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# Lipids as trophic markers in Antarctic krill. III. Temporal changes in digestive gland lipid composition of *Euphausia superba* in controlled conditions

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ABSTRACT: Two phytoplankton diets with markedly different fatty acid (FA) profiles (thraustochytrid and diatom over a range of concentrations) were fed to juvenile *Euphausia superba* Dana to evaluate the time lapse required for the development of trophic lipid signatures in the digestive gland (DG). Krill were collected after 5, 10 and 20 d of feeding on thraustochytrids. Krill fed on thraustochytrids for 20 d were also collected after a further 5, 10 and 20 d of feeding on diatoms. An accumulation of polar lipid (0.4 mg per DG) was observed after 5 d of feeding on thraustochytrids at every concentration of food. The deposition of triacylglycerol (0.25 mg per DG) required 10 d of feeding and only occurred at the 2 highest food concentrations. Amounts of thraustochytrid-derived FA, 14:0, 16:0 22:6ω3 and 22:5ω6, increased after 10 d of feeding. The final concentration of 22:6ω3 was dependent on food concentration. Though it was absent in the diet, the essential FA 20:5ω3 accumulated from the retroconversion of 22:6ω3 after 20 d. Changing the krill diet to diatoms resulted in a marked decline in thraustochytrid FA, particularly 14:0 and 22:6ω3, after 5 d, whilst diatom FA, 16:1ω7 and 20:5ω3, remained constant. Thraustochytrid signatures were completely erased from the FA profiles of DG after 20 d feeding on diatoms. Slow accumulation of storage lipid and short residence time of dietary FA suggest that lipid turnover rates are high in Antarctic krill DG.

KEY WORDS: *Euphausia superba* · Antarctic krill · Lipid class · Fatty acid · Digestive gland · Trophic signature · Temporal scale



## INTRODUCTION

In the Southern Ocean, one crucial link in the transfer of energy and carbon between primary producers and large predators is supported by the Antarctic krill *Euphausia superba* (hereafter 'krill'). The biomass of krill, which represents a major prey for birds, fish, seals, squids and whales (El-Sayed 1985), is estimated to be hundreds of millions of tons (Ross & Quetin 1988, Miller & Hampton 1989). Antarctic krill are described as opportunistic feeders which graze efficiently on diatom blooms in summer (Hopkins et al. 1993, Quetin et al. 1994, Frazer 1996, Perissinotto et al. 1997) and may rely on omnivory and carnivory when phytoplankton is scarce or absent. At depth during the night or in winter, exploited diets include detritus, metazoans and copepods (Price et al. 1988, Huntley et al. 1994, Atkinson & Snÿder 1997, Hernandez-León et al. 2001, Atkinson et al. 2002).

Processes for the acquisition, transfer and transformation of lipids, and of their essential building compounds known as polyunsaturated fatty acids (PUFA), likely play a predominant role in the control of production in marine consumers (Müller-Navarra et al. 2000). Moreover, at high latitudes lipids are involved in survival strategies of organisms during winter. In

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particular, seasonal proximate profiles of *Euphausia superba* suggest that lipids are substantial energy and carbon reserves accumulated in spring and summer when food is abundant (Fergusson & Raymont 1974, Clarke 1980, Hagen 1988, Quetin & Ross 1991, Hagen et al. 1996, 2001). However, Antarctic krill resort to several mechanisms, including protein catabolism, body shrinkage, lowered metabolic activity and use of heterotrophic food sources, to survive winter (Ikeda & Dixon 1982, Daly & Macaulay 1991, Quetin & Ross 1991, Huntley et al. 1994). Thus, the relative importance of lipid reserves in the overall energy budget is still not fully understood.

One aspect which remains under debate is the relationship between feeding and lipid composition in *Euphausia superba*. In copepods, experimental studies have demonstrated that lipid content provides an index of past feeding condition and that fatty acids (FA) are markers of trophic relationships (e.g. Håkanson 1984, Graeve et al. 1994). Observations of Antarctic krill, however, are contradictory. Several studies suggest that FA may be used as trophic markers in *E. superba* (Virtue et al. 1993b, Cripps & Atkinson 2000), and changes in lipid classes and FA composition in the field have been interpreted as resulting from regional differences in the available food (Bottino 1974, Mayzaud 1997, Cripps et al. 1999, Phleger et al. 2002). However, Cripps & Hill (1998) report similar FA compositions in krill collected in the field, despite regional and dietary changes. Stübing et al. (2003) also observed very little variation in lipid composition in controlled laboratory conditions based on different feeding regimes. Most previous studies have analysed whole animals, which might explain the contradictory results between the different studies, as physiological functions played by FA in various organs might interfere with trophic information (Clarke 1980, Kolokowska 1991, Pond et al. 1995, Virtue et al. 1996). Recently, we demonstrated that this major source of potential confusion could be avoided by focusing lipid analyses on digestive glands (DG). Controlled starvation and feeding on diatoms, flagellates, thraustochytrids and zooplankton induced distinct FA profiles in DG (Virtue et al. 1993a, Alonzo et al. 2003, 2005, this volume). The exposure time to food must be sufficient to establish significant trophic links, although its effect remains unknown.

This study aims to examine how diet-induced lipid signatures vary in krill DG over a range of feeding times. Feeding krill on 2 distinct phytoplankton diets, we addressed 3 issues: (1) How long does it take for krill to develop lipid signatures specific to controlled diets? (2) How long do such lipid signatures remain visible after the diet has changed? (3) How may the observed time scale be influenced by feeding rates?

## MATERIALS AND METHODS

Details of *Euphausia superba* collection, experimental conditions, measurement of ingestion and faecal pellet production, krill dissection, lipid analysis and data analysis are given in Alonzo et al. (2005).

**Food source.** Two distinct phytoplankton diets were fed to the krill: (1) spray-dried cells of the thraustochytrid *Schyzochitrium* sp. (Algamac-2000® purchased from Aquafauna Bio-marine), and (2) cultures in exponential growth of the diatom *Phaeodactylum tricornutum*. Diatoms were used in this study because of their importance in the Southern Ocean food webs. Thraustochytrids are also present in Antarctic waters (Bahnweg & Sparrow 1974) and were mainly used because their FA profiles are markedly different from those of diatoms (Table 1) (Alonzo et al. 2005).

The thraustochytrid FA profile is dominated by 4 major components representing 85% of total FA: the saturated FA (SFA) 14:0 and 16:0 and the PUFA 22:5ω6 and 22:6ω3 (hereafter 'DHA' for docosahexaenoic acid). These FA are found at much lower relative levels in diatoms, compared to thraustochytrids, or are absent (22:5ω6). Major diatom FA include the monounsaturated FA (MUFA) 16:1ω7 and the PUFA 20:5ω3 (hereafter 'EPA' for eicosapentaenoic acid).

**Diets.** ***Thraustochytrids:*** Lipid content and composition in krill DG was investigated over time and in relation to concentration of food. Food was diluted with 0.45 µm filtered seawater to achieve 4 protein concentrations referred to as 'low' (0.2 $mg^{-1}$), 'medium' (0.6 mg $l^{-1}$), 'high' (2.0 g $l^{-1}$) and 'saturating' (4.0 mg $l^{-1}$). Krill samples were collected at the beginning of

Table 1. Food quality: protein, lipid (percent of DW) and fatty acid composition (percent of total FA) of phytoplankton diets

| | Thraustochytrids | Diatoms |
|---|---|---|
| **Protein** | 39 | 11.7 |
| **Lipids** | 32 | 14.3 |
| Lipid:protein ratio | 0.82 | 1.23 |
| **Fatty acids** | | |
| 14:0 | 11.0 | 3.3 |
| 16:1ω7c | 7.3 | 22.8 |
| 16:0 | 38.5 | 11.2 |
| 18:1ω9c | 4.1 | 1.5 |
| 18:1ω7c | 0.0 | 2.9 |
| 20:5ω3 (EPA) | 0.6 | 33.7 |
| 22:5ω6 | 12.9 | 0.0 |
| 22:6ω3 (DHA) | 24.0 | 4.0 |
| Other | 1.5 | 20.5 |
| 16:1/16:0 | 0.2 | 2.0 |
| 18:1ω7c/18:1ω9c | 0.0 | 1.9 |
| EPA/DHA | 0.0 | 8.4 |
| Total ω7c | 7.3 | 25.7 |
| Total ω3 | 24.6 | 37.7 |

experiments on Day 0 (also referred to as 'initial condition'), and on Days 5, 10 and 20 of feeding.

***Thraustochytrids + diatoms:*** The effects of a shift in diet were inves-tigated by changing the krill diet from thraustochytrids to diatoms. Krill pre-fed for 20 d on the high level of thraustochytrids were transferred to low and high levels of diatoms. Further DG samples were collected 5, 10 and 20 d after this diet shift (referred to respectively as Days 25, 30 and 40). To examine whether the thraustochytrid signature was still visible after 20 d of feeding on diatoms, we compared 'thraustochytrids + diatoms' fed krill on Day 40 with krill fed low and high levels of diatoms for 20 d without a thraustochytrid pre-treatment.

## RESULTS

### Nutrition

Krill consumed food and produced faecal pellets in all feeding conditions (Table 2). Different patterns in ingestion and defecation were observed over time depending on the diet. In krill fed on thraustochytrids at any concentration, faecal pellet production decreased (by about 45 to 50 %) between Days 5 and 20, while mean ingestion rates were approximately constant. This observation suggests that animals were progressively acclimating to this food source. In krill fed diatoms, both ingestion and defecation rates decreased slightly (by about 25 %) over the course of experiments at the low concentration, whereas they remained constant over time at the high concentration.

Ingestion rates were correlated to food concentration, varying on average from 0.07 to 1.40 mg protein $d^{-1}$ $krill^{-1}$ with thraustochytrids and from 0.10 to 0.35 mg protein $d^{-1}$ $krill^{-1}$ with diatoms. At the high food concentration, protein ingestion rate was greater for thraustochytrids (0.65 mg protein $d^{-1}$ $krill^{-1}$) than for diatoms (0.35 mg protein $d^{-1}$ $krill^{-1}$), whereas lipid ingestion rates were approximately equal between food sources (average of 0.43 to 0.53 mg lipids $d^{-1}$ $krill^{-1}$).

### Lipid content and composition of digestive gland

Body wet weight (WW) was similar among krill exposed to the different feeding conditions. DG WW and lipid content showed significant differences in relation to both concentration of food and duration of feeding (Table 3).

DG lipid content increased over the course of experiments in all krill fed thraustochytrids, from 0.5 mg $g^{-1}$ of body WW on Day 0 up to 1.0 mg $g^{-1}$ of body WW at low and medium food concentrations and 1.2 to 1.4 mg $g^{-1}$ of body WW at high and saturating food concentrations (Fig. 1), though differences were not significant (N = 15, p = 0.28). A high value of 2.0 mg $g^{-1}$ of body WW was observed on Day 5, independent of food concentration. This was largely associated with an accumulation of polar lipids (PL), accounting for 80 % of total lipids. On Day 10, responses differed with the concentration of food. DG lipid content decreased to its final level at low and medium food concentrations, with the relative proportion of triacylglycerol (TAG) remaining at 28 % of total lipids, as observed on Day 0. DG lipid content increased up to 2.1 mg $g^{-1}$ of body WW at high and saturating food concentrations, while the relative proportion of TAG increased up to 40 % of total lipids.

After diets were shifted from thraustochytrids to diatoms, DG lipid content decreased from 1.0 to 0.5 mg $g^{-1}$ of body WW with the low food concentration, whereas it remained relatively constant with the high food concentration. The relative level of TAG decreased with both low and high diatom levels, accounting for 16 % of total lipids in 'thraustochytrids + diatoms' DG on Day 40 (Table 3, Fig. 2).

Table 2. *Euphausia superba* fed thraustochytrids or diatoms. Mean protein concentration in the experiment jars, protein and lipid ingestion rates, and faecal pellet production in relation to source and concentration of food and duration of feeding. Data are mean ± SD (Day 5: N = 3, Day 10: N = 5, Day 20: N = 10)

| Diet | Protein (mg $l^{-1}$) | Ingestion (mg $d^{-1}$ $krill^{-1}$) Protein | Lipids | Faecal pellets (mg DW $d^{-1}$ $krill^{-1}$) |
|---|---|---|---|---|
| **Thraustochytrids** | | | | |
| Low | | | | |
| Day 5 | 0.15 ± 0.05 | 0.06 ± 0.05 | 0.05 ± 0.04 | 0.09 ± 0.03 |
| Day 10 | 0.14 ± 0.03 | 0.07 ± 0.03 | 0.06 ± 0.03 | 0.05 ± 0.05 |
| Day 20 | 0.14 ± 0.03 | 0.07 ± 0.03 | 0.05 ± 0.03 | 0.05 ± 0.04 |
| Medium | | | | |
| Day 5 | 0.34 ± 0.09 | 0.28 ± 0.07 | 0.23 ± 0.06 | 0.06 ± 0.04 |
| Day 10 | 0.34 ± 0.06 | 0.28 ± 0.05 | 0.23 ± 0.05 | 0.05 ± 0.03 |
| Day 20 | 0.35 ± 0.05 | 0.28 ± 0.04 | 0.23 ± 0.03 | 0.05 ± 0.03 |
| High | | | | |
| Day 5 | 1.50 ± 0.13 | 0.60 ± 0.14 | 0.49 ± 0.12 | 0.24 ± 0.12 |
| Day 10 | 1.46 ± 0.10 | 0.65 ± 0.10 | 0.53 ± 0.09 | 0.16 ± 0.12 |
| Day 20 | 1.41 ± 0.10 | 0.70 ± 0.10 | 0.57 ± 0.08 | 0.13 ± 0.09 |
| Saturating | | | | |
| Day 5 | 2.94 ± 0.18 | 1.27 ± 0.19 | 1.04 ± 0.16 | 0.32 ± 0.04 |
| Day 10 | 2.80 ± 0.23 | 1.42 ± 0.23 | 1.16 ± 0.19 | 0.21 ± 0.13 |
| Day 20 | 2.70 ± 0.23 | 1.52 ± 0.23 | 1.24 ± 0.19 | 0.19 ± 0.11 |
| **Diatoms** | | | | |
| Low | | | | |
| Day 5 | 0.09 ± 0.01 | 0.11 ± 0.01 | 0.12 ± 0.01 | 0.24 ± 0.16 |
| Day 10 | 0.11 ± 0.02 | 0.10 ± 0.02 | 0.11 ± 0.02 | 0.24 ± 0.11 |
| Day 20 | 0.12 ± 0.03 | 0.08 ± 0.02 | 0.09 ± 0.03 | 0.18 ± 0.11 |
| High | | | | |
| Day 5 | 1.69 ± 0.04 | 0.39 ± 0.05 | 0.43 ± 0.05 | 0.36 ± 0.13 |
| Day 10 | 1.72 ± 0.08 | 0.35 ± 0.10 | 0.39 ± 0.11 | 0.30 ± 0.11 |
| Day 20 | 1.75 ± 0.08 | 0.32 ± 0.10 | 0.35 ± 0.11 | 0.36 ± 0.21 |

Table 3. *Euphausia superba* fed thraustochytrids, thraustochytrids + diatoms and diatoms (without thraustochytrid pretreatment). Wet weight of whole body (WB) and digestive gland (DG), lipid content and lipid class composition of DG in relation to diet and duration of feeding. Data are mean ± SD (N = 3)

| Diet | Wet weight (mg) | | Lipid content | Lipid classes (% of total lipids) | | |
|---|---|---|---|---|---|---|
| | WB | DG | (% WW of DG) | TAG | Sterols | PL |
| **Initial condition** | | | | | | |
| Day 0 | 242 ± 46 | 3.9 ± 1.2 | 3.2 ± 1.6 | 28.2 ± 5.9 | 1.7 ± 0.8 | 44.3 ± 17.6 |
| **Thraustochytrids** | | | | | | |
| Low | | | | | | |
| Day 5 | 292 ± 85 | 4.9 ± 1.8 | 13.5 ± 2.4 | 24.3 ± 9.6 | 1.8 ± 0.4 | 69.1 ± 9.9 |
| Day 10 | 258 ± 40 | 3.4 ± 2.5 | 4.4 ± 1.7 | 22.7 ± 3.3 | 0.4 ± 0.1 | 73.9 ± 4.6 |
| Day 20 | 242 ± 31 | 6.8 ± 3.2 | 3.7 ± 0.9 | 32.8 ± 9.4 | 1.1 ± 0.4 | 62.3 ± 6.5 |
| Medium | | | | | | |
| Day 5 | 230 ± 51 | 3.4 ± 1.3 | 13.6 ± 5.6 | 7.4 ± 1.7 | 1.1 ± 0.2 | 87.0 ± 2.3 |
| Day 10 | 238 ± 60 | 4.5 ± 1.5 | 5.4 ± 1.4 | 24.2 ± 11.3 | 0.8 ± 0.4 | 66.1 ± 12.3 |
| Day 20 | 271 ± 42 | 5.8 ± 2.3 | 4.8 ± 1.3 | 30.0 ± 10.5 | 0.9 ± 0.4 | 63.4 ± 5.7 |
| High | | | | | | |
| Day 5 | 256 ± 54 | 4.3 ± 1.5 | 10.3 ± 3.3 | 12.5 ± 1.9 | 1.4 ± 0.6 | 83.1 ± 1.9 |
| Day 10 | 299 ± 58 | 6.0 ± 1.3 | 10.6 ± 0.7 | 41.4 ± 16.6 | 0.2 ± 0.1 | 52.4 ± 18.4 |
| Day 20 | 253 ± 61 | 6.2 ± 3.1 | 5.0 ± 0.9 | 28.6 ± 17.5 | 0.8 ± 0.3 | 67.0 ± 12.0 |
| Saturating | | | | | | |
| Day 5 | 237 ± 57 | 5.3 ± 1.5 | 8.2 ± 2.9 | 11.3 ± 3.0 | 0.8 ± 0.3 | 84.9 ± 3.5 |
| Day 10 | 256 ± 52 | 6.7 ± 3.3 | 7.9 ± 0.4 | 43.4 ± 8.0 | 0.4 ± 0.2 | 47.1 ± 3.8 |
| Day 20 | 247 ± 45 | 5.8 ± 2.2 | 5.6 ± 1.1 | 36.0 ± 19.4 | 0.6 ± 0.2 | 57.9 ± 12.1 |
| **Thraustochytrids + diatoms** | | | | | | |
| Low | | | | | | |
| Day 25 | 257 ± 55 | 3.9 ± 1.4 | 4.0 ± 1.2 | 19.5 ± 4.6 | 0.9 ± 0.1 | 76.4 ± 4.4 |
| Day 30 | 256 ± 80 | 4.6 ± 2.0 | 4.0 ± 0.8 | 30.6 ± 4.8 | 0.6 ± 0.2 | 59.5 ± 4.5 |
| Day 40 | 247 ± 47 | 4.1 ± 1.1 | 3.0 ± 2.1 | 17.4 ± 3.5 | 0.9 ± 0.1 | 73.0 ± 6.9 |
| High | | | | | | |
| Day 25 | 292 ± 65 | 6.4 ± 2.2 | 3.7 ± 0.9 | 24.6 ± 4.2 | 0.8 ± 0.3 | 65.6 ± 7.8 |
| Day 30 | 265 ± 62 | 7.4 ± 4.6 | 6.4 ± 1.7 | 28.7 ± 0.6 | 0.3 ± 0.0 | 59.0 ± 4.2 |
| Day 40 | 332 ± 99 | 9.1 ± 3.8 | 3.7 ± 0.4 | 15.9 ± 2.7 | 0.5 ± 0.3 | 66.0 ± 7.8 |
| **Diatoms** | | | | | | |
| Low | | | | | | |
| Day 20 | 312 ± 129 | 9.7 ± 4.5 | 7.0 ± 0.8 | 31.8 ± 9.1 | 0.9 ± 0.1 | 64.9 ± 7.0 |
| High | | | | | | |
| Day 20 | 309 ± 121 | 9.2 ± 3.5 | 3.5 ± 1.4 | 7.7 ± 13.3 | 10.3 ± 8.6 | 79.9 ± 8.8 |

### Fatty acid profile of digestive gland

Major changes in the FA composition of the DG occurred during feeding experiments (Tables 4 & 5, Figs. 3 & 4). On an absolute basis, FA accumulated in the DG between Days 0 and 20 of feeding on thraustochytrids. On Day 5, a minimum total FA concentration of 130 mg $g^{-1}$ total lipids was observed as a result of the accumulation of lipids. Total FA concentrations on Day 20 were greater at high and saturating food concentrations (730 to 770 mg $g^{-1}$ of total lipids) than at low and medium concentrations (~500 mg $g^{-1}$ of total lipids). After the krill diet was shifted to diatoms, FA concentration in the DG stabilised at 500 mg $g^{-1}$ of total lipids, independent of food con-centration.

On Day 5, the observed decrease was much stronger in PUFA such as EPA and DHA than in SFA and MUFA (Fig. 3). As a consequence, the contribution of 14:0 and 16:0 increased markedly from 27% to 47–67% of total FA, depending on the food concentration, whereas 16:1ω7, 18:1ω7 and 18:1ω9 were unchanged and EPA and DHA decreased to nearly 0% (Table 4). On Day 10, all FA showed concentrations equivalent to the values observed on Day 0, with the exception of EPA at all food concentrations and DHA at the low food concentration. On Day 20, DG showed high proportions of all major thraustochytrid FA: 14:0 (10% total FA), 16:0 (24%) and DHA (from 11 to 19%, depending on the food concentration). Although EPA accounted for only 0.6% total FA in thraustochytrids (Table 1), it increased between Days 10 and 20 to 12% total FA in all DG, likely associated with retroconversion of DHA. Although 22:5ω6 was one of the most abundant FA in thraustochytrids (12.9%, Table 1), it was not accumulated in large amounts (from 1.1 to 3.1%, also depending on food concentration) in the DG of *Euphausia superba*.

After the krill diet was shifted to diatoms, trends in FA concentrations also reflected their abundance in the diets: thraustochytrid FA which were minor in *Phaeodactylum tricornutum*, such as 14:0 and DHA, showed a rapid decrease in krill DG (Fig. 4, Table 5). The MUFA 16:0 (present at 38.5% total FA in thraustochytrids and 11.2% in diatoms), decreased slightly in DG. Abundant FA in diatoms, such as 16:1ω7 and EPA, remained constant in DG between Days 20 and 40. As a result of the decline in thraustochytrid FA, proportions of 16:0, 16:1ω7 and EPA increased overall during feeding on diatoms.

### Fatty acid ratios in digestive glands

Fatty acid ratios, 16:1ω7c/16:0 and EPA/DHA (Tables 4 & 5), showed significant differences in krill DG ($p \leq 0.001$ in both cases) in relation to food source, concentration and incubation time, whereas 18:1ω7c/18:1ω9c did not ($p = 0.095$). When krill were fed on thraustochytrids, 16:1ω7c/16:0 remained at 0.1–0.3 from Day 0 to 20, independent of the food concentration. After the diet was shifted to diatoms, ratios did not change at the low food concentration, whereas

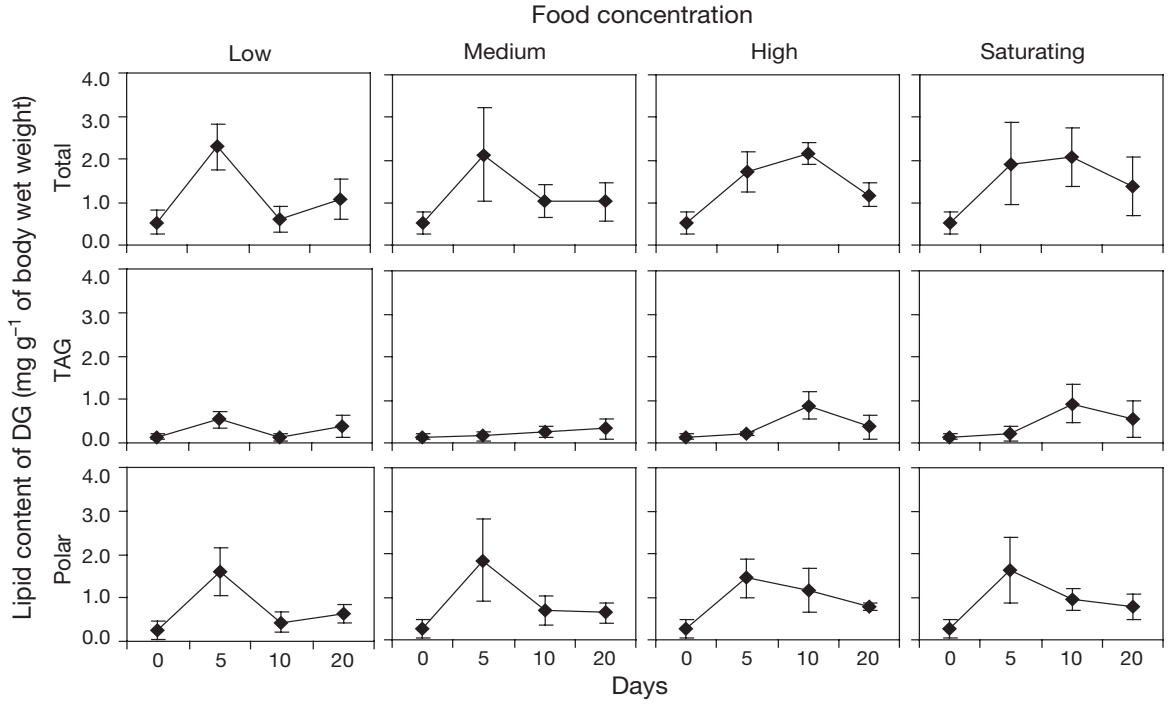


Fig. 1. *Euphausia superba* fed thraustochytrids. Changes in total lipid and lipid class content of the digestive gland in relation to thraustochytrid level (low, medium, high or saturating) and duration of feeding (5, 10 or 20 d). Error bars = SD (N = 3)

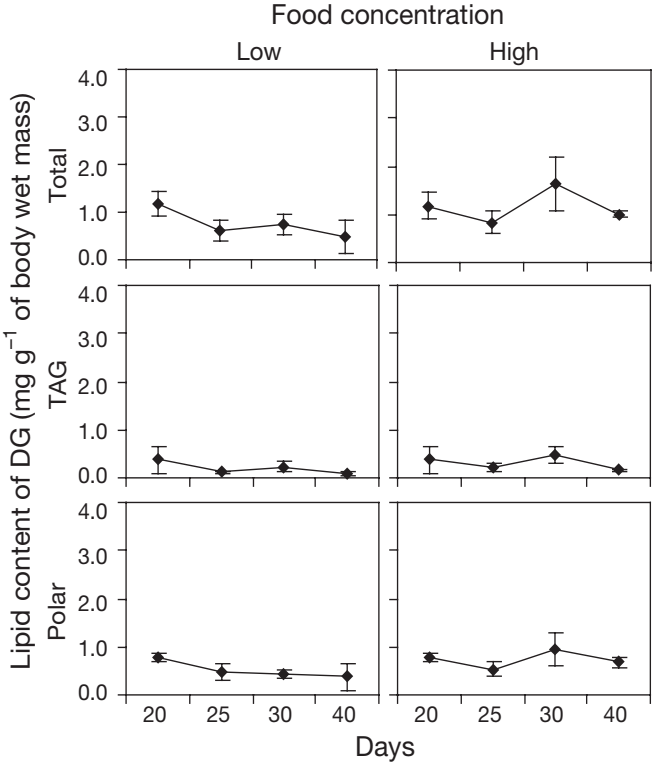


Fig. 2. *Euphausia superba* fed thraustochytrids (to Day 20) + diatoms (Day 20 to 40). Changes in total lipid and lipid class content of the digestive gland in relation to diatom level (low or high) and duration of feeding (25, 30 or 40 d). Day 20 = fed thraustochytrids at high level for 20 d. Error bars = SD (N = 3)

they quickly increased to 0.4 at the high concentration. Krill fed diatoms without a thraustochytrid pre-treatment showed a 16:1ω7c/16:0 value of 0.5. The 18:1ω7c/18:1ω9c ratio decreased progressively in DG to 0.8–1.0 with time and food concentration when krill were fed on thraustochytrids. Values increased progressively to 1.1–1.3 when krill diets were shifted to diatoms. These variations were not significant, due to the high variability of the ratio. Krill fed diatoms without a thraustochytrid pre-treatment showed a 18:1ω7c/18:1ω9c value of 1.1. In krill fed medium, high or saturating concentrations of thraustochytrids, EPA/DHA in DG changed from 2.2 on Day 0 to 0.0 on Day 5, before rising again to 0.6–0.9 on Day 20 when EPA started accumulating again. The pattern was different in krill fed a low concentration of thraustochytrids, with EPA/DHA progressively decreasing to 1.2. After the diet shift, the EPA/DHA ratio increased progressively, reaching a higher value with the high diatom concentration (3.3, the same as for krill fed diatoms without a thraustochytrid pre-treatment) than with the low concentration (1.7).

### Multivariate analysis of fatty acid profiles in digestive glands

The correlation of FA variables on principal component plans is described in Alonzo et al. (2005). DG

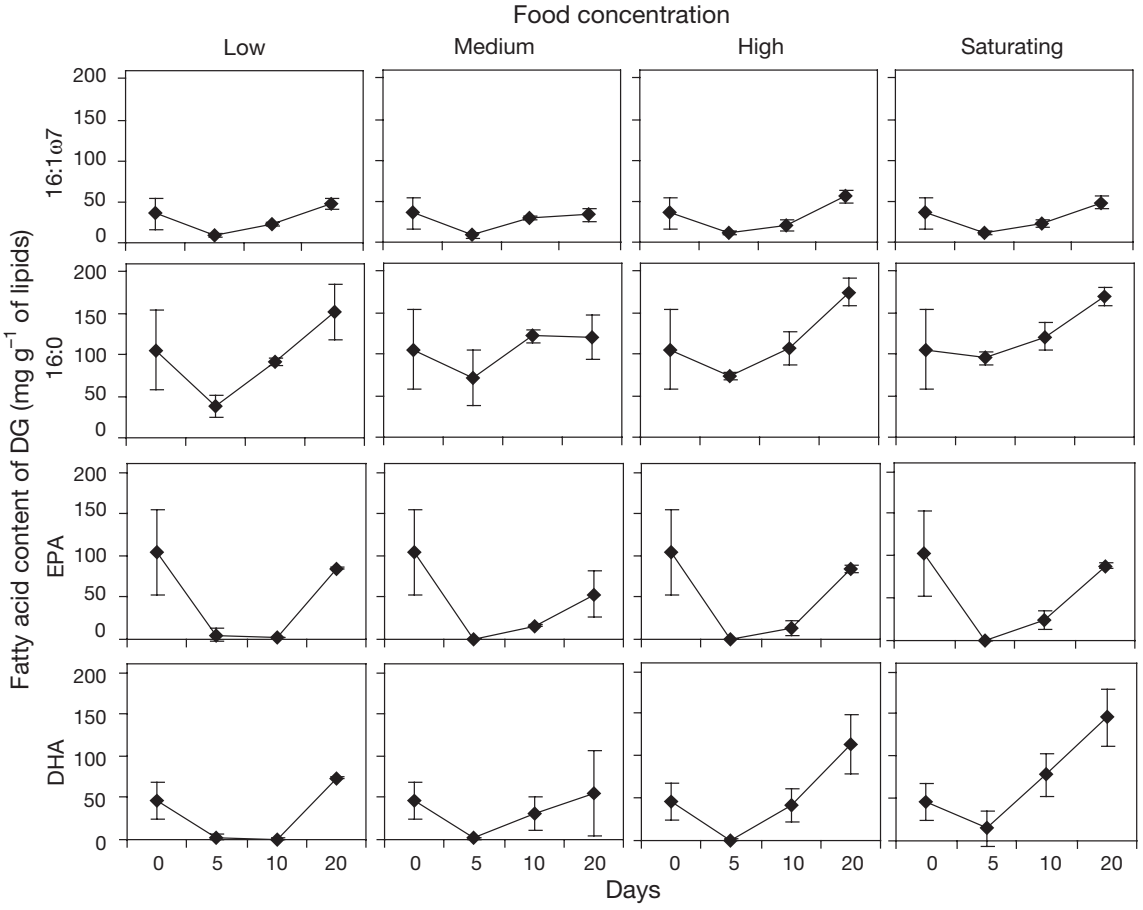


Fig. 3. *Euphausia superba* fed thraustochytrids. Changes in major FA content of the digestive gland in relation to food level (low, medium, high or saturating) and duration of feeding (5, 10 or 20 d). Error bars = SD (N = 3)

profiles of krill fed the various diets separated on the PC1 – PC2 plane (74% of the variance) in relation to food source, food level and duration of the feeding experiments (Fig. 5). On Day 5 of feeding on thraustochytrids, FA compositions were distributed within a small group characterised by low levels of all major FA (including 16:0, 16:1ω7, EPA and DHA). On Days 10 and 20, profiles of krill fed on high and saturating levels of thraustochytrids moved towards the top left corner of the plane, associated with higher DHA content. Krill fed diatoms for 20 d (control) were distributed along the PC1 axis (low DHA content). Krill fed at the low level were characterised by low amounts of all FA and tended to have positive scores along PC1, whilst krill fed on medium and high levels, characterised by high EPA and 16:1ω7 contents, showed negative scores. Krill fed on thraustochytrids and then on diatoms showed an intermediary ordination between the thraustochytrid- and diatom-fed groups. Krill with low concentrations of EPA and DHA partly overlapped with krill fed on low and medium levels of thraustochytrids for 10 d ('mixed' group). Within the thraustochytrids + diatoms group, krill fed on high levels of diatoms for 20 d located closest to the diatom-fed group (control).

## DISCUSSION

### Lipids as trophic markers in krill

In the wild, variability in the lipid content of *Euphausia superba* has been related to several factors, including sex, maturity stage and diet (Clarke 1980, Kolokowska 1991, Pond et al. 1995, Virtue et al. 1996, Mayzaud et al. 1998, Cripps et al. 1999, Phleger et al. 2002). However, the observation that different feeding regimes induced only slight variations in lipid class and FA composition in controlled conditions suggested that caution is needed when using lipids as dietary biomarkers in Antarctic krill (Stübing & Hagen 2003, Stübing et al. 2003). The absence of significant food signatures in FA profiles was interpreted as a consequence of major FA tracers being essential PUFA and

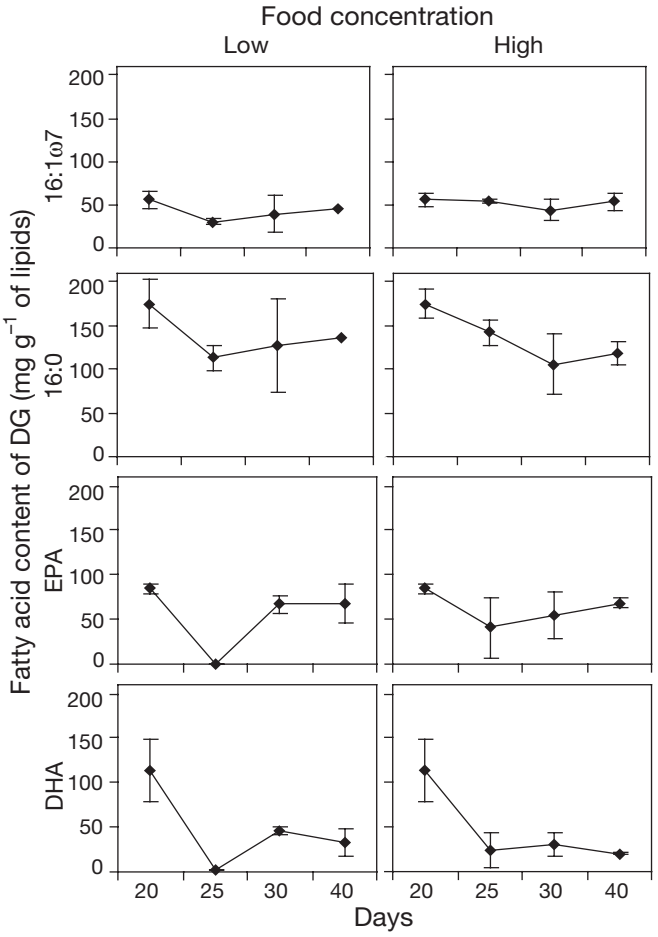


Fig. 4. *Euphausia superba* fed thraustochytrids (to Day 20) + diatoms (Day 20 to 40). Changes in major fatty acid content of the digestive gland in relation to diatom level (low or high) and duration of feeding (25, 30 or 40 d). Day 20 = fed thraustochytrids at high level for 20 d. Error bars = SD (N = 3)

therefore tightly conserved by organisms for physiological purposes. Some authors have suggested that the length of exposure to phytoplankton might significantly affect dietary signatures in krill lipids (Mayzaud et al. 1998). From this point of view, krill populations might reside in an area for too short a period to establish stable trophic links with local phytoplankton biomass. This could explain why lipid content might be poorly correlated with regional phytoplankton densities in the field.

In a companion study (Alonzo et al. 2005), we show that the failure of FA to provide trophic information is largely associated with the fact that most previous analyses were performed on whole krill. Using juvenile animals fed on distinct controlled herbivorous and carnivorous diets, we demonstrated that much of the changes observed in lipid class and FA composition occurred in the hepatopancreas (DG), rather than in other parts of the animals. These observations are in accordance with earlier findings that variability in FA profiles is high in krill DG during starvation (Virtue et al. 1993a). Our study concludes that lipid analyses aimed at identifying food components should be focused on the krill DG. Data on the distribution of lipids among organs are available for the abdominal muscle and the ovary (Clarke 1980), the DG (Virtue et al. 1993b, Alonzo et al. 2003) and various body sections (Seather et al. 1985, Mayzaud et al. 1998). These studies support the hypothesis that high concentrations of lipids are deposited in the DG of juveniles. These stores might serve as energy reserves to enable animals to survive periods of starvation, and be transferred later to the ovary in reproducing females. However, the temporal scales of changes in DG were unknown until the present study.

### Diet-induced changes in lipid class composition

In copepods, TAG content is associated with recent feeding conditions (over the last 3 d), whereas the presence of wax ester reflects longer-term trophic history lasting up to a week (Håkanson 1984). In our present study, feeding krill on thraustochytrids induced significant changes in the lipid class composition of the DG. A rapid increase in PL was observed after 5 d at all feeding levels. Accumulation of TAG in the DG, however, required at least 10 d of feeding and was only observed at the 2 highest feeding levels.

TAG has been described to be a short-term energy reserve for Antarctic krill (Lee et al. 1971), and is accumulated during spring and summer (Clarke 1984, Hagen et al. 1996, 2001). The role of PL, mainly phosphatidylcholine, as storage reserves has also been suggested (Hagen 1988, Seather et al. 1986, Hagen et al. 1996), but its importance remains questionable as PL show much less variability than TAG (Clarke 1980, Kolakoska 1991, Pond et al. 1995, Hagen et al. 2001). In the present study, TAG and PL content in DG varied within similar ranges (0.2 to 0.4 mg per DG). However, a much greater variability in TAG than in PL was observed in DG when krill were fed cryptomonads or diatoms compared to other diets (Alonzo et al. 2005). A maximum PL content of about 0.3 mg per DG, with greater relative amounts of TAG also occurring, was reached when krill were fed continuously for 20 d. Here, thraustochytrid-fed krill showed maximum TAG content on Day 10, and slightly lower content on Day 20. In general, lipid content did not reach levels equivalent to values observed in krill freshly caught from the wild (~1.96 mg per DG, Virtue et al. 1993b) in any of our experiments (this study and Alonzo et al. 2005). This difference resulted from lower feeding rates in our experiments compared to the field (Perissinotto et

Table 4. *Euphausia superba* fed thraustochytrids. Fatty acid composition (percent of total FA) of the digestive gland in relation to thraustochytrid concentration and duration of feeding. Data are mean ± SD (N = 3)

| | | Low | | | Medium | | | High | | | Saturating | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 5 | Day 10 | Day 20 | Day 5 | Day 10 | Day 20 | Day 5 | Day 10 | Day 20 | Day 5 | Day 10 | Day 20 |
| 14:0 | 5.6 ± 1.5 | 11.2 ± 1.8 | 9.9 ± 1.1 | 9.3 ± 0.4 | 14.9 ± 1.5 | 12.2 ± 2.6 | 9.9 ± 0.8 | 15.3 ± 1.0 | 14.1 ± 2.1 | 11.0 ± 0.5 | 12.8 ± 5.1 | 11.3 ± 2.0 | 9.9 ± 0.4 |
| 16:1ω7c | 7.0 ± 0.9 | 8.9 ± 0.2 | 9.0 ± 0.9 | 7.8 ± 0.4 | 6.0 ± 1.1 | 8.3 ± 2.2 | 7.2 ± 0.2 | 7.3 ± 0.9 | 6.0 ± 0.6 | 7.7 ± 1.7 | 4.6 ± 0.5 | 5.5 ± 0.9 | 6.2 ± 1.3 |
| 16:0 | 21.4 ± 1.2 | 35.6 ± 9.1 | 38.2 ± 2.5 | 24.8 ± 1.5 | 52.1 ± 2.0 | 36.2 ± 8.3 | 26.7 ± 5.0 | 46.6 ± 1.9 | 33.3 ± 5.4 | 24.0 ± 0.4 | 40.1 ± 9.6 | 29.8 ± 5.4 | 21.9 ± 0.4 |
| 18:1ω9c | 9.4 ± 0.4 | 10.4 ± 1.3 | 11.4 ± 0.7 | 8.4 ± 0.8 | 6.0 ± 2.0 | 8.8 ± 2.3 | 8.9 ± 1.3 | 8.0 ± 1.1 | 6.4 ± 0.8 | 8.8 ± 2.1 | 4.6 ± 0.6 | 5.0 ± 1.2 | 7.7 ± 0.3 |
| 18:1ω7c | 11.2 ± 0.7 | 10.5 ± 1.0 | 11.5 ± 0.7 | 8.2 ± 0.9 | 7.2 ± 1.9 | 9.1 ± 1.9 | 8.4 ± 1.2 | 8.5 ± 0.3 | 6.5 ± 0.1 | 7.0 ± 0.6 | 5.3 ± 0.4 | 5.6 ± 0.8 | 6.2 ± 0.6 |
| 20:5ω3 (EPA) | 20.8 ± 1.1 | 5.4 ± 8.4 | 0.6 ± 0.8 | 13.8 ± 2.2 | 0.0 ± 0.0 | 3.0 ± 4.9 | 11.1 ± 3.6 | 0.1 ± 0.1 | 3.8 ± 2.3 | 11.6 ± 1.5 | 0.1 ± 0.1 | 5.8 ± 2.7 | 11.4 ± 0.9 |
| 22:5ω6 | 0.0 ± 0.0 | 0.2 ± 0.2 | 0.1 ± 0.1 | 1.1 ± 0.6 | 0.4 ± 0.4 | 1.2 ± 1.6 | 1.5 ± 0.2 | 0.1 ± 0.2 | 3.1 ± 0.6 | 2.3 ± 1.0 | 0.5 ± 0.3 | 4.9 ± 1.1 | 3.1 ± 0.8 |
| 22:6ω3 (DHA) | 9.3 ± 1.4 | 2.6 ± 3.8 | 0.4 ± 0.4 | 11.4 ± 2.7 | 0.9 ± 0.8 | 5.8 ± 8.9 | 11.8 ± 2.9 | 0.6 ± 0.5 | 12.3 ± 4.0 | 15.5 ± 3.6 | 7.2 ± 10.1 | 18.8 ± 5.0 | 18.8 ± 3.5 |
| Other | 15.3 | 15.2 | 18.9 | 15.2 | 12.5 | 15.4 | 14.5 | 13.5 | 14.5 | 12.1 | 24.8 | 13.3 | 7.1 |
| 16:1ω7c/16:0 | 0.3 | 0.3 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 | 0.2 | 0.2 | 0.3 | 0.1 | 0.2 | 0.3 |
| 18:1ω7c/18:1ω9c | 1.2 | 1.0 | 1.0 | 1.0 | 1.2 | 1.0 | 0.9 | 1.1 | 1.0 | 0.8 | 0.2 | 1.1 | 0.8 |
| EPA/DHA | 2.2 | 2.1 | 1.5 | 1.2 | 0.0 | 0.5 | 0.9 | 0.1 | 0.3 | 0.7 | 0.0 | 0.3 | 0.6 |
| Total ω7c | 18.9 | 20.1 | 22.1 | 16.5 | 14.8 | 18.1 | 16.1 | 16.5 | 13.0 | 14.7 | 10.9 | 11.3 | 13.3 |
| Total ω3 | 33.1 | 9.3 | 2.1 | 28.0 | 1.3 | 10.0 | 25.5 | 1.2 | 17.9 | 30.0 | 7.7 | 28.0 | 32.8 |

Table 5. *Euphausia superba* fed thraustochytrids (to Day 20) + diatoms (from Day 20 to 40). Fatty acid composition (percent of total FA) of the digestive gland in relation to diatom concentration and duration of feeding. Day 20 = krill fed high thraustochytrid concentration for 20 d. Diatoms = fed low and high diatom concentrations for 20 d, without thraustochytrid pretreatment. Data are mean ± SD (N = 3)

| Fatty acid | | Low | | | | High | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 20 | Day 25 | Day 30 | Day 40 | Diatoms | Day 25 | Day 30 | Day 40 | Diatoms |
| 14:0 | 11.0 ± 0.5 | 13.6 ± 1.7 | 8.0 ± 1.1 | 6.2 ± 1.4 | 8.8 ± 2.1 | 10.7 ± 4.1 | 9.0 ± 0.5 | 8.2 ± 0.8 | 6.5 ± 0.9 |
| 16:1ω7c | 7.7 ± 1.7 | 9.9 ± 0.3 | 7.8 ± 0.6 | 7.0 ± 0.3 | 10.8 ± 1.1 | 10.9 ± 1.2 | 10.6 ± 1.0 | 11.2 ± 0.6 | 9.8 ± 1.6 |
| 16:0 | 24.0 ± 0.4 | 37.3 ± 2.5 | 25.0 ± 0.4 | 25.6 ± 1.4 | 22.6 ± 0.8 | 28.8 ± 4.7 | 25.4 ± 1.5 | 25.8 ± 1.0 | 19.9 ± 2.7 |
| 18:1ω9c | 8.8 ± 2.1 | 11.0 ± 0.6 | 10.0 ± 0.6 | 10.6 ± 0.5 | 9.2 ± 1.3 | 10.1 ± 1.5 | 9.6 ± 1.0 | 9.7 ± 1.3 | 8.4 ± 0.1 |
| 18:1ω7c | 7.0 ± 0.6 | 11.6 ± 1.0 | 10.2 ± 0.5 | 11.6 ± 1.3 | 9.9 ± 1.3 | 10.6 ± 1.1 | 9.9 ± 0.8 | 12.7 ± 0.9 | 10.5 ± 1.1 |
| 20:5ω3 (EPA) | 11.6 ± 1.5 | 0.1 ± 0.0 | 13.2 ± 1.1 | 14.0 ± 0.9 | 17.1 ± 2.2 | 7.9 ± 6.5 | 12.7 ± 1.9 | 13.6 ± 1.6 | 20.9 ± 2.2 |
| 22:5ω6 | 2.3 ± 1.0 | 0.2 ± 0.2 | 0.9 ± 0.3 | 0.5 ± 0.1 | 0.0 ± 0.0 | 0.7 ± 0.2 | 0.8 ± 0.3 | 0.2 ± 0.0 | 0.0 ± 0.0 |
| 22:6ω3 (DHA) | 15.5 ± 3.6 | 0.4 ± 0.2 | 9.1 ± 1.4 | 8.2 ± 0.9 | 5.4 ± 1.3 | 4.7 ± 3.5 | 7.1 ± 0.4 | 4.1 ± 0.6 | 6.3 ± 1.2 |
| Other | 12.1 | 15.9 | 15.8 | 16.3 | 9.6 | 15.6 | 14.9 | 14.5 | 10.5 |
| 16:1ω7c/16:0 | 0.3 | 0.3 | 0.3 | 0.3 | 0.5 | 0.4 | 0.4 | 0.4 | 0.5 |
| 18:1ω7c/18:1ω9c | 0.8 | 1.1 | 1.0 | 1.1 | 1.1 | 1.0 | 1.0 | 1.3 | 1.2 |
| EPA/DHA | 0.7 | 0.3 | 1.4 | 1.7 | 3.2 | 1.7 | 1.8 | 3.3 | 3.3 |
| Total ω7c | 14.7 | 22.8 | 18.5 | 20.6 | 21.4 | 23.1 | 21.0 | 24.6 | 20.9 |
| Total ω3 | 30.0 | 1.4 | 25.2 | 22.3 | 25.1 | 14.2 | 22.2 | 19.8 | 29.6 |

al. 1997). This suggests that lipids might accumulate more efficiently *in situ* than under laboratory conditions. Furthermore, lipid contents of wild krill also presumably reflect longer feeding periods during a whole season than in our 20 d experiments.

### Class-specific distribution of fatty acids

FA are not distributed randomly among lipid classes in *Euphausia superba* bodies. Hagen et al. (2001) and Stübing et al. (2003) showed that EPA and DHA are

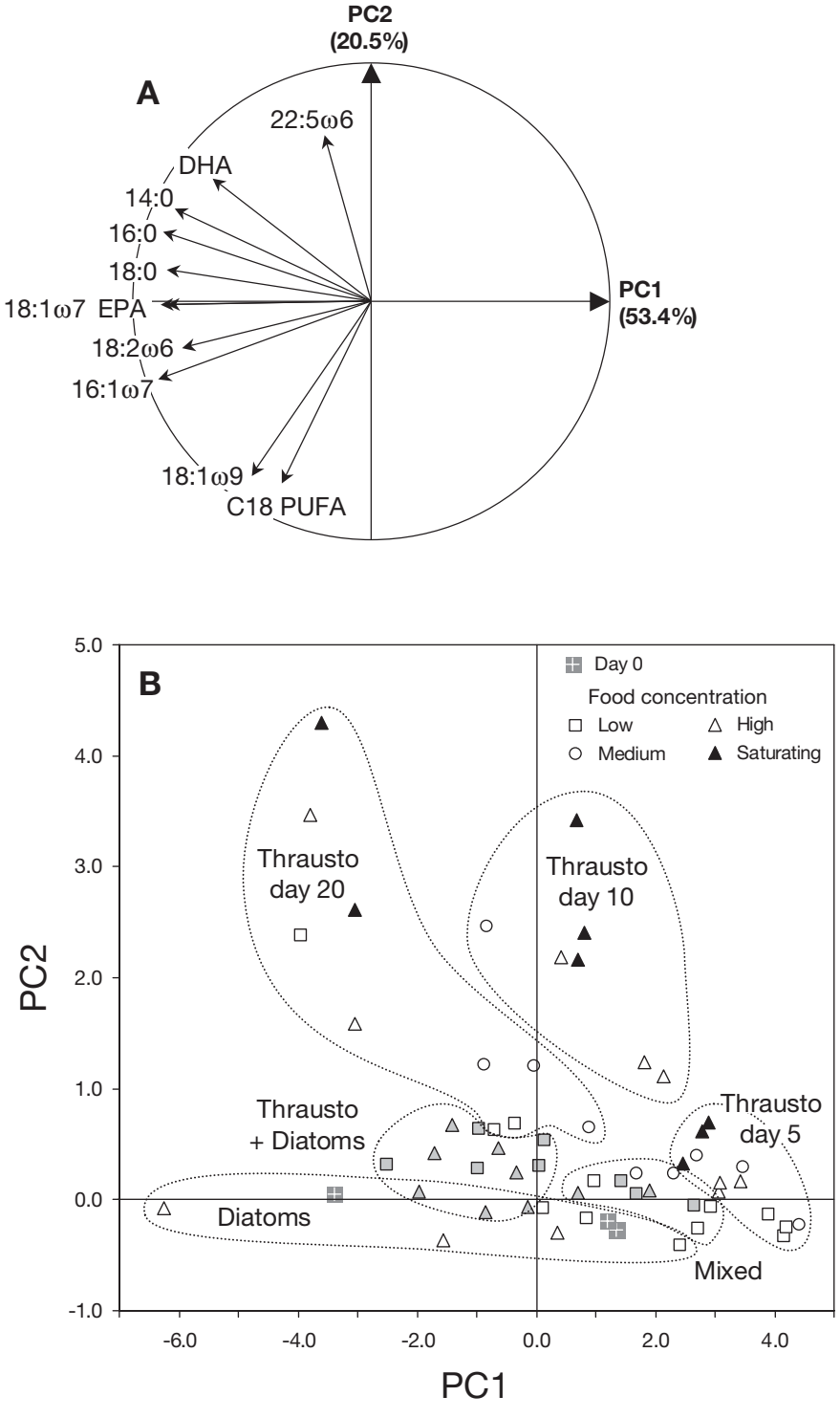


Fig. 5. *Euphausia superba*. Principal component analysis describing fatty acid profiles of the digestive gland (mg FA $g^{-1}$ lipid). (A) Projections of variables on the PC1 – PC2 plane. (B) FA profiles in the PC1 – PC2 plane for krill fed thraustochytrids, thraustochytrids + diatoms (grey labels) and diatoms. Dotted lines represent boundaries of groups, as labelled. The mixed group includes FA profiles of: (1) krill fed thraustochytrids at low and medium levels for 10 d and (2) krill fed thraustochytrids + diatoms. The PC2 axis is stretched relative to the PC1 axis for better visualisation

almost exclusively found in PL, representing almost half of the FA in this lipid class. Various FA, including 14:0, 16:1ω7 and 18:1ω9, show much higher proportions in TAG than in PL, accounting for more than 50% of total FA in the TAG class. One of the major FA in krill, 16:0, is present in approximately equal amounts in PL and TAG, as are all other minor FA. As PL and TAG were accumulated at different rates during our feeding experiments, differential distributions of FA among lipid classes might influence the development of dietary signatures on a temporal scale.

The distribution of FA among lipid classes in DG was not examined in the present study, but it is likely to be similar to that observed in whole krill. It could be expected that a high relative amount of TAG is accompanied by a lower level of EPA and DHA. This was not observed in our experiments, where maximum levels of these PUFA were found in feeding conditions which also induced the highest TAG proportion (Days 10 and 20, high and saturating levels of thraustochytrids). Similarly, EPA reached a maximum in krill fed diatom diets, which supported the second greatest accumulation of TAG among all controlled diets (Alonzo et al. 2005). The observation of much lower levels of 14:0 than 16:0 might be explained by the fact that 14:0 is specifically borne by TAG, whereas 16:0 is found in both TAG and PL (Hagen et al. 2001, Stübing et al. 2003). However, SFA are not considered to be phytoplankton biomarkers (Alonzo et al. 2003). These findings suggest that, if the class-specific FA distribution affects FA profiles, its influence would be rather small compared to those of PUFA abundance in the diet and food concentration. These factors were shown to greatly influence the intensity of the dietary signal in krill, i.e. how strictly dietary PUFA dominate over de novo synthesised SFA and MUFA in DG (Alonzo et al. 2005). In the present study, we show that the proportion of dietary PUFA in DG increases with both food concentration and time.

**Digestive gland fatty acids: markers of a trophic history**

In the literature presently available, data associating time scales with diet-induced changes in FA composition are scarce and are mostly for whole krill. Previous studies have used controlled experiments over a 5 mo feeding period (Virtue et al. 1993b), 16 d of intermittent feeding every 2 d (Cripps & Atkinson 2000) and 19 and 44 d feeding trials (Stübing et al. 2003). Virtue et al. (1993a) examined the FA composition of DG in the course of a 19 d starvation period. In the present study, we show that accumulation of dietary FA in the DG is a relatively slow process, commonly requiring 20 d for the observation of significant increases. These increases were often preceded by a temporary depression in FA abundance, which then recovered to initial levels after 10 d. EPA only returned to its initial level in DG of thraustochytrid-fed krill on Day 20, showing a delayed accumulation compared to other FA. As EPA was present at a low level in the food, the observed delay likely represents the time required for its retroconversion from the abundant DHA. A similar decrease in most FA was apparent 5 d after a diet shift from thraustochytrids to diatoms, with concentrations of diatom biomarkers increasing subsequently until Day 20. These observations suggest that FA turnover rates were high in DG of feeding krill. This is consistent with the results of Virtue et al. (1993a), who observed that 60% of FA present in DG were used during the first 3 d of starvation, and up to 90% after 12 d. As a consequence, the use of signature lipid analyses appears to provide information on food sources on both short and longer terms. Given the short residence time of dietary lipid signatures, changes in FA profiles may be marked as soon as after a 5 d exposure to phytoplankton.

In copepods, dietary-induced changes in FA profiles during the course of 42 d exposure to monoalgal phytoplankton food occurred more progressively than in the DG of *Euphausia superba* (Graeve et al. 1994). This is due to the presence of extensive wax ester reserves in copepods, which act as a buffer to food-related lipid variability, in a similar way to when whole Antarctic krill are examined.

## CONCLUSION

We show that lipids in DG may be used as trophic signatures to examine changes in food-web relationships of Antarctic krill. Under the conditions used in our study, lipid class and FA composition of krill DG responded rapidly. The biochemical changes observed were readily apparent after a 5 d period, and were generally markedly greater after longer (20 d) feeding periods. Therefore, the observation of high amounts of specific FA markers in field collected krill is more likely to reflect a longer (3 wk) period of feeding on the corresponding algae.

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