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What we do in the dark: Prevalence of omnivorous feeding activity in Arctic zooplankton during polar night

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Abstract

During the productive polar day, zooplankton and sea-ice amphipods fulfill a critical role in energy transfer from primary producers to higher trophic-level species in Arctic marine ecosystems. Recent polar night studies on zooplankton and sea-ice amphipods suggest higher levels of biological activity than previously assumed. However, it is unknown if these invertebrates maintain polar night activity on stored lipids, opportunistic feeding, or a combination of both. To assess how zooplankton (copepods, amphipods, and krill) and sea-ice amphipods support themselves on seasonally varying resources, we studied their lipid classes, fatty acid compositions, and compound-specific stable isotopes of trophic biomarker fatty acids during polar day (June/July) and polar night (January). Lipid storage and fatty acid results confirm previously described dietary sources in all species during polar day. We found evidence of polar night feeding in all species, including shifts from herbivory to omnivory. Sympagic-, pelagic-, and *Calanus* spp.-derived carbon sources supported zooplankton and sea-ice amphipods in both seasons. We provide a first indication of polar night feeding of sea-ice amphipods in the pelagic realm.

Seasonality is a periodic and predictable feature in almost all ecosystems, resulting in temporally distinct abiotic and biotic events. High-latitude environments have short productive seasons providing many organisms with limited time windows for growth, reproduction, and build-up of energy storage. In polar marine environments, the extremes of seasonal light availability result in highly pulsed primary production events (Leu et al. 2015) that in turn shape the life histories and trophic adaptations of herbivores as well as species higher in the food chain.

For Arctic marine zooplankton, adaptations to reduced food availability during winter include reduced feeding rates, shifts in dietary items, and/or overwintering in various states of arrested development. Many herbivorous metazoan zooplankton species hence accumulate large amounts of lipid reserves during the biologically productive season (Lee et al. 2006). Their reproductive strategies are well adapted to the Arctic seasonal cycle, ranging from using stored energy for reproduction to being reliant on food resources in order to produce offspring (Varpe et al. 2009). In contrast, omnivorous and carnivorous zooplankton consume food and reproduce year-round (Legeżyńska et al. 2012). Overwintering strategies of polar zooplankton range from diapause (a period of arrested development, surviving off stored energy pools) to what has been termed business-as-usual (continued feeding year-round, with no reduction in metabolism), with flexibility as an intermediate solution that combines energy stores and opportunistic feeding (Hagen 1999). In most studies, overall activity of the zooplankton community was assumed low during the polar night.

Recent polar night studies, however, showed higher than expected biological activity for many organisms in the absence of light and photosynthetic production (Berge et al. 2015). Some individuals in diapausing species such as

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Calanus spp. have been observed in surface waters as early as January (Berge et al. 2020; Espinasse et al. 2022), where alternative prey availability sustains polar night activity (Hobbs et al. 2020). Although overwintering strategies are less clear for ice-associated (sympagic) Arctic amphipods, the pelagic realm may serve as suitable habitat during polar night (Berge et al. 2012; Kunisch et al. 2020). As these findings demonstrate higher levels of activity than previously assumed, it remains to be seen if zooplankton and sympagic amphipods are maintaining activity on stored lipids alone, opportunistically feeding, or a combination of both.

This study explored potential seasonal diet changes for three Arctic crustacean zooplankton and two sympagic amphipod species with differing feeding modes and overwintering strategies. First, we studied their overwintering strategies in terms of their lipid storage patterns. Energy accumulations into lipids such as wax esters (WE), triacyclglycerols (TAG), and phospholipids are used as energy stores for zooplankton living in food-limited extremes (Hagen and Auel 2001). In our study, high investments in WE are interpreted as an overwintering strategy for longer term rest (i.e., diapause); or, to a lesser extent, consumption of organisms with high amounts of WE. Lipid accumulation via TAG can reflect opportunistic and year-round feeding behavior and shorter term energy storage (Hagen and Auel 2001), thus a business-as-usual strategy. Flexible strategies represent a mix of these two storage lipid classes, along with potential investments into membrane phospholipids.

Second, we investigated the fatty acid composition of the studied species as trophic biomarkers for specific diet sources. Two main sources of primary productivity in Arctic pelagic marine systems are sea-ice algae (often dominated by diatoms [Bacillariophyceae; Leu et al. 2020]) and phytoplankton (often diatoms, dinoflagellates, and others as main contributors; Booth and Horner 1997). Typical marker fatty acids in diatoms are 16:1(n-7) and 20:5(n-3) (Dalsgaard et al. 2003). Dinoflagellates have higher amounts of the 18:4(n-3) and 22:6(n-3) fatty acids (Viso and Marty 1993). During polar night, both diatoms and dinoflagellates can be found within the water column, though with lower fatty acid contributions to particulate organic matter (POM) when compared to polar day (Marmillot et al. 2020). Although 16:1(n-7) is the precursor of 18:1(n-7) through chain elongation, 18:1(n-9) can be produced by consumers and can serve as a biomarker for carnivory (Graeve et al. 1994*b*). Other trophic biomarkers, such as 22:1(n-11), are also used as an indication of carnivory on *Calanus* spp. in higher trophic level consumers (Graeve et al. 1994*a*). Bacterial production in the Arctic Ocean can, at times, be as high as primary production (Wheeler et al. 1996), and also occurs in the water column throughout the polar night (Iversen and Seuthe 2011). Thus, we included bacterial biomarkers which are represented by the odd-chained, branched fatty acids (15:0 and 17:0) (Parrish 2013).

Third, we investigated the seasonal patterns of the trophic biomarker fatty acids within each species. This was done by combining trophic biomarker fatty acid proportions with their carbon stable isotope values, or $\delta^{13}C_{FA}$. Diatom-associated [16:1(n-7), 20:5(n-3)] and dinoflagellate-associated [18:4(n-3), 22:6(n-3)] fatty acids can have higher $\delta^{13}C_{FA}$ values in ice algae than in phytoplankton due to carbon limitation in the sea-ice habitat (Fry and Sherr 1984). These carbon sources are then channeled through zooplankton and sympagic amphipods. We hence expected the higher $\delta^{13}C_{FA}$ values of ice-associated primary producers to be reflected in their consumers.

We hypothesized that changes in lipid classes and/or fatty acids of the study species during polar night would differ by overwintering strategies: diapause, flexibility, and business-as-usual. Following this, we investigated if fatty acid composition differed by season and habitat. We also hypothesized that mainly pelagic feeding zooplankton would have low seasonal variability in the $\delta^{13}C_{FA}$ values of trophic biomarker fatty acids because of the continued consumption of pelagic resources. In contrast, we hypothesized high seasonal variability in the $\delta^{13}C_{FA}$ values of trophic biomarker fatty acids in the sympagic amphipods—that were found away from the sea-ice habitat during polar night—because of an expected switch from ice-algae based carbon sources during the polar day to pelagic carbon sources during the polar night.

Methods

Study area and zooplankton sampling

Sampling was conducted during three expeditions in 2017 and 2018 in the Barents Sea and Arctic Ocean (Fig. 1). Two expeditions occurred in January 2017 and 2018 (polar night conditions) on the R/V Helmer Hanssen; the third expedition on the R/V Polarstern (PS106) occurred between 03 June 2017 and 14 July 2017 (polar day conditions).

During the January expeditions, zooplankton and sympagic amphipods were collected from various depths using a depth-stratified zooplankton Multinet sampler (Hydro-Bios, Kiel, Germany) equipped with five nets of 0.25 m^2 aperture each with a mesh size of 180 μm. Organisms were collected from 800 to 400 m and 400 to 100 m (January 2017), and 200 to 100 m (January 2018). Organisms were also sampled using pelagic nets towed to the surface (Table 1; Kunisch et al. 2020). No sea ice was encountered in January 2017; sampling occurred in-between smaller sea-ice fragments in the marginal ice zone in 2018.

In June–July 2017, sampling occurred in predominantly pre-bloom situations within 1st-year sea-ice areas. Four different types of nets were used to sample both pelagic and sympagic organisms. A zooplankton net (mounted to a remotely operated underwater vehicle, ROVnet) with 500 μm mesh size was operated at three specific depths: directly under ice, 5 and 10 m water depths (Wollenburg et al. 2020). A Surface and Under-Ice Trawl (SUIT, 0.15 mm mesh size) was also used directly under ice (Franeker et al. 2009). To sample the pelagic community, a rectangular midwater trawl (5.5 mm and

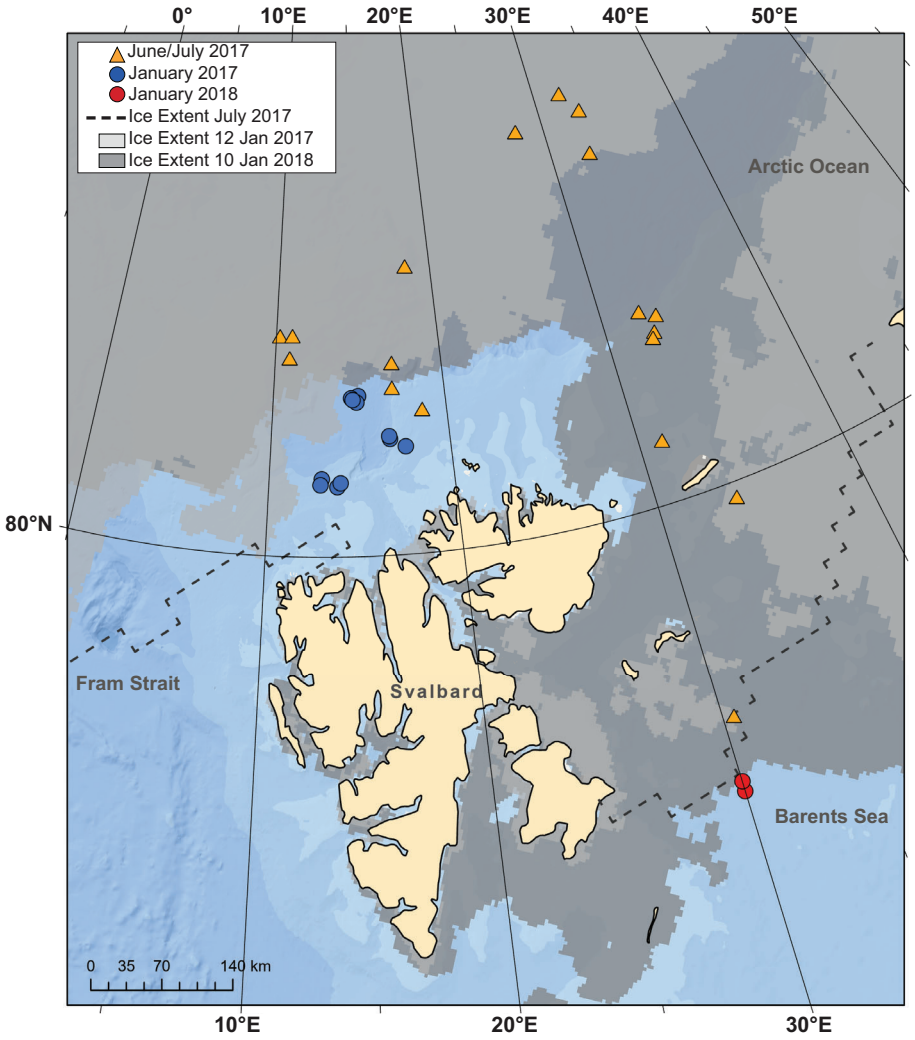


Fig. 1. Station locations (triangles—June/July, circles—January) denote zooplankton and sympagic amphipod collection sites in the area north and east of Svalbard in the European Arctic. Sea-ice extent data for 12 January 2017 and 10 January 2018 acquired from the University of Bremen, Germany (Spreen et al. 2008). Sea-ice extent data for the month of July 2017 are denoted by the black dashed line (data from the National Snow and Ice Data Center; Fetterer et al. 2017). Map created with ArcGIS Pro 2.8.2.

330 μm mesh sizes) and a bongo net (180 μm mesh size) targeted the upper 100–300 m of the water column.

At each station, collected organisms (Table 1) were sorted by species and placed into precombusted and pre-weighed 8-mL borosilicate vials or wrapped in aluminum foil (not precombusted) and placed into air-tight bags. Samples were frozen at −80°C until analysis. Our interest was in the seasonal differences within species, and not among life stage within species. Hence, we attempted to collect adult life stages for each species. For *Calanus glacialis* we collected overwintering copepodite stages CV and adult females in January 2017 and CIV in January 2018. We found no statistical differences in our variables of interest between *C. glacialis* stages CV and adult females; hence findings were pooled within the species.

Fatty acid and lipid class analysis

All laboratory analyses were conducted at the Alfred Wegener Institute in Bremerhaven, Germany. Prior to lipid extraction, organisms were removed from −80°C freezers and freeze-dried for 24 h. Samples were mechanically homogenized, and lipids were extracted using dichloromethane/methanol 2:1 v/v (Folch et al. 1957). Total lipid mass of each sample was determined gravimetrically. Lipid class analysis was conducted using high-performance liquid chromatography on aliquots of the extracted lipids (Graeve and Janssen 2009). Extracted lipids were converted into fatty acid methyl esters by transesterification using methanol with a 3% solution of concentrated sulfuric acid. Fatty acid methyl esters were quantified with an internal standard, tricosanoic acid methyl ester (23:0) (Supelco, Germany), that was added prior to lipid extraction. Detection limits were based on a

Table 1. Sample information for species sampled during January 2017, June/July 2017, and January 2018. Species were analyzed for lipids, fatty acids, and carbon isotope values of fatty acids. Water masses are defined as follows: Atlantic water: temperature (T) > 3°C, salinity (S) > 35 (Rudels et al. 2000); modified Atlantic water: $T > 2$°C, $S > 34.91/0$°C < $T < 2$°C, $34.4 < S < 34.91/T < 0$°C, $S < 34.676$ (Schlichtholz and Houssais 1999; Rudels et al. 2000); polar surface water: T approximately −1°C, S 34.2–34.3 (Rudels et al. 2000); Arctic water $T < 0$°C, $S < 34.7$ (Oziel et al. 2016).

Date	Station	Latitude (°N)	Longitude (°E)	NET	Sampled depth (m)	Water mass	Species	N
11 Jan 2017	NS1	80.60	13.68	WP2	100-0	Atlantic water	*Calanus glacialis*	35
		80.64	13.85	Pelagic trawl	155-0		*T. libellula*	1
	NS4	80.67	12.84	MIK	317-0	Atlantic water	*Apherusa glacialis*	1
		80.62	12.77	Pelagic trawl	245-0		*T. libellula*	1
12 Jan 2017	NS6	81.38	14.95	Multi net	800-400	Modified Atlantic water	*A. glacialis*	1
13 Jan 2017		81.35	14.67	Pelagic trawl	199-0	Modified Atlantic water	*Gammarus wilkitzkii*	2
							T. inermis	11
							T. libellula	4
		81.33	14.84	MIK	160-0	Modified Atlantic water	*A. glacialis*	6
							G. wilkitzkii	1
							T. libellula	2
		81.37	14.53	WP2	100-0		*C. glacialis*	25
	NS9	81.00	16.65	MIK	400-0	Atlantic water	*A. glacialis*	5
		81.03	16.62	Multinet	400-0		*G. wilkitzkii*	1
14 Jan 2017	NS10	80.93	17.50	MIK	250-0	Atlantic water	*G. wilkitzkii*	4
10 Jun 2017	27	81.91	10.22	ROV	Under ice	Under ice	*A. glacialis*	30
							C. glacialis	37
11 Jun 2017	28	81.91	10.99	ROV	Under ice	Under ice	*A. glacialis*	20
15 Jun 2017	32	81.72	10.86	ROV	Under ice	Under ice	*A. glacialis*	3
					5		*T. libellula*	1
25 Jun 2017	45	78.11	30.48	ROV	Under ice	Under ice	*A. glacialis*	15
					10		*C. glacialis*	60
				By hand	Surface		*A. glacialis*	1
27 Jun 2017	49	79.88	33.89	BONGO	238-0	Arctic intermediate water	*C. glacialis*	30
							G. wilkitzkii	1
							T. inermis	12
							T. libellula	13
29 Jun 2017	50	80.55	31.24	SUIT	Under ice	Under ice	*A. glacialis*	6
							G. wilkitzkii	1
							T. inermis	2
01 Jul 2017	63	81.46	32.81	SUIT	Under ice	Under ice	*A. glacialis*	20
	64	81.41	32.62	RMT	100-0	Modified Atlantic water	*C. glacialis*	30
							T. inermis	3
02 Jul 2017	65	81.59	33.24	SUIT	Under ice	Under ice	*T. inermis*	3
	66	81.66	32.32	SUIT	Under ice	Under ice	*A. glacialis*	5
							G. wilkitzkii	1
							T. inermis	21
							T. libellula	2
05 Jul 2017	70	83.11	32.80	SUIT	Under ice	Under ice	*G. wilkitzkii*	1
							T. libellula	2
06 Jul 2017	72	83.49	33.12	SUIT	Under ice	Under ice	*A. glacialis*	11
							G. wilkitzkii	2
07 Jul 2017	73	83.68	32.05	SUIT	Under ice	Under ice	*A. glacialis*	19
							T. libellula	5

(Continues)

Table 1. Continued

Date	Station	Latitude (°N)	Longitude (°E)	NET	Sampled depth (m)	Water mass	Species	N
08 Jul 2017	74	83.48	27.91	SUIT	Under ice	Under ice	*A. glacialis*	15
							C. glacialis	30
							G. wilkitzkii	4
		83.46	28.12	RMT	100-0	Arctic intermediate water	*T. libellula*	4
10 Jul 2017	76	82.49	18.36	SUIT	Under ice	Under ice	*A. glacialis*	20
							G. wilkitzkii	2
							T. libellula	1
		82.49	18.27	RMT	100-0	Arctic intermediate water	*T. libellula*	1
11 Jul 2017	79	81.66	17.03	SUIT	Under ice	Under ice	*A. glacialis*	5
							T. inermis	11
							T. libellula	1
12 Jul 2017	80	81.45	16.95	SUIT	Under ice	Under ice	*G. wilkitzkii*	2
							T. inermis	4
13 Jul 2017	83	81.25	18.61	RMT	100-0	Modified Atlantic water	*T. inermis*	6
							T. libellula	1
09 Jan 2018	B34	77.55	30.01	Pelagic trawl	108-0	Arctic intermediate water	*T. libellula*	5
10 Jan 2018	B34	77.47	29.99	Multinet	200-100	Arctic intermediate water	*C. glacialis*	10

certified reference material (Supelco 37 Component fatty acid methyl ester mix). Fatty acids are presented in shorthand notation, that is, *A*:*B*(*n*-*x*), where *A* indicates the number of carbon atoms in the straight fatty acid chain, *B* represents the number of double bonds present, *n* represents the position of the terminal methyl group, and *x* denotes the position of the 1st double bond from the terminal end. Proportions of individual fatty acids are expressed as mass percentages of total fatty acid content. Although all fatty acids were examined for analysis, we also highlighted known trophic biomarker fatty acids (Table 2).

Table 2. Trophic markers identified in fatty acid contributions of pelagic and sympagic food sources. Storage lipids are comprised of WE and TAG, while membrane lipids are comprised of phospholipids and other biomembranes.

Fatty acid	Trophic biomarker*	Lipid class, function
16:1 (n-7)	Diatoms	TAG†, storage†
20:5 (n-3)	Diatoms	Phospholipids-, biomembranes†, TAG†
18:1 (n-9)	Carnivory	TAG‡
18:4 (n-3)	Dinoflagellates	Storage§
22:6 (n-3)	Flagellates	Phospholipids*, biomembranes†
22:1 (n-11)	*Calanus* spp.	Storage*
$\sum 15:0, ai15:0, i15:0, 17:0$	Bacteria	nd

nd, not determined.
*Graeve et al. (1994*b*), Falk-Petersen et al. (2000), Dalsgaard et al. (2003), and Parrish (2013).
†Scott et al. (1999, 2002).
‡Lee et al. (2006).
§Kattner and Hagen (1995).

Compound-specific stable isotope analysis

Carbon stable isotope ratios of fatty acid methyl esters ($\delta^{13}C_{FA}$) of tissue samples were analyzed using a Trace Ultra gas chromatograph (GC), a GC Isolink system and Delta V Plus isotope ratio mass spectrometer (IRMS), connected via a Conflo IV interface (Thermo Scientific Corporation). Samples were injected in splitless mode and separated on a DB-FFAP column (60 m, 0.25 mm I.D., 0.25 μm film thickness) using temperature programming. The limit of detection was comparable to the methods described above. The $\delta^{13}C_{FA}$ values were calibrated using certified, referenced standards of 14:0 ($\delta^{13}C$: −29.98‰), 16:0 ($\delta^{13}C$: −30.74‰), 18:0 ($\delta^{13}C$: −23.24‰), and 20:0 ($\delta^{13}C$: −30.68‰) (supplied by Indiana University, USA). To ensure accuracy and precision (±0.8‰ for GC-IRMS), certified standards were analyzed before and after sample runs in the GC-IRMS. All reported $\delta^{13}C_{FA}$ values are relative to Vienna Pee Dee Belemnite using the standard notation $\delta^{13}C_{FA}$ (‰) $= [(R_{sample}/R_{standard}) - 1] \times 1000$, where R is the corresponding ratio of $^{13}C/^{12}C$.

Multivariate and univariate statistical analyses of fatty acid and lipid class data

Data analysis was conducted in R version 4.0.3 (R Core Team 2022) using both the tidyverse (Wickham et al. 2019)

and vegan (Oksanen et al. 2020) packages. For lipid class analysis, a correspondence analysis (CA) on the relative proportions (mass % of sum) was conducted to determine how various lipid classes contributed to dissimilarity among species. We focused on two neutral lipids (WE and TAG), and two polar phospholipids (phosphatidylethanolamine [PE] and phosphatidylcholine [PC]). To test for seasonal differences between lipid class proportions within species, we used the Kruskal–Wallis (KW) rank sum tests followed by post hoc Dunn's test for pairwise comparisons, because the data did not have the normal distributions needed for parametric tests.

The following environmental variables were included to determine their importance in the fatty acid compositions within species: water mass, season (polar day/night), and location. For water mass, temperature (T) and salinity (S) data were derived from conductivity–temperature–depth (CTD) profiles from PS106 (Heuzé et al. 2018; Nikolopoulos et al. 2018) and the polar night expeditions. We included CTD data from the location nearest to the sampled zooplankton, often from the same station. Profiles of T and S were averaged over the sampled net depth (e.g., if a zooplankton tow was 100-0 m). These values were then made categorical and binned into distinct water masses (described in Table 1). Because the ROVnet and SUIT targeted shallower depths directly under sea ice, the water properties of these depths were different than where a CTD began sampling at 10–20 m below surface (Castellani et al. 2020). Species sampled under ice via the ROVnet or SUIT were therefore binned into a separate category, under ice. Sampled months were binned into two different seasons: polar night (January) and polar day (June and July). Location groups were defined by bottom depths: shelf < 400 m, shelf break/slope stations between 401 and 2500 m, and basin stations > 2500 m (Bluhm et al. 2020).

To identify the importance of the environmental parameters on fatty acid composition within species, permutation tests on forward model selection were conducted on canonical correspondence analysis (CCA). CCA models were conducted on the significant fatty acids (determined by the vegan *envfit* function at $\alpha = 0.05$). Seasonal differences in the trophic biomarker fatty acids (Table 2) and their respective $\delta^{13}C_{FA}$ values were univariately tested using KW rank sum tests followed by a post hoc Dunn's test for pairwise comparisons.

Results

Zooplankton

C. glacialis stored lipids primarily as WE in both seasons (Fig. 2; Table 3) with higher relative amounts of WE in January

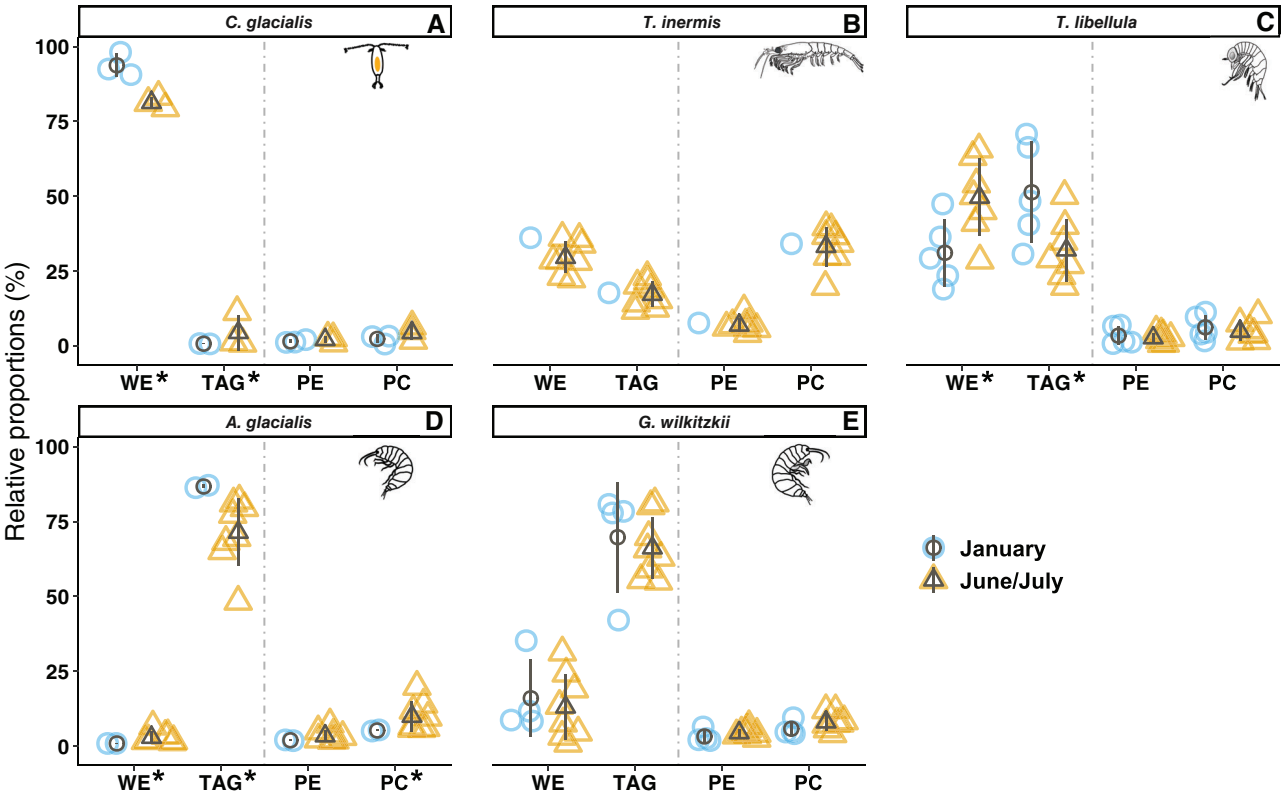
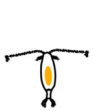


Fig. 2. Seasonal lipid class differences organized by zooplankton (**A**–**C**) and sympagic amphipod (**D**,**E**) species. Displayed are WE, TAG, PE, and PC. Lipid class data are presented as relative proportions (mass %) of total lipids. Shapes and colors represent samples (note many individuals/sample; *see* Table 2): blue circles represent January and orange triangles, June/July. Note that in all lipid classes in January *T. inermis* lack corresponding means and standard deviations (gray shapes/lines) because there was only 1 sample (of 11 pooled individuals, Table 2). Asterisk indicates statistical differences.

Table 3. Overview of the studied species. Information on zooplankton overwintering strategies is based on Hagen (1999) and updated based on our own findings on a flexible overwintering strategy for *Calanus glacialis* and feeding during polar night for all studied species. For *Apherusa glacialis* and *Gammarus wilkitzkii*, diet while found in association with the sea-ice habitat is listed. *See* discussion for further interpretation of our polar night findings. Percentages of total lipids, WE, TAG, PE and PC between the two sampled periods are shown (mean ± 1 SD).





	Calanus glacialis		*T. inermis*		*T. libellula*		*Apherusa glacialis*		*Gammarus wilkitzkii*	
Polar day diet	Primarily herbivorous		Herbivorous–omnivorous		Carnivorous		Herbivorous–omnivorous*,†		Carnivorous*,†	
Overwintering strategy	Flexibility		Flexibility‡		Business-as-usual‡		Flexibility		Business-as-usual	
Polar night diet (this study)	Omnivorous		Omnivorous		Carnivorous		Omnivorous		Carnivorous	
	June/July	**January**	**June/July**	**January**	**June/July**	**January**	**June/July**	**January**	**June/July**	**January**
Individuals/sample	37 ± 13	23 ± 13	8 ± 7	11	3 ± 4	2 ± 1	13 ± 9	3 ± 3	2 ± 1	2 ± 1
Total lipids/dry weight (%)	nd	22.7 ± 14.7	nd	7.3	nd	25.4 ± 25.1	nd	48.1 ± 36	10.4 ± 4.6	20 ± 14.8
WE (%)	81.3 ± 1.9	93.7 ± 3.9	29.6 ± 5.3	36.2	49.7 ± 12.9	31.14 ± 11.2	2.9 ± 1.9	0.9 ± 0.007	13 ± 11	16 ± 13
TAG (%)	4.5 ± 6	0.8 ± 0.1	17.2 ± 4.3	17.8	32.1 ± 11	51.4 ± 17	71.4 ± 11.3	86.7 ± 0.6	66.2 ± 10.2	69.8 ± 18.5
PE (%)	2.1 ± 1.1	1.6 ± 0.5	7 ± 2.3	7.7	2.7 ± 1.6	3.5 ± 3.1	3.4 ± 1.9	2 ± 0.2	4.5 ± 1.3	3.2 ± 2.1
PC (%)	4.5 ± 2.4	2.4 ± 1.5	33.2 ± 7	34.2	4.9 ± 3.2	6.3 ± 4.1	9.9 ± 5.1	5.2 ± 0.3	8.1 ± 2.8	5.8 ± 2.5

nd, not determined.
*Poltermann (2001).
†Werner and Auel (2005).
‡Hagen (1999).

than in June/July (KW–Dunn $p = 0.03$). Total lipids per dry mass was low for both sampled January seasons (12% and 33%, respectively). The contribution of the carnivory biomarker 18:1(n-9) in *C. glacialis* was higher in January than in June/July (KW–Dunn $p = 0.03$). Fatty acids acquired from de novo biosynthesis [22:1(n-11), 22:1(n-9), and 20:1(n-11)] (Graeve et al. 1994*b*) and two diatom-associated fatty acids [16:4(n-1) and 20:5(n-3)] significantly contributed to the CA patterns (Fig. 4). However, permutation test results determined that none of the environmental variables significantly explained fatty acid composition. $\delta^{13}C_{FA}$ values ranged from −34.8‰ to −26.8‰ for diatom-associated fatty acids [16:1(n-7), 20:5(n-3)] and −33.6‰ to −25.3‰ for dinoflagellate-associated fatty acids [18:4(n-3), 22:6(n-3)] (Fig. 5). In comparison, the *Calanus* spp.-specific fatty acid 22:1(n-11) had $\delta^{13}C_{FA}$ values ranging from −29.4‰ to −26.1‰, which did not differ seasonally (Fig. 5).

T. inermis exhibited similar proportions of WE, TAG, and phospholipids in January compared to June/July with highest investments into WE and PC (Fig. 2; Table 3). The diatom marker 20:5(n-3) and carnivory biomarkers 16:0 and 18:1(n-9) contributed most to the fatty acid composition of *T. inermis*, in both seasons (Fig. 3). *T. inermis* accumulated similar amounts of the diatom-associated 20:5(n-3) in both seasons. Diatom- and dinoflagellate-associated fatty acids significantly contributed to the CA patterns, namely 16:1(n-7), 16:4(n-1), 20:5(n-3), 18:4(n-3), and 22:6(n-3) (Fig. 4). Permutational test results determined that none of the tested environmental variables significantly explained differences in seasonal fatty acid patterns. $\delta^{13}C_{FA}$ values ranged from −38.7‰ to −25.2‰ for the diatom-associated fatty acids (Fig. 5).

T. libellula amassed mostly neutral lipids in both seasons. WE were more abundant in June/July than in January (KW–Dunn $p = 0.02$). As these are relative contributions, the opposite was true for TAG, which were more abundant during January opposed to June/July (KW–Dunn $p = 0.02$). In comparison, *T. libellula* also accumulated phospholipids (PE and PC), yet in lower proportions and without seasonal differences (Fig. 2). January *T. libellula* accumulated an average of 25% total lipids/dry mass, though the range of total lipids was wide

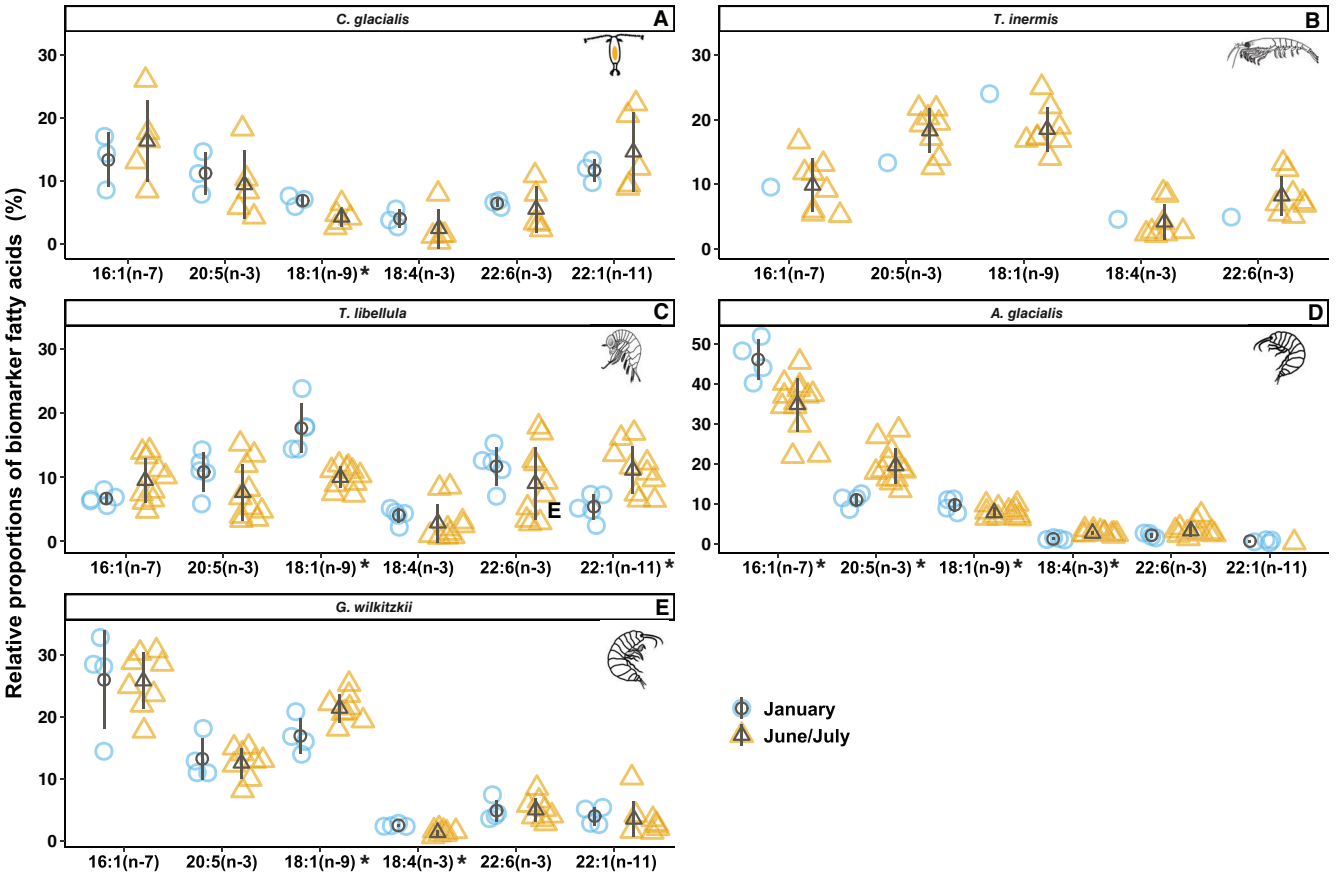


Fig. 3. Relative composition of biomarker fatty acids (mass % of total fatty acids), in zooplankton (**A**–**C**) and sympagic amphipod (**D**,**E**) species. Shapes and colors represent samples (note many individuals pooled by sample, *see* Table 2): blue circles, January and orange triangles, June/July. Gray shapes represent means, vertical gray lines represent corresponding standard deviations of the mean. Note that in all fatty acids in January *T. inermis* and 22:1(n-11) in June/July *Apherusa glacialis* lack corresponding means and standard deviations (gray shapes/lines) because there was only one sample (of many pooled individuals, Table 2). Asterisk indicates statistical differences.

between samples (Table 3). The amounts of the carnivory biomarker 18:1(n-9) in *T. libellula* were higher (KW–Dunn $p = 0.001$) in January than in June/July (Fig. 3). The opposite was true for the *Calanus* spp. trophic biomarker [22:1(n-11)] of which *T. libellula* had higher amounts in June/July than in January (KW–Dunn $p = 0.005$; Fig. 3). This difference was evident in all *Calanus* spp. fatty acids combined (Σ 20:1, 22:1, KW–Dunn $p = 0.005$). The 20:1 and 22:1 fatty acids also contributed to the overall patterns and a clear seasonal separation in the *The. libellula* CA (Fig. 4). Permutational test results indicated that only season explained ($p = 0.002$) these patterns. The mean $\delta^{13}C_{FA}$ values of the carnivory marker 18:1(n-9) ranged from −32.5‰ to −28.5‰ between June/July and January with no seasonal differences (Fig. 5). The dinoflagellate-associated 18:4(n-3) had higher $\delta^{13}C_{FA}$ values in January than in June/July (KW–Dunn $p = 0.04$).

Sympagic amphipods

TAG was the dominant lipid class for *Apherusa glacialis* regardless of season with higher proportions in January than in June/July (KW–Dunn $p = 0.02$). Energetic investments into other lipid classes, such as WE and phospholipids (PC), were comparatively low (Fig. 2). The diatom-associated 16:1(n-7) was particularly abundant in *A. glacialis* in both seasons (Fig. 3) with higher contributions in January than in June/July (KW–Dunn $p = 0.003$). In June/July, *A. glacialis* had higher contributions of the diatom-associated 20:5(n-3) and dinoflagellate-associated 18:4(n-3) compared to January (KW–Dunn $p = 0.002$ for both fatty acids). Only one June/July sample had the *Calanus* spp. biomarker 22:1(n-11), while all January *A. glacialis* samples had this fatty acid biomarker. *A. glacialis* also had higher proportions of the carnivory biomarker 18:1(n-9) in January than in June/July (KW–Dunn $p = 0.04$), though proportions were overall low in both seasons. Reflecting the above patterns, the *A. glacialis* CA resulted in clear separation by season along axis 1 (Fig. 4). January *A. glacialis* fatty acid patterns were driven by the contribution of 22:1(n-11). Permutational tests indicated that season ($p = 0.005$) and location ($p = 0.02$) explained *A. glacialis* fatty acid patterns. There were higher mean $\delta^{13}C_{FA}$ values of the two diatom-associated fatty acids and one dinoflagellate-associated fatty acid in June/July than in January (KW–Dunn test results: 16:1(n-7) $p = 0.04$, 20:5(n-3) $p = 0.002$, 22:6(n-3) $p = 0.04$, Fig. 5).

Gammarus wilkitzkii were similar to *A. glacialis* in storing lipids mostly as TAG during both seasons but also—in low

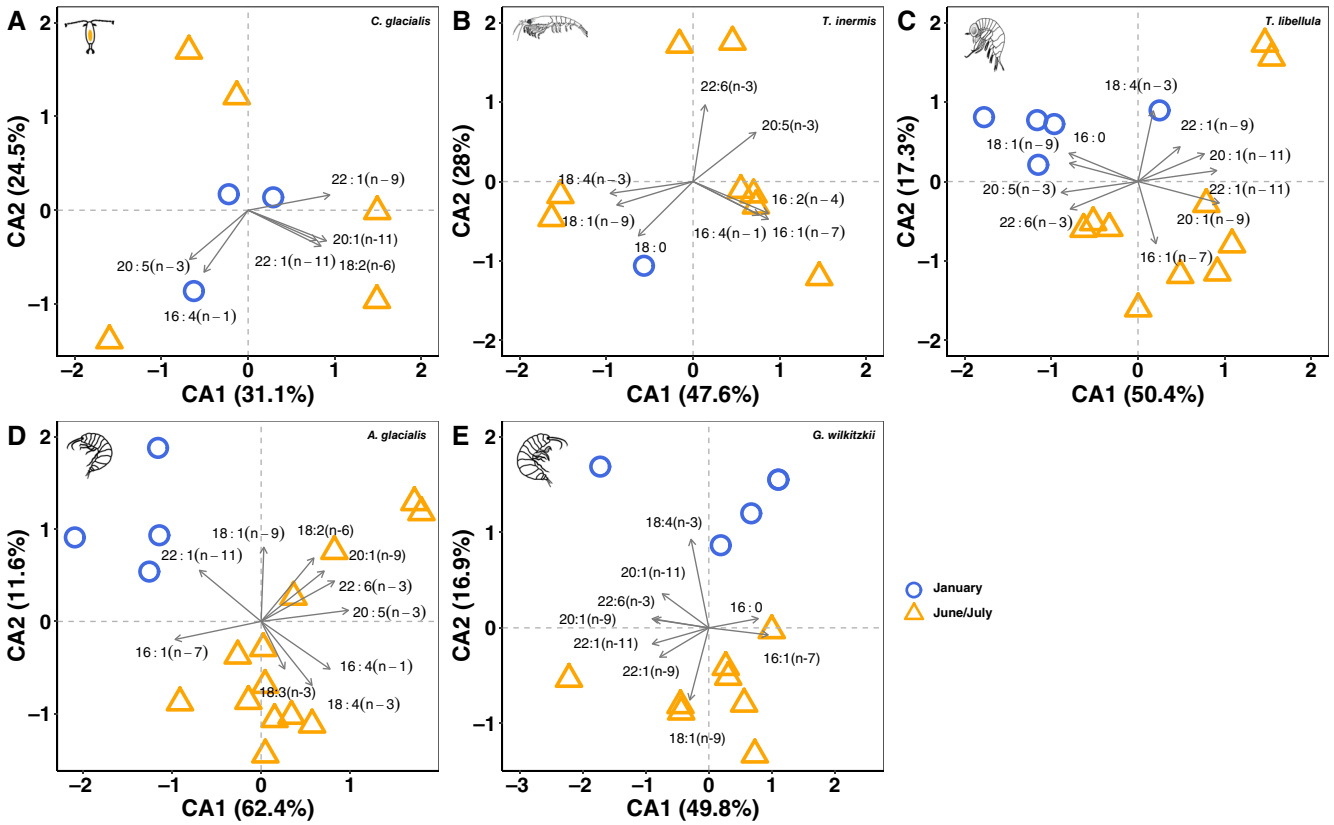


Fig. 4. CA biplots on all fatty acids, displayed for each crustacean species (**A**–**E**), from all stations. Arrows depict directionality of significant and trophic biomarker fatty acids (*see* Table 2 for descriptions) in the ordination plane. Shapes and colors indicate time of year: blue circles—January, orange triangles—June/July.

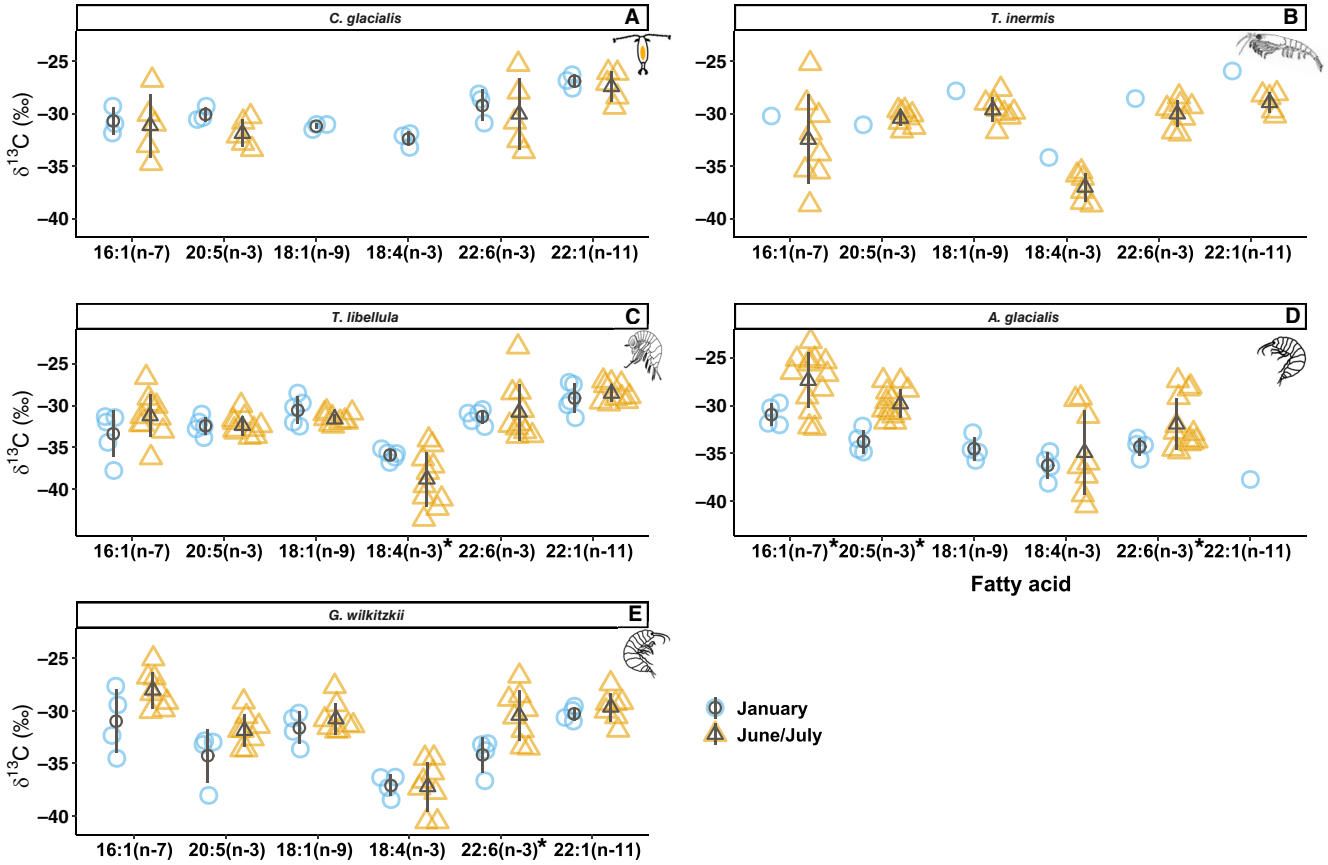


Fig. 5. $\delta^{13}C_{FA}$ values of biomarker fatty acids, organized by crustacean species (**A–E**). Shapes and colors represent samples (note many individuals pooled/sample, *see* Table 2): blue circles, January and orange triangles, June/July. Gray shapes represent means, vertical gray lines represent corresponding standard deviations of the mean. Note that in all fatty acids in January *T. inermis* and 22:1(n-11) in January *Apherusa glacialis* lack corresponding means and standard deviations (gray shapes/lines) because there was only one sample (of pooled individuals; Table 2). Asterisk indicates statistical differences.

proportions—as WE and phospholipids (Fig. 2; Supporting Information Fig. S1). We found no differences in lipid class composition between January and June/July. Relative contributions of seasonal fatty acids were highest for 16:1(n-7) and 18:1(n-9) (Fig. 3). January *G. wilkitzkii* accumulated higher proportions of the dinoflagellate-associated 18:4 (n-3) compared to June/July (KW–Dunn $p = 0.003$, Fig. 3), which also contributed to its seasonal separation in fatty acids (Fig. 4). June/July *G. wilkitzkii* accumulated higher amounts of the carnivory biomarker 18:1(n-9) (KW–Dunn $p = 0.02$), and was further characterized by zooplankton feeding, indicated by the contributions of the 20:1 and 22:1 monounsaturated fatty acids (MUFA) along with the other carnivory biomarker 16:0 (Figs. 3 and 4). Although a seasonal separation along axis 2 in the *G. wilkitzkii* CA was evident, permutational tests indicated that none of the environmental variables were significant. *G. wilkitzkii* had higher mean $\delta^{13}C_{FA}$ values of the dinoflagellate-associated 22:6(n-3) in June/July (−30.4‰) than in January (−34.2‰, KW–Dunn $p = 0.02$). There were no seasonal differences in the $\delta^{13}C_{FA}$ values for the other diatom markers, 16:1(n-7) and 20:5(n-3) (Fig. 5).

Discussion

Flexible feeding strategies—*C. glacialis*, *T. inermis*, *A. glacialis*

C. glacialis, *T. inermis*, and *A. glacialis* have been commonly considered to be primarily herbivorous, largely relying on the productive seasons (sympagic and pelagic) during spring and summer. In contrast, our results rather suggest flexible feeding strategies. Overwintering lipid storage patterns, coupled with their seasonal fatty acid patterns, indicate that these species transitioned into omnivory during polar night. The $\delta^{13}C_{FA}$ values of biomarker fatty acids of diatoms and dinoflagellates in *C. glacialis*, *T. inermis*, and *A. glacialis* suggest a mixed contribution of ice-algal and phytoplankton carbon in the studied seasons.

C. glacialis followed previously reported trends (reviewed in Berge et al. 2020), accumulating high amounts of WE in order

to survive food-limited periods. However, the total lipids per dry mass in the January-sampled *C. glacialis* were in fact considered lipid poor (Hirche and Kattner 1993). Low lipid levels in winter *Calanus* spp. are, however, not unusual as a large WE pool might instead be allocated for early gonad development and/or molting preparation (Hirche and Kattner 1993; Freese et al. 2017). Low lipid levels can also indicate surface-dwelling, non-diapausing individuals within our study area (Daase and Søreide 2021), suggesting that *Calanus* spp. that lack adequate lipid stores to overwinter would then remain active near the surface to feed or anticipate the phytoplankton spring bloom (Espinasse et al. 2022).

Throughout the productive season, *T. inermis* converts large portions of their consumed diet into storage lipids, mainly WE and TAG (Falk-Petersen et al. 2000). We instead found that *T. inermis* had higher concentrations of PC (a membrane phospholipid) in both seasons compared to the other species in our study (Supporting Information Fig. S1), and similar to the Antarctic euphausiids (Hagen and Auel 2001). Total lipids/dry mass was quite low compared to the other studied species, as *T. inermis* can drastically reduce their lipid stores in winter (Falk-Petersen et al. 2000). Euphausiids have the capacity to cyclically shrink their bodies and sexually regress due to food limitations or as an overwintering survival mechanism (Kawaguchi et al. 2007).

January *A. glacialis* had lipid levels comparable to deep overwintering *Calanus* spp. (Falk-Petersen et al. 2009), and similar total lipids/dry mass to previously sampled January *A. glacialis* at depth (Berge et al. 2012), potentially aiding *A. glacialis* in neutral buoyancy at depth (Campbell and Dower 2003). We conclude *A. glacialis* has adequate lipid stores to perform seasonal vertical migrations as recently suggested (Berge et al. 2012). Compared to *C. glacialis* and *T. inermis*, *A. glacialis* had high TAG deposits, particularly in January, indicative of continued feeding activity during a food-limited period.

In *Calanus* spp., saturated fatty acids are the main dietary precursors of the de novo synthesized 20:1 and 22:1 fatty acids and alcohols, which provide high amounts of energy within WE (Kattner and Hagen 1995). The significantly higher contributions of the dietary precursors 16:0 and 18:0 in January than June–July *C. glacialis* suggests that this lipid synthesis pathway was most likely active during polar night. As fatty acid assimilation time in *Calanus* spp. ranges between days to weeks (Graeve et al. 2005), we suggest that January *C. glacialis* was omnivorous, based on the significantly higher proportions of the carnivory biomarker 18:1(n-9) and saturated fatty acids (16:0 and 18:0) during polar night. Our data clearly indicate that the concept of diapause at depth should be replaced with a flexible overwintering mode, as *C. glacialis* occurs throughout the water column and not only at depth in winter (Berge et al. 2020). Furthermore, *C. glacialis* can be rather carnivorous outside the spring bloom, feeding on metazoan prey (Cleary et al. 2017). Because calanoid copepods lack the enzymes needed to bioconvert 18:3(n-3) into polyunsaturated fatty acids (Bell and Toucher 2009), we assume that the diatom- and dinoflagellate-associated polyunsaturated fatty acids and their carbon content all stem from dietary sources for *C. glacialis*. Both in our study area and in other parts of the Arctic, the $\delta^{13}C_{FA}$ values of marker fatty acids of diatoms and dinoflagellates are higher in sea ice-associated particulate organic matter (iPOM) than in phytoplankton POM (pPOM) (Wang et al. 2014; Kohlbach et al. 2016). During June/July 2017 in our study area, the mean $\delta^{13}C_{FA}$ value of 20:5(n-3) in iPOM (−27.4‰) was higher than for pPOM (−35.2‰; Kunisch et al. 2021). A value of 31.9‰ in June/July *C. glacialis* suggests a mixed diet consisting of both pelagic and sympagic carbon sources (Kohlbach et al. 2016).

We found that *T. inermis* overwinters on a combination of stored lipids and alternative food sources, supporting previous winter studies (Huenerlage et al. 2015). Depending on season and body size, *T. inermis* are opportunistic foragers, acting as omnivores–carnivores (Huenerlage et al. 2015). The January data indicated opportunistic omnivory, with the presence of the *Calanus* spp. fatty acids and the carnivory biomarker 18:1 (n-9), and further supported by the insignificance of the tested environmental variables on fatty acid composition. The wide range of $\delta^{13}C_{FA}$ values in 16:1(n-7) during June/July, along with the year-round presence of the *Calanus* biomarker 22:1 (n-11), is suggestive of seasonally varying carbon sources for *T. inermis*.

Previous winter studies on ice-associated *A. glacialis* diet found high contributions of the diatom-associated 16:1(n-7) (37%, Werner and Auel 2005). We instead found relatively higher contributions of 16:1(n-7) in the January pelagic *A. glacialis*. Fatty acid turnover for *A. glacialis* is unknown, but in a benthic freshwater gammarid amphipod, *Pallaseopsis quadrispinosa*, turnover for 16:1(n-7) was higher than for other fatty acids, suggesting a rapid assimilation of this fatty acid from a diatom-fed diet (Taipale et al. 2021). During November/December in the northern Barents Sea, 16:1(n-7) proportion was in fact low (mean, 7%) in both iPOM and pPOM (Kohlbach et al. 2021). However, 16:0 in iPOM and pPOM had similar mean proportions in November/December (24–26%) when compared to June/July iPOM (34%) and pPOM (26%) (Kohlbach et al. 2021; Kunisch et al. 2021). 16:0—a fatty acid precursor in the diatom C16 pathway—from algal diet can be biosynthesized into 16:1(n-7) in *Calanus* copepods (Dalsgaard et al. 2003). Thus, we cannot rule out that this pathway exists for other primarily herbivorous species, and that *A. glacialis* fed on pelagic diatoms during the polar night, though the overall abundance of pelagic diatoms is low at that time (Berge et al. 2015). Ice-associated *A. glacialis* are also classified as detrivorous, ingesting crustacean remains (Poltermann 2001). We found comparably small, seasonal contributions of the *Calanus* spp. biomarkers (20:1 and 22:1 MUFA) in *A. glacialis* diet. In our study area, there were relatively high amounts of dead copepods in the water column in

January (Daase and Søreide 2021), potentially serving as a food source for *A. glacialis* during polar night. Season and location were significant environmental variables explaining *A. glacialis* fatty acid composition, indicating that differences in habitat influenced their seasonal diet. The seasonal differences in the $\delta^{13}C_{FA}$ values of the diatom- and dinoflagellate-associated fatty acids in *A. glacialis* suggests a mixed contribution of both ice-algal and phytoplankton carbon. The variation in the June/July $\delta^{13}C_{FA}$ values could be attributed to sampling *A. glacialis* earlier and in prebloom situations, further demonstrating some degree of dietary plasticity before the onset of sea-ice algal blooms. However, June/July *A. glacialis* sampled from sea ice had the highest $\delta^{13}C_{FA}$ values in the diatom marker 16:1(n-7), suggesting an elevated contribution of ice algal carbon to their diet. The presence of 22:1(n-11) $\delta^{13}C_{FA}$ in January *A. glacialis* is suggestive of *Calanus* spp. carbon supporting the flexible overwintering strategies of these species. We conclude that the sea-ice habitat seasonally supports *A. glacialis*, while the polar night food web (in addition to adequate lipid stores) could also support a more active overwintering strategy in the pelagic realm (Kunisch et al. 2020).

Business-as-usual—*The. libellula*, *G. wilkitzkii*

The wide range in the relative proportions of WE and TAG for *T. libellula* demonstrates that this opportunistic carnivore has some degree of dietary plasticity at the individual level. WE are a major lipid class of *T. libellula* during winter, comprising ≥ 77% of their body mass (Kraft et al. 2015) indicative of their WE-rich *Calanus* spp. prey. January *T. libellula* in our study had considerably fewer WE (31%) and a higher proportion of TAG (51%) indicating prey with year-round feeding activity (Lee et al. 2006). This could also explain the overlap of lipid composition between *T. libellula* and *G. wilkitzkii* (Supporting Information Fig. S1), further supporting the previously stated trophodynamic similarities between these species (Auel and Werner 2003). The range of total lipids/dry mass in January *G. wilkitzkii* is suggestive of continuous feeding activity for those individuals found in open water. Similar to *T. libellula*, *G. wilkitzkii* had similar proportions of WE throughout the year, which is again attributed to the consumption of *Calanus* copepods rather than lipid storage (Scott et al. 2001). With relatively high investments into TAG during both sampling periods, we suggest year-round feeding activity for *G. wilkitzkii*, regardless of habitat.

T. libellula fatty acid composition was influenced by season, similar to previous findings (Mayzaud and Boutoute 2015). The diatom-associated 16:1(n-7) in June/July diet points towards consumption of herbivorous grazers (Dalpadado et al. 2008). In January, diatom- and dinoflagellate-associated fatty acids were also present in *T. libellula*—along with lower amounts of 22:1(n-11), the *Calanus* spp. biomarker, and higher amounts of 18:1(n-9), the carnivory biomarker. Similar to the lipid results, the January fatty acid findings could be indicative of other herbivorous prey species (instead of *Calanus* spp.) available in our study area. This is supported by the seasonal differences in the $\delta^{13}C_{FA}$ values of diatom- and dinoflagellate-associated fatty acids in *T. libellula*, suggestive of seasonally varying carbon sources in their consumed herbivorous zooplankton prey. At the same time, *Calanus* spp. fatty acids were still present in *T. libellula*, demonstrating the importance of *Calanus* copepods as an important food source throughout the year (Dalpadado et al. 2008; Kraft et al. 2013).

The insignificant seasonal effects on *G. wilkitzkii* fatty acid composition supports our lipid class findings. Toward the end of winter (March/April) the decrease in total lipids was associated with an increase in the relative amount of the carnivory marker 18:1(n-9) (Werner and Auel 2005). In our study, January and June/July *G. wilkitzkii* had similar amounts of diatom-associated fatty acids, with lower amounts of 18:1(n-9) in January *G. wilkitzkii*. These differences could be attributed to sampling times, as we sampled *G. wilkitzkii* much earlier in the winter (January), compared to Werner and Auel (2005). Our June/July fatty acid composition findings are comparative to previous in-ice studies, in that *G. wilkitzkii* assimilated diatom-associated fatty acids along with high contributions of 18:1(n-9) (Scott et al. 1999). Ice-associated *G. wilkitzkii* has a broad diet (Poltermann 2001), including the direct ingestion of microalgae, partially explaining the presence of diatom- and dinoflagellate-associated fatty acids (Scott et al. 2001; Werner and Auel 2005). The presence of *Calanus* spp. biomarkers in *G. wilkitzkii* in our study, however, is evidence for copepod consumption throughout the year. There were significantly higher $\delta^{13}C_{FA}$ values in 22:6(n-3) in June/July than in January, demonstrating the differences in organic source material (sea-ice-derived carbon when found with the sea-ice habitat during June/July). However, seasonal differences in the other diatom- and dinoflagellate-associated $\delta^{13}C_{FA}$ values were not as pronounced in *G. wilkitzkii* (when compared to the other sea-ice amphipod, *A. glacialis*). Our findings further support a business-as-usual strategy for *G. wilkitzkii*, even when found away from the sea-ice habitat.

Limitations and knowledge gaps of trophic marker approaches

There are limitations to interspecific comparisons in our trophic biomarker approach. *Calanus* spp. are unique in that they can elongate saturated fatty acids into MUFA (e.g., 14:0 into 20:1), as well as elongate 16:1(n-7) into 22:1 (Dalsgaard et al. 2003). Diatom- and dinoflagellate-associated fatty acids in *T. libellula* diet are assumed to be routed through prey, while *G. wilkitzkii* are thought to directly ingest microalgae even though they are mostly carnivorous (Poltermann 2001). We therefore largely limited our comparisons of seasonal carbon pathways ($\delta^{13}C_{FA}$ values) primarily within species. Recent genetic work has found that in fact many marine invertebrates have the ability to de novo synthesize *n*-3 Polyunsaturated fatty acids (PUFA, also known as omega-3 fatty acids),

challenging the trophic marker concept (although no calanoid copepods, euphausiids or amphipods were included in the study; Kabeya et al. 2018). In our study, *n*-3 PUFA [18:4(n-3), 20:5(n-3), 22:6(n-3)] were assumed to originate from primary producers and passed onto consumers unmodified. It remains unknown how much *n*-3 PUFA could be biosynthesized as opposed to assimilated in our study species. Studies using essential amino acid signatures and fatty acid studies incorporating molecular analysis, in addition to tracer-based experiments, could provide insight into the dietary-driven strategies of zooplankton species found in seasonally disparate environments.

Spatial variability in the $\delta^{13}C_{FA}$ values of carbon end members iPOM and pPOM in June/July 2017 (Kunisch et al. 2021) likely affect our comparisons of these values as carbon baselines to consumer zooplankton and sympagic amphipods. Because we sampled in pre-bloom situations in June/July, it is possible that the $\delta^{13}C_{FA}$ values in zooplankton and sympagic amphipods still reflected a late-winter signal. Although carbon turnover rates remain unknown in our species, isotopic turnover (the time it takes for stable isotopes in tissues to be replaced by stable isotopes from diet) in bulk $\delta^{13}C$ can be up to 77 d for coastal Arctic amphipods during winter and a third of that in spring (Kaufman et al. 2008). If such slow turnover had occurred in our studied species, our January $\delta^{13}C_{FA}$ values could still include signatures from a late autumn phytoplankton bloom. Yet even if that were to be the case, we argue that our seasonal comparisons are still valid since we essentially are comparing feeding activities between high- and low-productive periods.

It is important to acknowledge that $\delta^{13}C_{FA}$ values can be affected by metabolic processes, lipid dynamics, and—largely unquantified—isotopic fractionation (Bec et al. 2011). Comparing consumer $\delta^{13}C_{FA}$ values to baseline POM sources assumes isotopic equilibriums within species and between seasons. However, the overall influences of these processes in our study species are unknown and many processes likely affect the $\delta^{13}C_{FA}$ values, as zooplankton and sympagic amphipods all possess unique traits (such as partially surviving off stored energy pools) to survive periods of low food availability. Combinations of multiple independent trophic biomarkers such as essential amino acid carbon isotopic fingerprints may be used to constrain assumptions about the susceptibility of trophic biomarkers to these variations. For example, the general patterns in the importance of sympagic and pelagic diatoms and dinoflagellates for June/July *A. glacialis* was confirmed in a study using the ^{13}C signature of essential amino acids and demonstrated that the under-ice diatom, *Melosira arctica*, could have significantly contributed to the diatom-associated fatty acid signal (Vane et al. 2023).

Seasonal food web drivers—Considerations and recommendations

Our findings suggest overwintering on a combination of both stored energy reserves and food intake in our study species. We found that for *C. glacialis*, *T. inermis*, and *G. wilkitzkii*, certain environmental factors such as season or water mass did not matter in terms of their fatty acid composition throughout the year. However, for other species, there were both seasonal (*T. libellula* and *A. glacialis*) and location (*A. glacialis*) differences in their fatty acid composition. Based on what is known on the overwintering strategies of these studied species, we find a broad flexibility for ice-associated amphipods in the absence of presumed winter sea-ice habitat, and for *C. glacialis*, opportunistic feeding during winter.

The key to making opportunistic polar night feeding possible for zooplankton and sympagic amphipods may lie in the advective nature in our study area. The Atlantic water inflow (via the deep Fram Strait) serves as a large source of nutrients and zooplankton biomass throughout the year (Codispoti et al. 2013; Basedow et al. 2018). Although some of the sampled species were collected directly under-ice, Atlantic water is a predominate feature within much of our study area (Rudels et al. 2000). In January 2018, water mass characteristics near our sampling areas in the Barents Sea were likely influenced by the polar front, a region where Atlantic water and Arctic water mix (Oziel et al. 2016). These oceanographic settings suggest relatively higher quantities of food for all the species in our study area than elsewhere in the Arctic, though questions remain on the quality of food for herbivorous species, where higher quality foods (omega-3 rich) generally enhance individual fitness. Furthermore, food quality can change $\delta^{13}C_{FA}$ values, linked to the isotopic turnover of lipids (Chamberlain et al. 2006).

The microbial loop has the potential to sustain polar food webs during winter (Manganelli et al. 2009), as it plays a fundamental role in the uptake and reworking of dissolved organic carbon (Calleja et al. 2013). Bacterial inputs to zooplankton and sympagic amphipods were only somewhat resolved in our study, as there was evidence of bacterial fatty acids in all our studied species (Supporting Information Fig. S2). Although contributions of these bacterial biomarkers were low (≤ 3%), we found seasonal fluctuations in their $\delta^{13}C_{FA}$ values. Seasonal fluctuations suggest differential carbon sources, unknown metabolic effects within our studied species, or both. Despite the low abundance of bacterial markers, we were able to determine corresponding $\delta^{13}C_{FA}$ values demonstrating the utility of combining biomarker approaches to track bacterial inputs to the food web even at relatively low contributions.

Conclusions

We found evidence of polar night feeding activity in all studied species, regardless of dietary differences or presumed overwintering strategies. Based on our findings, we infer a more flexible overwintering strategy for the primarily herbivorous *C. glacialis* and *A. glacialis* in the Atlantic water inflow area than previously assumed, and that pelagic *G. wilkitzkii*

has a business-as-usual strategy during polar night. For *A. glacialis*, we confirm a seasonal variation in their $\delta^{13}C_{FA}$ values of diatom- and dinoflagellate-biomarker fatty acids, suggesting that dietary fatty acids reflect the variability of primary producer communities in both pelagic and sympagic habitats. Furthermore, we confirm that *Calanus* spp. also functions as a carbon source (via the presence of *Calanus* spp. trophic biomarkers) supporting all other species in our study, regardless of dietary preferences. The notion of polar night as a dormant phase should be phased out, at least in the Atlantic inflow region to the Arctic.

Data availability statement

The full dataset can be found in the data repository PANGAEA.

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Conflict of Interest

None declared.

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