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Interannual and between species comparison of the lipids, fatty acids and sterols of Antarctic krill from the US AMLR Elephant Island survey area

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Abstract

Antarctic euphausiids, *Euphausia superba*, *E. tricantha*, *E. frigida* and *Thysanoessa macrura* were collected near Elephant ¦ Island ¦ during 1997 and 1998. Total lipid was highest in *E. superba* small juveniles (16 mg g^{-1} wet mass), ranging from 12 to 15 mg in other euphausiids. Polar lipid (56–81% of total lipid) and triacylglycerol (12–38%) were the major lipids with wax esters (6%) only present in *E. tricantha*. Cholesterol was the major sterol (80–100% of total sterols) with desmosterol second in abundance (1–18%). 1997 *T. macrura* and *E. superba* contained a more diverse sterol profile, including 24-nordehydrocholesterol (0.1–1.7%), *trans*-dehydrocholesterol (1.1–1.5%), brassicasterol (0.5–1.7%), 24-methylenecholesterol (0.1–0.4%) and two stanols (0.1–0.2%). Monounsaturated fatty acids included primarily 18:1($n-9$)*c* (7–21%), 18:1($n-7$)*c* (3–13%) and 16:1($n-7$)*c* (2–7%). The main saturated fatty acids in krill were 16:0 (18–29%), 14:0 (2–15%) and 18:0 (1–13%). Highest eicosapentaenoic acid [EPA, 20:5($n-3$)] and docosahexaenoic acid [DHA, 22:6($n-3$)] occurred in *E. superba* (EPA, 15–21%; DHA, 9–14%), and were less abundant in other krill. *E. superba* is a good source of EPA and DHA for consideration of direct or indirect use as a food item for human consumption. Lower levels of 18:4($n-3$) in *E. tricantha*, *E. frigida* and *T. macrura* (0.4–0.7% of total fatty acids) are more consistent with a carnivorous or omnivorous diet as compared with herbivorous *E. superba* (3.7–9.4%). The polyunsaturated fatty acid (PUFA) 18:5($n-3$) and the very-long chain (VLC-PUFA), C_{26} and C_{28} PUFA, were not present in 1997 samples, but were detected at low levels in most 1998 euphausiids. Interannual differences in these biomarkers suggest greater importance of dinoflagellates or some other phytoplankton group in the Elephant Island area during 1998. The data have enabled between year comparisons of trophodynamic interactions of krill collected in the Elephant Island region, and will be of use to groups using signature lipid methodology.

Keywords: Antarctica; *Euphausia superba*; Fatty acid; Krill; Sterol; Triacylglycerol

1. Introduction

Antarctic krill (*Euphausia superba* Dana) provide 30–90% of the diet for marine carnivores in the Southern Ocean and have an estimated standing stock biomass of about 500 million metric tons (Ross and Quetin, 1988). The global fishery for krill peaked prior to 1990 at about 500 thousand tons per year. The current fishery is about 100 thousand tons per year. This present low level is due primarily to lack of demand (Nicol and Endo, 1999). The success of Antarctic krill reflects their

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ability to adapt to major differences in seasonal food supply. Krill are mainly herbivorous and feed on phytoplankton in the summer. In winter, krill feed on ice algae and probably bacteria and marine detritus, as well as depleting body protein for energy (Virtue et al., 1996). High recruitment and early spawning occur during years where there is a high pack ice concentration of long duration (Siegel and Loeb, 1995).

The Southern Ocean is a complex ecosystem including planktonic herbivores (krill, salps, copepods) fed upon by squid, seals, baleen whales, birds and fish (Quetin and Ross 1991; Loeb et al., 1997). Lipid biomarkers, including lipid class, sterol and fatty acid spectra, have been used increasingly to help understand marine trophodynamics (e.g. Nichols et al., 1984; Sargent et al., 1987). Recent studies using the lipid signature approach have helped to clarify aspects of Antarctic ecology not visible by conventional techniques (Falk-Petersen et al., 1999; Phleger et al., 1999, 2000; Nelson et al., 2000, 2001).

There is a growing literature on Antarctic krill (*E. superba*) lipids (Saether et al., 1985; Quetin and Ross, 1991; Pond et al., 1995; Virtue, 1995; Hagen et al., 1996; Virtue et al., 1996; Mayzaud et al., 1998; Cripps et al., 1999). Fatty acids have been used extensively as bioindicators in *E. superba* (e.g. Virtue et al., 1993b), with sterols used to a lesser extent. More limited detail is available for *E. tricantha* and *E. frigida* (Phleger et al., 1998) and *Thysanoessa macrura* (Reinhardt and Van Vleet, 1986; Kattner et al., 1996; Falk-Petersen et al., 1999). However, few studies have examined interannual changes in krill lipid composition for animals collected from specific locations.

The purpose of this study is therefore to examine comparatively lipid classes, specific sterols and fatty acid biomarkers of Antarctic krill *E. superba* and other less-studied euphausiids, including *E. tricantha*, *E. frigida* and *T. macrura*. The availability of krill collected in both 1997 and 1998 in the oceanographic region near Elephant Island was possible as the area has been intensively surveyed for zooplankton by the United States Antarctic Marine Living Resources (US AMLR) Program. It is noted for high biological productivity and rich krill populations that experienced major fluctuations in density depending on sea ice extent and temperature (Loeb et al., 1997; Brierly et al., 1999). According to Loeb et al. (1998), 1993 and 1998 were 'salp years' with *Salpa thompsoni* numerically dominant (56–89% of total zooplankton), post-larval *Thysanoessa macrura* second in abundance (8–14%) followed by post-larval krill and copepods. The years 1995 and 1996 were 'copepod years' with copepods (presumably *Metridia gerlachei*) dominant taxa. Larval *T. macrura* ranked fourth and second in abundance during February–March 1995 and 1996, respectively. Post larval *T. macrura* and chaetognaths switched in order of abundance during these years. In contrast, *S. thompsoni* ranked sixth and eighth and contributed $<1.5\%$ of total zooplankton. February–March 1994 and March 1997 appear to be 'transition periods (years)' between 'copepod years' and 'salp years'. During 'transition years', copepods were numerically dominant, followed in order by *S. thompsoni*, post-larval *T. macrura* and *Euphausia frigida* (Loeb et al., 1998). 'Salp years' appear to correlate with the 4–5-year period of the Antarctic Circumpolar wave, which propagates changes in sea surface temperatures and wind stress direction (White and Peterson, 1996). An objective of our study is to utilize the lipid composition data to help clarify trophodynamics, including examining possible interannual changes in feeding.

2. Materials and methods

2.1. Sample description

Krill were obtained as part of the AMLR Field Study conducted annually in the Elephant Island region of the Antarctic Peninsula located between 60–62.5°S and 53–59°W. (Loeb et al., 1997; Martin, 1997/8). Specimens were collected by Isaacs–Kidd midwater trawl fitted with a 505 μm mesh plankton net from the R/V *Yuzhmorgeologiya* during January and February, 1997 and 1998. The net was obliquely towed to 170 m depth for approximately 30 min at a speed of 2 knots, or to 10 m above the bottom in shallower waters. Samples were frozen in liquid nitrogen immediately after sorting on board ship. They were then transported frozen (dry ice) by air to CSIRO Marine Research, in Hobart, Tasmania, where they were maintained at -70 °C prior to analysis. 1997 krill were 1.00–1.34 g fresh mass for *E. superba* (five and six pooled individuals for each sample, respectively), 0.01 g for *E. tricantha* (one only) and 0.20–0.70 g for *T. macrura* (20 and 70 pooled for each sample, respectively). Krill for 1998 were

1.29–1.39 g fresh mass for *E. superba* adults (two pooled for each sample), 0.28–0.38 g for *E. superba* juveniles (large), 0.22–0.31 g for *E. superba* juveniles (small) (two pooled for each sample), 0.70–0.86 g for *E. tricantha* (three to four pooled for each sample) and 0.24–0.28 g for *E. frigida* (six to eight pooled for each sample).

2.2. Lipid extraction and analysis

Oil (lipid) analyses were conducted using methods described in Nichols et al. (1998a,b,c) and Phleger et al. (1998, 1999). Briefly, samples were extracted using a single phase Bligh and Dyer (1959) procedure. Oil yield was determined gravimetrically. An aliquot of the oil was analyzed by TLC-FID to determine lipid class composition (Volkman and Nichols, 1991). Fatty acid and sterol profiles were obtained by capillary GC and GC-MS analysis following *trans*-methylation and saponification of aliquots of the oil.

2.3. Statistical analyses

Fatty acid profiles (mg g^{-1} wet mass) of individual samples were compared by cluster analysis using Pearson's correlation coefficient and average linkage (Fig. 1). Pearson's correlation coefficient and non-metric multidimensional scaling (MDS) were also used to compare FA profiles (full profiles) in two dimensions, using the Kruskal Loss Function. All multivariate analyses were conducted using SYSTAT 9 (SYSTAT, Inc., Evanston, IL, USA).

3. Results

3.1. Lipid classes

Polar lipids were the major lipid class in all euphausiids in 1998 (56–81% of total lipid; Table 1). Triacylglycerols (TAG) were the second most abundant lipid class (22–38% in *Euphausia superba*, 16% in *E. tricantha* and 12% in *E. frigida*). *E. tricantha* was the only euphausiid with wax esters (WE) (6% of total lipid). WE were below detection (<0.5%) in the other species. In all animals, sterols (ST) accounted for 4–7% of total lipids, with low free fatty acids (FFA) (1–3%). Total lipid was highest in *E. superba* small juveniles (15.9 mg g^{-1} wet mass) and ranged from 12.4–14.6 mg g^{-1} in all other euphausiids (Table 1).

3.2. Sterols

Cholesterol was the major ST in all krill (80–100% of total ST) with highest values in *E. tricantha* (94–100%) and *E. frigida* (97%) (Table 2). Desmosterol was the second most abundant ST (0–18% of total ST), and was highest in 1997 *E. superba* (18%), but markedly less in 1998 *E. superba* (2–4%). Although there was 6% desmosterol in 1997 *E. tricantha*, none was detected in 1998 *E. tricantha* (Table 2). Desmosterol was also not detected in *E. frigida*. The 1997 *Thysanoessa macrura* and *E. superba* samples were the only samples with ST other than cholesterol and desmosterol. These included primarily 24-nordehydrocholesterol (0.1–1.7%), *trans*-dehydrocholesterol (1.1–1.5%), brassicasterol (0.5–1.7%) and 24-methylenecholesterol (0.1–0.4%). Low levels of stanols (0.1–0.2% cholestanol and brassicastanol) were only detected in 1997 *T. macrura* and *E. superba*, but not in the 1998 krill (Table 2).

3.3. Fatty acids

Polyunsaturated fatty acids (PUFA) were 39–45% of the total FA in all *E. superba* samples, but were somewhat lower in 1998 *E. tricantha* (30%) and 1998 *E. frigida* (31%), with 11% total PUFA in 1997 *E. frigida* (Table 3). Eicosapentaenoic acid [EPA, 20:5($n-3$)] and docosahexaenoic acid [DHA, 22:6($n-3$)] were the two major PUFA in all samples (Table 3). Highest EPA and DHA values were detected in *E. superba* (15–21% and 9–14%, respectively). These two PUFA were generally lower in abundance in *E. frigida* (4–18% EPA; 5–9% DHA) than in *E. tricantha* (12–18% EPA; 14–16% DHA). Ratios of EPA/DHA for 1997 and 1998 *E. superba* were similar (1.4–1.6; Table 3, Fig. 2) whereas this ratio was somewhat lower for 1997 *E. tricantha* (1.2 vs. 0.9 for 1998). In contrast, the EPA/DHA ratio for *E. frigida* was lowest (0.7) in 1997 and highest (2.0) in 1998 of all samples analyzed.

Arachidonic acid [AA, 20:4($n-6$)] was 1% of total FA in 1997 and 1998 *E. tricantha*, and only 0.4–0.6% in 1997 and 1998 *E. frigida*. In contrast, AA was not detected in *E. superba* and *T. macrura*. Although levels of the PUFA 18:4($n-3$) were 4–9% in *E. superba* from both years, it was only

Table 1
Percentage lipid class composition of 1998 Antarctic euphausiids[a]

	n	Wax ester	Triacylglycerol	Free fatty acid	Sterol	Polar lipid	Lipid as mg g^{-1} wet mass	Lipid/individual (mg g^{-1})
E. superba (adult)	4	–	26.0±7.4	1.3±0.3	6.1±0.9	66.6±6.3	14.5±4.3	7.2±2.1
E. superba (juv-large)	2	–	38.4±3.4	1.1±0.5	4.1±0.8	56.4±2.1	14.6±4.3	14.6±4.3
E. superba (juv-small)	2	–	22.1±0.3	1.8±0.7	4.0±0.2	72.0±0.6	15.9±2.7	8.0±1.3
E. tricantha	3	5.8±1.8	16.2±7.4	3.2±1.0	7.4±1.1	67.5±6.4	12.4±3.5	3.9±1.6
E. frigida	3	–	11.6±2.6	1.8±0.3	5.8±0.6	80.8±3.3	13.9±0.9	2.1±0.2

[a] Presented as mean±S.D.; –, below detection.

Table 2
Percentage sterol composition of 1997 and 1998 Antarctic euphausiids[a]

Sterol	1997			1998				
	E. superba ($n=2$)	*E. tricantha*	*T. macrura* ($n=2$)	*E. superba* (adult, $n=4$)	*E. superba* (juv-large, $n=2$)	*E. superba* (juv-small)	*E. tricantha* ($n=3$)	*E. frigida* ($n=3$)
24-Nordehydrocholesterol	0.1±0.2	–	1.7±2.1	–	–	–	–	–
24-Nordehydrocholestanol	–	–	tr	–	–	–	–	–
Occelasterol	–	–	0.1±0.1	–	–	–	–	–
trans-Dehydrocholesterol	1.1±0.4	–	1.5±1.0	–	–	–	–	–
Cholesterol	80.0±2.5	94.3	81.2±15.3	92.8±5.0	86.9±0.9	88.6	100.0±0.0	96.8±2.8
Cholestanol	0.1±0.1	–	0.1±0.1	–	–	–	–	–
Desmosterol	18.2±1.8	5.7	6.5±1.3	1.7±1.3	3.8±0.5	2.9	–	–
Brassicasterol	0.5±0.0	–	1.7±1.8	–	–	–	–	–
Brassicastanol	–	–	0.2±0.2	–	–	–	–	–
24-Methylenecholesterol	0.1±0.1	–	0.4±0.2	–	–	–	–	–
Other	–	–	6.7	5.5	9.3	8.5	–	3.2

[a] Presented as mean±S.D.; –, below detection; tr, trace (below integration).

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