Condition of larval (furcilia VI) and one year old juvenile *Euphausia superba* during the winter–spring transition in East Antarctica

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ABSTRACT

Antarctic krill, *Euphausia superba*, is an important species in the Southern Ocean ecosystem. Information on krill condition during winter and early spring is slowly evolving with our enhanced ability to sample at this time of year. However, because of the limited spatial and temporal data, our understanding of fundamental biological parameters for krill during winter is limited. Our study assessed the condition of larval (furcilia VI) and one year old juvenile krill collected in East Antarctica (115°E–130°E and 64°S–66°S) from September to October 2012. Krill condition was assessed using morphometric, elemental and biochemical body composition, growth rates, oxygen uptake and lipid content and composition. Diet was assessed using fatty acid biomarkers analysed in the krill. The growth rate of larvae was 0.0038 mm day with an inter-moult period of 14 days. The average oxygen uptake of juvenile krill was 0.30 ± 0.02 μl oxygen consumed per mg dry weight per hour. Although protein was not significantly different amongst the krill analysed, the lipid content of krill was highly variable ranging from 9% to 27% dry weight in juveniles and from 4% to 13% dry weight in larvae. Specific algal biomarkers, fatty acids ratios, levels of both long-chain ($\geq C_{20}$) monounsaturated fatty acids and bacterial fatty acids found in krill were indicative of the mixed nature of dietary sources and the opportunistic feeding capability of larval and juvenile krill at the end of winter.

1. Introduction

Estimates of krill production are hampered by our lack of understanding of their winter growth rates and survival. A vital step in successful krill recruitment, and hence total population size, is larval survival through the first winter (e.g. Atkinson et al., 2004; Loeb et al., 1997; Siegel and Loeb, 1995). However, the overwintering mechanisms of krill, particularly of their larval stages, are still poorly understood (Flores et al., 2012; Meyer, 2012).

Studies investigating the response of krill to short and long-term starvation (Auerswald et al., 2009; Meyer and Oettl 2005; O'Brien et al., 2010; Virtue et al., 1997), and to changes in temperature (Quetin et al., 1994) and day-length (Brown et al., 2010; Teschke et al., 2008, 2011) have helped us to understand the physiology of krill in extreme conditions. Laboratory and field-based research efforts have identified a number of possible over-wintering physiological strategies for adult and larval krill.

Overwintering mechanisms proposed for adult krill include reduced metabolism, shrinkage, utilisation of body lipids, and switching to alternative food sources. Depression of metabolism during winter is a very efficient energy saving mechanism (Meyer, 2012). However, lowered respiration rates and lipid reserves alone cannot sustain krill throughout the winter. Thus feeding activity, although very low, is needed to survive the winter season (Meyer et al., 2010). Opportunistic feeding and combustion of body stores in combination with reduced metabolic activity ensure that the animals maintain base line condition during the winter season. Body shrinkage is an alternative way for krill to conserve energy in extreme conditions when lipid reserves are almost depleted. With

the onset of spring, when metabolic rates increase and lipid levels are low due to winter depletion, sea-ice biota and spring blooms are important food sources. This food supply fuels lipid accumulation in order to induce gamete development to undergo successful spawning (Ross and Quetin, 2000; Schmidt et al., 2012; Siegel, 1988).

Recruitment success off West Antarctica is linked to winter sea-ice cover, with low ice years related to poor recruitment or low biomass the following summer (Atkinson et al., 2004; Loeb et al., 1997). These observations suggest that sea ice is an important overwintering habitat for larval krill in particular (e.g. Daly, 2004; Flores et al., 2012; Frazer et al., 2002; Meyer et al., 2003), but the mechanisms involved are poorly understood, especially for the transition phase between winter and spring. The degree to which krill depends on ice biota seems to decrease with increasing age (Daly, 2004; Meyer, 2012; Meyer et al., 2009). Most of our knowledge on the relationship between krill and sea ice is derived from studies in the Atlantic sector of the Southern Ocean and information from East Antarctica is very limited (O'Brien et al., 2011; Virtue et al., 2010).

The physiological mechanisms in larval krill are a function of food supply. In all seasons larvae show a positive functional response in metabolic and feeding activity with increasing food availability, suggesting that food is a key factor involved in the seasonal variability in physiological functions (Meyer et al., 2009). Similar to adults, body shrinkage is not a general overwintering strategy but rather a flexible behaviour to overcome severe conditions such as low lipid levels resulting from low food supply (Meyer, 2012). Although larvae can only endure very short term starvation, adults are able to survive long periods of food shortage (Meyer and Oettl, 2005; O'Brien et al., 2011).

The difficulties involved in collecting samples during winter have previously hampered our understanding of the physiological state of krill. Very little information is available about larval krill condition from East Antarctica (O'Brien et al., 2011). Here we use a combination of complementary methods (elemental and biochemical composition, signature lipid profiling, instantaneous growth and oxygen activity rates) to investigate the condition of furcilia VI larval and one year old juvenile *Euphausia superba* collected during the winter–spring transition in East Antarctica.

2. Materials and methods

2.1. Sampling

Sampling was conducted as part of the Sea Ice Physics and Ecosystems eXperiment 2 (SIPEX-2) (14 September–4 November 2012) off East Antarctica (115°E–130°E and 64°S–66°S). Krill (furcilia VI larvae and one year old juveniles) were collected directly under the ice via either hand dip nets, plankton nets or using a modified commercial fish pump (Aqua-life BioStream) from ice floe station 6 (13–14th October 2012, 65° 14′S, 120° 08′E) and station 8 (29th–1st October 2012, 64° 08′S, 116° 44′E).

Freshly caught krill were used for instantaneous growth and oxygen uptake rate measurements. Samples of whole krill from the same sites were stored at −86 °C until further analysis of dry weight (DW), body carbon, nitrogen (C, N), and body lipid and protein content. Whole krill were also frozen for lipid class and fatty acid compositional analysis. Before freezing, digestive gland size, carapace length and total length of each krill were measured. Due to logistical difficulties of sampling krill under sea ice from an ice floe, the number of animals caught was low and not all parameters could be measured at each station and for both developmental stages.

2.2. Morphometrics

The total length of krill (mm) was measured from the front of the eye to the tip of the telson. The carapace length was measured from the tip of the rostrum to the posterior notch of the carapace, whereas the digestive gland (DG) was measured through the carapace along its longest axis by using digital callipers. The size of the DG (relative to carapace length) was calculated for each individual to provide an indication of its recent feeding history.

2.3. Elemental and biochemical composition

Individual larvae were freeze-dried for 24 h and weighed on a Mettler UM 3 microbalance for determination of DW. Elemental (C and N) and biochemical body composition (protein and lipid) were analysed from bulked samples of either 10 larvae or 2 juveniles. The individual dried krill were pooled and homogenised in 1 mL of Milli-Q water by sonication in an ice bath, shock frozen by dipping the tube in liquid nitrogen and then stored at −86 °C for further analyses of body C, N, protein, and lipid content. For C and N analyses, 0.2–0.5 mg of krill powder was used and analysed as described elsewhere (e.g. Meyer et al., 2003). Total body protein was measured by incubating 0.8–1 mg of larval powder in 1 mL of 1 mol L^{-1} NaOH for 2 h at 60 °C. After centrifugation at a relative centrifugal force of 3g for 5 min, the supernatant was used for determination of protein according to Lowry et al. (1951), with bovine serum albumin as a standard, in a microplate reader. Lipid content of samples from station 6 was determined gravimetrically according to Hagen (2000).

2.4. Lipid sample preparation

Krill samples used for lipid class analysis were freeze-dried to calculate dry mass, then extracted overnight using a modified Bligh and Dyer (1959) one-phase methanol–chloroform–water extraction (2:1:0.8 v/v/v). The phases were separated by addition of chloroform–water (final solvent ratio, 1:1:0.9 v/v/v methanol–chloroform–water). The total solvent extract (TSE) was concentrated using rotary evaporation at 40 °C then stored in chloroform in 1.5 ml glass vials with Teflon-lined caps at −20 °C until analysis. Total lipid content was determined gravimetrically after evaporation of CHCl$_3$ under a stream of nitrogen until dry.

For lipid class composition, an aliquot of the total lipid extract was analysed using an Iatroscan MK V TH10 thin-layer chromatography-flame ionisation detector (TLC-FID) analyser (Tokyo, Japan) (Ackman, 1981, Volkman and Nichols, 1991). The FID was calibrated for each lipid class (phosphatidylcholine, cholesterol, cholesteryl oleate, oleic acid, squalene, TAG (derived from fish oil), wax ester (derived from orange roughy, *Hoplostethus atlanticus*, oil) and diacylglyceryl ether (DAGE; derived from shark liver oil; 0.1–10 µg range)). Peaks were quantified using SIC-480 Scientific Software. TLC-FID results are generally reproducible to +/− 10% of individual class abundances (Volkman and Nichols, 1991).

An aliquot of the total lipid extract was trans-methylated to produce fatty acid methyl esters as described in Virtue et al. (1996). Samples were analysed by gas chromatography (GC) using an Agilent Technologies 7890B GC (Palo Alto, California, USA) filtered with an Equity™-1 fused silica capillary column (15 m × 0.1 mm i.d., 0.1 µm film thickness), an FID, a split/splitless injector and an Agilent Technologies 7683B Series auto sampler and injector. Helium was the carrier gas. Samples were injected in splitless mode at an oven temperature of 120 °C. After injection, the oven temperature was raised to 270 °C at 10 °C min^{-1} and finally to 310 °C at 5 °C min^{-1}. GC results are subject to an error of ± 5% of individual component area. GC–mass spectrometric (GC–MS) analyses were performed on a Finnigan Thermoquest DSQ

GC–mass spectrometer fitted with an on-column injector using Thermoquest Xcalibur software (Austin, Texas, USA).

2.5. Instantaneous growth rate (IGR)

Krill were used for growth experiments using the IGR method (Virtue et al., 2010). Immediately after capture, live krill were maintained individually at 0.5 °C. Jars were checked for moults at 24 h intervals. Growth increments (% growth per moult) were calculated by measuring the length of both left and right uropod exopodites on the fresh krill and their discarded exoskeletons using a digital microscope camera equipped with image analysis software. Growth increments (GI) were calculated using the formula:

$$GI = 100 \times \frac{\left(\frac{LU - LM}{LM}\right) + \left(\frac{RU - RM}{RM}\right)}{2}$$

where LU is the total length of the animal's left uropod, LM is the length of the moulted left uropod, RU is the length of the animal's right uropod, and RM is the length of the moulted right uropod. Where one uropod was damaged, GI was calculated based on the undamaged side only. If both left and right uropods were damaged, the animal was excluded from the study.

2.6. Oxygen uptake rates

Oxygen consumption rates (mg/mL) were measured every minute during a 24 h period using an OXY 4-Channel Oxygen Meter (Presens, Germany) and Oxygen Sensor Spots (PSt3 spots, Presens, Germany) attached to the inside of 100 ml flasks (Schott DURAN, Germany). For each sensor, two-point calibrations were performed, and sodium sulphite and aeration were used to calibrate the 0% and 100% air saturation points, respectively. Prior to measurements, all eight flasks were filled with 0.2 μm filtered seawater at in situ temperature, aerated for 10 min and left to outgas for another 15 min to avoid oxygen super-saturation. Air bubbles adhering to the sensor spots were removed using forceps. Six bottles were used with individual freshly caught juvenile krill. Two bottles were left empty as controls. After closing without any air entrapped, all flasks were lowered into a tank filled with filtered seawater and chilled at in situ temperature.

For calculations of oxygen consumption rates in each respiration chamber, linear regressions of oxygen concentrations versus time were calculated. Regressions were then corrected for oxygen saturation changes inside the control chambers. Data were expressed as volume of oxygen consumed per mg dry weight (DW) per hour (μL O_2/mg DW/h).

2.7. Statistical analyses

For testing of significant differences, one-way analyses of variance (ANOVA) were conducted using the programme R. A significance level of 99% was used in interpreting results. Data are reported to ± standard deviation. PRIMER (Plymouth Routines in Multivariate Ecological Research), a software package for analysing multivariate ecological data, was used to assess the groupings and investigate relationships within the fatty acid data set. Parametric, principal component analysis (PCA) and nonparametric cluster analysis was used.

3. Results

3.1. Environmental conditions at ice floe stations 6 and 8

At both stations 6 and 8, sea surface water temperatures ranged from −1.87° to −1.80 °C and surface water salinities remained constant at 34.2 ppt throughout the voyage. Air temperature increased over time throughout the sampling period, ranging from −21.71 °C to −7.45 °C. The average ice thickness from all stations was 1.38 m and ranged from 0.75 to 2.18 m, while the mean ice thickness at stations 6 and 8 was 1.29 m and 2.16 m, respectively. The average snow cover over the ice was 0.46 m (Toyota et al., this volume) and ranged from 0.01 to 1.0 m (Ugalde et al., this volume). Mean chlorophyll *a* (Chl *a*) concentration in the bottom 10 cm of ice cores was 4.10 ± 5.42 μg L^{-1}, which constituted an average of 28% of the Chl *a* in the total ice core volume. The mean Chl *a* concentrations of ice cores at stations 6 and 8 were 3.29 and 0.57 μg L^{-1}, respectively. Mean Chl *a* concentration in near-surface water sampled directly under the ice was 0.16 ± 0.07 μg L^{-1} (Ugalde et al., this volume; Jia et al., this volume). Diatoms dominated the algal community within the sea ice, often associated with different copepod species at various ontogenetic stages. A detailed description of the ice algal communities is described by Ugalde et al., (this volume). The zooplankton and associated heterotrophic organisms within sea ice and under-ice water is described by Wallis et al. (this volume).

3.2. Condition of larvae and juvenile krill

Table 1 summarises the body length, dry weight and elemental composition (carbon and nitrogen content) of larvae and juvenile krill sampled at stations 6 and 8. Larvae and juveniles at station 6 were slightly larger, heavier and had higher body carbon content than animals at station 8, but these differences were not significant.

Although krill protein content did not differ significantly, body lipid content of larval and juvenile krill was highly variable between and within stations, with station 6 having significantly higher levels than station 8 (Table 2). The lipid class composition was determined for larval and juvenile krill sampled at station 8 (Table 3). Storage lipids (triacylglycerol, TAG) in juvenile krill accounted for $29.8\% \pm 6.6\%$ ($n=9$) of total body lipid, whereas membrane lipids (polar lipid, PL) were $59.4 \pm 5.4\%$ ($n=9$) of the total body lipid. In larvae, TAG accounted for $6.9 \pm 1.4\%$ ($n=11$) of total body lipid, and PL made up $76.1 \pm 3.0\%$ ($n=11$) of total body lipid (Table 3).

Due to the limited number of animals available, growth was only measured in larvae, and oxygen uptake rates and the digestive gland size were only analysed in juveniles. The average growth rate of larvae was 0.0038 mm d^{-1} ($n=35$) with an inter-moult period (IMP) of 14 days and a growth increment (GI) of 4.3%. Average oxygen uptake rates of juvenile krill were 0.30 ± 0.02 μl O_2 mg^{-1} DW h^{-1} ($n=6$). The relative digestive gland size (as % carapace length) of juvenile krill did not differ significantly between stations, showing an average of $31.8 \pm 7.4\%$ ($n=36$) (Fig. 1).

3.3. Fatty acid composition of krill

3.3.1. One year old juveniles

The major fatty acids in one year old juvenile krill in order of dominance were 16:0, 20:5ω3 (eicosapentaenoic acid, EPA), 18:1ω9, and 22:6ω3 (docosahexaenoic acid, DHA) (Table 4). Polyunsaturated fatty acids (PUFA) accounted for 41% of the total fatty acids, with a PUFA/saturated fatty acids (SFA) ratio of 1.4. The ratio of 16:1ω7/16:0 was 0.3 for juveniles suggesting diatoms were

Table 1
Mean body length, dry weight, percentage of carbon, nitrogen, and carbon:nitrogen ratio of one year old juvenile and furcilia larval stage VI *Euphausia superba* (from station 6 and 8). The range of data is given in parentheses.

Krill stage	Length (mm)	Weight (mg)	Carbon %	Nitrogen %	Carbon:nitrogen ratio
Station 6					
Furcilia ($n=30$)	9.7 (8.2–10.2)	1.1 (0.8–1.3)	37.8 (35.1–43.7)	8.5 (7.4–9.3)	4.5 (4.1–5.8)
Juvenile ($n=10$)	30.0 (23.0–31.0)	24.7 (19.6–36.8)	43.9 (40.7–46.2)	9.4 (8.0–11.3)	4.7 (3.6–5.4)
Station 8					
Furcilia ($n=10$)	9.1 (8.2–10.2)	0.9 (0.7–1.2)	34.1 (32.1–38.2)	9.1 (8.2–9.9)	4.0 (3.9–4.7)
Juvenile ($n=15$)	24.9 (17.9–26.6)	12.7 (7.9–28.7)	39.4 (36.6–41.7)	9.9 (9.3–10.7)	3.9 (3.8–4.4)

Table 2
Total lipid and protein composition of one year old juvenile and furcilia larval stage VI *Euphausia superba* (from station 6 and 8) expressed as mean % ± SD (Standard Deviation) dry weight.

	Juvenile	Furcilia VI
Station 6 total lipid	26.8 ± 5.2 ($n=12$)	12.6 ± 3.1 ($n=6$)
Protein	42.0 ± 4.4 ($n=12$)	33.4 ± 1.7 ($n=6$)
Station 8 total lipid	12.3 ± 2.1 ($n=15$)	4.1 ± 0.4 ($n=11$)
Protein	50.6 ± 11.7 ($n=7$)	45.8 ± 3.7 ($n=6$)

Table 3
Lipid class composition of one year old juvenile and furcilia larval stage VI *Euphausia superba* (from station 8), expressed as mean % of total lipid ± SD (Standard Deviation).

Lipid Class (%)	Juvenile ($n=9$)	Furcilia VI ($n=11$)
Hydrocarbon	0.9 ± 0.3	4.6 ± 0.9
Free fatty acid	4.7 ± 1.5	8.0 ± 1.1
Sterol	5.1 ± 1.3	4.5 ± 0.6
Triacylglycerol	29.8 ± 6.6	6.9 ± 1.4
Polar lipid	59.4 ± 5.4	76.1 ± 3.0

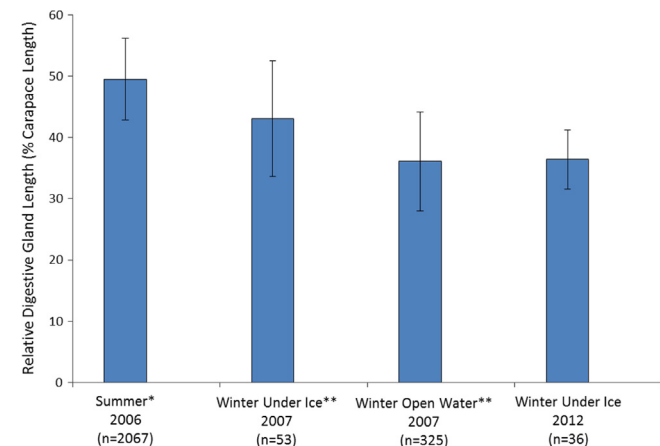Polar lipid (includes phospholipids and glycolipids).

Fig. 1. Relative digestive gland length (% Carapace Length) of juvenile *Euphausia superba* captured in East Antarctica from under-ice and open water zones in Sep–Oct 2007 (**O'Brien et al., 2011) and under-ice in Sep–Nov 2012 (this study) in comparison with relative digestive gland length of adults caught in summer in East Antarctica (*Virtue et al., 2010).

Table 4
Fatty acid composition of one year old juvenile and furcilia larval stage VI *Euphausia superba* (from station 8), expressed as mean % ± SD (Standard Deviation) of total fatty acids.

Fatty acid composition (mean % total fatty acids ± SD)

Fatty acid	Juvenile	Furcilia VI
14:0*	6.3 ± 1.1	0.7 ± 0.6
16:1ω7c*	5.2 ± 1.0	1.8 ± 0.4
16:0 *	19.0 ± 0.9	18.0 ± 0.9
18:3ω6	0.5 ± 0.2	0.4 ± 0.0
18:4ω3*	2.8 ± 1.2	2.4 ± 0.2
18:2ω6	2.8 ± 0.2	3.2 ± 0.1
18:3ω3	0.9 ± 0.1	2.3 ± 0.2
18:1ω9c*	13.0 ± 2.0	9.8 ± 0.5
18:1ω7c*	6.9 ± 0.5	5.9 ± 0.3
18:0 *	1.8 ± 0.4	1.9 ± 0.7
20:4ω6	0.8 ± 0.2	0.8 ± 0.1
20:5ω3* EPA	19.0 ± 2.1	26.0 ± 1.1
20:4ω3	0.4 ± 0.1	0.6 ± 0.1
20:1ω9c	0.6 ± 0.1	0.8 ± 0.1
21:5ω3	0.4 ± 0.1	0.8 ± 0.1
22:6ω3* DHA	13.0 ± 1.8	20.0 ± 0.3
22:5ω3	0.5 ± 0.1	0.5 ± 0.1
Other	6	3.8
Total SFA	29.3 ± 1.7	21.9 ± 2.2
Total MUFA	29.7 ± 1.7	20.0 ± 0.8
Total LC-MUFA ≥ C₂₀	2.2 ± 0.2	1.5 ± 0.1
Total PUFA	41.0 ± 3.3	58.0 ± 1.5

SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), LC-MUFA (long-chain monounsaturated fatty acids ≥ C_{20}), PUFA (polyunsaturated fatty acids).
'Other' includes fatty acids < 0.5%: 14:1, i15:0, a15:0, 15:0, i16:0, 16:1ω9c, 16:1ω7t, 16:1ω5c, 16:1ω13t, 16:0FALD, i17:0, 17:1ω8c+a17:0, 17:1, 17:0, i18:0, 18:1ω7t, 18:1ω5c, 18:1, 18:1FALD, 18:0FALD, 19:1, 20:3ω6, 20:2ω6, 20:1ω11c, 20:1ω7c, 20:1ω5c, 20:0, 21:0, 22:5ω6, 22:4ω6, 22:1ω11c, 22:1ω9c, 22:1ω7c, 22:0, 23:0, 24:1ω11c, 24:1ω9c, 24:0.

  * Included in PCA analyses.

3.3.2. Furcilia VI

Furcilia VI larval krill fatty acid profiles were dominated by 20:5ω3, 22:6ω3, and 16:0 (Table 4). PUFA accounted for 58% of the total fatty acids, with a PUFA/SFA ratio of 2.6. The ratio of 16:1ω7/16:0 was 0.1 which was significantly lower than in the juvenile krill. The flagellate marker 18:4ω3 made up 2.4% of the furcilia total fatty acids. Furcilia also had lower levels (1.5%), than in juveniles of the LC-MUFA. Fatty acids of bacterial origin were again lower than for furcilia VI, and accounted for 0.6% of total fatty acids.

3.3.3. Principle component analysis of krill fatty acids profiles

In terms of the fatty acid profiles, one year old juvenile and furcilia VI groups separated from each other having a Bray–Curtis similarity of 82% (ANOSIM-R > 0.99) (Fig. 2). PCA and SIMPER analyses showed that levels of 20:5ω3, 22:6ω3, 14:0 18:1ω9 and 16:1ω7 contributed most of the dissimilarity (78.4%) of the fatty acid profiles between these two life stages of krill.

not a major food source. The common flagellate marker 18:4ω3 made up 2.8% of the juvenile total fatty acids. Juveniles had low levels of long-chain monounsaturated fatty acids (≥ C₂₀, LC-MUFA, 2.2%) such as 20:1ω9, 22:1ω9, 22:1ω11 and 24:1ω9, which are known copepod biomarkers. The bacterial markers 15:0, a15:0, i15:0, 17:0, i17:0 17:1ω8c and 17:1 together made up 1.4% of the juvenile fatty acids.
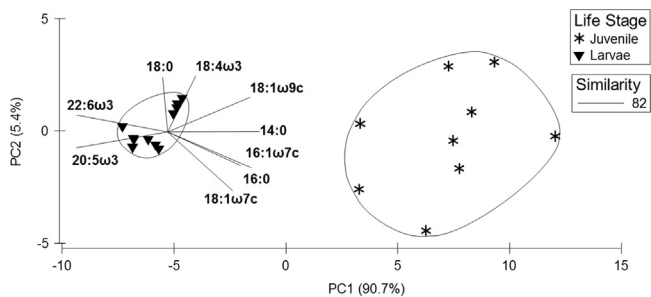
Fig. 2. First and second principle components using signature fatty acid profiles from one year old juvenile and furcilia larval stage VI *Euphausia superba* (from station 8) East Antarctica (including all fatty acids > 0.5% total fatty acid), with 82% similarity clusters. Fatty acids with eigenvector coefficient > |0.15| are included.

4. Discussion

4.1. Sea ice

Significant changes have occurred in patterns of sea-ice seasonality in West Antarctica since 1979 (Stammerjohn et al., 2012), with wide-ranging climatic, biological and biogeochemical consequences (Ducklow et al., 2007). By contrast, patterns of change and variability in sea-ice seasonality are relatively complex across East Antarctica, where sea-ice coverage is strongly seasonal and occurs in a relatively narrow band even at maximum extent (Massom et al., 2013). The ice extent in East Antarctica varies from approximately 500 km to 1300 km north of the coast in September, depending on longitude. Other sectors of the Southern Ocean experience maximum ice extent of up to several thousand kilometres from the coast. This proximity of the sea ice zone to the open ocean in some areas of East Antarctica means that the entire pack-ice zone is often affected by ocean swell (Kohout et al., 2015), and is highly mobile and often deformed as a result of the frequent passage of storm systems (Worby et al., 2008).

Moreover, recently-published three-dimensional maps developed from data collected using an autonomous underwater vehicle, from the SIPEX-2 and other voyages, suggest that up to approximately 75% of springtime sea-ice volume on average is deformed in regions of East Antarctica and the Antarctic Peninsula (Williams et al., 2015). They also suggest that Antarctic sea ice may be substantially thicker due to this deformation than previously thought. Although krill abundance has been directly related to sea-ice extent in the Antarctic Peninsula region (Atkinson et al., 2004), other studies show that it may also be affected by sea ice thickness and deformation as it affects both habitat and food availability (Frazer et al., 1997, 2002; Massom et al., 2006; Meyer et al., 2009). A reduction in light penetration due to thick and deformed sea ice may limit sea-ice algal biomass, with flow on consequences for krill and other zooplankton feeding on sea-ice biota. By the same token, gaps between deformed ice blocks, afford a protective environment for juvenile krill.

4.2. Krill condition

Krill, occasionally in high concentrations, have been observed to be closely associated with the under-surface of the ice in winter and early spring (Hamner et al., 1989; Stretch et al., 1988). It has been reported that they are able to efficiently scrape algal cells from sea ice (Daly, 1990; Marschall, 1988; Stretch et al., 1988). In the present study using a remotely-operated vehicle, larval and juvenile krill were observed feeding on sea-ice associated organisms and using gaps between ice blocks formed by ice deformation to feed and hide (from us and presumably predators). During our study, furcilia VI larvae and one year old juveniles were sampled under pack ice in the transition phase between winter and spring, in East Antarctica. All animals were actively feeding with visible green digestive glands, and were considered to be in good feeding condition. Furcilia VI and juveniles from station 6 were slightly larger and heavier compared to those in station 8. Protein and lipid usually account for most of the body C and N in zooplankton, and since the C:N ratio for protein is about 3, C:N ratio can be used as an indicator of lipid storage. The C:N ratios found in the present study are all within the range of previous studies (e.g. Daly, 2004; Meyer, et al., 2009). Larval growth rates generally decrease during autumn, reaching a minimum in early winter, and increase to maximum rates by late winter (Quetin and Ross, 2003), reflecting the variable seasonal pattern of food availability during this period (Meyer, 2012). The growth increment (GI) of 4.3% found in krill in this study was lower than the GI of late furcilia stages observed in the Antarctic Peninsula area (∼10%) at the same time of the year i.e., September (Quetin et al., 1996), but higher than that observed in the Lazarev Sea (1.3–4.1%) (Meyer et al., 2009). The morphometric data as well as the elemental composition of krill in the present study suggest that they are in good to moderate physiological condition. We further suggest that these krill could cover their metabolic demands of 0.30 µl O_2 mg^{-1} DW h^{-1} for year one juveniles and gain enough energy for positive growth i.e., GI=4.3% in furcilia VI larvae (Daly, 2004).

The highly variable body lipid content in larvae and juveniles, of 4–13% and 13–27% DW^{-1} respectively, could be a reflection of the high degree of patchiness in food availability at this time of the year (Hagen et al., 1996; Ju and Harvey, 2004; Mayzaud et al., 1998; Meyer, 2012; O'Brien et al., 2011). This patchiness may result from variable ice thickness due to deformation processes. A positive correlation was found between ice thickness and lipid levels between the two stations sampled. Juvenile and larval krill from station 8 had less than a half and a third of total lipid, respectively, compared to those analysed from station 6. The sea ice at station 8 was almost twice as thick and had a significantly deeper snow cover than that at station 6. There was also a positive correlation between sea-ice thickness and Chl *a* levels in the sea ice. Chl *a* levels in station 8 ice cores were six times lower that than those found in ice cores from station 6 (Ugalde et al., this volume). Thinner ice with less snow would allow more light to penetrate, resulting in higher primary production and in turn a higher biomass of sea-ice biota for krill to feed on.

A body lipid content of 3.5% DW^{-1} is thought to be essential for krill survival (Hagen et al., 2001). Hence, the larvae with a mean body lipid content of 4% DW^{-1} and storage lipids (TAG) of only 7% would not be able to withstand periods without food for very long (Meyer and Oettl, 2005; O'Brien et al., 2011; Ross and Quetin, 1988). The majority of larvae, in the present study, seem to channel dietary resources into somatic growth rather than into storage lipids, evident by their high levels of phospholipids which are required for cell and organelle membrane development. The metabolic needs of larval krill in this study were being met from nutrients in the sea ice rather than the water column, which was confirmed via both carbon and nitrogen stable isotope analysis (Jia et al., this volume).

The size of the digestive gland in krill is a good indicator of recent feeding history. The digestive gland shrinks rapidly when krill are starved, and increases in size on feeding (Shin, 2000). The relative length of the digestive gland (as % of carapace length) of juvenile krill in the present study was, on average, 32%. This is similar to the sizes of digestive glands reported for krill feeding in the open water in the pack ice zone in East Antarctica (36%), while in the same study, krill feeding under the ice were reported to have significantly larger digestive glands (43%) (O'Brien et al., 2011) (Fig. 1). The average digestive gland size of adult krill caught in the summer in East Antarctica was 49% of carapace length

(Virtue et al., 2010). Despite being relatively larger than those in this study, direct comparison of digestive gland size cannot be made between adult and sub adult krill due to changes in body shape

4.3. Krill diet

Fatty acid biomarkers can be used to help understand energy pathways in trophic systems (Phleger et al., 1998). Phytoplankton synthesise all required fatty acids de novo, while heterotrophic organisms obtain essential fatty acids from their diet. Due to this fundamental difference, fatty acid analysis allows trophic relationships to be investigated and is particularly useful in dietary studies of lower trophic organisms. Controlled feeding experiments have revealed significant differences in the fatty acid profiles of krill fed on different diets (Alonzo et al., 2003; Hagen et al., 2007; Virtue et al., 1996), hence providing evidence of the usefulness of this signature lipid method for assessing potential nutrient sources in field studies. Well-established trophic indices and fatty acid markers have been used here to provide dietary information on larval and juvenile krill during the winter–spring transition in the pack-ice zone.

Using Principle Component Analysis of krill fatty acid profiles obtained in this study (Table 4), juvenile and furcilia larvae were separated from each other with a Bray–Curtis similarity of 82% (ANOSIM-R > 0.99) (Fig. 2). The fatty acids that contributed 78.4% of this separation were 20:5ω3 (EPA), 22:6ω3 (DHA), 14:0, 18:1ω9 and 16:1ω7. This separation between the two life stages was driven partially by the differences in relative levels of triacylglycerol and phospholipid between larvae and juveniles. Larval krill had higher relative levels of phospholipid than juveniles. Higher PUFA levels (of total fatty acids) occur in phospholipids, particularly EPA and DHA, and these key components constitute the membrane structure of cells and organelles. Triacylglycerol, on the other hand, has much lower PUFA levels, but a higher level of the saturated fatty acid 14:0. Levels of 18:1ω9 and 16:1ω7 are both solely dietary driven and hence are found in higher levels in triacylglycerol.

EPA and DHA are useful as food biomarkers as they largely cannot be synthesised by marine zooplankton and hence must be dietary derived (Phleger et al., 1998). EPA is typically found in higher proportions in diatoms (Volkman et al., 1998), whereas flagellates generally contain higher DHA proportions relative to EPA (Brown et al., 1997). When diatoms are the dominant dietary component, the ratios of 16:1ω7/16:0 reach >1 (Nichols et al., 1986; Skerratt et al., 1998). Although we found high levels of EPA in krill, the ratio of 16:1ω7/16:0 was only 0.1 in furcilia and 0.3 in juveniles, suggesting that diatoms were not a major food source at this time of year. Both larval and juvenile krill examined in the present study had much higher levels of DHA (20% and 13% respectively) compared to levels (9%) observed in animals collected in summer. This indicates the greater importance of microheterotrophs, including flagellates, in the winter to spring transition diet. Additional evidence for an enhanced flagellate contribution to the diet is the presence of the common flagellate fatty acid marker, 18:4ω3, which made up 2.4% and 2.8% of the larval and juvenile krill total fatty acids, respectively.

Ratios of 18:1ω9/18:1ω7 can be used to indicate trophic position in food chains and a high 18:1ω9/18:1ω7 ratio suggests carnivory as the predominant feeding mode. Both larval and juvenile krill in the present study had much higher 18:1ω9/18:1ω7 ratios (1.7 and 1.9 respectively) than those reported in adult krill caught in mid-summer and feeding solely on phytoplankton blooms i.e., 0.4 (Virtue et al., 1996).

High levels of LC-MUFA, 22:1ω11 and 20:1ω9, may be indicative of a diet containing copepods (Dahl et al., 2000). Juveniles had

low relative levels (2.2%) of LC-MUFA. Furcilia also had low levels (1.5%), slightly lower than those found in juveniles. The bacterial markers (15:0, a15:0, i15:0, 17:0, i17:0 17:1ω8c and 17:1) made up 1.4% and 0.6% of juvenile and furcilia total fatty acids respectively. These bacterial markers, although present in low abundance, were appreciable and indicate a diet containing detrital material.

To date, we have very limited data on the diet of larval and juvenile krill during the winter–spring transition, particularly from East Antarctica. The relationship between krill condition, winter sea-ice extent, thickness and degree of deformation remains unclear. There has only been one other study conducted in East Antarctica on krill condition in late winter (O'Brien et al., 2011), and, similar to the present study, the sample size was small. This current study provides additional crucial data to help further unravel the complex relationship between the condition of larval and juvenile krill and sea ice in East Antarctica. Our combined environmental, biological and biochemical findings suggest that flagellates and detrital material are important components of krill diet during the winter–spring transition. Our findings also suggest that sea-ice thickness may influence krill condition as a result of nutrients becoming less accessible as the ice deforms and thickens.

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