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Antarctic harpacticoids exploit different trophic niches: a summer snapshot using fatty acid trophic markers (Potter Cove, King George Island)

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ABSTRACT: Unraveling food webs is a first step toward understanding of ecosystem functioning and a requirement to forecast climate-induced ecosystem responses. In this study, the organisms under examination were benthic copepods (order Harpacticoida) inhabiting a fjord-like environment on the southern coastline of King George Island at the northwestern tip of the Antarctic Peninsula, one of the most rapidly warming regions on Earth. Despite increased understanding of Antarctic food web structures, little is known about the feeding ecology of benthic copepods in these systems. A fatty acid trophic marker strategy was used to unravel the diet composition of Antarctic harpacticoid copepod species or assemblages collected from distinct habitats in summer. Their diverse storage fatty acid composition revealed the occupation of different trophic niches associated with their specific lifestyles, i.e. endobenthic or epiphytic with (*Alteutha* spp.) or without (*Harpacticus* sp.) frequent water column excursions. Moreover, the prevalence of biosynthesized ω7 long-chain monounsaturated fatty acids in *Harpacticus* sp. and ω9 fatty acids in *Alteutha* spp. further suggested adaptations to particular habitats in polar ecosystems, as different dietary precursors — 16:1ω7 (microphytobenthos, epiphytic diatoms) or 18:1ω9 (flagellates) — fuel these elongation pathways.

KEY WORDS: Copepods · Diet · Storage lipids · Epiphytic · Polar

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INTRODUCTION

Knowledge of trophic relationships among species is required to understand ecosystem functioning, biochemical fluxes and their changes under climatic warming (Clarke et al. 2012). This is especially true for the Antarctic Peninsula, which is one of the most rapidly warming regions on Earth, having experienced a 2°C increase in the annual mean temperature and a 6°C rise in the mean winter temperature since 1950 (Turner et al. 2005, Ducklow et al. 2007). Previous efforts to unravel trophic interactions have focused on Antarctic krill *Euphausia superba*, a keystone species in the Southern Ocean (Falk-Petersen et al. 2000, Schmidt et al. 2003, Stübing et al. 2003), and on Amphipoda (Graeve et al. 2001, Nelson et al. 2001, Nyssen 2005). Although the energy flow in summer tends to be channeled through krill, other branches at the basis of the food web deserve more investigation with respect to their feeding ecology, as they have an important role in stabilizing communities. For example, the degree of omnivory, feeding on more than one trophic level, exerts a strong effect on ecosystem functioning and stability (Clarke et al. 2012). One of these branches includes copepod species belonging to the order Harpacticoida (Copepoda). So far, their feeding ecology in Antarctic food webs has not received much attention and there is a

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tendency to lump the different species into one group. This low resolution may underestimate the actual food web complexity as polar harpacticoid copepods, in parallel with their temperate and tropical relatives, likely show differences in life-history strategies, habitat utilization and trophic niches (De Troch et al. 2005, 2008).

Climate-induced diet shifts or alterations in feeding ecology may be caused by direct changes of the food sources or by indirect effects. Direct changes in primary production have been reported along the western Antarctic Peninsula (WAP), with a decline in summer chlorophyll *a* and a shift in primary production farther south (Montes-Hugo et al. 2009), although local differences have also been observed (Prézelin et al. 2004, Schloss et al. 2012). Decreased sea-ice cover allows an increase in the deepening of the summer wind-mixed layer, which has resulted in decreased production with a shift in balance from diatoms to smaller flagellates (Walsh et al. 2001). Enhanced stability of the water column due to increased freshwater input may stimulate primary productivity. Recently, at King George Island, large phytoplankton blooms have been reported in areas with a historically low biomass (Schloss et al. 2014). In contrast, the associated sediment-loaded glacial runoff may increase turbidity in the upper water column with negative effects on phytoplankton development (Klöser et al. 1994). Additional indirect alterations associated with the loss of sea-ice habitat include the loss of its high standing stocks of bacteria, protists and invertebrates and its link to higher trophic levels (Arndt & Swadling 2006). Moreover, the thick macroalgal forests that dominate the sub- and intertidal hard bottom communities along the WAP are highly influenced by climate-induced alterations, i.e. increased temperatures, glacial retreat, ice scouring and changes in light availability (Zacher et al. 2009, Quartino et al. 2013, Deregibus et al. 2016). Alterations in their primary productivity may affect the entire coastal food web (Deregibus et al. 2016), considering their central importance as a refuge for associated fauna (Huang et al. 2006), their food value for herbivores and benthic organisms (Graeve et al. 2002, Campana et al. 2009), and contribution to the particulate and dissolved organic matter in coastal food webs (Iken et al. 2011, Quartino et al. 2013).

This study primarily aimed to identify the diet composition of harpacticoid copepod species or assemblages from different habitats (epiphytic and endobenthic) in the coastal environment of an Antarctic island (Potter Cove, King George Island) during summer. Second, a feeding experiment was performed to elucidate the role of resource (diatom) availability in the copepods' response to elevated temperature as indicated by its energy balance, i.e. the dynamics of membrane and storage lipids. Diatoms are most efficiently grazed upon by first-level consumers in Antarctic food webs, likely due to their optimal size range (Moline et al. 2004), and represent the most efficient pathways for carbon transfer to higher trophic levels, resulting in short food chains typical for polar oceans (Moline et al. 2004, Kattner & Hagen 2009).

To unravel the feeding ecology, fatty acids (FAs) were used as dietary tracers, i.e. the so-called fatty acid trophic marker (FATM) approach (Dalsgaard et al. 2003). Most FAs are of dietary origin and are incorporated, largely unmodified, into the consumer's lipid pool. Screening of storage FA composition is accurate for diet untangling (Fraser et al. 1989, Dalsgaard et al. 2003), while membrane and storage FAs in copepods, especially with respect to the presence of the essential FAs eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3), may provide information on their fitness and nutritive value for higher trophic levels.

In addition to FA tracers, the consumer's bulk carbon isotopic signature also informs on the nature of the food sources (Fry 2006). In contrast to FAs, which can be preferentially assimilated and rapidly stored, the stable isotope composition typically integrates over long timescales (Schmidt et al. 2003) and was therefore used as a complementary tracer in this study. In the feeding experiment, ^{13}C prelabeled diatoms were used to estimate the amount of carbon assimilated by the consumer and thus to monitor the carbon flow from food to copepod under various diet and temperature treatments.

In summary, we aimed to resolve the following questions: (1) Do harpacticoid copepods occupy different trophic niches as inferred from their storage FA composition? (2) How important is the diet (diatom spp. composition) for temperature acclimatization?

MATERIALS AND METHODS

Diatom culturing

The diatom strains were obtained from the diatom culture collection (BCCM/DCG) of the Belgian Coordinated Collection of Micro-organisms (http://bccm.belspo.be; accession numbers DCG 0485 [*Navicula*

sp.], DCG 0421 [*Nitzschia* sp.] and DCG 0423 [*Cylindrotheca fusiformis*], hereafter referred to as *Navicula*, *Nitzschia* and *Cylindrotheca*). The diatoms were non-axenically grown to higher densities in filtered (0.5 µm) and autoclaved natural seawater (salinity 30) supplemented with f/2 medium (Guillard 1975) at 15 ± 1°C under a 12:12 h light:dark period (25–50 µmol photons m^{-2} s^{-1}). All diatoms were labeled with ^{13}C by adding 16.8 mg $NaH^{13}CO_3$ (99%, ^{13}C, Cambridge Isotope Laboratories) per 100 ml of culture medium and were harvested after 15 d of growth (exponential growth phase). Centrifugation removed the ^{13}C labeled supernatants from the cultures. After 3 h storage at −80°C, 3 washing cycles with MilliQ water and subsequent centrifugation removed the remaining ^{13}C and salts from the cultures. After freeze-drying the diatoms, triplicate samples for later bulk stable isotope and FA profiling were stored at −20°C and −80°C, respectively. The atomic% ^{13}C of the freeze-dried diatoms increased to 25.4 ± 0.9%, 26.9 ± 0.2% and up to 38.8 ± 0.5% for *Navicula*, *Nitzschia* and *Cylindrotheca*, respectively. Aliquots of 20 mg freeze-dried *Navicula*, *Nitzschia* and *Cylindrotheca* were prepared for later use in the feeding experiment (mono diet), while the aliquots for the mixed diet treatments contained approximately 7 mg of each diatom species.

Copepod collection

The macroalgae-associated harpacticoid community was sampled on the intertidal rocky shore of Potter Península (Peñón Uno), 25 de Mayo/King George Island, South Shetland Islands, Antarctica (62° 15′ S, 58° 41′ W) during low tide (2–3 January 2014). Macroalgae, including *Phaeurus antarcticus*, *Desmarestia menziesii* and *Ascoseira mirabilis*, were collected from the lower tidal pools (6.5 ± 0.6°C and a salinity of 32 *in situ*) and thoroughly rinsed in a bucket of filtered seawater (100 µm). Subsequent sieving (250 µm) of the water concentrated the epiphytic harpacticoid copepods, while the residual water, containing macroalgal epibiota, was filtered through a GF/F filter (Whatmann, pre-combusted at 450°C) for later FA analysis.

The harpacticoid community was predominantly *Alteutha potter* (Peltidiidae family) (described by Veit-Köhler & Fuentes 2007), characterized by different developmental stages (Table 1), and copepods of the genus *Harpacticus*. Additionally, a larger-sized but less abundant *Alteutha* sp. was present. Triplicate samples of *A. potter*, *Harpacticus* sp. (both 80–100 ind. $sample^{-1}$) and duplicates of *Alteutha* sp. (20 ind. $sample^{-1}$) were washed in filtered seawater (0.20 µm) and stored at −20°C for later lipid fractionation and FA screening. Moreover, triplicate samples of *A. potter* and *Harpacticus* sp. were stored at −20°C for bulk stable isotope (SI) analysis (20 ind. $sample^{-1}$), with separate analysis of adult *Harpacticus* sp. females and their egg sacs (minimum 30). In addition, the sediment-associated harpacticoid community (interstitial, burrowing and epibenthic species) was extracted from sediment samples, collected at the entrance of Potter Cove perpendicular to Punta Elefante at a depth of 16 m using Van Veen grabs (cf. sampling location in Veit-Köhler 2005). As species-specific abundances appeared too low for FA analysis, the collected specimens were pooled into one replicate 'endobenthic' harpacticoid copepod sample.

Table 1. Life stages of the *Alteutha potter* population at the moment of sampling, expressed as relative abundance (%), based on a subsample of 80 randomly picked individuals

Life stage	%
CII	7
CIII	9
CIV	15
Adult ♂	40
Adult ♀	29

Feeding experiment

Tissue culture flasks contained 80 to 100 *A. potter* individuals incubated in 500 ml filtered seawater (0.7 µm), collected from the shoreline. Copepods in the mono diet treatments were offered ^{13}C-labeled and freeze-dried *Navicula*, *Nitzschia* and *Cylindrotheca* diatom cells as a single food source, standardized towards weight in the mixed diet treatments. Food quantity in each experimental unit was considered to be ad libitum (20 mg $unit^{-1}$). These diet series were incubated outdoors in a water tank under the Dallmann laboratory at Carlini station (Potter Cove, King George Island) research station (sheltered conditions) and indoors in a climate room where the temperature was on average 2°C higher. Temperature was recorded daily around midday and the experiment was terminated after 10 d. Copepods were sorted to assess mortality. The surviving individuals were washed in filtered seawater (0.20 µm), left for 6 h at their respective temperature to allow gut clearance and stored at −20°C for bulk SI analysis (20 ind.

sample^{-1}), and lipid fractionation and FA analysis (minimum 24 ind. sample^{-1}). In parallel with the feeding experiment, in- and outdoor control treatments contained the ^{13}C-enriched freeze-dried food sources in filtered seawater (0.7 µm), to test for potential ^{13}C leakages during the experiment. After 10 d of incubation, these algal residues were stored at −20°C for bulk SI analysis.

Bulk SI analysis

The carbon SI ratios and carbon content of copepods and diatoms were determined in 3 biological replicates for each treatment, using an isotope ratio mass spectrometer (type Europa Integra) at the Davis Stable Isotope Facility (University of California, USA). Internal reference materials were calibrated against the international reference material USGS-41, with a long-term standard deviation of 0.2‰ for ^{13}C. Stable isotope ratios are given in the δ notation with Vienna PeeDee Belemnite (VPDB) as the reference standard and expressed in units per thousand (‰), according to the standard formula $\delta^{13}C = [(R_{sample} / R_{VPDB}) - 1] \times 10^3$, where R is the ratio of $^{13}C/^{12}C$ and $R_{VPDB} = 0.01118$.

However, the atomic% ^{13}C values were used to calculate the assimilation of freeze-dried diatoms by the copepods. Atomic% ^{13}C values of freeze-dried algae from the control treatments were slightly lower compared with the pre-incubation values, possibly because of ^{13}C leakage over the course of the experiment. To avoid underestimation of the assimilated carbon, % ^{13}C values of the control lines were used when estimating the fraction (f) of copepod carbon derived from the ^{13}C labeled diet:

$$f = \frac{a^{13}C_{cop,treatment} - a^{13}C_{cop,control}}{a^{13}C_{enriched\ food} - a^{13}C_{natural\ food}} \quad (1)$$

where $a^{13}C_{cop,treatment}$, $a^{13}C_{cop,control}$, $a^{13}C_{enriched\ food}$ and $a^{13}C_{natural\ food}$ are the isotopic compositions of copepods fed ^{13}C-enriched food, control copepods, and enriched and not-enriched food, respectively. Multiplication of this fraction with the mean copepod carbon content resulted in the amount of assimilated carbon per individual copepod.

Lipid fractionation, FA derivatization and FA analysis

Details on lipid extraction, fractionation into neutral, glyco- and polar lipids, and FA derivatization have been described previously (Werbrouck et al. 2016a,b). FA analyses were performed using a gas chromatograph (Hewlet Packard 6890N) coupled to a mass spectrometer (HP Agilent 5973) as in De Troch et al. (2012) with fatty acid methyl ester (FAME) 19:0 (Sigma-Aldrich) as the internal standard. The FAMEs were identified by comparing the retention times and mass spectra with authentic standards and mass spectral libraries (Wiley, own library) and analyzed using MSD ChemStation software (Agilent Technologies). Quantification of individual FAMEs was accomplished using a component FAME 37 and bacterial acid methyl ester (BAME) mix (Sigma-Aldrich) and completed with individual FAME standards (Larodan). Shorthand FA notations of the form A:BωX were used, where A represents the number of carbon atoms, B gives the number of double bonds and X gives the position of the first double bond counting from the terminal methyl group. Copepod FA content was standardized per individual, while the FA profiles of the diatoms are presented as relative concentration (%).

Data analyses

A principal coordinate analysis (PCO) based on a Bray-Curtis similarity matrix visualized the relative FA composition of field-collected harpacticoid samples and macroalgal epibiota (overall FA profile) that correlated at least 80% with one of the 2 PCO axes. Furthermore, several biomarker FAs (classes or ratios) were used as dietary tracers (Table 2) and compared among harpacticoid copepod species with 1-way permutational ANOVA (PERMANOVA). All univariate data analyses were based on a Euclidean resemblance matrix.

Data resulting from the feeding experiment, i.e. copepod survival, carbon content and assimilation, membrane and storage FA content, and membrane- and storage-associated EPA and DHA content, were tested with 2-way PERMANOVA tests (effects of temperature and food). Multivariate analysis informed on FA compositional changes in membrane and storage lipids (arcsine square-root transformation of relative FA data). In particular, a non-metric multidimensional scaling method (nMDS) (Bray-Curtis similarity matrix) visualized the samples based on their membrane and storage FA composition and subsequent 2-way ANOSIM tests indicated whether grouping according to temperature and diet was significant. In case of significant grouping, SIMPER identified the FAs contributing

Table 2. Fatty acids (FAs) used as dietary tracers to unravel the trophic niches of *Alteutha* spp., *Harpacticus* sp. and the endobenthic harpacticoid community

FA biomarker	Group	Source
∑bacterial FAs (%)[a]	Bacteria	Dalsgaard et al. (2003)
16:1ω7/16:0	Bacillariophytes	Dalsgaard et al. (2003)
∑C16/∑C18	Bacillariophytes	Dalsgaard et al. (2003), Falk-Petersen et al. (1998)
16:1ω7/18:4ω3	Diatoms ↔ dinoflagellates	Stübing et al. (2003), Graeve et al. (1994a,b)
EPA/DHA	Diatoms ↔ dinoflagellates	Stübing et al. (2003), Graeve et al. (1994b)
18:1ω9 + 18:4ω3 (%)	Prymnesiophytes	Dalsgaard et al. (2003)

[a]i-15:0, a-15:0, 15:0, i-16:0, i-17:0, 17:0, 18:1ω7c

most to the observed differences. In the food diversity experiment with *A. potter*, one replicate sample from the 3°C *Cylindrotheca* treatment (polar fraction) and one replicate from the 3°C *Nitzschia* treatment (neutral fraction) were omitted from the multivariate analyses as they appeared as extreme outliers on the nMDS plots. All the analyses were performed within PRIMER v6 with PERMANOVA add-on software (Clarke & Gorley 2006).

RESULTS

Seasonal snapshot of food preference: FA dietary tracers

The storage FA composition of both species from the genus *Alteutha* clearly differed from the other groups, i.e. *Harpacticus* sp., the endobenthic harpacticoid community and the macroalgae-associated epibiota (based on the full FA profile), as shown by PCO axis 1 (72.6%) (Fig. 1). The FAs that correlated with the genus *Alteutha* were the *Phaeocystis* markers 18:1ω9 and C18 polyunsaturated FAs (PUFAs, 18:2ω6, 18:3ω3 and 18:4ω3), but also 20:1ω9, C20 PUFAs (20:2ω6, 20:3ω3, 20:3ω6, 20:4ω3 and 22:2ω6) and the essential FA 20:4ω6 (ARA). PCO axis 2 (14.2%) separated the endobenthic harpacticoid community mainly based on the saturated FAs (SFAs) 16:0 and 18:0. FAs 16:1ω7 and 16:4ω4 characterized the genus *Harpacticus* while the macroalgal epibiota were specified by 14:0, i-15:0, 16:1ω9 and 20:0.

The proportion of bacterial FA markers was highest in *Harpacticus* sp., followed by the endobenthic harpacticoids, *Alteutha* sp. and *A. potter* (pseudo-$F_{3,5}$ = 458, p < 0.001; all pairwise p < 0.05) (Table 3). Concomitantly, the 16:1ω7/16:0 and ∑C16/∑C18 ratios, characteristic for bacillariophytes, were most pronounced in *Harpacticus* sp., followed by endobenthic harpacticoids and both *Alteutha* spp. (pseudo-$F_{3,5}$ = 433 and 1105 respectively, both p < 0.01; all pairwise p < 0.05). However, the 16:1ω7/18:4ω3 and EPA/DHA

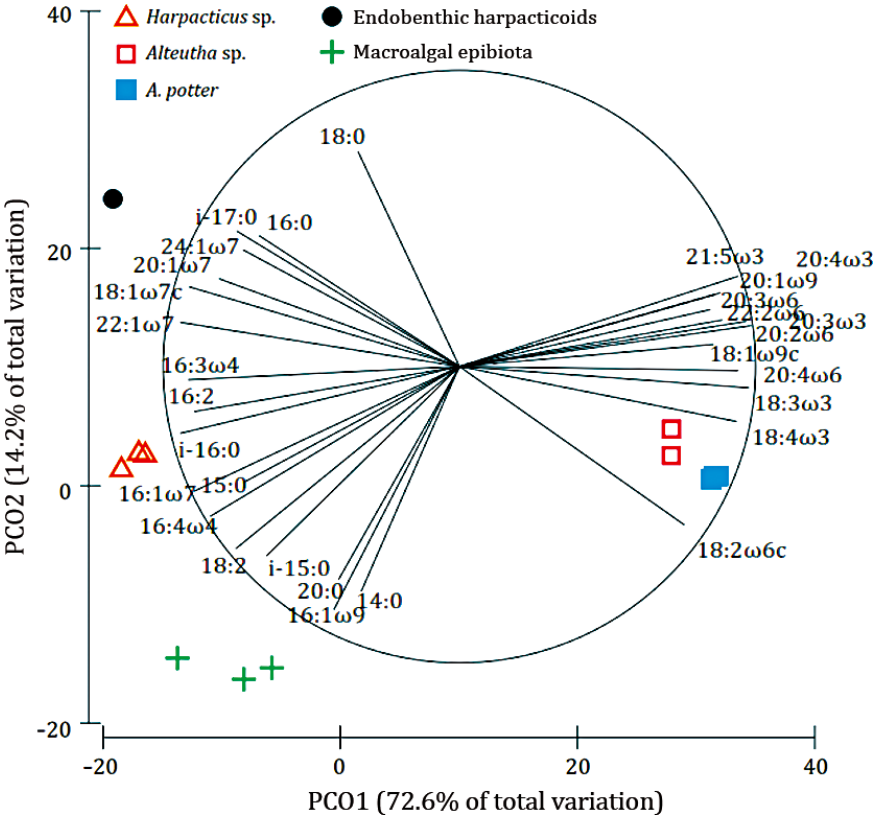

Fig. 1. Principal coordinate (PCO) analysis of the relative storage fatty acid composition of field-collected harpacticoids and macroalgal epibiota (overall profile). Vectors represent specific biomarkers correlating >80% with the first 2 PCO axes

Table 3. Fatty acids (FAs) applied as dietary tracers (mean ± SD) for harpacticoid storage FA composition. Endobenthic refers to the pooled sample of endobenthic harpacticoid copepods

FA biomarker	*Harpacticus* sp.	*Alteutha* sp.	*A. potter*	Endobenthic
∑bacterial FAs (%)	8.1 ± 0.4	2.1 ± 0.3	1.2 ± 0.1	7.7
16:1ω7/16:0	0.9 ± 0.1	0.05 ± 0.003	0.07 ± 0.003	0.3
∑C16/∑C18	1.8 ± 0.1	0.3 ± 0.001	0.2 ± 0.01	1.2
16:1ω7/18:4ω3	2.0 ± 0.2	0.04 ± 0.003	0.03 ± 0.002	5.0
EPA/DHA	2.6 ± 0.1	11.9 ± 0.6	11.2 ± 0.5	2.8
18:1ω9 + 18:4ω3 (%)	6.4 ± 0.3	22.7 ± 0.3	26.6 ± 0.1	4.3

ratios, both indicators for a diatom- versus dinoflagellate-based diet, showed the opposite response. The 16:1ω7/18:4ω3 ratio highlighted a preference for endobenthic harpacticoids and *Harpacticus* sp. for diatoms, while the ratios in both *Alteutha* spp. appeared extremely low (pseudo-$F_{3,5}$ = 772, p < 0.01; pairwise p < 0.05). In contrast, the EPA/DHA ratios were higher in the *Alteutha* spp. compared with the other harpacticoid copepods (pseudo-$F_{3,5}$ = 344, p < 0.01; pairwise p < 0.05). This pattern was attributed to the relatively low abundance of DHA in the storage lipids of *Alteutha* spp. (Table S1 in the Supplement at www.int-res.com/articles/suppl/m568p059_supp.pdf). The contribution of prymnesiophytes as part of the diet (18:1ω9 + 18:4ω3) was in the order *A. potter* > *Alteutha* sp. > *Harpacticus* sp. > endobenthic harpacticoid copepods (pseudo-$F_{3,5}$ = 4886, p < 0.01; all pairwise p < 0.05).

Feeding experiment

Temperature regimes and food characterization

Water temperatures in the tanks fluctuated throughout the experiment and were 3.2 ± 1.2°C versus 0.9 ± 1.8°C in the in- and outdoor tanks, respectively. Details on daily fluctuations are displayed in Fig. S1.

The diatoms used in the feeding experiment clustered significantly per species based on their relative FA composition (ANOSIM, global R = 0.998, p = 0.001; all pairwise R = 1). This indicated that their characteristic FA profiles were preserved after the freeze-drying procedure (Table S2).

Responses of *A. potter* to different temperature and food scenarios

Copepod survival, carbon content and assimilation. Both temperature and food affected survival of *A. potter* (pseudo-$F_{1,22}$ = 33, pseudo-$F_{3,20}$ = 6.6, respectively, both p < 0.01; no interaction effect) (Fig. 2). Incubation at 3°C (93 ± 6%, mean ± SD) promoted higher survival than at 1°C (78 ± 12%). Furthermore, a *Nitzschia* diet resulted in lower copepod survival (76 ± 16%) compared with *Navicula* (92 ± 6%) or *Cylindrotheca* (90 ± 8%) as the sole food source (both pairwise p < 0.01). Copepod carbon content was slightly higher after incubation at 1°C (6.8 ± 0.7 µg C copepod^{-1}, mean ± SD) compared with 3°C (6.2 ± 0.4 µg C copepod^{-1}), but the trend was not significantly different (Fig. 2). The natural $\delta^{13}C$ composition of *A. potter* was more depleted (–31.3 ± 0.1‰) compared with *Harpacticus* sp. (–21.3 ± 0.2‰) and their egg sacs (–20.8‰) and increased only slightly (on average by 120‰) after experimental incubation, indicating low assimilation of the diatoms (Fig. 3B). Carbon assimilation depended only on the offered food type (pseudo-$F_{3,20}$ = 21.6, p < 0.01) and was highest for the *Navicula* diet (0.09 ± 0.02 µg C copepod^{-1}), followed by the mixed diet (0.06 ± 0.01 µg C copepod^{-1}) and the *Nitzschia* diet (0.05 ± 0.01 µg C copepod^{-1}). The lowest assimilation was observed in the treatments with *Cylindrotheca* as the sole food source (0.03 ± 0.004 µg C copepod^{-1}) (all pairwise p < 0.05).

Lipid class dynamics. Regardless of the offered food source, membrane and storage FA contents in *A. potter* declined approximately by factors of 3 and 4, respectively, after 10 d of incubation (Fig. 3A). Glycolipids comprised only a minor proportion of the total FA content (<10%) and were therefore omitted from further analyses. Only temperature affected the mem-

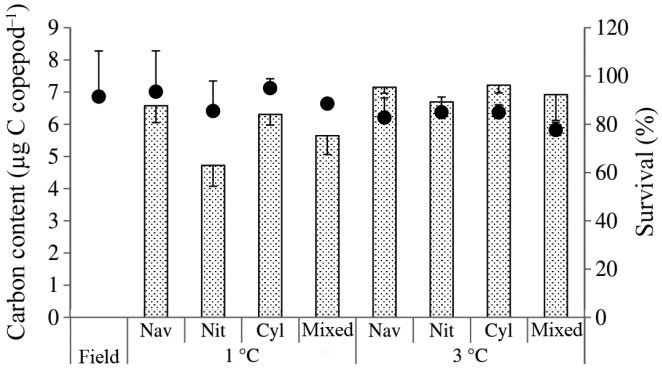

Fig. 2. Carbon content of *Alteutha potter* (µg C copepod^{-1} + SD) (black dots) and survival (% – SD) (bars) collected from the field (Field), and after feeding with *Navicula* (Nav), *Nitzschia* (Nit), *Cylindrotheca* (Cyl) or a mixture of the 3 diatom species (Mixed) at 1°C and 3°C

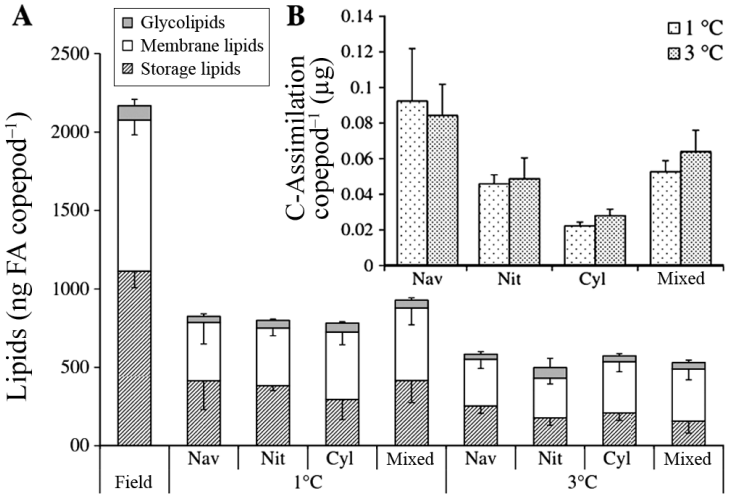

Fig. 3. (A) Lipid profile (ng FA copepod^{-1}) of *Alteutha potter* on treatment with *Navicula* (Nav), *Nitzschia* (Nit), *Cylindrotheca* (Cyl) and a mixture of the 3 diatom species (Mixed) as food at 1°C and 3°C. Data are mean +1 SD for glycolipids and mean −1 SD for membrane and storage lipids. (B) Assimilation of the different diatom species (µg carbon copepod^{-1} + 1 SD) by *A. potter* at 1°C and 3°C

brane and storage FA content (pseudo-$F_{1,22}$ = 9.8 and 18, respectively, both $p < 0.01$) with the strongest reductions observed after incubation at 3°C. The EPA and DHA contents had the same temperature response, regardless of their association with membrane (pseudo-$F_{1,22}$ = 11.7 and 6.2, respectively) or storage lipids (pseudo-$F_{1,22}$ = 20 and 17.5, respectively) (all $p < 0.05$). However, the overall depletion of EPA associated with membrane and storage lipids (by factors of 3.5 and 4, respectively) was stronger compared with DHA (by factors of 2 and 3, respectively) (Fig. S2 in the Supplement).

Membrane and storage FA composition. Temperature and food affected both the membrane (R = 0.929, p = 0.001 and R = 0.635, p = 0.001, respectively) and storage (R = 0.652, p = 0.001 and R = 0.682, p = 0.001, respectively) FA composition of *A. potter* (2-way ANOSIM). The original copepod membrane FA composition altered slightly more after 3°C exposure compared with the 1°C treatments (Fig. 4A). A 1-way ANOSIM for the effect of temperature regime (global R = 0.605, p = 0.001) confirmed this trend based on the higher pairwise R-value detected for the comparison of field versus 3°C (R = 0.976, p = 0.003), compared with field versus 1°C (R = 0.932, p = 0.002). Mainly 16:0, 18:0, EPA and DHA were affected by the temperature regime (SIMPER, Table 4). The initial membrane FA composition altered substantially even in the presence of a mixed food source (Fig. 4A). However, a mixed diet buffered the temperature impact to some extent, as the membrane FA compositions at 1°C and 3°C were more similar for the mixed diet treatments, i.e. pairwise R-value was smallest (R = 0.741 versus 1 for the mono diets). Despite significant changes at the level of the individual FA, the membrane composition remained similar at the FA-class level (Table 4).

Additionally, the original storage FA composition of *A. potter* altered significantly after experimental incubation, but no obvious grouping according to the temperature regime emerged (Fig. 4B). A mixed diet dampened temperature-induced changes in storage FA composition, but only to a minor extent (R = 0.222

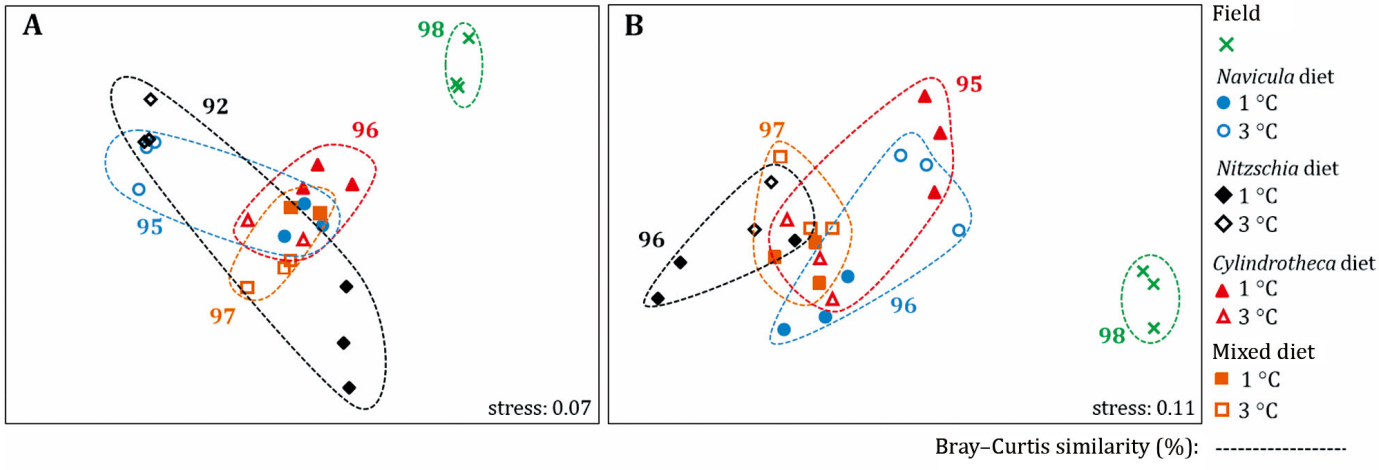

Fig. 4. Non-metric multidimensional scaling (Bray-Curtis similarity) of arcsine square-root transformed relative fatty acid data for (A) membrane lipids and (B) storage lipids of *Alteutha potter* after incubation with different diatom species at 1°C and 3°C

Table 4. Alterations in FA ratios (± SD) and the major FA classes (% ± SD) in the membrane and storage FA composition of *A. potter* on treatment with *Navicula* (Nav), *Nitzschia* (Nit), *Cylindrotheca* (Cyl) and a mixture of the 3 diatom species (Mixed) as food at 1°C and 3°C

	Field	1°C				3°C			
		Nav	Nit	Cyl	Mixed	Nav	Nit	Cyl	Mixed
Membrane lipids									
SFAs	18.2 ± 1.2	18.2 ± 1.5	15.2 ± 0.8	20.3 ± 1.3	18.3 ± 1.4	19.2 ± 2.3	20.5 ± 0.6	17.9 ± 1.8	17.0 ± 1.6
MUFAs	8.7 ± 0.4	10.4 ± 0.4	10.8 ± 0.3	10.5 ± 0.2	10.3 ± 0.1	9.9 ± 0.1	10.7 ± 0.9	9.6 ± 0.3	9.8 ± 0.3
PUFAs	73.1 ± 1.0	71.4 ± 1.2	74.0 ± 0.6	69.2 ± 1.5	71.4 ± 1.4	70.9 ± 2.2	68.8 ± 0.3	72.6 ± 2.0	73.2 ± 1.6
HUFAs	68.4 ± 1.0	68.1 ± 1.2	71.1 ± 0.5	65.7 ± 1.4	67.9 ± 1.3	67.6 ± 2.3	65.5 ± 0.3	69.5 ± 2.0	70.1 ± 1.5
DHA/EPA	0.7 ± 0.03	1.0 ± 0.1	1.1 ± 0.03	1.0 ± 0.04	1.0 ± 0.03	1.2 ± 0.03	1.2 ± 0.04	1.2 ± 0.02	1.2 ± 0.02
16:0/18:0	1.5 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	0.9 ± 0.2	1.0 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.6 ± 0.1
Storage lipids									
SFAs	11.0 ± 0.7	13.3 ± 2.1	13.9 ± 1.5	12.3 ± 0.8	12.2 ± 0.4	13.0 ± 0.7	13.4 ± 0.6	12.3 ± 0.7	12.9 ± 0.7
MUFAs	9.3 ± 0.3	12.0 ± 0.5	12.9 ± 1.4	11.4 ± 0.4	11.9 ± 0.3	11.7 ± 0.4	11.8 ± 0.5	11.9 ± 0.5	11.4 ± 0.3
PUFAs	79.6 ± 1.0	74.8 ± 2.2	73.2 ± 2.8	76.3 ± 0.4	75.9 ± 0.6	75.4 ± 1.0	74.8 ± 1.0	75.8 ± 0.3	75.7 ± 0.7
HUFA	41.5 ± 1.0	44.9 ± 0.6	45.4 ± 1.9	44.5 ± 0.9	45.9 ± 1.3	44.8 ± 1.1	46.0 ± 0.03	45.6 ± 0.2	44.1 ± 1.1

versus 0.25 for *Nitzschia* and 1 for the other mono diets). The SFA, monounsaturated FA (MUFA) and highly unsaturated FA (HUFA, i.e. FAs ≥20 carbon atoms and ≥3 double bonds) classes increased at the end of the experimental incubation, while the PUFA (i.e. FAs >1 double bond) class declined, regardless of the treatment (Table 4). Detailed membrane and storage FA compositions are available in Table S3 in the Supplement.

DISCUSSION

Diet inferred from FA trophic markers

Unraveling the diet composition of benthic, invertebrate grazers poses more challenges compared with pelagic zooplankton due to the diversity of benthic primary producers (Kelly & Scheibling 2012). Moreover, benthic copepods are also capable of FA modification, which possibly obscures the signature of dietary FATMs (De Troch et al. 2012, Kelly & Scheibling 2012). In addition to their bioconversion capacity (De Troch et al. 2012), benthic copepods have a wide dietary spectrum composed of diatoms (Pinto et al. 2001, Azovsky et al. 2005), flagellates (Harris 1977, Norsker & Støttrup 1994), cyanobacteria (Caramujo et al. 2008), detritus (Hicks & Coull 1983) and, to some extent, bacteria (Cnudde et al. 2013). Despite these challenges, the Antarctic harpacticoid community had a diverse storage FA composition, revealing the exploitation of different trophic niches associated with distinct lifestyles (habitats).

Endobenthic harpacticoid copepods

In endobenthic harpacticoids, the storage FAs were dominated by bacterial FAs (odd-numbered and branched FAs) and SFAs (16:0 and 18:0), indicative of decaying material and thus reliance on a detritus-based food web (Suhr et al. 2003). However, the substantial presence of the diatom markers 16:1ω7 and 20:5ω3 pointed at microphytobenthos, or alternatively detrital diatoms, as another important food source. These FATM outcomes are supported by Pasotti et al. (2015), who classified endobenthic harpacticoids from Potter Cove as deposit feeders and omnivores based on their $\delta^{13}C$ and $\delta^{15}N$ SI compositions.

Phytal harpacticoid copepods

Both *Harpacticus* and *Alteutha* are typically part of phytal assemblages (Fraser 1936, Hicks 1977). However, in this study, their storage FA composition suggested the exploitation of different trophic niches. The higher ratios of ∑C16/∑C18 and 16:1ω7/18:4ω3 observed in *Harpacticus* sp. compared with *Alteutha* spp. suggested a stronger reliance on diatoms of the former. Nonetheless, 16:1ω7 in *Harpacticus* sp. was only modest (10%) and the presence of bacterial markers (8%) and 18:4ω3 (5%) prevented the classification of this species as an exclusive diatom feeder. The diversity of trophic markers suggested the harpacticoid's reliance on a heterogeneous food source, likely macroalgal epibiota whose FA screening was indicative of bacteria, diatoms and flagel-

lates. Epiphytes may constitute an important food source for harpacticoid copepods, even more than their substrate (Mascart et al. 2013). Similar to many Antarctic amphipod species, *Harpacticus* sp. may exploit epiphytic diatoms and filamentous endo-/epiphytes, which are widespread throughout the macroalgae community along the WAP (Aumack et al. 2011, Majewska et al. 2016). Also, the natural $\delta^{13}C$ composition of *Harpacticus* sp. (–21.3‰) supports the copepod's reliance on epiphytic diatoms (–21.8‰ in Aumack 2010).

Despite the low 16:1ω7/18:4ω3 ratio in *Alteutha*, the high EPA/DHA ratio was not indicative of a dinoflagellate-composed diet. In particular, the low storage-associated DHA levels (1–2%) did not support a dinoflagellate-composed diet as dinoflagellates usually contain high DHA levels (Graeve et al. 1994b, Falk-Petersen et al. 1998, Kelly & Scheibling 2012). Noticeable were the levels of 20:4ω6 in both membrane (7–8%) and storage (3–5%) lipids of *Alteutha* spp., as this essential FA occurs usually in much lower amounts (Werbrouck et al. 2016a,b). Although macroalgae are seldom the prime food source of harpacticoid copepods (De Troch et al. 2008, Cnudde et al. 2015), *Alteutha potter*'s FA profile was in line with the previously reported FA composition of polar rhodophytes and phaeophytes. Generally, 18:4ω3, EPA and ARA dominate the FA composition of Phaeophyta, followed by 18:3ω3 and 18:2ω6. FA 16:0 and EPA, together with 16:1ω7 and ARA, are indicative of Rhodophyta (Graeve et al. 2002).

Alternatively, the high amounts of 18:3ω3 and 18:4ω3 in the storage lipids of *Alteutha* spp. suggested the assimilation of planktonic food sources. In particular, 18:4ω3 is indicative of cryptomonads (Dalsgaard et al. 2003, Arndt & Swadling 2006) and, together with 18:1ω9, may be a signature of feeding on a decaying *Phaeocystis* bloom (Graeve et al. 2001, Dalsgaard et al. 2003). The prymnesiophyte *Phaeocystis antarctica* is among the first to occur in spring (September, October, November) within coastal Antarctic waters, and is succeeded in time by other major functional groups of diatoms and cryptomonads (Walsh et al. 2001). Consequently, a summer sampling may reflect the full impact of a potential prymnesiophyte bloom on the storage accumulation process of *Alteutha* spp.

These FATM outcomes corresponded to the $\delta^{13}C$ composition, as the depleted value of *A. potter* (–31.3‰) was within the $\delta^{13}C$ range of red and brown macroalgae reported for the WAP (Dunton 2001, Aumack 2010, F. Pasotti pers. comm.). In addition, a consumer relying on pelagic primary production (–28‰) is typically more ^{13}C depleted, while significant inputs from microphytobenthos (–13‰) or ice-algae (–18 ‰) would yield more enriched ^{13}C values (Hobson et al. 1995, Pasotti et al. 2015). *A. potter* occurs year-round in plankton surveys in Potter Cove with maximum abundances observed in winter under the sea-ice (Veit-Köhler & Fuentes 2007). Despite this tight sea-ice association, the depleted $\delta^{13}C$ data did not support any contribution of sea-ice algae to the diet of *A. potter*.

Ecology of *A. potter*

The high numbers of copepodites, adults and molts suggested that the *A. potter* population was actively reproducing and growing at the time of sampling (summer). The association of copepodites (stage II, III and IV) with intertidal macroalgae, while consistently absent in previous plankton surveys in Potter Cove (Veit-Köhler & Fuentes 2007), support the assumption that macroalgae, abundant in- and outside the cove (Klöser et al. 1996, Quartino & Boraso de Zaixso 2008), function as a nursery habitat for the offspring. The numerous red droplets dispersed throughout the body of *A. potter* were a remarkable feature and may reflect the presence of carotenoids (Schneider et al. 2016). The red coloration in copepods is thought to be an inducible trait and involved in partner attraction or protection against UV radiation-induced free radicals, as carotenoids may function as antioxidants (Olson & Owens 1998, Hansson 2000). Possibly, both are relevant for *A. potter* considering the numerous mating pairs observed and their occurrence in tidal pools on the peninsula, which may be more UV-exposed. Furthermore, carotenoids support the production of steroid hormones and are thus involved in reproduction (Olson & Owens 1998). The red pigmentation may also act as a camouflage strategy. *A. potter* individuals previously collected from the plankton were described as bright amber-colored (Veit-Köhler & Fuentes 2007), while the individuals associated with macroalgae in the current study were red. Color change can follow upon dietary switching, as reported for an Antarctic amphipod species (Tucker & Burton 1988).

The higher FA content in *A. potter* (325 ng FA µg C^{-1}) compared with *Harpacticus* sp. (160 ng FA µg C^{-1}) was likely due to the presence of lipid droplets. These presumed lipid bodies are generally composed of neutral lipids (Dahms et al. 1990, Williams & Biesiot 2004) such as di- and triacylglycerols and wax

esters (WE), surrounded by phospholipids, glycolipids and/or sterols, frequently in association with proteins (Murphy 2001). Accumulation of lipids in polar copepods provides energy for metabolic maintenance during periods of low food availability and for reproductive processes before the onset of the spring phytoplankton bloom, so that juvenile development is synchronized with favorable environmental conditions (Hagen & Schnack-Schiel 1996). The peak of ovigerous females observed in the plankton after breakup of the sea ice (October) and summer (end of December) (Veit-Köhler & Fuentes 2007) suggests that this strategy may be adopted by *A. potter* as well.

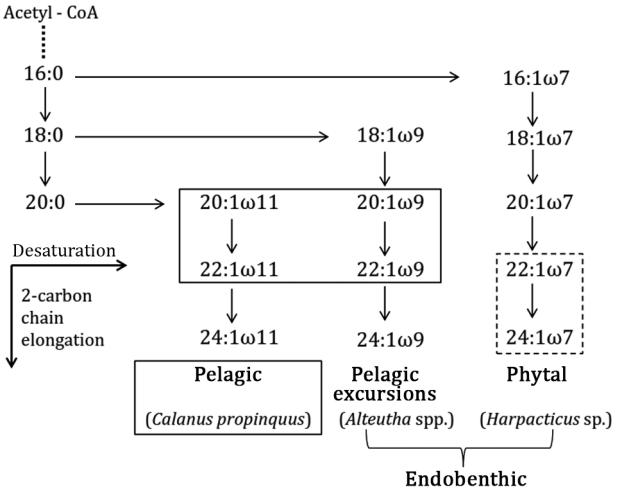

Fig. 5. Biochemical pathways in harpacticoid copepods in particular habitats, including an extension of the ω7 pathway (dashed box) (modified after Dalsgaard et al. 2003)

Long-chain MUFAs reflect trophic niche

It was remarkable to see that the long-chain (LC) MUFAs (20:1ω7/9, 22:1ω7/9 and 24:1ω7/9) occurred only in trace amounts at the moment of sampling. LC-MUFAs (20:1ω9 and 22:1ω11) are typically de novo produced by herbivorous calanoid copepods in large amounts (Fraser et al. 1989, Hagen & Auel 2001) and incorporated as WEs in large lipid stores to outlast a 4–9 mo period without feeding (Kattner & Hagen 1995, Pond et al. 2012).

Despite their trace amounts, the diversity of LC-MUFAs in all Antarctic harpacticoid species was remarkably high compared with temperate harpacticoids, where only 20:1ω9 was detected in trace amounts (De Troch et al. 2012, Cnudde et al. 2013, Werbrouck et al. 2016a,b). This possibly reflects an adaptation to high-latitude environments, as similar observations were made for Arctic harpacticoids (Budge et al. 2008). These LC-MUFAs may be products of elongation events, given their low occurrence in macroalgal epibiota. The higher prevalence of 16:1ω7 in *Harpacticus* sp. and 18:1ω9 in *Alteutha* spp., supplied through different food sources, may explain the occurrence of LC-MUFA ω7 in the storage lipids of *Harpacticus* sp. and ω9 in *Alteutha* spp. (Fig. 5). Both ω7 and ω9 LC-MUFAs were equally present in the storage lipids of endobenthic harpacticoids with their precursors likely introduced through microphytobenthos (16:1ω7) or settled flagellates (18:1ω9). Bentho–pelagic coupling may be particularly tight in high-latitude environments, as a larger proportion of biogenic material may reach the bottom than at lower latitudes (Corbisier et al. 2004). The ecological relevance behind chain elongation is the increased calorific value of FAs, for example to compensate for the species' inability to produce WEs, as suggested by Kattner & Hagen (1995) for the Antarctic calanoid *Calanus propinquus*. It is assumed that marine copepods share a common, though flexible, lipid biosynthetic pathway ('Type I fatty acid synthetase pathway' Sargent & Henderson 1986) that leaves enough potential for the different species to use those pathways best suited to fulfill the metabolic requirements (Kattner & Hagen 1995) in their particular habitat.

Lipid dynamics of *A. potter*

The overall food uptake by *A. potter* was low (0.02–0.1 µg C copepod^{-1}) compared with those reported by Cnudde et al. (2011) (range 0.2–0.5 µg C copepod^{-1}). This explained the lack of a food effect at the level of the copepod carbon content. Furthermore, the decrease in FA content suggested nutritional stress, despite the ad libitum diatom supply. Although freeze-drying possibly reduces the nutritional value of the food and lower digestibility (Albentosa et al. 1997), it preserved the differences in FA composition among the diatom species. Nonetheless, other food characteristics, such as the cell morphology, the external epiflora and the presence of exudates, may be affected by freeze-drying procedures and influence food perception by harpacticoids (Cnudde et al. 2011). Moreover, the morphology of *A. potter* suggests adaptation to clinging to a surface, as illustrated by their large maxilliped (Veit-Köhler & Fuentes 2007). Therefore, the lack of a substratum in

the experimental units may have impeded natural feeding behavior.

More likely, diatoms did not contribute to *A. potter*'s natural diet, at least during summer as appeared from the field FA and $\delta^{13}C$ compositions. Consequently, the outcome of the experiment highlighted the response of *A. potter* to the combined effects of nutritional and temperature stress rather than any effect of resource availability in the adaptation to elevated temperature.

Food stress and elevated temperature strongly affected *A. potter*'s energy balance as the membrane and storage FA contents were 3 to 4 times lower. This illustrated the highly dynamic nature of the lipid reserves and thus likely their short turnover times. Vulnerability to food stress might especially peak during energy-costly events such as molting. Active feeding occurs only during the intermolt, while feeding declines and stops prior to and during the molt, respectively (Sánchez-Paz et al. 2006). Elevated temperature enhanced FA depletion of membrane and storage lipids, likely the consequence of increased metabolic rates (Gillooly et al. 2001). Furthermore, elevated temperature was related to FA compositional changes in the membrane lipids, in line with the response observed in temperate harpacticoids (Werbrouck et al. 2016b), i.e. a decline in the 16:0/18:0 ratio and an increase in the DHA/EPA ratio. Also, nutritional stress selected for DHA and against EPA in the membrane lipids of *A. potter*, which may reflect the scarcity of DHA compared with EPA in the natural diet. Furthermore, a mixed diet buffered alterations in membrane FA composition. However, the effect was only modest and did not result in fitness benefits such as higher survival.

Storage depletion occurred selectively at the FA-class level. PUFAs dominated the storage lipids, and consequently, were also relatively more metabolized compared with SFAs, MUFAs and HUFAs, regardless of the temperature regime. Increased mortality at 1°C compared with 3°C was peculiar and may have been caused by the larger temperature fluctuations in the outdoor tanks. However, intertidal organisms are regularly exposed to large temperature fluctuations in their natural environment (Williams & Somero 1996, Zacher et al. 2009). In particular, organisms inhabiting tide pools in Potter Península may experience water temperatures up to 14°C, which may be reached within a few hours on sunny days during austral summer (Klöser & Arntz 1994). In the context of these temperature fluctuations, food availability and thus a balanced energy household are fundamental requirements for stress adaptation and tolerance (Sokolova et al. 2012).

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