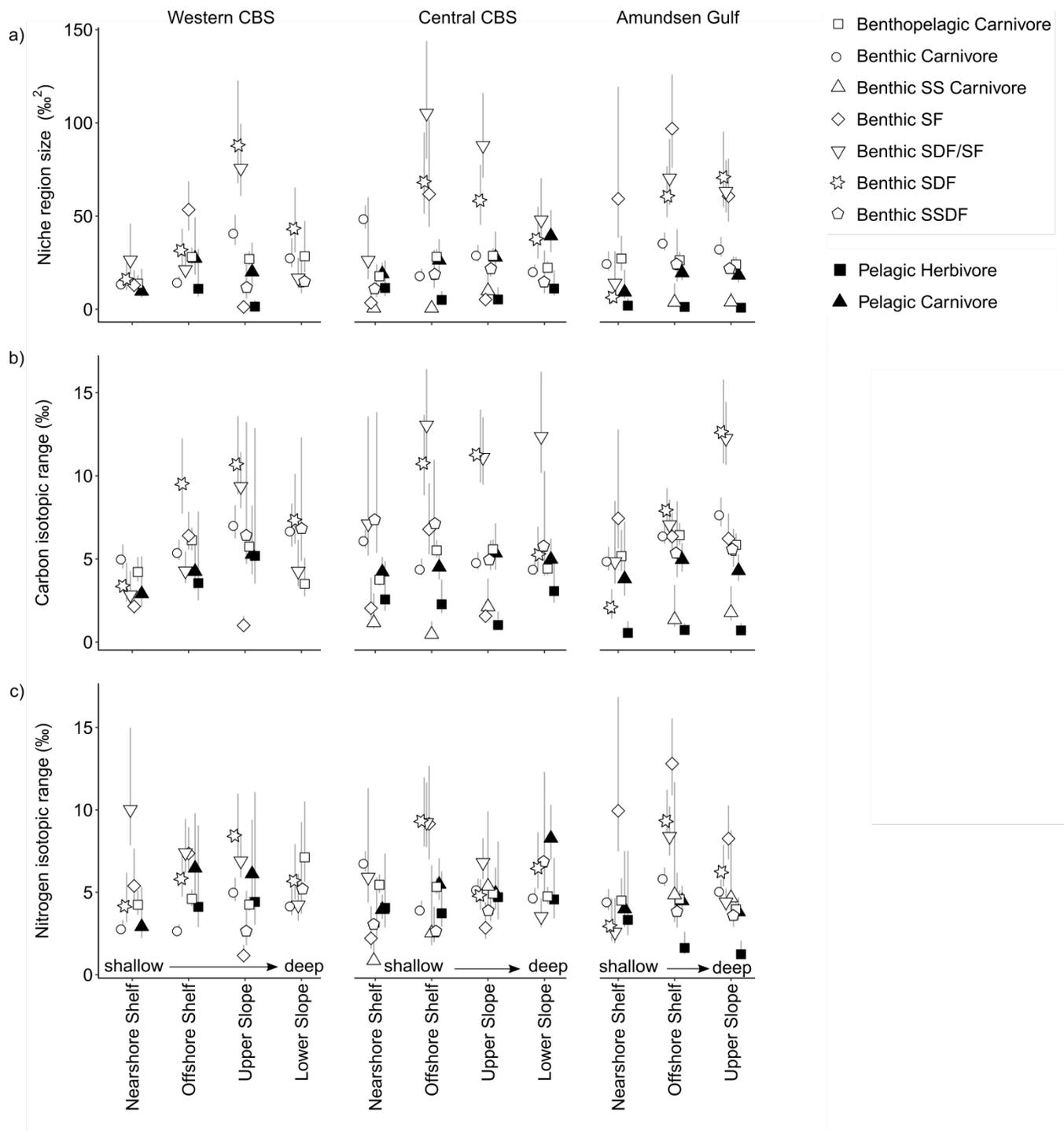


Fig. S2-3. Isotopic niche metrics for each functional group across water mass assemblages, and within longitudinal regions. Each point represents the mode of the posterior distribution, and whiskers represent 95% credible intervals. Low sample size ($n = 2$) prevented the calculation of niche metrics for pelagic herbivores and pelagic carnivores in the western CBS/Lower slope assemblage. Instead, the arithmetic means ± 1 standard deviation are presented for these groups. Lines illustrate trends among discrete water mass assemblages (not a continuous scale).

Table S2-1. Isotopic niche metrics calculated for each regional community. Niche metrics are reported as the mode and 95% credible intervals (CI) of the posterior distribution, each of which comprised 10,000 iterative calculations.

Water mass assemblage	Region	Niche region size (‰ ²)		Mean $\Delta^{13}\text{C}_{\text{pel}}$ (‰)		Carbon isotopic range (‰)		Mean adj. $\delta^{15}\text{N}$ (‰)		Nitrogen isotopic range (‰)	
		Mode	95% CI	Mode	95% CI	Mode	95% CI	Mode	95% CI	Mode	95% CI
<i>Nearshore shelf</i>											
	Western CBS	53.49	47.28, 60.87	3.78	3.58, 3.98	6.36	5.85, 7.01	4.01	3.70, 4.32	9.68	8.90, 10.64
	Central CBS	54.47	50.31, 59.43	3.80	3.67, 3.94	6.48	6.11, 6.89	4.39	4.23, 4.56	7.78	7.33, 8.28
	Amundsen Gulf	71.00	61.72, 82.69	5.86	5.54, 6.17	8.62	7.83, 9.62	3.01	2.69, 3.32	8.68	7.91, 9.73
<i>Offshore shelf</i>											
	Western CBS	54.37	49.76, 59.43	3.82	3.67, 3.97	6.74	6.34, 7.19	3.57	3.40, 3.74	7.75	7.30, 8.28
	Central CBS	72.05	66.54, 78.48	4.30	4.13, 4.48	8.48	8.01, 8.99	4.96	4.78, 5.14	8.80	8.32, 9.36
	Amundsen Gulf	85.97	80.00, 92.54	5.46	5.31, 5.62	8.59	8.17, 9.05	3.88	3.71, 4.06	9.55	9.09, 10.08
<i>Upper slope</i>											
	Western CBS	62.49	57.23, 68.46	5.21	5.03, 5.39	8.10	7.62, 8.67	5.87	5.71, 6.03	7.00	6.58, 7.49
	Central CBS	92.11	85.62, 99.45	5.08	4.92, 5.24	8.48	8.07, 8.96	5.70	5.52, 5.88	9.71	9.22, 10.26
	Amundsen Gulf	84.67	78.52, 91.47	6.00	5.80, 6.20	10.50	9.97, 11.10	4.63	4.48, 4.77	7.91	7.50, 8.34
<i>Lower slope</i>											
	Western CBS	73.39	62.74, 87.25	4.87	4.56, 5.19	7.50	6.74, 8.53	5.40	5.03, 5.78	8.81	7.88, 9.98
	Central CBS	69.80	64.18, 76.23	4.59	4.44, 4.75	7.34	6.93, 7.82	6.04	5.83, 6.24	9.34	8.81, 9.96

Examination of the potential influence of lipids and tissue type on conclusions drawn from $\delta^{13}\text{C}$ data

The need to analyse different tissues across taxa could have implications for comparing $\delta^{13}\text{C}$. A particular concern is that variability in the lipid content among consumer tissues can alter bulk $\delta^{13}\text{C}$ (DeNiro & Epstein 1977). Lipid extraction is often suggested as a solution to avoid confusion between isotopic variability caused by lipids and variability caused by dietary shifts (e.g., Logan et al. 2008; although see Mohan et al. 2016). Decisions not to extract lipids from tissues or mathematically correct $\delta^{13}\text{C}$ values for possible differences in lipid content among tissues, therefore, have the potential to confound food web interpretations. Lipids were not extracted from animal tissues in this study. Similarly, animals containing carbonate-rich tissues could have higher $\delta^{13}\text{C}$ compared to other animals, irrespective of differences in feeding, if inorganic carbon removal during acid treatment was incomplete (e.g., Rau et al. 1991, Cloern et al. 2002). Consequently, variation in tissue type and tissue lipid content could affect interpretations of $\delta^{13}\text{C}$ -derived isotopic metrics if they were linked to spatial variation in $\delta^{13}\text{C}$ observed across regions and water mass assemblages.

Data on lipid content, post-acidification carbonate content, and tissue-specific differences in $\delta^{13}\text{C}$ were not available to directly quantify their implications for interpreting $\delta^{13}\text{C}$. However, the issue for this particular study was not whether or not two individuals or taxa could be compared directly, but whether specific tissue types or lipid content would systematically bias the results from one regional community or functional group. In other words, is the greater difference between benthic and pelagic $\delta^{13}\text{C}$ ($\Delta^{13}\text{C}_{\text{pel}}$) in the Amundsen Gulf really just a consequence of the lipid content or tissue types analysed for animals in those communities?

To assess the potential for systematic bias resulting from differing tissue types among communities, we calculated the proportional representation of tissue types in each regional community and compared those to regional variation in $\Delta^{13}\text{C}_{\text{pel}}$. Refer to Stasko et al. (2017) for tissue types analysed for each taxon. A greater divergence between benthic and pelagic functional groups along the $\delta^{13}\text{C}$ continuum in the Amundsen Gulf and in the upper slope could be caused by the lipid content of benthic and pelagic functional groups if: a) *Calanus hyperboreus* (the pelagic baseline) had relatively higher lipid content in those communities; b) benthic organisms had relatively lower lipid content in those communities; or, c) both. Spatial variation in the mean difference in C:N between *C. hyperboreus* and all functional groups ($\Delta\text{C:N}$) was thus examined for each regional community, using C:N as a proxy for lipid content (e.g., Logan et al. 2008). Mean $\Delta\text{C:N}$ was estimated from posterior distributions derived from the same Bayesian protocol used to calculate isotopic niche metrics (described in *Methods* of this study). Linear regression was used to test for a significant relationship between mean $\Delta^{13}\text{C}_{\text{pel}}$ and mean $\Delta\text{C:N}$ for all benthic functional groups. Furthermore, we examined the relationships between C:N and $\delta^{13}\text{C}$ for individual species. Lipid extraction is considered unnecessary for fish muscle when C:N ratios are < 4 (e.g., Post 2007, Logan et al. 2008). More than 99 % of fish had C:N ratios < 4 , rendering extraction unnecessary. The 1% of fish with C:N > 4 were randomly distributed among regional communities, such that they

would not have confounded conclusions regarding regional differences in $\delta^{13}\text{C}$. In contrast, C:N ratios were > 4 for 31.3 % of invertebrates, after removing inorganic carbon (Stasko et al. 2017). We regressed $\delta^{13}\text{C}$ against C:N for 13 widespread invertebrate taxa (total n per taxon = 30 to 145; C:N from 0.9 to 25.8) to examine the potential influence of lipid on $\delta^{13}\text{C}$ values for invertebrates with C:N > 4 .

The proportional representation of tissue types was not substantially different among communities, and did not appear to cause a systematic bias that was consistent with our inferences of spatial patterns in $\delta^{13}\text{C}$ data (Table S2-2, Fig. S2-4). For example, both the upper and lower slope habitats of the Western CBS, and the upper slope habitat of the Amundsen Gulf had similar tissue type distributions, despite large differences in $\delta^{13}\text{C}$ -derived niche metrics (Fig. S2-4). Mean $\Delta\text{C:N}$ did not follow the same spatial patterns as mean $\Delta^{13}\text{C}_{\text{pel}}$ (Fig. S2-5), and mean $\Delta\text{C:N}$ was not significantly linearly related to $\Delta^{13}\text{C}_{\text{pel}}$ ($p = 0.35$, $r^2 = 0.01$, $F_{1,63} = 0.87$), suggesting lipid content did not bias conclusions regarding regional differences in $\Delta^{13}\text{C}_{\text{pel}}$. In addition, C:N, as a proxy for lipid content, did not explain substantial variation in $\delta^{13}\text{C}$ among tested invertebrates ($r^2 < 0.31$; Table S2-3). Taken together, these results indicate that lipid extraction would not have significantly altered our results. Our conclusion is consistent with other stable isotope studies on Arctic marine invertebrates (e.g., McTigue & Dunton 2014). We therefore conclude that although lipid content and tissue type may have altered $\delta^{13}\text{C}$ values for individual organisms, regional variation in mean $\Delta^{13}\text{C}_{\text{pel}}$ at the community and functional group level cannot be explained by spatial variation in lipid content or tissue types. Our dataset is also consistent with studies on Arctic marine benthic communities that have identified ecologically-driven patterns in food web structure from $\delta^{13}\text{C}$ data despite variation in tissue composition among taxa (e.g., Iken et al. 2005; McTigue & Dunton 2014, Renaud et al. 2015). Plots of raw C:N versus $\delta^{13}\text{C}$ values are provided for reference (Fig. S2-6).

It should be noted that lipid adjustment using a generic mathematical model has been suggested as a relatively easy solution to variation in lipid content (e.g., Post 2007). However, generic models assume that the difference between lipid and protein $\delta^{13}\text{C}$ is constant, when in fact lipid $\delta^{13}\text{C}$ can vary by species (Mohan et al. 2016). Moreover, generic models do not account for significant variation in species- and population-specific relationships between C:N and $\delta^{13}\text{C}$ (e.g., Logan et al. 2008, Fagan et al. 2011). Therefore, the use of generic lipid adjustment models may be inappropriate for studies such as this one that involve a broad spectrum of phylogenetic groups, where models may introduce more unquantified variation than they remove (Mohan et al. 2016).

LITERATURE CITED

- Cleorn JE, Canuel EA, Harris D (2002) Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. *Limnol Oceanogr* 47: 713-729.
- DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* 197:261-263

- Iken K, Bluhm B, Gradinger R (2005) Food web structure in the high Arctic Canada Basin: Evidence from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. *Polar Biol* 28:238-249
- Logan JM, Jardine TD, Miller TJ, Bunn SE, Cunjak RA, Lutcavage ME (2008) Lipid corrections in carbon and nitrogen stable isotope analyses: Comparison of chemical extraction and modelling methods. *J Anim Ecol* 77:838–846
- McTigue ND, Dunton KH (2014) Trophodynamics and organic matter assimilation pathways in the northeast Chukchi Sea, Alaska. *Deep Sea Res Part II Top Stud Oceanogr* 102:84–96
- Mohan SD, Connelly TL, Harris CM, Dunton KH, McClelland JW (2016) Seasonal trophic linkages in Arctic marine invertebrates assessed via fatty acids and compound-specific stable isotopes. *Ecosphere* 7:e01429
- Post DM (2007) getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* 152: 179-189
- Rau GH, Hopkins TL, Torres JJ (1991) ^{15}N : ^{14}N and ^{13}C : ^{12}C in Weddell Sea invertebrates: implications for diet and trophic structure. *Mar Ecol Prog Ser* 77: 1-6
- Renaud PE, Løkken TS, Jørgensen LL, Berge J, Johnson BJ (2015) Macroalgal detritus and food-web subsidies along an Arctic fjord depth gradient. *Front Mar Sci* 2:31

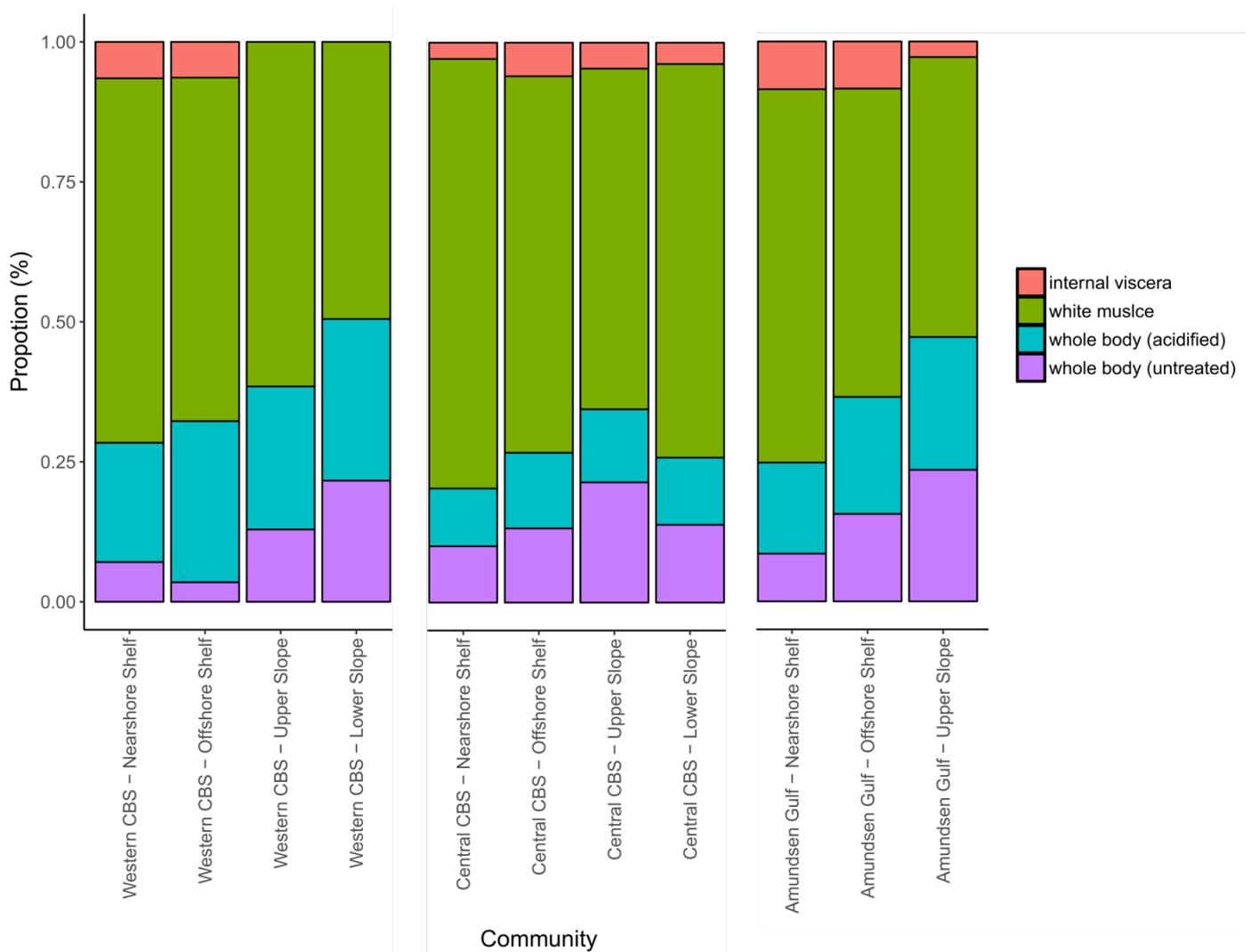


Fig. S2-4. Proportions of tissue types analysed in each regional community, including internal viscera (shell removed; e.g. starfish, some bivalves), white muscle, whole body after acidification to remove inorganic carbon, and untreated whole body (animals without exoskeleton, e.g., polychaetes).

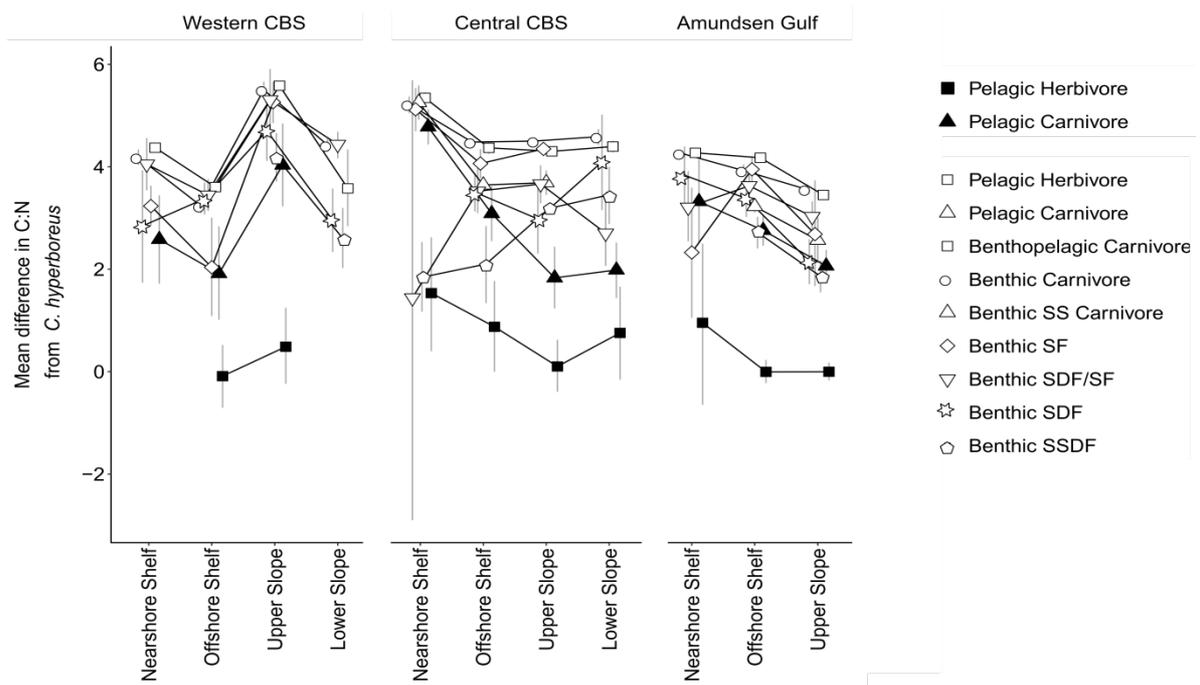


Fig. S2-5. Mean difference between C:N ratios of the baseline pelagic zooplankter *Calanus hyperboreus* and individuals in each of nine functional groups. Each point represents the mode of the posterior distribution of 10,000 estimates derived from the same Bayesian procedure used to derive the trophic niche metrics from nicheROVER. Lines illustrate trends among discrete water mass assemblages (not a continuous scale).

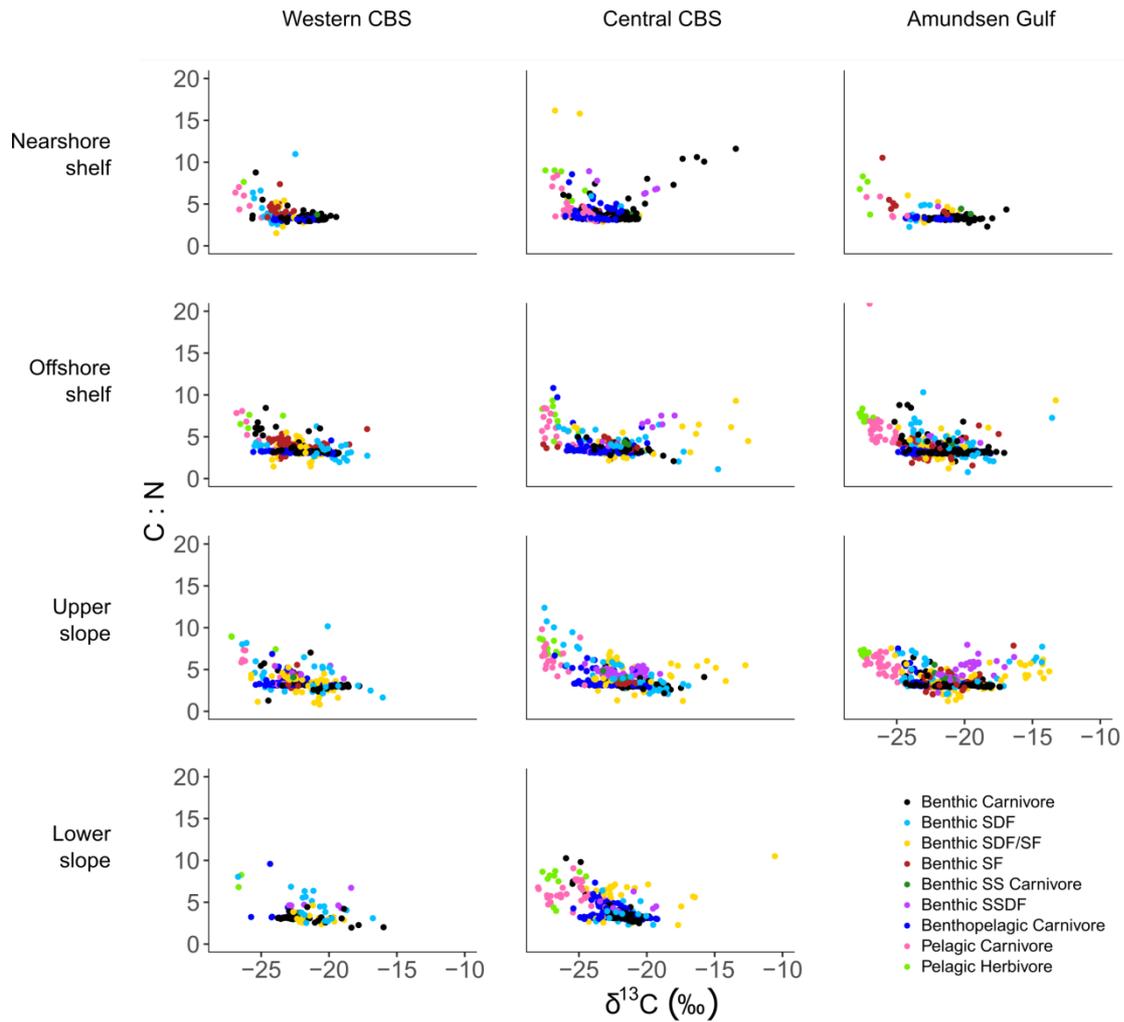


Fig. S2-6. Plots of C:N ratios against unadjusted $\delta^{13}\text{C}$ for all individuals included in the study, divided by longitudinal study region (Western CBS, Central CBS, and Amundsen Gulf) and by water mass assemblage (nearshore shelf, offshore shelf, upper slope, and lower slope). Individuals are coloured by functional group. See also Stasko et al. (2017). Three individuals of the crinoid *Heliometra glacialis* had $20 > \text{C:N} > 26$, but are not shown to maximize visibility of the majority of data (two from the offshore shelf of the western CBS, one from the upper slope of the Amundsen Gulf).

Table S2-2. Mean, maximum, and minimum proportions of each tissue analysed in regional communities.

Tissue	Mean	Max.	Min.
Internal viscera	0.05	0.09	0.00
White muscle	0.62	0.77	0.49
Whole body (acidified)	0.19	0.29	0.10
Whole body (untreated)	0.14	0.24	0.03

Table S2-3. Results for linear regressions of C:N and $\delta^{13}\text{C}$ for 13 widespread invertebrate taxa included in the study, as well as mean, standard deviation, maximum, and minimum observed C:N for each species.

Taxon	n	Intercept	Slope	p	r²	F	Mean	SD	Max.	Min.
<i>Argis dentata</i>	70	8.74	-0.73	0.32	0.01	1.00	3.25	0.20	4.65	2.98
<i>Calanus hyperboreus</i>	51	0.05	0.03	0.53	0.01	0.39	7.75	0.84	9.43	6.13
<i>Eualus gaimardii</i>	141	10.53	-1.31	<0.01	0.19	32.45	3.40	0.41	5.89	2.97
<i>Heliometra glacialis</i>	92	4.11	0.18	<0.01	0.12	12.73	4.27	3.80	25.84	1.97
<i>Molpadia sp.</i>	45	4.86	0.53	0.01	0.15	7.84	5.27	0.94	8.05	2.33
<i>Ophiacantha bidentata</i>	108	3.05	0.67	<0.01	0.12	14.12	3.78	1.21	9.39	1.30
<i>Ophiocten sericeum</i>	30	0.87	1.02	<0.01	0.31	12.61	3.34	0.92	6.18	1.53
<i>Ophiopleura borealis</i>	143	5.00	0.49	<0.01	0.11	17.72	4.22	2.02	17.53	0.90
<i>Pontaster tenuispinus</i>	106	6.08	0.23	0.24	0.01	1.38	3.55	1.24	10.26	1.22
<i>Sabinea septemcarinata</i>	48	15.03	-2.36	0.02	0.11	5.88	3.34	0.15	3.95	2.96
<i>Sclerocrangon ferox</i>	145	18.18	-3.18	<0.01	0.10	15.27	3.22	0.13	3.92	2.97
<i>Similipecten greenlandicus</i>	49	1.73	0.33	0.40	0.02	0.73	4.30	0.57	6.02	3.19
<i>Themisto libellula</i>	37	2.92	-0.32	<0.01	0.23	10.19	6.22	1.11	8.54	4.37