

On the use of lipid biomarkers in marine food web analyses: An experimental case study on the Antarctic krill, *Euphausia superba*

Dorothea Stübing¹ and Wilhelm Hagen

Marine Zoology, University of Bremen, P.O. Box 33 04 40, D-28334 Bremen, Germany

Katrin Schmidt

Institute for Baltic Sea Research Warnemünde, Seestrasse 15, D-18119 Rostock, Germany

Abstract

The application of marker fatty acids to trace the feeding habits of *Euphausia superba* (krill) has produced contradictory results. We examined the effects of various diets on the fatty acid composition of larval, juvenile, and adult *E. superba* collected in April 1999 in the southwest Lazarev Sea and in April 2001 in the Bellingshausen Sea. Specimens were fed four different diets (mixed phytoplankton, mixed ice algae, the ice diatom *Fragilariopsis cylindrus*, and mixed copepod assemblages) or starved for up to 44 d. Total lipid content, lipid classes, and fatty acid composition showed very little variation in juvenile and adult krill with the different feeding regimes. Furcilia lipids were much more strongly influenced by the fatty acid signatures of their food. No stage-specific food preferences were detected in the larvae, and spatial patterns were mirrored by all furcilia stages. Comparison of the fatty acid profiles of the offered food with those of the subsequently excreted feces indicated preferential assimilation of polyunsaturated fatty acids by *E. superba*.

The analysis of lipid compositions has been applied successfully to reveal food web relationships in marine ecosystems. This trophic biomarker concept is based on observations that specific dietary lipid components, particularly fatty acids, are incorporated into the consumers' lipids largely unmodified (Sargent and Whittle 1981; Sargent et al. 1987; Graeve et al. 1994b). This approach can provide information where the classical gut content analysis fails (e.g., soft-bodied organisms, advanced digestion). Instead of a snap-shot impression, biomarkers integrate the trophic information over a longer time scale of several weeks. However, lipid signatures usually do not have the precision to identify species-specific interactions. Rather, they provide trophic information on the level of larger taxonomic groups.

Experimental studies have further consolidated the trophic biomarker approach. Clear changes in fatty acid compositions could be induced by different phytoplankton diets in Arctic copepods (Graeve et al. 1994b) and even traced up to secondary consumers, such as juvenile North Sea cod (St. John and Lund 1996). In recent years, an increasing number

of studies have applied this method to identify trophic relationships in various marine ecosystems: among benthic species and communities (e.g., Graeve et al. 1997), in a variety of Arctic and Antarctic zooplankton groups (e.g., Scott et al. 1999; Falk-Petersen et al. 2002), and most extensively in polar copepods (e.g., Graeve et al. 1994a, Kattner and Hagen 1995; Scott et al. 1999) and euphausiids (Hagen and Kattner 1998; Kattner and Hagen 1998; Phleger et al. 1998; Virtue et al. 2000; see also Falk-Petersen et al. 2000 for review).

In Antarctic krill, *Euphausia superba*, investigations of feeding habits via fatty acid compositions produced contradictory results. Virtue et al. (1993a) found significant differences between adult krill fed on diatoms versus the flagellate *Phaeocystis*. Another feeding experiment with copepods yielded a strong increase in polyunsaturated fatty acids as compared to field krill (Cripps and Atkinson 2000). In other investigations, this approach did not provide such clear results. Cripps and Hill (1998) found that the fatty acid compositions of copepods clearly reflected dietary preferences and changing food availability, but there were no such relationships detectable for *E. superba*. Based on a multi-seasonal data set, Hagen et al. (2001) concluded that the influence of dietary fatty acids on the lipid composition of *E. superba* is rather small. On the other hand, numerous studies traced feeding habits in *E. superba* with the biomarker approach (Virtue et al. 1997; Cripps et al. 1999; Cripps and Atkinson 2000; Falk-Petersen et al. 2000; Phleger et al. 2002).

Therefore, one major objective of this study was to clarify the applicability of fatty acids as trophic markers for *E. superba* and discuss the reasons for possible limitations in various ontogenetic stages. This study presents a comprehensive experimental approach to evaluate the influence of dietary fatty acids on the lipid composition of *E. superba*. Females, juveniles, and furciliae were caught in two autumn seasons

¹ Corresponding author (stuebing@uni-bremen.de).

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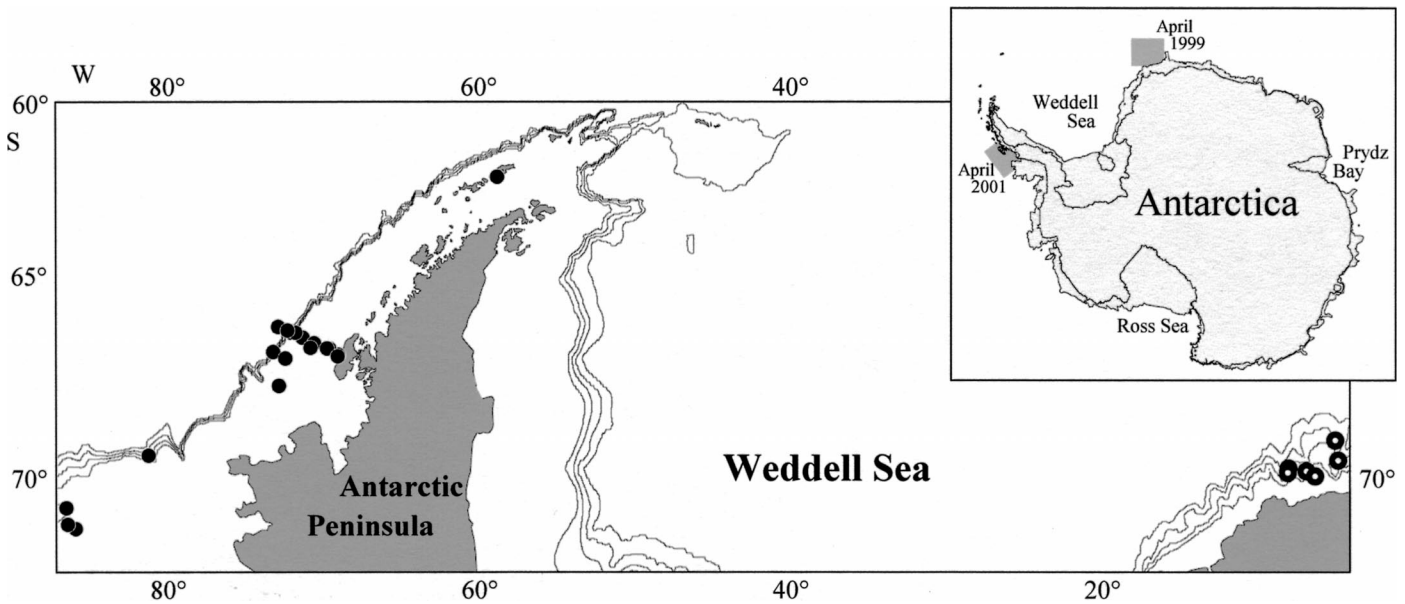


Fig. 1. Map of the investigation areas. Open circles mark stations sampled in the southwest Lazarev Sea in autumn 1999; filled circles indicate stations in the Bellingshausen Sea in autumn 2001.

and fed controlled diets (mixed phytoplankton, ice algae, or copepods) or starved for up to 44 d. Experimental data were compared with those of field samples, and the results are discussed with regard to lipid metabolism and ontogenetic differences.

Methods

Sampling—*E. superba* specimens were sampled during two autumn cruises with RV *Polarstern* (14–20 April 1999, open circles, and 18 April–1 May 2001, filled circles, Fig. 1) on transects across the shelf break in the southwestern Lazarev Sea and in the Bellingshausen Sea, respectively. In 1999, slow vertical bongo net tows (335- μ m mesh, 5-liter closed cod end) were carried out in the top 150 m at night. In 2001, zooplankton was sampled by double oblique rectangular midwater trawl (RMT 1 + 8) hauls (mesh size 325 and 4,500 μ m, 20-liter closed cod end), vertical bongo net tows, or a hand-hauled Apstein net. Zooplankton was thus obtained in excellent condition and immediately transferred to the cool lab for sorting. After a \pm 24-h defecation period in 1- μ m-filtered seawater *E. superba* specimens were either frozen at -80°C or transferred to the experimental containers. Prior to freezing, developmental/maturity stage, sex, and length (tip of rostrum to end of telson) were recorded, and the animals were briefly rinsed with deionized water and blotted dry. Samples of juveniles and adults represent individual specimens, whereas 5–95 furciliae were pooled for each sample, depending on size and availability.

Experiments—Adult and juvenile *E. superba* were maintained in aerated 170-liter tanks and furcilia larvae in 18-liter containers. The experiments were carried out in a cold room (0 – 2°C) in dim light. Three batches of about 50 mixed juvenile and adult krill were incubated with freshly caught

copepods or sea ice biota or were starved in 1- μ m-filtered seawater. The treatments for furciliae (about 200 per batch) were starvation or a diet of ice biota, a monoalgal culture of the common Antarctic ice diatom *Fragilariopsis cylindrus*, or mixed phytoplankton. Ice biota were obtained by slowly thawing brown pieces of sea ice in at least a threefold volume of filtered seawater and subsequently screening through a 55- μ m sieve. Copepods were picked out daily from bongo net tows, excluding damaged animals and large carnivorous species such as *Pareuchaeta* spp. Thus, the copepod diet consisted of varying species compositions dominated at first by the more southern species *Calanus propinquus* and *Calanoides acutus* and later by *Calanus simillimus* and *Metridia gerlachei*. Both the mixed phytoplankton and the ice biota were dominated by diatoms: the first by *Fragilariopsis*, *Chaetoceros*, and *Pseudonitzschia* species; the latter also by *Fragilariopsis* spp. and *Chaetoceros* spp. as well as by small resting spores or cysts. The food concentrations approximated $480 \pm 220 \mu\text{g C L}^{-1}$ for copepods, $105 \pm 35 \mu\text{g C L}^{-1}$ for phytoplankton, 350 ± 120 (furciliae) or $220 \pm 80 \mu\text{g C L}^{-1}$ (juveniles and adults) for ice algae, and $110 \pm 40 \mu\text{g C L}^{-1}$ for *F. cylindrus*. Every 48 h, animals were transferred to a new batch of food or filtered seawater. Animals in poor condition, as well as fecal strings, were removed and frozen in dichloromethane/methanol under a nitrogen atmosphere at -80°C . Food uptake was monitored by chlorophyll measurements and counting of the phytoplankton cells and the copepods. Subsamples of the food were frozen in dichloromethane/methanol for fatty acid analyses.

Krill from these experiments were used either for lipid analyses (present study) or for stable isotope measurements (Schmidt et al. 2003).

Lipid analyses—After lyophilization for 48 h, the samples were weighed and total lipid was extracted with dichloro-

methane/methanol (DCM/MeOH) (2:1 [v/v] + 0.01% butylhydroxytoluene [BHT] as antioxidant) and determined gravimetrically (Hagen 2000). In order to obtain a correction value for the BHT, 10 blank aliquots of extraction solvent were treated the same way as the samples, and the mean blank mass (=0.33 mg) was subtracted from the total lipid mass of each sample. Because of the interference of BHT with sterol esters (*see below*), it was not added as antioxidant to the 2001 samples.

Neutral lipid classes were analyzed in duplicate by thin-layer chromatography (TLC)–flame ionization detection (FID) on an Iatroscan Mk V according to Fraser et al. (1985). Because different lipid classes give different FID responses, two mixtures of commercial standards (Sigma) that approximated the lipid class compositions of the analyzed samples were prepared for calibration: phosphatidylcholine:triolein:cholesterol:cholesteryl oleate:oleic acid at 49:35:8:5:3 (v/v) for juveniles and adults and 64:25:6:4:1 (v/v) for furciliae. Because BHT co-runs with sterol esters, a dilution series of the BHT blanks was analyzed by Iatroscan, and the sterol ester amounts of the 1999 samples were corrected by the appropriate values.

Because the separation of polar lipids remained unsatisfactory by TLC-FID, the phospholipid composition was determined by high-performance (HP) TLC-scanning densitometry (modified after Olsen and Henderson 1989). Five microliters of the total lipid extracts was applied by means of a CAMAG Linomat IV in duplicate or triplicate on pre-developed HPTLC plates (silica gel 60, Merck). The plates were developed in a horizontal chamber with isopropanol:methylacetate:chloroform:methanol:0.25% KCl (25:25:25:10:9, v/v) for 17 min and dried for 30 min in an evacuated desiccator. The plates were then immersed for 5 s in a postchromatographic derivatization reagent with a CAMAG Chromatogram Immersion Device III and charred at 200°C for 20 min. The derivatization reagent was prepared by dissolving 1.2 mg manganese(II)-chloride in 180 ml of Aqua bidest and adding 180 ml methanol and 12 ml concentrated sulfuric acid. The lipid bands were quantified with a CAMAG TLC-Scanner 3 at 550 nm wavelength and calibrated using commercial standards for each detected lipid class.

For the fatty acid analysis of single lipid classes, aliquots of the total lipid extracts were spotted on self-coated glass plates (silica gel Merck H60, film thickness 750 μm) by a CAMAG Linomat IV. In order to keep the run time as short as possible, neutral and polar lipids were developed separately. The developing solvent was evaporated with nitrogen, and the lipid bands were visualized by iodine vapor. The bands were scraped off with a Teflon spatula and extracted according to the total lipid extraction. The purity of the isolated lipid classes was verified by TLC. Lipids were hydrolyzed, and the fatty acids were converted to their methyl ester derivatives (FAME) in methanol containing 3% concentrated sulfuric acid at 80°C for 4 h (Kattner and Fricke 1986). After cooling, 4 ml of Aqua bidest. were added, and FAMES were extracted with hexane (3 \times 1.7 ml), analyzed in a gas chromatograph (HP 6890A) equipped with a DB-FFAP column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness) using temperature programming and he-

lium as the carrier gas. FAMES were detected by flame ionization and identified by comparing retention time data with those obtained from standard mixtures.

Statistics—Analysis of variance was performed to detect significant differences between the means of the different experimental treatments. Using the SPSS software package for Macintosh, a one-way ANOVA was calculated and the Games–Howell post hoc test was applied for multiple comparisons.

Multivariate statistical analyses were applied to the percent fatty acid composition of the total lipids of *E. superba*. They were carried out with the software “Primer.” The Bray–Curtis index was used to calculate a similarity matrix, which was the basis for the cluster analysis (hierarchical agglomerative, group average linkage).

Results

Dry mass and total lipid content—Length, dry mass, and total lipid content of the *E. superba* used in the feeding/starvation experiments are given in Table 1. There was no optimal random distribution of the krill specimens to the various treatments; that is, the body length varied between treatments, with the largest animals in the field group representing time zero in the experiments. Accordingly, body mass and total lipid content also showed pronounced variations. In order to allow comparisons between the different treatments irrespective of length-dependent variations, values were calculated for standardized specimens of a certain length. For this purpose, regressions of dry mass and lipid mass versus total length were calculated separately for each time point of each experiment. Dry mass and lipid mass, respectively, of a medium-sized animal (females 44 mm, juveniles 31 mm) were then derived from these regressions. Figure 2 shows the development of dry mass and total lipid for such hypothetical animals in the different treatments. Dry mass and total lipid decreased during the experiments, indicating that the krill were not doing well in our experiments. Female dry mass decreased most under starvation conditions and least on the ice algae diet (Fig. 2a). Total lipid showed the reverse, with the strongest initial decline in individuals fed on ice algae (Fig. 2b). Although the dry mass continued to drop, lipid mass increased slightly toward the end of the ice algae experiment after 44 d.

Whereas the dry mass of females steadily decreased in the course of all experiments, this was only true for the starving juveniles. After an initial loss, juveniles feeding on copepods or ice algae increased again in body mass (Fig. 2c), almost entirely because of lipid accumulation (Fig. 2d).

Because of experimental constraints (a limited number of larvae per treatment, only 5–9 individuals could be frozen in DCM/MeOH), dry mass data were not available for the furcilia experiments from April 1999. Dry mass and total lipid mass strongly increased in furciliae feeding on the Antarctic ice diatom *F. cylindrus* (Fig. 2e,f). Although the slope of dry mass increase was steeper during the first 10 d of feeding, total lipid increased more rapidly between days 10 and 17, indicating that somatic growth is fueled first before substantial lipid accumulation occurs.

Table 1. *Euphausia superba*. Standard length (SL), dry mass (DM), and total lipid (TL), absolute and percent dry mass, of females, juveniles, and furciliae used in the different treatments. *n*, number of samples; n.d., not determined.

	Females					Juveniles					Furciliae				
	SL (mm)	DM (mg)	TL (mg)	TL (% DM)	<i>n</i>	SL (mm)	DM (mg)	TL (mg)	TL (% DM)	<i>n</i>	Stage	DM (mg)	TL (mg)	TL (% DM)	<i>n</i>
Field	50±2	208.9±27.4	74.0±18.5	35.1±5.8	11	33±3	60.5±15.5	22.8±7.3	34.7±5.4	19	FIII	0.47±0.07	0.07±0.02	13.8±2.6	8
Copepods	47±3	184.4±39.1	66.5±19.6	35.5±4.4	6	32±2	57.1±10.0	21.5±4.7	37.5±2.6	9	—	—	—	—	—
Ice algae	46±2	148.3±34.7	59.4±28.5	35.5±10.0	8	31±1	46.7±3.6	16.3±2.3	34.8±3.1	5	FIII	n.d.	0.06±0.02	n.d.	4
<i>F. cylindrus</i>	—	—	—	—	—	—	—	—	—	—	FIII-V	0.99±0.21	0.15±0.05	14.8±2.5	3
Phytoplankton	—	—	—	—	—	—	—	—	—	—	FIV-V	n.d.	0.17±0.04	n.d.	7
Starvation	45±3	144.7±14.3	53.1±2.7	37.0±3.0	3	31±2	46.6±15.1	16.4±7.3	34.2±5.1	10	FIII	n.d.	0.06±0.02	n.d.	3

After 19 d, the furciliae feeding on a diet of mixed ice biota and those under starvation did not show any significant changes in their lipid content, whereas the phytoplankton-fed animals showed a significant ($p \leq 0.001$) increase in lipid mass (Fig. 2f). Concomitantly, these furciliae continued to grow and molt, reaching stage IV to V at the end of the experiment after 44 d (Table 1).

Lipid class composition—Lipid class compositions were very uniform for females and juveniles and did not show any food-related differences. The variability of individuals from the same treatment was within the same range as for individuals from different treatments. Therefore, the means were averaged over all experiments (Fig. 3). Triacylglycerol (TAG) was the major lipid class with 20% of dry mass (DM) in juveniles and slightly less in females. In furcilia III–IV larvae, the proportion of TAG was <5% DM. Their lipids were dominated by phosphatidylcholine (PC) with >7% DM, which is equivalent to 47% of total lipids. The second prominent phospholipid, phosphatidylethanolamine (PE), was present in fairly equal levels of ~3% DM in all stages.

Fatty acid composition—Juveniles and adults: As for the lipid class compositions, the fatty acid compositions also did not show major differences between the experimental treatments (Table 2). This might be partly because of the similarity of the algal diets used in the feeding experiments, which all showed clear diatom fatty acid signals. However, the proportion of the essential polyunsaturated fatty acid (PUFA) 20:5(n-3) is lowest in the mixed phytoplankton and highest in the pure ice diatom culture (Fig. 8). Accordingly, one objective of our study was to find out whether it is possible to discriminate between feeding on phytoplankton or ice diatoms by applying fatty acid analyses (or stable isotope analyses, Schmidt et al. 2003).

The lipids of both juveniles and females were dominated by the fatty acids 16:0 and 20:5(n-3), followed by 18:1(n-9) and 14:0 with similar proportions of 12–16% of total fatty acids (TFA). Fatty acids 22:6(n-3) and 18:1(n-7) ranked next with 6–8% TFA. For females, only one significant difference could be detected: 22:1(n-9), one of the marker fatty acids for calanid copepods (e.g., Kattner and Hagen 1995), exhibited a significant increase in individuals feeding on copepods, as compared to the field samples. For juveniles, both 22:1 isomers reached significantly higher levels in the copepods treatment than in the field animals and the other treatments. Juveniles feeding on ice algae showed significantly higher proportions of 18:1(n-9) accompanied by lower levels of 20:5(n-3), as compared to field samples or animals feeding on mixed phytoplankton or starving ($p \leq 0.05$). This result is contrary to the expected changes because 20:5(n-3), with up to 33% TFA, is by far the dominant fatty acid of the ice algae, and 18:1(n-9) is only a minor constituent (Fig. 8).

Dietary influences on the fatty acid composition of total lipid extracts can be masked by the presence of structural lipids, which are supposed to have a relatively stable fatty acid profile (Sargent et al. 1987). Because there was no clear response observable for the total lipids from postlarval krill, particularly females, of the various feeding regimes (Table

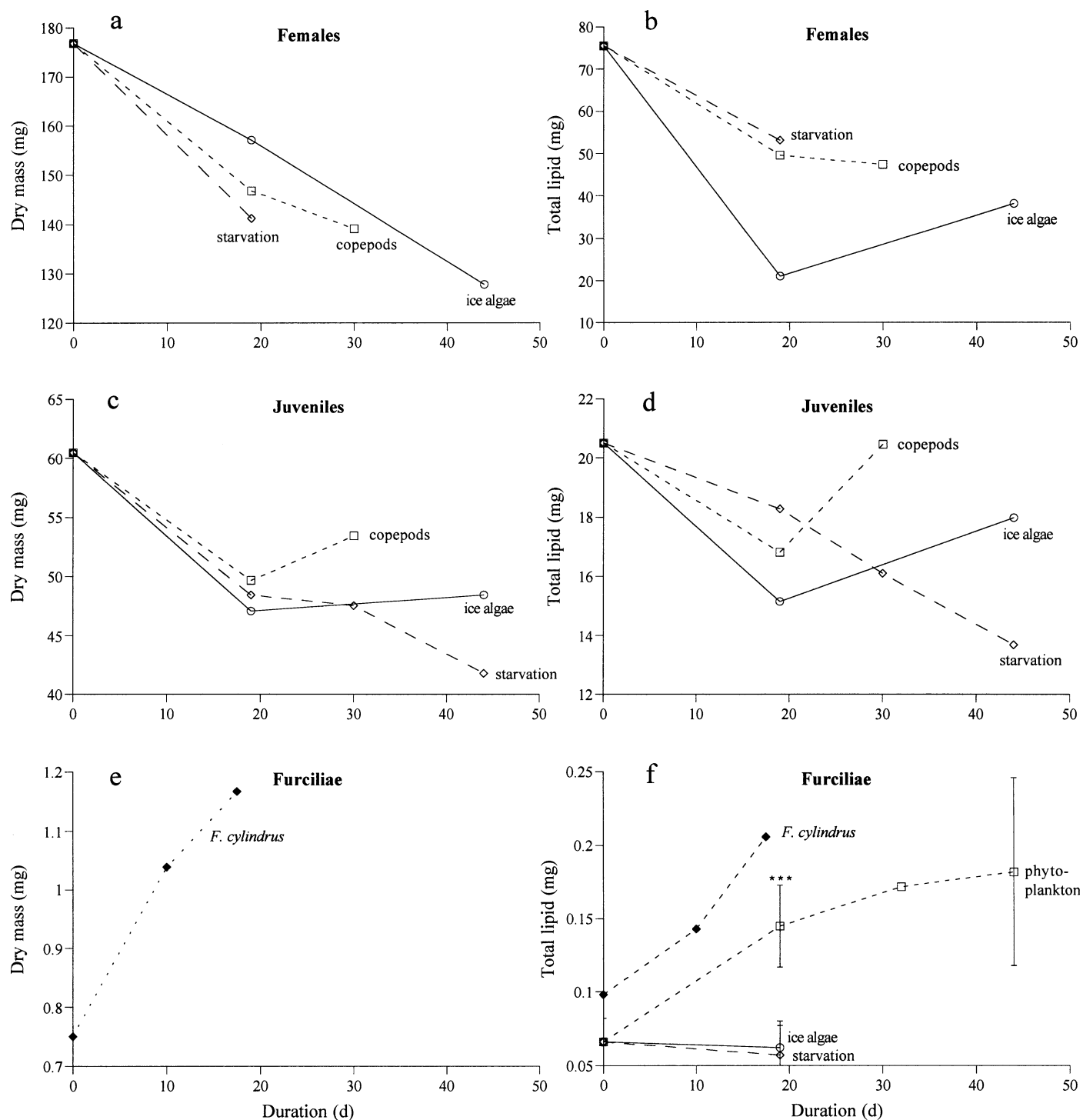


Fig. 2. *Euphausia superba*. (a,b) Development of dry mass and total lipid mass during the feeding/starvation experiments for a standardized female of 44 mm body length, (b,c) a standardized juvenile of 31 mm, and (e,f) for furciliae (not standardized). The significant increase in total lipid of furciliae feeding for 19 d on phytoplankton is *** $p \leq 0.001$.

2), the fatty acid compositions were analyzed separately for the dominant lipid classes. This is based on the assumption that the fatty acid composition is characteristic for each lipid class and that dietary influences are primarily mirrored in the storage lipids. Because TAG is the primary storage lipid of *E. superba* and phosphatidylcholine (PC) serves, besides

its structural function in membranes, as an additional energy source (Hagen et al. 1996, 2001), the fatty acid composition of these two lipid classes should best reflect the animals' feeding habits. Figure 4 compares the dominant fatty acids of the main lipid classes TAG, PC, and phosphatidylethanolamine (PE). In contrast to the hypothesis, the fatty acid

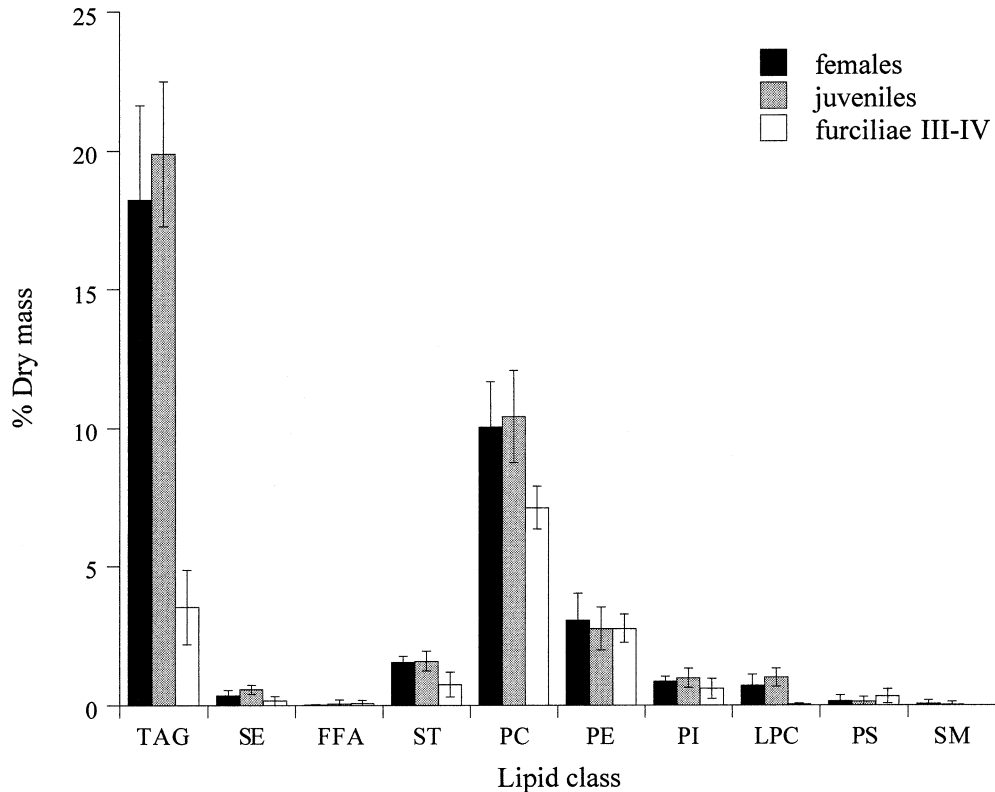


Fig. 3. *Euphausia superba*. Lipid class composition (means and standard deviations) of females, juveniles, and furciliae averaged over all treatments. SE, sterol ester; TAG, triacylglycerol; FFA, free fatty acids; ST, sterol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; LPC, lysophosphatidylcholine; SM, sphingomyelin.

compositions of TAG and PC were quite uniform and showed only very little variation with the different feeding regimes, both in juveniles and females. PE however, which is purely a membrane lipid and should thus be stable, was found to have the strongest variability, although not correlated with the typical dietary fatty acids.

Furciliae: Whereas no clear changes in the fatty acid profiles of juvenile and female krill could be induced by different feeding regimes, there was a higher variability exhibited by the furciliae. As Fig. 2f shows, from the 1999 experiments, the phytoplankton-fed furciliae should be most interesting because they were the only ones that obviously fed and grew during the experimental period. Compared to the field samples, their lipids increased significantly in the diatom marker 16:1(n-7) and in 18:1(n-7) (Table 2), the latter probably resulting from chain elongation of the former fatty acid. However, the portion of 20:5(n-3), the second diatom marker, decreased by almost 5%. This can be explained by the comparatively moderate levels of this long-chain PUFA in the lipids of the mixed phytoplankton culture (~18% TFA) compared to the ice algae (Fig. 8). However, the other two treatments also had observable changes in the fatty acid composition, although the total lipid content did not change significantly (Fig. 2f). The most pronounced changes concerned the two 18:1 isomers: whereas the (n-9) isomer decreased significantly, the (n-7) moiety showed a

significant increase during 19 d of feeding on ice algae. Significant decreases compared to the field and the phytoplankton-fed individuals also were observed for the flagellate marker 18:4(n-3). This decrease was even stronger during starvation. Ice algae were the only diet which did not induce a decline in the proportion of 20:5(n-3), indicating that the furciliae had been feeding on a diet rich in this PUFA (i.e., ice algae) in the field.

In 2001, a large number of furciliae could be sampled at different locations, and varying fatty acid compositions could be related to different feeding histories. For the later furcilia stages (III–VI), multivariate statistics were applied on the percent compositions of all identified fatty acids of the total lipids to detect similarities. Three groups were identified by cluster analysis (Fig. 5a). Although most of the larvae showed fatty acid profiles typical of feeding on diatoms (Table 2; Fig. 5b), there were clear deviations toward a more flagellate-dominated diet at two stations. Group I comprises animals from station 301, the northernmost off-shore station off Adelaide Island (Fig. 1). They were characterized by a relatively low lipid content of $12.0 \pm 2.4\%$ of dry mass (DM) and accordingly high levels of PUFAs (Table 2). The ratios 16:1(n-7)/18:4(n-3) and 20:5(n-3)/22:6(n-3) (=EPA/DHA) were intermediate and did not point to a diatom- or a flagellate-dominated diet. Group II represents furciliae IV–VI with a mean lipid content of about 20% DM and the lowest PUFA levels. They were caught on station

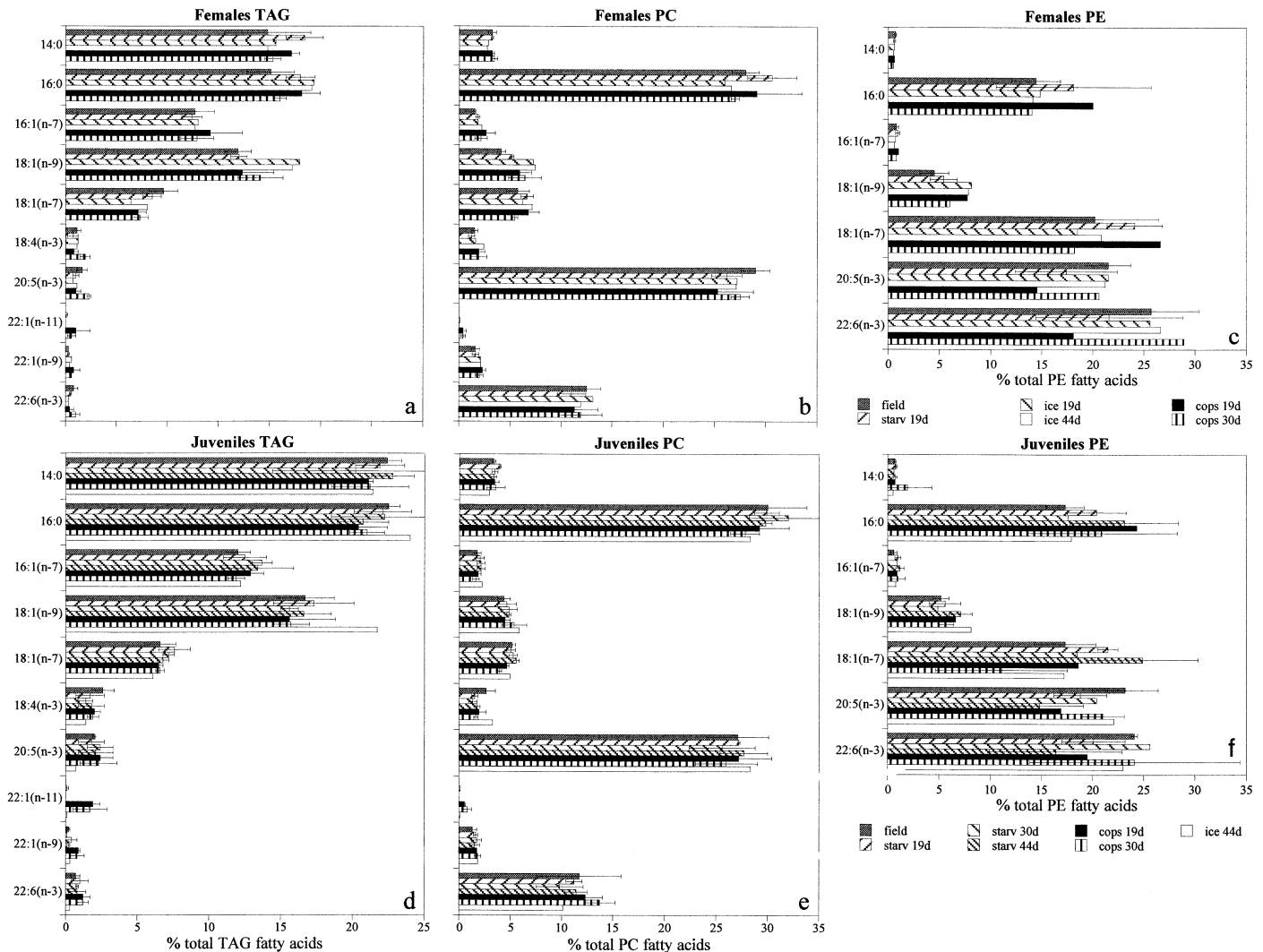


Fig. 4. *Euphausia superba*. Percentage fatty acid compositions of the three dominant lipid classes of (a–c) females and (d–f) juveniles compared for all experiments. TAG, triacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; starv, starvation; cops, feeding on copepods; ice, feeding on ice algae.

328, also on the northernmost transect, but further inshore and 2 weeks later than those from station 301. Their trophic indices were very low and clearly pointed to a flagellate-dominated diet. The last group is a mixture of larval stages III–V from different stations and from the feeding experiment with *F. cylindrus*. They share a high lipid content of $18.9 \pm 4.6\%$ DM and very high diatom/flagellate marker ratios.

In Fig. 5b, furciliae samples were plotted according to their ratios 16:1(n-7)/18:4(n-3) and EPA/DHA to discriminate between diatom (high values) versus flagellate feeding histories (low values). The same groups were identified by the multivariate analyses (Fig. 5a). The high variability in the diatom-feeding group is from differences in the total lipid content. The two samples with lower EPA/DHA ratios contained only 13.7 and 11.4% DM lipid compared to $21.0 \pm 2.6\%$ in the six samples with high ratios. The intermediate group was characterized by very low lipid content, and a 16:1(n-7)/18:4(n-3) ratio of about 1 points to a mixed diet

consisting of diatoms and flagellates. In the third group, both ratios were very low, clearly indicating a flagellate-dominated diet.

In order to find out in which lipid class the flagellate marker fatty acids are accumulated, detailed analyses of the fatty acid compositions of the three main lipid classes (PC, PE, and TAG) were carried out for the four furcilia samples from station 328. Most of the flagellate marker 18:4(n-3) is accumulated in TAG (65%), with about one third in PC and only minor amounts in PE (Fig. 6). The second flagellate marker 22:6(n-3) is predominantly located in the phospholipids, especially in PC. Other typical TAG fatty acids are 14:0 and 16:2(n-4), which occur only in minor amounts in the phospholipids. Fatty acids 18:0 and 18:1(n-7) have the highest share in PE.

The feeding experiment with the Antarctic ice diatom *F. cylindrus* was carried out with furciliae caught at station 301 (group I, Table 2, Fig. 5). Their low lipid content and the weak diatom signal in their fatty acids represented an ideal

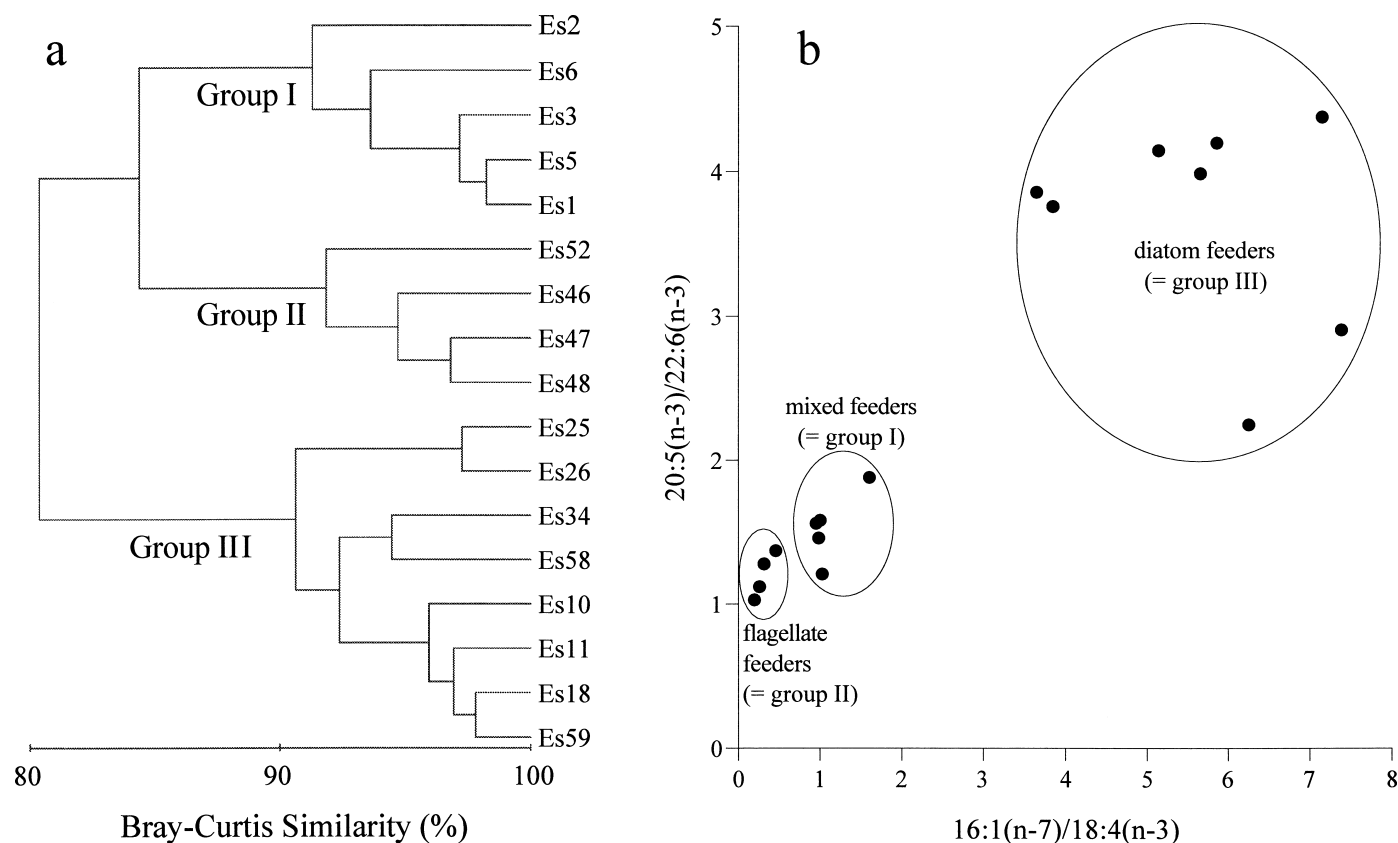


Fig. 5. *Euphausia superba*. Groupings of furciliae III–VI caught at different stations in 2001 (a) according to their percentage composition of the total fatty acids by cluster analysis and (b) according to their ratios of 20:5(n-3)/22:6(n-3) versus 16:1(n-7)/18:4(n-3).

background signature for this experiment. The development of the dominant fatty acids after 10 and 17 d of feeding is illustrated in Fig. 7. In the course of the experiment, the diatom markers 20:5(n-3) and 16:1(n-7), which are the major constituents of the lipids of *F. cylindrus* (Fig. 8b), increased markedly. The saturated short-chain fatty acids 14:0 and 16:0, as well as the 18:1 moieties, also showed a moderate increase in mass, but this did not cause a shift in the percent composition (Table 2). The absolute amount of 22:6(n-3) did not change throughout the experimental period, resulting in a relative decrease of over 50% from 15 to 7% TFA. Fatty acid 18:4(n-3) was largely depleted when feeding on *F. cylindrus*, which is poor in this fatty acid.

Biotransformation of fatty acids during gut passage—Comparisons of fatty acid compositions of food sources with those of the feces can help deduce whether specific dietary fatty acids are preferentially assimilated by *E. superba* (Fig. 8). Because the fatty acid composition of the prey copepods varied markedly with time, no means were calculated. Instead, the signature of the copepod assemblage, which had not been eaten by the end of the experiment, was compared with that of the fecal strings that were produced during the preceding 4 d. Whereas the short-chain saturated fatty acids 14:0 and 16:0 tended to be more abundant in the fecal strings of krill than in the prey copepods, the energy-rich long-chain polyunsaturated essential fatty acids 20:5(n-3) and 22:6(n-3) had been assimilated and occurred in low

amounts in the feces. The two 22:1 isomers, typical marker fatty acids for calanid copepods, however, were apparently not selectively assimilated by the krill. They occurred in similar levels both in the prey copepods and in the feces (Fig. 8a). The lipids of both the mixed ice biota and the ice diatom *F. cylindrus* were clearly dominated by 20:5(n-3) followed by 16:1(n-7) (Fig. 8b,c). The fecal strings were depleted of both these marker fatty acids, as well as of the shorter chain polyunsaturated fatty acids 16:3(n-4) and 16:4(n-1). Again, the saturated moieties 16:0 and 18:0 were found in higher concentrations in the feces, and also 18:1(n-9) was not assimilated from the food. The lipids of the diatom-dominated phytoplankton mixture were also primarily composed of the typical diatom fatty acids, but with a more even distribution and a higher level of 16:0 (Fig. 8d). The essential fatty acids 20:5(n-3) and 22:6(n-3) had been extracted from the phytoplankton, resulting in a higher portion of short-chain saturated moieties in the fecal strings.

Discussion

The present study produced contradictory results on the potential of fatty acids as trophic biomarkers in krill. Whereas the fatty acid compositions of neither total lipids nor the storage lipid classes of female and juvenile *E. superba* were much influenced by different diets, those of larval krill were. The reasons for possible limitations of the biomarker approach and potential ontogenetic differences are outlined be-

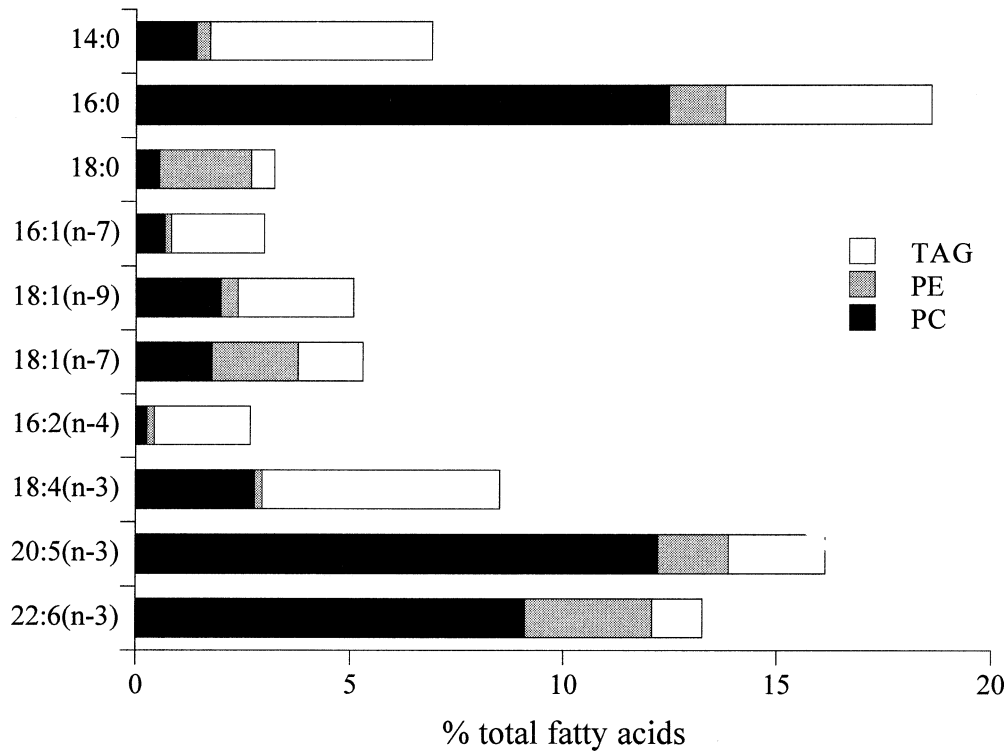


Fig. 6. *Euphausia superba*. Percentage contribution of the three main lipid classes to the total fatty acid composition of furciliae IV-VI from group II (see Fig. 5). TAG, triacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

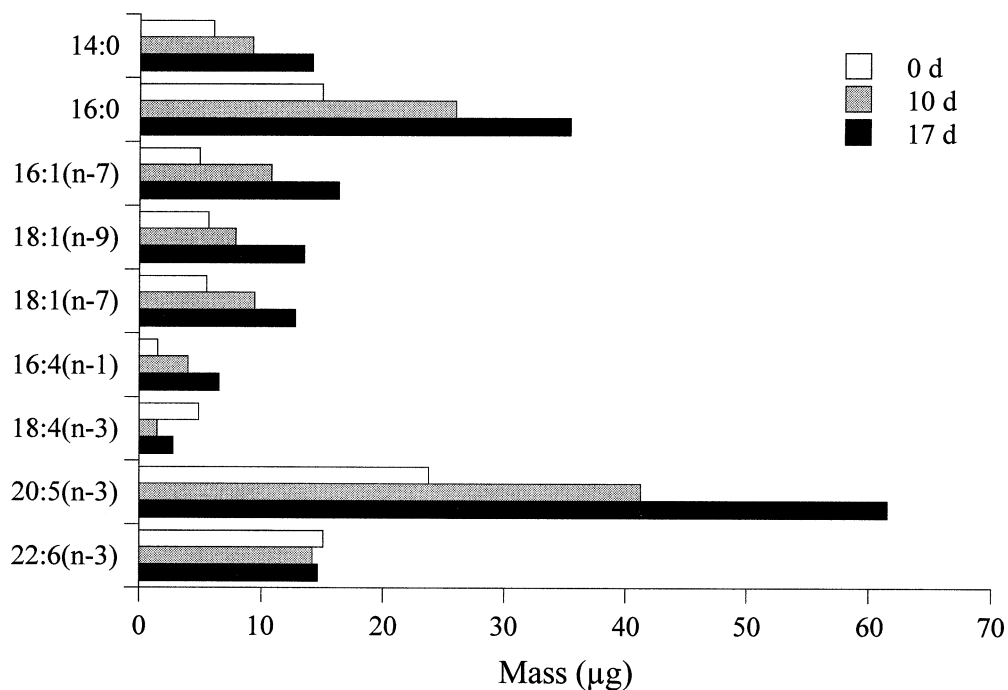


Fig. 7. *Euphausia superba*. Development of the main fatty acids of furciliae III-V feeding on *Fragilariopsis cylindrus* for up to 17 d.

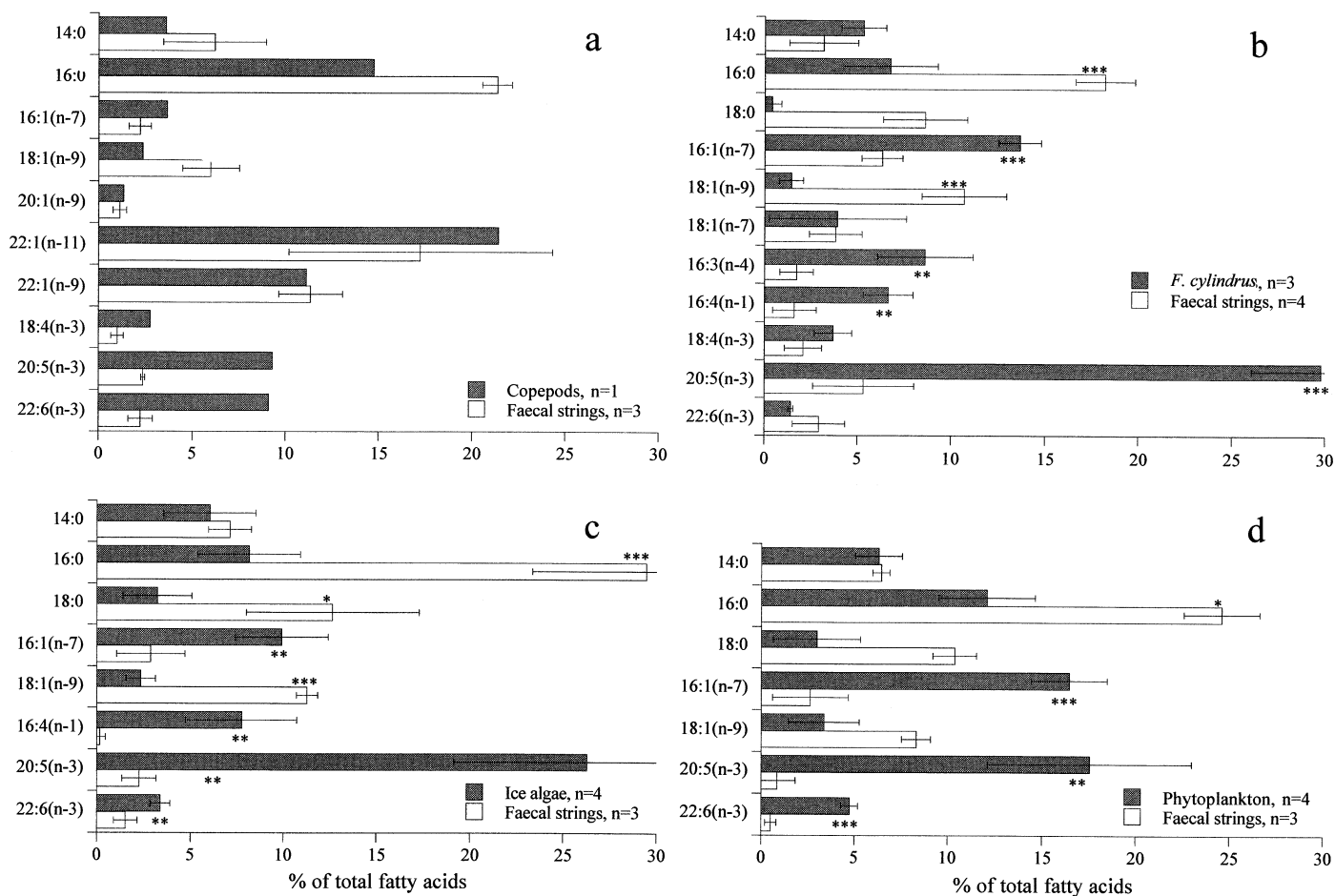


Fig. 8. *Euphausia superba*. Comparison of the main fatty acids from the different food sources with those of the feces. Significant differences between means are * $p \leq 0.05$, ** $p \leq 0.01$, or *** $p \leq 0.001$.

low and the specific marker fatty acids are discussed in terms of their respective usefulness for tracing feeding habits of the Antarctic krill.

Juvenile and adult krill—Our results are in line with the findings of a companion study on stable isotopes (Schmidt et al. 2003), which were measured in *E. superba* specimens from the same experiments as in the present study. Juvenile and adult *E. superba* did not significantly shift toward a heavier carbon (C) and nitrogen (N) signal when feeding on copepods or ice algae or when starving for 20 d. Only after 30 d had the juveniles from the two feeding regimes significantly increased in their $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios. The poor response of postlarval krill, with regard to both lipid and stable isotope signals, to different diets can be attributed to various reasons. Although molting rates were higher than in starving krill (Schmidt et al. 2003) and faecal strings were produced, most of the experimental juveniles and adults from the 1999 treatments were apparently not feeding efficiently on the offered food, as indicated by their decreasing dry and lipid masses.

Parallel to our studies, Atkinson et al. (2002) have conducted feeding experiments with juvenile and adult *E. superba* sampled at the same location and time. They found

very low feeding rates even after prolonged exposure to high food concentrations and a metabolic reduction of 60–80% compared to summer values. Thus, the juvenile and adult krill from our study were apparently already adjusting to winter conditions and did not efficiently use the food offered in the experiments. At the least, the diet was not utilized for further accumulation of storage lipid but rather for short-term energy requirements. This weakens the conclusions that can be drawn from our observed results of the feeding experiments.

However, there appears to be a generally low influence of dietary lipids on the fatty acid signatures of juvenile and adult krill, irrespective of feeding and metabolic activity. During a summer cruise to the South Shetland Islands (February 2000), adult krill were sampled at various stations, and POM fatty acids were compared to those of *E. superba*. Even from clearly deviating stations with strong diatom versus flagellate signatures, the krill lipids did not reflect these differences (data not shown). This is consistent with findings from Cripps and Hill (1998), who, along a transect from pack ice to the open ocean, investigated the effect of various food regimes on the fatty acid compositions of five Antarctic copepod species as well as *E. superba*. In contrast to the copepods, the fatty acid compositions of krill were essen-

tially the same at all stations. Hagen et al. (2001) drew similar conclusions from a multiseason data set, where the lipids of adult *E. superba* did not show pronounced dietary fatty acid signals. However, juveniles, to a certain extent, did reflect seasonal changes in algal composition, especially by variations of the marker fatty acid for flagellates, 18:4(n-3).

The comparatively large lipid reservoir of juvenile and adult krill seems to buffer short-term diet-induced variations. In addition, dietary lipids are probably modified to a larger degree; thus, a specific overall pattern is apparently maintained by the krill.

Larval krill—In contrast to the small effect of dietary lipids on the fatty acid compositions of juvenile and adult *E. superba*, furciliae lipids are markedly influenced by their food. Changes in fatty acid composition could be induced experimentally, by offering controlled diets, as well as detected in field samples from different locations. Trophic marker fatty acids were indicative of either diatom or flagellate feeding. Intermediate signatures combined with low lipid content demonstrated an opportunistic feeding behavior when overall phytoplankton biomass was presumably low.

The contrasting results between larval and postlarval krill are in line with findings by Hagen et al. (2001). Furciliae showed much stronger seasonal variations in the levels of phytoplankton marker fatty acids than adult krill. The same has been found for *Thysanoessa macrura*, another abundant Antarctic euphausiid (Hagen and Kattner 1998). The greater extent to which furciliae fatty acid compositions are influenced by dietary lipids could be due in part to their nonreduced feeding rates and metabolic activities in autumn (Meyer et al. 2002). It had already been shown that krill larvae do not enter dormancy, but continue to grow and feed during the winter (Daly 1990; Quetin and Ross 1991). The first winter is one of the most critical periods in the life cycle of *E. superba*. In contrast to adult and juvenile krill, the energy reserves of the furciliae are not sufficient to ensure survival during the long winter months without feeding.

However, irrespective of these activity differences, the low lipid levels facilitate the detection of dietary lipids. Future investigations must show whether these ontogenetic differences are due to different pathways of lipid metabolism (e.g., different degrees of modification of ingested fatty acids).

Trophic marker fatty acids—Marker fatty acids are a valuable tool for the identification of trophic relationships among polar plankton. It can provide information where the classical gut content analyses fail (e.g., soft-bodied organisms, advanced digestion) and integrates the trophic information over a longer time scale of several weeks. This approach has been applied in various studies on the trophic ecology of *E. superba* (Virtue et al. 1993a, 1997; Cripps and Hill 1998; Cripps et al. 1999; Cripps and Atkinson 2000; Phleger et al. 2002).

However, a lot of factors influence the fatty acid composition other than food (e.g., Sargent and Henderson 1995). Most dominant fatty acids were shown to clearly accumulate with increasing lipid levels, independent of the diet (Hagen et al. 2001). Therefore, fatty acids as trophic markers should

be used with caution, and comparisons should only be made between specimens with a similar total lipid background.

In the following section we discuss the specific marker fatty acids for various plankton groups with regard to their applicability to trace feeding habits in *E. superba*. Measurements of preferential assimilation of specific fatty acids (i.e., comparison of the fatty acid compositions of the food with those of the feces) have helped to evaluate the actual usefulness of the taxon-characteristic fatty acids as trophic markers.

Diatoms: *E. superba* is still considered to feed primarily on phytoplankton (e.g., Mayzaud et al. 1998), although many recent studies illustrate the opportunistic feeding behavior of the Antarctic krill that use any kind of food available (e.g., Atkinson and Snýder 1997; Perissinotto et al. 1997). Phytoplankton blooms in the Antarctic Ocean are principally dominated by diatoms (e.g., Clarke and Leakey 1996), although temporally, dinoflagellates and *Phaeocystis* can be abundant as well (Kang and Fryxell 1993; Clarke and Leakey 1996).

Diatoms are particularly rich in 16:1(n-7) and 20:5(n-3) (=EPA) (e.g., Nichols et al. 1993; Dunstan et al. 1994), and these fatty acids are considered trophic markers for this algal group (Sargent et al. 1987; Graeve et al. 1994a,b). Our comparative analyses of the diatom food with the subsequently excreted feces showed that both fatty acids were extracted from the food and thus confirmed their potential as trophic markers. However, autoxidation processes cannot be completely excluded. The highly unsaturated fatty acids are less stable than the saturates (SFAs) and monounsaturates (MUFAs). They are therefore more susceptible to oxidation, and their proportions would diminish in favor of the SFAs and MUFAs. However, the inconsistent behavior of similar fatty acids (e.g., the portions of 16:1[n-7] being always lower in the feces than in the food in contrast to 18:1[n-9]) suggests that chemical processes are an unlikely source of modification. To our knowledge, there is no quantitative study on the transformation of fatty acids during gut passage. Our conclusion is in accordance with findings by Olsen et al. (1991) that cod feces can contain significant amounts of polyunsaturated fatty acids (PUFAs).

EPA usually is one of the dominant fatty acids in the total lipids of *E. superba*. Like the second dominant essential long-chain PUFA 22:6(n-3), it is tightly conserved (i.e., losses via catabolism are low) (Sargent and Whittle 1981; Sargent and Henderson 1995). It is therefore only of limited use with regard to providing trophic information. In contrast, 16:1(n-7) can clearly be considered a trophic marker for diatoms because it is subjected to larger variations and can apparently be depleted when krill feed on other food sources. Although most marine animals also have the potential to synthesize 16:1(n-7) de novo, the strong uptake of 16:1(n-7) from the food (as demonstrated by its low abundance in the fecal strings) as well as its pronounced seasonal and regional variability in *E. superba* lipids (see also Hagen et al. 2001) confirm its suitability as a trophic marker in Antarctic krill.

In addition to 16:1(n-7), various C16 PUFAs also seemed to be preferentially assimilated from the food. However, their

proportions of *E. superba*'s total fatty acids remained rather small (<4% on average).

Despite clear differences in the fatty acid signatures of ice algae and phytoplankton (particularly of high levels of EPA in the former), the lipid biomarker approach is not sensitive enough to quantify the respective contributions of sympagic versus planktonic diatoms in the feeding history of *E. superba*.

Flagellates: The lipids of dinoflagellates usually contain high amounts of 18:4(n-3) and 22:6(n-3) (=DHA) (Sargent et al. 1987; Graeve et al. 1994b). Prymnesiophytes, of which *Phaeocystis* spp. can occur in high abundances in the Antarctic Ocean, and cryptomonads can be rich in these fatty acids as well (Sargent et al. 1987; Volkman et al. 1989; Virtue et al. 1993a). Fatty acid 18:5(n-3) has also been described as a potential marker for dinoflagellates (Mayzaud et al. 1976) or *Phaeocystis* (Virtue et al. 1993a). However, 18:5(n-3) was not detected in any of our krill samples. It is usually present in very low concentrations in zooplankton (e.g., Fraser et al. 1989; Virtue et al. 2000), and very few studies reported the occurrence of this fatty acid in krill (Mayzaud et al. 1976; Virtue et al. 2000; Phleger et al. 2002). A possible explanation for its low abundance was presented by Ghioni et al. (2001). They studied the fate of radio-labeled 18:5(n-3) in cultured fish cells and proposed its rapid conversion to 18:4(n-3), which can be further elongated and desaturated to 20:5(n-3). However, in the mixed phytoplankton culture that was fed to the krill furciliae, no 18:5(n-3) and only small amounts of 18:4(n-3) (<1% of total fatty acids) were detected. This is consistent with the low numerical abundance (<2%, data not shown) of flagellates as determined by the cell counts.

We did not carry out feeding experiments with flagellates. In an experimental study on a calanoid copepod feeding on a dinoflagellate species, assimilation efficiencies for different dietary lipid components were calculated (Harvey et al. 1987). The authors observed a very high assimilation efficiency for polyunsaturated fatty acids, with the flagellate markers 18:4(n-3) and 22:6(n-3) being almost completely removed from the food during the gut passage.

In *E. superba*, 18:4(n-3) is predominantly accumulated in the triacylglycerols (Hagen et al. 2001; this study). Because it seems to be rapidly metabolized when not refueled by exogenous sources, it is a valuable short-term trophic marker, in contrast to the efficiently retained long-chain polyunsaturated fatty acid DHA.

Copepods: It is now well established that *E. superba* can resort to carnivory during periods of phytoplankton shortage (Huntley et al. 1994; Atkinson and Snýder 1997; Perissinotto et al. 2000; Atkinson et al. 2002). Particularly, copepods would represent an abundant high-energy food source because they can contain >50% of their dry mass as lipids (e.g., Hagen and Schnack-Schiel 1996). The C20 and C22 monoenes are typical components, both as fatty acids and fatty alcohols of the wax esters and triacylglycerols of herbivorous calanid copepods (e.g., Kattner and Hagen 1995). However, these components are not accumulated in *E. superba* lipids (Phleger et al. 1998; Hagen et al. 2001; this

study). In contrast, other euphausiids and oil-rich fish preying on these copepods incorporate these moieties into their body lipids. They occur in significant proportions, especially in the triacylglycerols of *Meganyctiphanes norvegica* (e.g., Virtue et al. 2000), as well as in those of North Atlantic herring and North Sea sprat (see Sargent and Henderson 1995 for review). Hence, in contrast to the carnivorous *M. norvegica*, calanid copepods do not appear to constitute a regular food source for *E. superba*.

As the present study has demonstrated, fecal strings of krill feeding on copepods rich in the two 22:1 isomers contain high levels of these fatty acids, indicating that they are not assimilated. A quantitative determination of the amount of these fatty acids in the prey copepods before and after gut passage was not possible. Therefore, it remains unclear whether their low abundance in krill body lipids is due to their generally low absorption or whether the assimilated fatty acids are readily metabolized for energy production instead of deposition. Similar results have been published for small cod juveniles feeding on zooplankton rich in the C20 and C22 monoenes (Olsen et al. 1991). Until a certain age, their body lipids did not contain these fatty acids, and the authors suggested a limited ability to digest neutral lipids in the early life stages. High levels of wax esters, as well as 20:1(n-9) and 22:1(n-11) moieties in the fish feces confirmed their conclusion. The apparent weak assimilation of the long-chain monoenes makes these fatty acids unsuitable as trophic markers to detect feeding on calanid copepods by *E. superba*.

Cripps and Atkinson (2000) proposed that the ratio of polyunsaturated to saturated fatty acids (PUFA/SFA) is a useful indicator for carnivorous (high values) versus herbivorous feeding in krill. However, there is a clear relationship between the total lipid content and the levels of polyunsaturated fatty acids (PUFA) in Antarctic euphausiids (data not shown). Nevertheless, there is some individual variability, which could be attributable to differences in feeding histories. This underlines the importance of such indices only being applied to individuals with comparable lipid content. Sargent and Henderson (1995) reviewed literature on the origin and transfer of marine (n-3)-PUFAs and stated that diet is not the only factor responsible for variations in lipid composition. They emphasize the importance of total oil levels. They also point out that the lower the percentage of neutral lipid in total zooplankton lipids, the higher the content of (n-3) PUFA in total lipid.

The dietary imprint of copepods on *E. superba* lipids is rather small. But even if *E. superba* does switch to copepod prey during times of low phytoplankton availability, it remains doubtful whether they can efficiently utilize this lipid-rich food. It cannot be decided yet whether krill can enzymatically degrade and efficiently absorb copepod wax esters. The low portions of the monounsaturated C20 and C22 fatty acids in krill lipids and their high levels in the fecal material suggest a poor ability of *E. superba* to digest these high-energy lipids of copepods.

Starvation—Juvenile and adult krill show a high tolerance toward suboptimal feeding conditions and can survive long periods without food (Ikeda and Dixon 1982). The authors

observed a considerable reduction in metabolic activity of starved *E. superba* compared to wild specimens, but no change in chemical composition (C and N). This indicates that both body lipid and protein are used as energy sources in starving krill. Similar results have been found by Virtue et al. (1997). They reported a significant decrease of lipid mass per animal during 130 d of starvation, while the relative lipid portion in percentage of body dry mass remained constant, although it should be kept in mind that the initial lipid levels of the experimental specimens were already quite low. The percent composition of both lipid classes and fatty acids also showed no significant changes. In an earlier study by the same authors, the effect of short-term starvation on the lipid content and composition of the digestive gland of *E. superba* was examined (Virtue et al. 1993b). Again, absolute lipid levels significantly decreased, but on a lipid class basis, relative levels remained the same. However, there was a change concerning the fatty acid composition. The portions of the long-chain PUFAs 20:5(n-3) and 22:6(n-3) decreased in favor of 16:0. Our results agree with these literature data: Although absolute lipid levels decreased during starvation, the relative compositions remained largely unchanged in juvenile and adult krill. That there is no preferential utilization of either high-energy long-chain PUFAs or low-cost short-chain saturates further illustrates the balanced lipid metabolism of postlarval *E. superba*.

The concept of body shrinkage in *E. superba* during periods of food limitation (e.g., Ikeda and Dixon 1982; Nicol et al. 1992) agrees well with these findings that the relative biochemical compositions do not change. Shrinkage apparently occurs via a reduction in cell volume rather than the resorption of whole cells, as deduced by microscopic counts of cell nuclei (McGaffin et al. 2002). This implies an even reduction of the various cell constituents, including proteins as well as storage and membrane lipids, except for nucleic material.

We emphasize that these rather stable total lipid levels and relative lipid compositions under laboratory starvation conditions are not in contradiction to the pronounced seasonal lipid dynamics observed in the wild (Hagen et al. 1996, 2001; Falk-Petersen et al. 2000). This is a matter of different scales: laboratory investigations observe a reduction of absolute lipid mass by a factor of two or less (Virtue et al. 1997; this study), whereas those of field samples can vary by a factor of 10.

To our knowledge, the effects of starvation on the lipid biochemistry of *E. superba* furciliae have not been examined so far. Similar to the results of the feeding experiments, starvation also had a stronger effect on the furcilia larvae than on juvenile and adult krill. There was a significant reduction of the contribution of storage lipid (triacylglycerol and phosphatidylcholine) typical fatty acids, resulting in a relative increase of membrane lipid fatty acids. This suggests that larvae follow a different strategy than juvenile and adult krill. These furciliae utilize their lipid reserves and do not metabolize their body mass for energy production. Findings by Frazer et al. (1997) on stable isotopes of *E. superba* larvae (mainly Furcilia VI) corroborate this conclusion. During 8 weeks of starvation, there was no isotopic change toward a heavier $\delta^{15}\text{N}$ signal because of the excretion of isotopically

light ammonium. This indicates that krill larvae do not catabolize their body nitrogen during starvation.

This study revealed only a weak influence of dietary fatty acids on the lipid compositions of juvenile and adult *E. superba*. This might be partly attributable to the late season and thus reduced feeding and metabolic activities, although weak trophic effects on the fatty acid compositions of post-larval *E. superba* have also been found for the summer season. The large lipid deposits seem to buffer variations in dietary lipid supply; hence, a specific fatty acid pattern is maintained.

The fatty acid compositions of larval krill, however, are much more clearly influenced by their food. An unaltered incorporation of dietary fatty acids in furciliae body lipids together with lower lipid levels allows the deduction of feeding habits through fatty acid analyses.

It is emphasized that the relative lipid compositions are strongly dependent on the total lipid contents. Trophic indices derived from marker fatty acids therefore should not be interpreted as absolute values but viewed in the physiological context of the animals.

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