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Seasonal differences in citrate synthase and digestive enzyme activity in larval and postlarval Antarctic krill, *Euphausia superba*

Abstract Antarctic krill maintain large population sizes despite dramatic seasonal fluctuations in food availability, but the mechanisms for this are still debated. The aim of this study was to compare seasonal differences in enzyme activity and respiration rates of larval and postlarval krill to provide insights into their overwintering strategies. Respiration rates, activity of the metabolic enzyme citrate synthase (CS), and those of the digestive enzymes laminarinase and total proteinase were measured in austral summer west of the Antarctic Peninsula, and in autumn in the southwestern Lazarev Sea. The 100-fold difference in chlorophyll *a* concentrations between the two studies is representative of the classic transition from a summer bloom to sparse winter conditions. Correspondingly, adult krill showed reduced respiration rates and CS activity in autumn. However, their digestive enzyme activity was significantly higher, suggesting more efficient assimilation of food at low food levels. Similar-sized larvae showed no summer–autumn differences in respiration rates and enzyme activity, supporting suggestions that they need to feed and grow year-round. However, trends in enzymatic activity varied between the larval stages measured, implying ontogenetic changes in body structure and function.

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Introduction

The Antarctic krill, *Euphausia superba*, plays a central role in the Antarctic pelagic ecosystem. Krill utilise phytoplankton directly and in turn serve as the main food for several top predators such as whales, seals, and birds. Overwintering success of krill is a key factor that determines its population size (Siegel and Loeb 1995), but there is uncertainty over how they survive the winter when much of their habitat is ice covered and pelagic food is scarce. Suggested mechanisms for adult krill involve either non-feeding strategies (reduced metabolism, lipid utilisation, shrinkage in size) or switching to alternative foods (carnivory, ice algae, detritus). Quetin and Ross (1991) suggest that a threefold reduction in metabolic rate is the most important energy-saving mechanism for adults.

However, a different overwintering strategy might be expected for larval krill. Their early furcilia stages usually appear at the end of the austral summer, develop during their first dark season, and moult to juveniles before December (Siegel and Kalinowski 1994). The need to feed during winter is suggested by their continued development in the field (Marr 1962; Daly 1990), low lipid reserves (Hagen 1988; Hagen et al. 2001), and thus their inability to tolerate long starvation periods (Quetin and Ross 1991; Ross and Quetin 1991). The differing biochemical composition, energy requirements, and behaviour of krill larvae, juveniles, and adults throughout winter suggest that they have fundamentally different overwintering strategies (Quetin et al. 1994).

A central question with these so-called overwintering strategies is whether a seasonal change in metabolic rate simply reflects a change in ingestion rate (and would thus exist in summer conditions of rich and sparse food) or whether there are more fundamental biochemical adaptations. For postlarval krill caught in summer, Ikeda and Dixon (1984) compared metabolic rates during starvation with those fed at bloom concentrations. Respiration rates of starving krill were about one-third

of those with a daily Carbon (C) ration of 10%: a similar reduction to that found in summer–winter comparisons (Ikeda and Dixon 1984; Kawaguchi et al. 1986; Ross and Quetin 1991). This implies that the substantial energy savings from a lower winter metabolism could just reflect the fact that adults often cannot feed rapidly (see Quetin et al. 1996). In contrast, larval feeding, respiration, and growth rates in winter may be commensurate with summer values.

A problem with measuring energy budgets of krill is the possibility that laboratory confinement can lead to laboratory artefacts (Quetin et al. 1994; Ritz 2000). An alternative way of examining biochemical adaptations that does not involve laboratory experimentation is the analysis of enzymatic activity. Previous studies of marine invertebrates have shown that the activity of the key metabolic enzyme citrate synthase (CS), the "condensing enzyme" in the Krebs cycle, is correlated with metabolic activity (Torres and Somero 1988; Thuesen and Childress 1993). Clarke and Walsh (1993) demonstrated that CS activity has the potential for defining the nutritional condition of copepods. However, nutritional condition depends also on the ability to utilise food efficiently, and sufficient activity levels of digestive enzymes are a prerequisite for processing this food, particularly under a variable food regime (Saborowski and Buchholz 1999).

In this study, we compared the activities of CS and the digestive enzymes laminarinase and total proteinase among larval and adult krill and between summer (a bloom west of the Antarctic Peninsula) and late autumn (very low food concentrations in the SW Lazarev Sea). This comparison, between a summer bloom and the onset of winter, represents the extremes of phytoplankton availability that krill can encounter. On one hand it could reflect the seasonal transition from summer to the onset of winter, but on the other hand it could reflect regional and short-term changes observable during summer (e.g. Clarke and Leakey 1996). In any case, comparisons of enzymatic activity between ontogenetic stages of krill from periods of high and low food availability can provide some insights into their different responses.

Materials and methods

The sampling in austral summer (January to March 2000) was at the Rothera Time Series (RATS) monitoring station near the British Antarctic Survey Research Station at Rothera Point (67°34′S, 68°07′W, Adelaide Island, Western Antarctic Peninsula). The autumn study (14–20 April 1999) in the southwestern Lazarev Sea (69°43′S to 69°70′S and 4°38′W to 6°44′W) was surveyed with R.V. "Polarstern". The environmental conditions of both sampling areas are summarised in Table 1. The difference in the chl *a* concentration represents a classic contrast between the environment in a mid-summer, open water bloom and the sparse phytoplankton assemblage just before winter.

Summer sampling off Rothera

Larvae were collected from an inflatable boat using a 250-μm mesh handnet with a 1-litre closed cod end. The net was towed vertically

Table 1 Environmental condition off Rothera in summer 2000 and in the southwestern Lazarev Sea in autumn 1999. Data from summer were from monthly monitoring and were kindly made available by the British Antarctic Survey

	Summer	Autumn
Chl *a* concentration	5–25 μg l^{-1}	0.06–0.09 μg l^{-1}
Temperature	–0.6 to 0.5°C	–1.8 to 0°C
Sea ice cover	None	Sparse broken floes

from 200 m to the surface, as most of the larvae encountered during daylight resided below 100 m depth. Adult krill was sampled with an Isaac-Kidd-Midwater trawl from the R.V. "Laurence Gould" from the environs of Rothera. Back in the laboratory at Rothera, one fraction of freshly caught krill was frozen immediately (larvae on a 200-μm mesh, adults individually) and stored at –80°C for later analyses of dry mass (DM) and enzyme activity. The other fraction was used for measuring oxygen uptake rates. Ontogenetic stages were identified according to Fraser (1936). The dominant larval stages used for all analyses and experiments were calyptopis stage III (CIII) and furcilia stage I (FI).

Autumn sampling in the southwestern Lazarev Sea

On R.V. "Polarstern", larval and adult krill were captured using a 350-μm mesh Bongo net with a 5-l closed cod end. The net was towed vertically from 150 m to the surface. The larvae were mainly furcilia stage III (FIII), so only this stage was used for all analyses and experiments (described above).

Oxygen uptake rate measurements

To determine respiration rates of larval krill during both seasons, eight individuals of CIII and four individuals of FI or FIII were incubated in sealed 100-ml glass bottles filled with filtered seawater (0.45 μm pore size) at ambient temperatures for 15 h. Seven to eight replicates were carried out for each larval stage and four controls were run simultaneously without larvae. Adult krill were incubated in sealed 12-l glass flasks for 15 h. Six replicates contained two individuals each and six controls were run without krill.

In the autumn study, oxygen concentrations were measured with Winkler titrations (Strickland and Parsons 1972). These were carried out with a 716 DMS Titrino (METROM), either on the entire content of the 100-ml incubation bottles (larvae) or on three subsamples of 50 ml (adults). In the summer study, the couloximetry method was used, by taking three replicate subsamples of 1 ml with a syringe pipette and using the method described by Peck and Whitehouse (1992).

The decrease of oxygen concentration within all experiments was less than 10%, which has been shown not to affect larval respiration (Johnson et al. 1984). To compare respiration rates between seasons we normalised the results to 0°C, the average ambient temperature, using a Q_{10} value of 2.0 (Ikeda 1985). The daily C loss from respiration was calculated after Ikeda et al. (2000) using a respiratory quotient of 0.97.

Measurements of dry mass and enzyme activity

DM was measured using replicates of 15 CIII, 4 FI and 2 FIII larvae, and 1 adult. The samples were freeze dried and weighed on a Mettler UM3 microbalance following the procedure described by Donnelly et al. (1990).

For measuring enzyme activity larvae were dissected into a thoracic part for determination of digestive enzyme activity, and an abdominal part for analysis of CS activity. In adults the stomach and midgut gland were dissected for digestive enzyme analysis and the fourth abdominal segment was removed to assay CS activity.

Each dissected part was transferred into pre-weighted microtubes. The wet weight was analysed by using a microbalance before sonicating in 1 ml of demineralised water with a cell disrupter (Branson Sonifier B12, microstic 101-148-063) at about 30% of maximum power. The extracts were centrifuged at 15,000 g for 15 min at 4°C. The supernatants were transferred to new tubes and used for the determination of enzyme activity. In total, 8–13 replicates of larvae and 9 replicates of adult krill were used.

CS (E.C. 4.1.3.7) was determined after Stitt (1984). Standard assays were run as follows: 520 µl of buffer (50 mmol l^{-1} Tris/HCl, supplemented with 100 mmol l^{-1} KCl and 1 mmol l^{-1} EDTA, adjusted to pH 7.5), 20 µl DTNBA (6.0 mmol l^{-1} buffer), 20 µl Acetyl-CoA (6.0 mmol l^{-1} distilled water), and 20 µl sample (supernatant) were placed in a semi-microcuvette. After 5 min of pre-incubation at 25°C the reaction was initiated by addition of 20 µl oxaloacetate (12.0 mmol l^{-1} distilled water) and monitored continuously at 412 nm. The molar turnover was calculated by using the extinction coefficient $\epsilon_{412} = 13.6$ l $mmol^{-1}$ cm^{-1}.

Laminarinase (endo-1,3-β-glucanase) was determined with the dye-labelled substrate CM-Curdlan-RBB (Loewe Biochemica GmbH, 04101). To 250 µl of 0.2 mol l^{-1} CPB, pH 6.0, 50 µl of sample and 100 µl of substrate (4 mg ml^{-1}) were added to initiate the reaction. After incubation at 35°C for 30 min the reaction was stopped with 100 µl of 2 mol l^{-1} HCl and cooled on ice for 15 min. The reaction mixtures were centrifuged for 10 min at 15,000 g. The supernatant was transferred into a microcuvette and read at 600 nm in a spectrophotometer. Controls were run in parallel and the activity (A) was expressed as change of absorption per unit time and wet weight (WW; $A = \Delta E_{600}$ min^{-1} g^{-1} WW).

Total proteinase was determined with azocasein-Na-salt as substrate (Serva, 14391) as described by Donachie et al. (1995) and Buchholz and Saborowski (1996). In brief, the reaction mixture contained 200 µl of 0.2 mol l^{-1} citrate-phosphate buffer (CPB) pH 6.0 and 20 µl of sample. After pre-incubation in microreaction caps for 5 min at 35°C, the reaction was started with 50 µl of azocasein solution (1% in CPB). After 30 min of incubation at 35°C the reaction was stopped with 500 µl of trichloroacetic acid (TCA, 8% in distilled water). The precipitate was separated by centrifugation (5 min, 15,000 g) and the absorption of the supernatant was read at 366 nm in a spectrophotometer. Controls were run in parallel in which the samples were applied to the reaction together with TCA. The activity was expressed as the change of absorption with time and in relation to the sample WW ($A = \Delta E_{366}$ min^{-1} g^{-1} WW).

Statistics

Where the criteria for normal distribution and equal variances were met, pairwise comparison was carried out with a t-test. Where these criteria were not met, a Mann–Whitney rank sum test was used. Comparison of more than two data sets was with a one-way ANOVA followed by a pairwise multiple comparison procedure (Tukey test). The significance level was set at $P = 0.05$. All statistical analyses were done with the PC program SigmaStat 2.03 (SPSS Inc.)

Results

Larvae

During austral summer CIII and FI dominated, whereas FIII prevailed in autumn. Mean DMs of these three stages were 0.21 mg, 0.34 mg, and 0.38 mg, respectively (Table 2).

The respiration rates were similar between all larval stages and seasons, ranging from 0.72 to 0.80 µl O_2 mg^{-1} DM h^{-1}. This corresponds to an average daily C loss of 3% of body C day^{-1} (Table 2).

The CS activity was lowest in CIII (0.48 U g^{-1} WW) and highest in FIII (3.02 U g^{-1} WW). The differences between CIII and FI and CIII and FIII were statistically significant (Fig. 1). No significant seasonal differences in CS activity were evident between larvae of similar DM, FI and FIII.

The activity of the digestive enzyme laminarinase was highest in CIII stages and progressively decreased in FI and FIII (Fig. 2a). The differences were not statistically significant between CIII and FI but were so between CIII and FIII ($P < 0.05$).

Total proteinase activity increased significantly between CIII and FI stages (Fig. 2b). FIII stages from the autumn had the lowest activities, which did not differ significantly from those of CIII stages.

Adults

Adult krill from both seasons had similar DMs (Table 2), and respiration rates were significantly higher in summer than in autumn (0.58 µl O_2 mg^{-1} DM h^{-1} vs 0.20 µl O_2 mg^{-1} DM h^{-1}, $P < 0.001$). The oxygen consumption corresponded to a daily loss of 1.5% of body C in summer and 0.5% of body C in autumn.

The CS activity in the abdominal muscle of krill was 10.9 U g^{-1} WW in summer and was significantly higher

Table 2 Dry mass, respiration rate, and daily respiratory loss of body carbon of freshly caught larvae and adult krill in summer 2000 off Rothera and in autumn 1999 in the southwestern Lazarev Sea. For comparison of respiration rates between the different seasons the data were normalised to 0°C

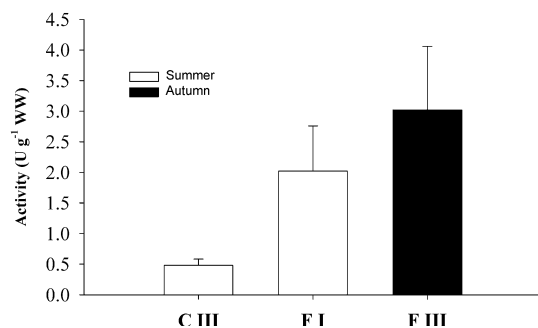| Stages	Summer			Autumn		
	Dry mass (mg)	Respiration rate (µl O_2 mg^{-1} DM h^{-1})	Respiration carbon loss (% body C day^{-1})	Dry mass (mg)	Respiration rate (µl O_2 mg^{-1} DM h^{-1})	Respiration carbon loss (% body C day^{-1})
Calyptopis III	0.21 ± 0.04, ($n = 287$)	0.72 ± 0.14, ($n = 10$)	2.7			
Furcilia I	0.34 ± 0.07, ($n = 201$)	0.80 ± 0.47, ($n = 11$)	2.8			
Furcilia III				0.38 ± 0.03, ($n = 61$)	0.73 ± 0.22, ($n = 16$)	2.6
Adults	209.2 ± 22.4, ($n = 22$)	0.58 ± 0.03, ($n = 7$)	1.5	214.3 ± 76.9, ($n = 21$)	0.20 ± 0.01, ($n = 5$)	0.5

Fig. 1 *Euphausia superba*. Citrate synthase activity of krill larvae, calyptopis III (*CIII*) and furcilia I (*FI*) in summer and furcilia III (*FIII*) in autumn. Number of replicates: CIII, $n = 10$; FI, $n = 8$; and FIII, $n = 13$
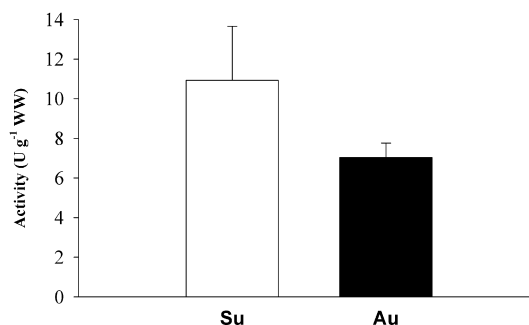

Fig. 3 *E. superba*. Citrate synthase activity of adult krill from summer (*Su*) and autumn (*Au*). Number of replicates for both seasons: $n = 9$
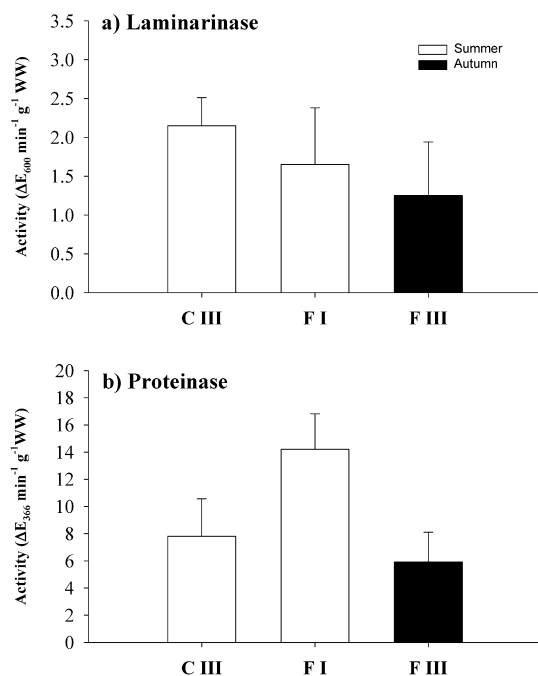

Fig. 2a, b *E. superba*. Digestive enzyme activity (**a** laminarinase, **b** proteinase) of krill larvae, *CIII* and *FI* in summer and *FIII* in autumn. Number of replicates: CIII, $n = 10$; FI, $n = 8$; and FIII, $n = 13$

Stomach

Fig. 4a, b *E. superba*. Digestive enzyme activity (**a** laminarinase, **b** proteinase) in the stomach of adult krill from summer (*Su*) and autumn (*Au*). Number of replicates for both seasons: $n = 9$

than values found in autumn (7.0 U g^{-1} WW, $P < 0.001$, Fig. 3).

The digestive enzymes laminarinase and proteinase were determined in the stomach (Fig. 4) and in the midgut gland separately (Fig. 5). In general, in the stomach, activities were about twice those in the midgut gland. In both organs laminarinase as well as proteinase showed significantly higher activity in autumn than in summer.

Discussion

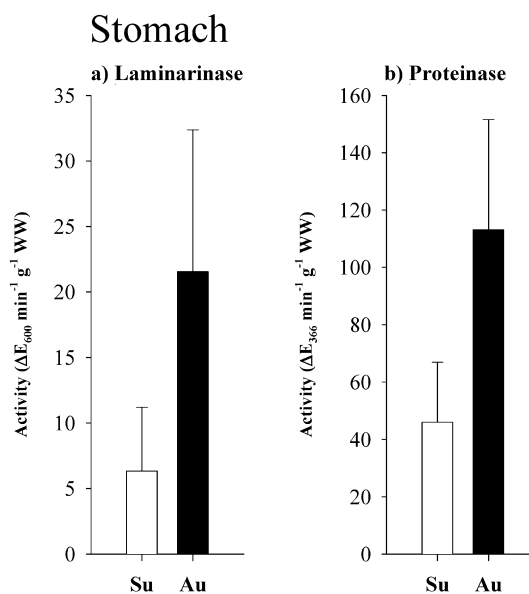CS activity and respiration rates of larval and adult krill showed systematically different trends between the summer bloom and low food concentrations at the onset of winter. Values of equivalent-sized larvae were not significantly different, but respiration rates of adults in autumn were about one-third of summer values. This difference for adults is about the same as that found by Ikeda and Dixon (1984) for summer-caught postlarvae during starvation or feeding at bloom concentrations. Therefore our findings may not necessarily indicate an "overwintering strategy" but could simply reflect food availability, being available to larvae in autumn, perhaps within narrow cavities of the ice, but not to the adults (Atkinson et al. 2002; Meyer et al. 2002).

Whether the seasonal and regional differences in this article should be interpreted as part of the summer-to-winter transition in krill physiology, or just responses to high and low food levels remains under debate. However, the results provide support for a growing appreciation that larval and adult krill have fundamentally different survival mechanisms (see Quetin et al. 1996). For adults, the high activities of digestive enzymes
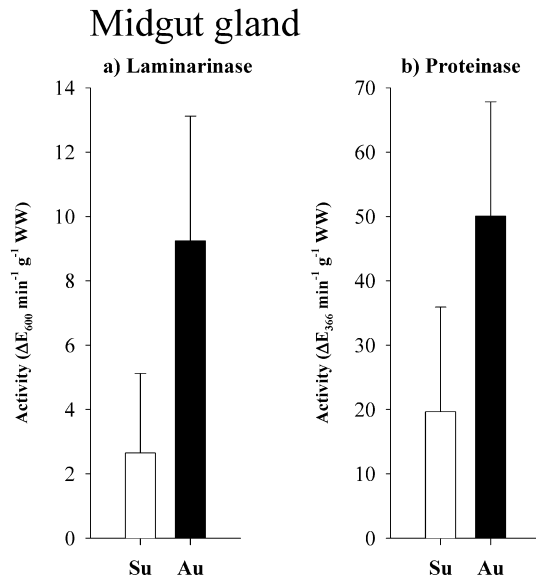
Midgut gland

Fig. 5a, b *E. superba*. Digestive enzyme activity (**a** laminarinase, **b** proteinase) in the midgut gland of adult krill from summer (*Su*) and autumn (*Au*). Number of replicates for both seasons: $n = 9$

despite low respiration rates and CS activity further suggests that, when little food is entering the gut, it is processed more efficiently than in a summer bloom.

Respiration rates and CS activity

Quetin and Ross (1991) discussed a marked reduction in metabolic rate as the most important energy-saving mechanism for adult krill in winter. Our study showed that they had autumn respiration rates < 30% of summer values. Furthermore, CS activity in the abdominal muscle decreased significantly in autumn. CS is a key enzyme of the tricarboxyl acid cycle, a major pathway of aerobic energy metabolism, which is located in the mitochondria. Thuesen and Childress (1993) demonstrated a high correlation between oxygen consumption rates and CS activity in deep-sea pelagic worms, as did Torres and Somero (1988) in Antarctic mesopelagic fishes. Thus, a decrease of both respiration and CS activity is a strong indication for metabolic reduction. In our autumn study chl *a* concentration was approximately 100-fold less than values during the summer bloom at Rothera. This raises the question of whether the metabolic reduction is simply caused by the decrease in food supply and slow growth (Buchholz 1989) during this time of the year or whether krill adapt to winter conditions in a more specific way. Feeding experiments during the autumn study showed that juveniles and adults had clearance rates < 25% of summer values and they failed to respond even after prolonged exposure to high food concentration (Atkinson et al. 2002). This is a result that would favour an adaptive seasonal strategy rather than short-term acclimation to low food levels, but it is still an open question.

Krill larvae showed continuous growth and development during winter (Marr 1962; Daly 1990) and have only low lipid reserves (Hagen 1988; Hagen et al. 2001), which implies that they have to feed. Therefore, it is likely that krill larvae, in contrast to the adults, do not reduce their metabolism. Investigations on metabolic enzymes in larval stages are scarce and studies on fish larvae have shown that CS activity is dependent on growth during ontogeny (Clarke et al. 1992; Power and Walsh 1992). In krill larvae, significant morphological changes appear between calyptopis and furcilia instars, related to the development of the swimming and feeding appendages (Marschall 1985a, b). Since swimming and feeding require much energy, the proportion of mitochondria in the tissue and thus CS activity might increase from calyptopis to furcilia stages. Hence, because of the similarity in morphology of furcilia instars compared to the calyptopis (Ullrich and Storch 1993) and the similar DM of FI (summer) and FIII (autumn), only these two stages were considered for a seasonal comparison.

In the larval stages studied, the oxygen uptake rates did not differ significantly and, unlike in adults, no variation between summer and autumn was evident. The rates measured in both seasons are similar to those measured by Ikeda (1981, 1984) in summer; in addition, the activity of the metabolic key enzyme CS showed no significant differences between FI and FIII from different seasons. Thus, in contrast to results found for the adults, similar respiration rate and CS activity in furcilia instars from summer and autumn indicate no metabolic reduction between seasons. Feeding experiments during the autumn study demonstrated that FIII larvae showed high clearance rates at in situ food concentrations and were able to utilise high food concentrations when they were available (Meyer et al. 2002).

Digestive enzyme activity

The potential of utilising particular food compounds is reflected by the appearance of specific enzymes. We limited the number of enzymes studied to two extracellular enzymes, laminarinase and proteinase. The first catalyses the degradation of 1,3-beta-D-glucosidic linkages of laminarin, a major compound in phytoplankton species (McConville et al. 1986; Haug and Myklestad 1973), and the ability to digest protein-rich diets is represented by the latter.

Among larval krill the two digestive enzymes showed different trends between CIII, FI, and FIII. The enzyme activity between larvae stages varies due to morphological changes in feeding appendages and/or anatomical changes of digestive organs during ontogeny and in general due to the different needs of larval stages to grow. However, their relative importance remains unclear.

The trend in laminarinase activity, a decrease from CIII to FIII, reflects possibly different diets between

calyptopes and furcilia, because of differences in mouthpart morphology (Wolfe and Felgenhauer 1991; Ullrich and Storch 1993). The first larval stages (CI–CIII and FI) lack a filtering basket as described for adult krill. The filtering basket becomes functional from the FII stages onwards (Barkley 1940; Kils 1983). Therefore feeding of early larval stages may differ from that of FIII stages as well as adult krill and might influence enzyme activity. The different trend in proteinase between larval stages could be explained either by differences in morphology of the gut (Ullrich et al. 1991; Ullrich and Storch 1993), as suggested by differences in the equivalent protozoea and zoea of decapods (Williamson 1982), or alternatively by differences in food quantity or type between the summer and autumn studies. The main food source of furcilia larvae are microalgae and they show high ingestion and turnover rates when food is available (Meyer et al. 2002). Jones et al. (1997) reported that larvae that principally consume a diet of microalgae contain a high percentage of proteolytic enzymes. Assuming that protein is a more essential nutrient than carbohydrates to fuel metabolism and growth, a strategy of maintaining high proteolytic activity seems highly efficient to gain the most energy and amino acids and thus may explain the increase of proteinase compared to laminarinase in FI.

The pattern in digestive enzyme activity in adult krill between seasons seems to be much clearer. Pakhomov et al. (1997) reported that phytoplankton diet alone is not always sufficient to meet the estimated metabolic costs of krill during summer and increasing evidence is appearing for omnivorous as well as carnivorous feeding behaviour of adult krill (Price et al. 1988; Atkinson and Snÿder 1997; Cripps and Atkinson 2000; Hernandez-Leon et al. 2001). The principal digestive organs in euphausiids are the stomach and the midgut gland. Because of the complex digestive process, enzyme activity may vary between the two organs (Saborowski and Buchholz 1999). We found higher digestive enzyme activity in the stomach than in the midgut gland, a difference noted previously (Buchholz 1989; Saborowski and Buchholz 1999), probably reflecting a higher concentration of digestive juice. In either organs of adult krill the laminarinase and proteinase activity was higher in autumn than in summer. Conversely, chl a concentration and metabolic rates in adult krill were significantly lower in autumn than in summer.

Enzyme activities have often been used as an indicator for feeding activity in marine zooplankton (Båmstedt et al. 2000). However, the interpretation of the relation between enzyme levels and feeding activity is difficult because the results have been inconsistent. Mayzaud and Conover (1975) and Mayzaud and Poulet (1978) found a strong positive correlation between digestive proteases and carbohydrases and available food. Likewise, Cox and Willason (1981) found that glucanase activity in some zooplankters became reduced after starvation. In contrast, negative correlations have been found, for example, by Hassett and Landry (1983),

Samain et al. (1985), and Mayzaud et al. (1985). These were discussed as an adaptive strategy to process food efficiently at low food concentrations and in patchy food environments.

Such inconsistencies in the relationship between digestive enzyme activity and feeding rate have also been found for *E. superba*. McConville et al. (1986) reported that the glucanase activity was negatively related to food availability and roughly doubled after starvation, while Garrison et al. (1986) found high levels of digestive enzymes in late winter krill. These findings, like our own, are suggestive of an increase in enzyme concentrations at low food levels. In contrast, Saborowski and Buchholz (1999) showed that in starving *E. superba*, digestive enzyme activity rapidly decreases in the stomach and to a lesser extend also in the midgut gland. However, after advanced starvation of several days, activities recovered again but did not reach initial values. The factors controlling digestive enzyme concentrations in Antarctic krill thus remain uncertain.

Enzyme concentrations in zooplankton's gut are likely to depend on the digestion and enzyme breakdown rate, gut throughput rate, food quantity, and the type of enzyme as well as the rate at which it is secreted. In krill, it is known that gut residence time depends on food quality (Pond et al. 1995) and gut passage time is inversely related to food concentration (Quetin and Ross 1985). During the autumn study, adult krill had very low feeding rates (Atkinson et al. 2002) and thus probably also long gut passage times. Assuming that total enzyme activity is related to gut turnover, then longer residence time is likely to produce higher enzyme concentrations for a given rate of enzyme secretion. Thus it is possible that in our summer study the concentration of digestive enzymes and thus their activity was simply diluted by the mass of food present or the elevated gut passage time. The alternative is that they also resulted from higher rates of enzyme secretion, or protein expression towards more active isoforms (Harris et al. 1986).

In conclusion, adult *E. superba* had lower metabolic rates in autumn than in summer, as shown by decreased respiration rates and CS activity. Simultaneously, they maintained or even improved their ability to utilise food, since the activities of digestion in the stomach and the midgut gland were elevated. In contrast, larval krill showed no metabolic reduction between summer and autumn, as determined in stages with similar morphology and dry mass, FI and FIII.

These differences may be explained in two ways. First they may reflect an adaptational strategy of adult krill to deal with winter periods of food shortage by reducing feeding rates and thus respiration losses, as well as increasing enzyme secretion to process the food more efficiently. The larvae, on the other hand, have food available during the winter (probably within the sea ice).

An alternative explanation is possible, one that invokes no seasonal physiological adaptation. This explanation is essentially one of an invariant seasonal physiology, but driven solely by variations in food

availability. If food is available in autumn/winter to larvae but not to postlarvae, then larvae would suffer no significant seasonal differences in CS activity, respiration rate, or digestive enzyme activity. However great variations in available food for adult krill were seen in this study. This resulted in low feeding rates during autumn (Atkinson et al. 2002) coupled with low respiration rates and slow gut passage time, which in turn led to higher digestive enzyme concentrations. Nevertheless, feeding experiments during the autumn study showed that juveniles and adults failed to respond even after 11 days' exposure to high food concentration. These findings would favour an adaptive seasonal strategy. Whichever explanation is the more likely remains an open question, but in either case the results underline the profound differences in the biochemistry and physiology of larval and adult krill sampled from the same locations.

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