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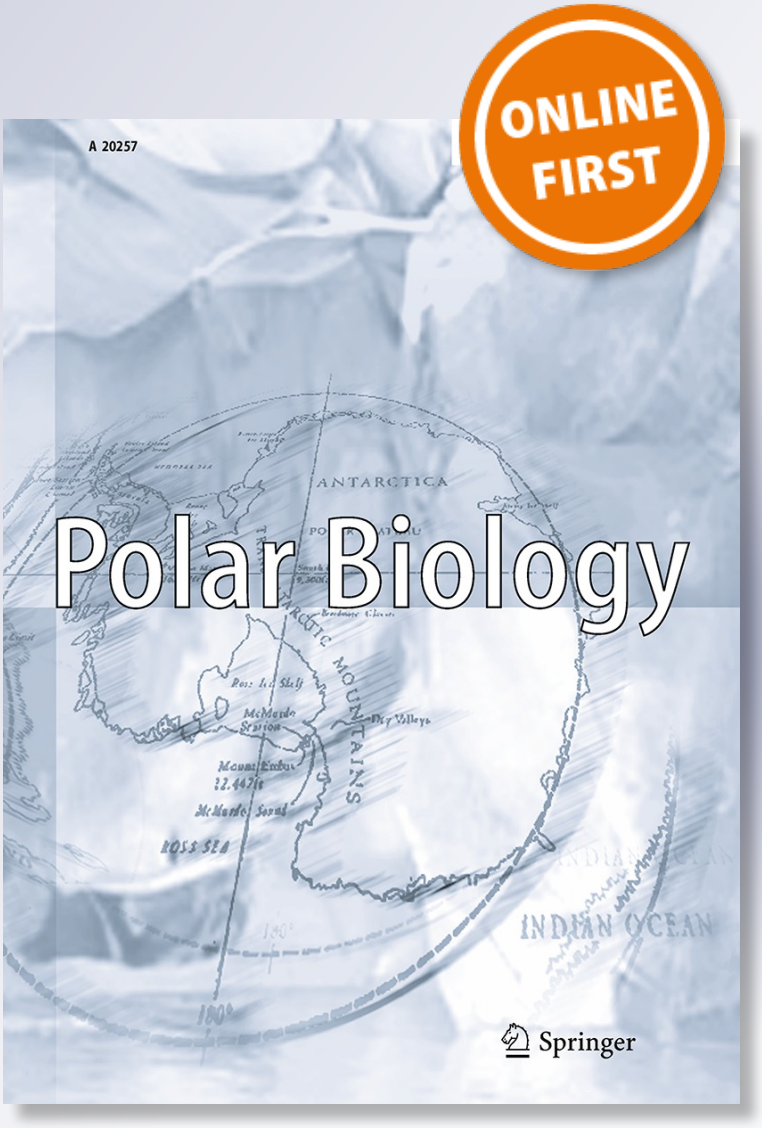

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ORIGINAL PAPER

Physiological response of adult Antarctic krill, *Euphausia superba*, to long-term starvation

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Abstract Adult *Euphausia superba* survive winter without or with little feeding. It is not exactly known whether the scarcity of food or an internal clock, set by the natural Antarctic light regime, are responsible for non-feeding. Our research questions were therefore the following: (1) How will physiological and biochemical conditions of krill change during long-term starvation at constant light regime? (2) If and how do enzyme activities change during such starvation? (3) What is the influence of food availability versus that of light regime? To answer these questions, adult krill were starved under laboratory conditions for 12 weeks with constant light regime (12:12; dark/light) and the impact on physiological functions was studied. Initial experimental condition of krill resembled the condition of late spring krill in the field with fully active metabolism and low lipid reserves. Metabolic activity and activities of enzymes catabolising lipids decreased after the onset of starvation and remained low throughout, whereas lipid reserves declined and lipid composition changed. Mass and size of krill decreased while the inter-moult period increased. Depletion of storage- and structural metabolites occurred in the order of depot lipids and glycogen reserves after onset of starvation until proteins were almost exclusively used after 6–7 weeks of starvation. Results confirmed various proposed overwintering mechanisms such as metabolic slowdown, slow growth or shrinkage and use of lipid reserves. However, these changes were set in motion by food shortage only, i.e. without the trigger of a changing light regime.

Keywords Metabolic activity · Overwintering · Energy metabolite · Lipids · Enzyme activity · Light regime

Lutz Auerswald and Bettina Meyer have contributed equally to this work.

L. Auerswald (✉)
Branch: Fisheries Management, Department of Agriculture, Forestry and Fisheries, Private Bag X2, 8012 Rogge Bay, Cape Town, Republic of South Africa
e-mail: LutzA@daff.gov.za

L. Auerswald
Department of Animal Sciences, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa

B. Meyer · M. Teschke
Scientific Division Biological Oceanography, Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

W. Hagen
BreMarE Bremen Marine Ecology, Marine Zoology, University of Bremen, P.O Box 330440, 28334 Bremen, Germany

S. Kawaguchi
Australian Antarctic Division, 203 Channel Highway, Kingston, TAS 7050, Australia

S. Kawaguchi
Antarctic Climate and Ecosystems Cooperative Research Centre, Private Bag 80, Hobart, TAS 7001, Australia

Introduction

Antarctic krill, *Euphausia superba*, clearly dominates the group of Euphausiacea (krill) in terms of biomass. It is a key species in the Antarctic marine food web, because it serves as direct link between primary producers and apex predators (Everson 2000). Krill fisheries yield the largest tonnage in the Southern Ocean (Kawaguchi and Nicol 2007).

The natural habitat of *E. superba* is characterised by extreme seasonality in terms of daylight intensity and duration. In addition, winter sea ice covers an area 4.5 times

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larger than in summer (Parkinson 2004). As a result, food (phytoplankton) availability is also strongly seasonal. For most of the krill population, almost complete darkness in winter alternates with near constant availability of daylight in summer. This, in turn, means almost no algal food in the water column in winter with <0.1 μg L^{-1} Chl *a* and primary production lower than 1 mg Cm^{-2} day^{-1}, in contrast with massive phytoplankton blooms of >1,000 mg Cm^{-2} day^{-1} in spring and early summer, resulting in Chl *a* concentrations of more than 10 μg L^{-1} (Atkinson et al. 2002; Vernet et al. 2012). In addition, the decline in algal food availability during autumn can be very rapid (Vernet et al. 2012).

Because of its long life span of 5–7 years (Siegel 2000; Nicol 2000), krill must survive several long Antarctic winters. As a consequence, krill has to live during this period without or with little feeding, before metabolite stores can be replenished during Antarctic spring. Accordingly, physiological, biochemical and elemental composition as well as metabolic activity vary strongly during the course of the year, as has been revealed by numerous field studies (for a review, see Meyer 2012). Summarised, these studies provided sufficient data to develop hypotheses on the main physiological and biochemical survival mechanisms during the adverse winter periods.

A suit of overwintering strategies is used by adult krill: accumulation of energy reserves during summer, reduced metabolic activity and feeding as well as use of lipid stores in winter, switch to non-phytoplankton food and reduced growth—or even shrinking (Falk-Petersen et al. 2000; Meyer 2012). Return of spent females and males to immature stage ("rejuvenation") was suggested as another possible adaptation (Siegel 2012). Accumulation of energy reserves in summer and metabolic depression in winter are regarded as the most efficient mechanisms to save energy and survive winter without food uptake (Hagen et al. 1996; Atkinson et al. 2002; Meyer 2012). Other studies revealed that matching life-history stages with environmental events, such as sea ice retreat and algal spring bloom, is important for krill survival and reproduction (Siegel and Loeb 1995; Meyer 2012). As an essence from previous studies, it can be concluded that the two main drivers of krill physiology during the course of the year are as follows: (1) light regime and (2) food availability.

During summer, genes controlling feeding, digestion and respiration, amongst others, are up-regulated compared with winter conditions (Seear et al. 2009, 2012), and the light regime seems of paramount importance to trigger these adjustments of *E. superba* to the drastic seasonal changes in Antarctic waters. The presence of food alone is not sufficient to trigger feeding activity (Atkinson et al. 2002; Teschke et al. 2007). Recent research indicated that endogenous (inherent) mechanisms exist to synchronise the seasonal and circadian rhythmicity of metabolic and physiological output (Teschke et al. 2007, 2008, 2011; Mazzotta et al. 2010), triggered by light (Seear et al. 2009; Teschke et al. 2011; Brown et al. 2013). On the other hand, it was clearly shown in short-term starvation experiments that food availability does play a role: even after only 18 days without food, krill switched to a mode that reduced energy consumption and initiated consumption of body lipids (Atkinson et al. 2002; Auerswald et al. 2009). A combined regulation of metabolism by the triggers, light and food, seems therefore likely.

It is impractical to comprehensively investigate in the field to what extent light regime and food availability, respectively, contribute to the changes in krill's life style during seasonal transition. Such stimuli cannot be differentiated in the field, and results from cruises remain snapshots, especially since they usually cover only short periods of time which often originate from different areas and seasons. Well-defined laboratory-based simulation experiments that cover long periods are therefore a prerequisite to elucidate the mechanisms that lead to changing physiological condition of adult krill.

In this regard, we previously investigated "constant-proportion enzymes" that represent different catabolic pathways and that previously allowed revealing metabolic shifts in insects (Auerswald and Gäde 2000). In krill, these enzymes were useful to describe a shift from a more protein-based metabolism to the consumption of lipid reserves (Auerswald et al. 2009; Meyer et al. 2010).

In the present study, we investigated long-term effects of starvation under controlled laboratory conditions. In previous studies, such laboratory experiments were done with changing light regimes and feeding conditions. Here, we kept light regime constant and compared between starved and fed groups. The aims of the present study were to find out (1) how physiological and biochemical conditions of krill change during long-term starvation at constant light regime, (2) if and how enzyme activities change during such starvation, including constant-proportion enzymes, (3) what the influence is of food availability versus that of light regime.

Materials and methods

Krill sampling and maintenance

Live Antarctic krill were collected on 7 February 2005 (66°15′S, 74°45′E) and 3 March 2006 (66°02′S, 79°32′E) using a rectangular mid-water trawl (RMT 8) (Baker et al. 1973) onboard research vessel *Aurora Australis*. Krill were transferred to 200-L tanks and maintained onboard ship with a continuous supply of seawater in a cold laboratory (0 °C, dim light and no food). After arriving in Hobart, Tasmania, krill were delivered directly to the Australian Antarctic Division (AAD) krill aquarium and kept in a

1,670-L holding tank, which was connected to a 5,000-L chilled seawater recirculation system. The seawater was maintained at 0.5 °C and was recirculated every hour through an array of filtration devices. Lighting was provided by fluorescent tubes, and a controlled-timer system was used to set a natural photoperiod corresponding to that of the Southern Ocean at 66°S and 30 m depth. A detailed description of the aquarium system is given by Kawaguchi et al. (2010).

Experimental set-up

A total of 306 krill specimens of both genders were transferred individually into 250-mL jars with small holes to allow water exchange in a 1,000-L flow-through seawater system maintained at 0 °C (Kawaguchi et al. 2006). In the first 3 weeks of acclimation, each animal was fed with a mixture of the pennate diatom *Phaeodactylum tricornutum*, the flagellate *Isochrysis* sp., and the flagellate *Pavlova* sp. Water flow in the tank was closed (for 5 h) to enable krill to feed on the algal mixture. These algae had been used successfully in several experimental trials at the AAD prior to this study, and they guarantee good condition of krill in long-term laboratory experiments with low mortality and high feeding rates (King et al. 2003). The food mixtures were injected daily into each jar to correspond to a final Chl *a* concentration of 10 µg L^{-1}. All animals were fed for the first 2 weeks of the experiment. Subsequently, 225 of these animals were starved for 12 weeks (starved group), the other 81 continued to be fed (control group). Ten randomly selected starved specimens were sampled every week and ten fed specimens every third week. Each jar was checked daily for moults and dead animals. Sub-sampled krill were first used to measure oxygen uptake rates. Thereafter, wet mass and length were measured; an aliquot of haemolymph was withdrawn using a Hamilton syringe, before snap-freezing the animals in liquid nitrogen for further analysis of elemental and biochemical composition as well as for determination of enzyme activities.

Analysis of dry mass (DM) and body composition (carbon, nitrogen, protein, lipid, glycogen)

To determine fresh mass (FM), whole krill, the dissected hepatopancreas and the abdomen, separated from the cephalothorax by scalpel, were weighed. Subsequently, individual parts or whole krill were ground, using mortar and pestle, to a powder in liquid nitrogen. After lyophilisation for 24 h, whole krill or parts thereof were reweighed, DM calculated and the sample ground to a powder in liquid nitrogen. For analyses of carbon (C) and nitrogen (N), aliquots of 0.2–0.5 mg from each krill homogenate were analysed as described elsewhere (Meyer et al. 2002a, Meyer et al. 2003). For determination of total body protein in krill, the Bio-Rad DC Protein Assay was used, which is a modification of the Lowry et al. (1951) assay. For this assay, another aliquot of powder (2 mg) was incubated in 1 mL 1 N NaOH for 2 h at 60 °C, then diluted to 0.5 N NaOH and centrifuged at 2,000*g* for 5 min. The supernatant was used for determination of protein content, using a microplate reader (Bio-TEK Synergy HT). The standard curves were prepared with bovine serum albumin in 0.5 N NaOH. This method for determination of the total body protein content in krill was preferred to that of Bradford (1976), since the latter underestimates the protein level in krill up to threefold (Meyer et al. 2010).

Total lipids were extracted from 10 to 15 mg of krill homogenate in dichloromethane/methanol (2:1 v/v) and total lipid (TL) content determined gravimetrically (after Hagen 2000). Lipid class compositions of the abdomen were analysed in duplicate by thin-layer chromatography—flame-ionisation detection on an Iatroscan Mark V (Fraser et al. 1985). Single compound standards were used for calibration, with dipalmitoyl phosphatidylcholine, cholesterol, triolein and oleic acid palmityl ester. For fatty acid analysis of abdomen, hepatopancreas and haemolymph, total extracted lipids were hydrolysed and the fatty acids converted to their methyl ester derivatives (FAMEs) according to Kattner and Fricke (1986). FAMEs were detected and quantified via flame ionisation in a gas chromatograph (HP 7890A) equipped with a programmable temperature vaporizer injector (Gerstel CIS4 in solvent vent mode) and a DB-FFAP column (30 m length, 0.25 mm inner diameter). Peaks were identified by comparing retention time data to those of standard mixtures.

Measurements of metabolic rates

The rates of oxygen consumption and ammonium production were measured by incubating individual krill in 2.5-L sealed glass bottles with filtered seawater (0.2 µm pore size, 0 °C). In each experiment, four bottles containing krill and three control bottles without krill were used. All experimental bottles were incubated at 0 °C for 12–24 h in a water bath located in a constant temperature room. After incubation, sub-sampling was carried out rapidly by inserting a glass tube attached to a silicon tube and siphoning the mixed contents of the bottles into 50-mL Winkler bottles for oxygen determination according to Atkinson et al. (2002) and into 15-mL Falcon tubes for analysis of ammonia content. For oxygen determination, three replicate sub-samples were taken from each experimental bottle. Oxygen concentration was determined after immediate fixing for Winkler titrations, as described by Meyer et al. (2002a), using a 702 SM Titrino (Metrohm).

During all experiments, individual krill were freely swimming in the incubation bottles and the decrease in oxygen concentration was <10 %, which is not believed to affect krill behaviour and hence respiration rates (Johnson et al. 1984). Ammonium concentration was analysed photometrically by the phenol-hypochlorite method according to Solorzano (1969).

Determination of enzyme activities

At stated times of the starvation experiment, either whole krill or the fifth abdominal segment of frozen animals (dissected carefully on a cooling element to avoid thawing) was homogenised in a pre-weighed and pre-cooled glass potter (Tissue grind pestle, Kontes Glass Company, US) in ice-cold deionised water at a ratio of 100 mg FM mL^{-1}. The homogenate was centrifuged at 5,000*g* and 4 °C and the supernatant transferred into a fresh tube. From this supernatant, maximum activity of malate dehydrogenase (MDH; EC 1.1.1.37) was determined according to Teschke et al. (2007), whereas activity of citrate synthase (CS; EC 4.1.3.7) was determined according to Stitt (1984) using previously described modifications (Meyer et al. 2002b, Meyer et al. 2010). Activities of 3-hydroxyacyl-CoA dehydrogenase (HOAD; EC 1.1.1.35), glyceralaldehyde-3-phosphate dehydrogenase (GAPDH; EC 1.2.1.12) were measured as described in Auerswald and Gäde (1999). Total esterases (carboxylic ester hydrolases; EC 3.1.1.) were measured as described by Knotz et al. (2006), and activity of lipases (glycerol ester hydrolases; EC 3.1.1.) was determined according to Prim et al. (2003). In situ activity at experimental temperature (0 °C) was established for each enzyme as described in detail in Meyer et al. (2010).

Statistics

For testing significance of differences using STATISTICA 8.0, Kruskal–Wallis ANOVA with post hoc Dunn test was used for comparison of data from starved krill with pre-starvation values (t_0). Data sets that were not normally distributed were logarithmised before analysis. Significance level was generally set at $p < 0.05$.

Results

Physical parameters and growth

Mortality in both krill groups was low during 12 weeks of experimentation, but slightly higher in starved than in fed krill (Table 1). Initial mean FM of all specimens was 492 ± 141 mg which declined consistently throughout the experiment in both groups (Fig. 1a). The decline was more pronounced in starved krill after 12 weeks, and their mass was reduced by 52 % to 236 ± 31 mg, whereas fed animals reduced 34 % of their mass to 323 ± 32 mg. Mean length at the start of the experiment was 40.1 ± 3.7 mm and declined in both groups during the second half of the experiment (Fig. 1b). Starved krill reduced their lengths by 18 %, fed krill by only 12 %. Relative size of hepatopancreas declined after 12 weeks from an initial 7.5 ± 2.1 % FM by 40 % to 4.5 ± 0.3 % FM in starved krill and by 26 to 5.5 ± 1.0 % FM in fed krill (Fig. 1c). Relative hepatopancreas size was always substantially larger in fed krill. Inter-moult periods (IMP) increased with each moult cycle in starved krill (Table 1), and the last full IMP was 5 days longer than the first one (+19 %). In fed krill, there was only a slight increase in the IMP of 0.9 days or 3 % from the first to the fourth full IMP.

Elemental and biochemical composition of whole animals

DM of fed whole krill increased during the first 6 weeks of the experiment and remained constant from then onwards (Fig. 2a). At the end of the experiment, DM was 15 % higher compared with initial values. DM of starved krill decreased and ended 12 % lower than the initial DM (Fig. 2a).

Table 1 Mortality and duration of inter-moult periods (IMP) of adult *E. superba* during chronic starvation

	Mortality[#] %	IMP number			
		1	2	3	4
Starved	7.6	26.3 ± 1.6	27.2 ± 2.3*	29.6 ± 2.7*	31.3 ± 2.6*
n		202	142	78	14
Fed	6.2	26.6 ± 1.7	27.2 ± 1.8	27.0 ± 1.5	27.5 ± 1.6
n		77	60	44	21

Values are given as mean ± S.D. IMP is expressed as days

* Indicates significant difference to first IMP using Levene's ANOVA followed by post hoc Dunnett test

[#] Calculated for complete duration of experiment; animals killed for analysis not included

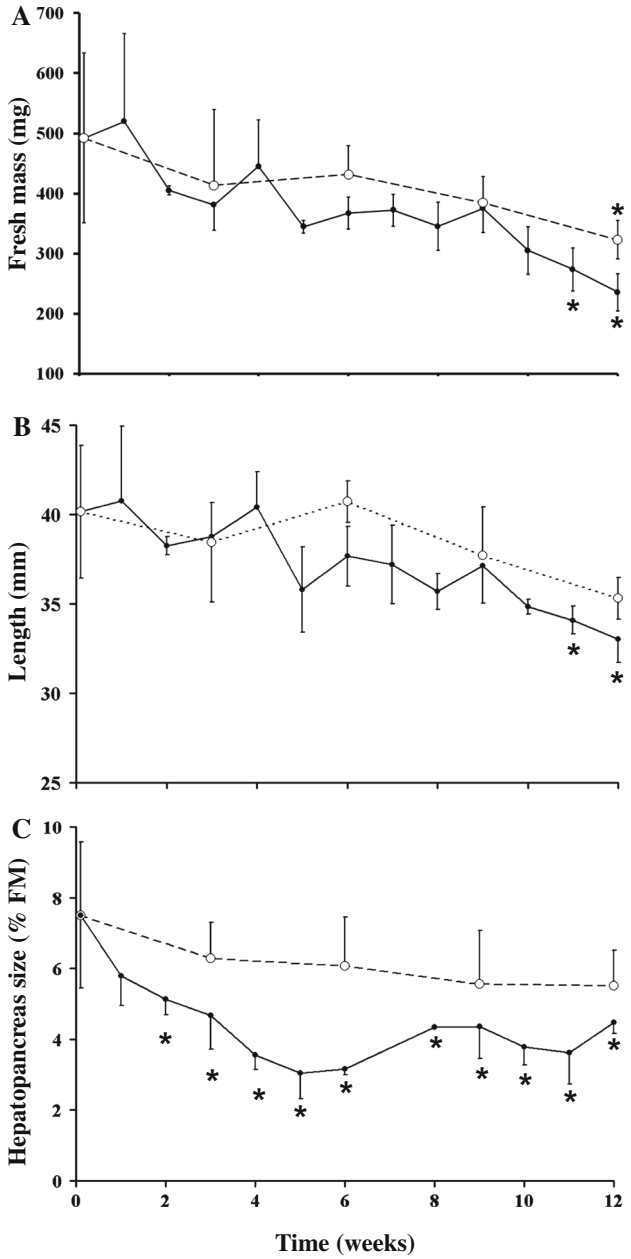


Fig. 1 Changes in fresh mass **a**, total length **b** and relative hepatopancreas size **c** of fed (*empty circle*) and starved (*full circle*) adult krill during chronic starvation. Values are mean ± S.D., $n = 2$–8. *Indicates significant difference to values at the start of the experiment (t_0) using Kruskal–Wallis ANOVA with post hoc Dunn test

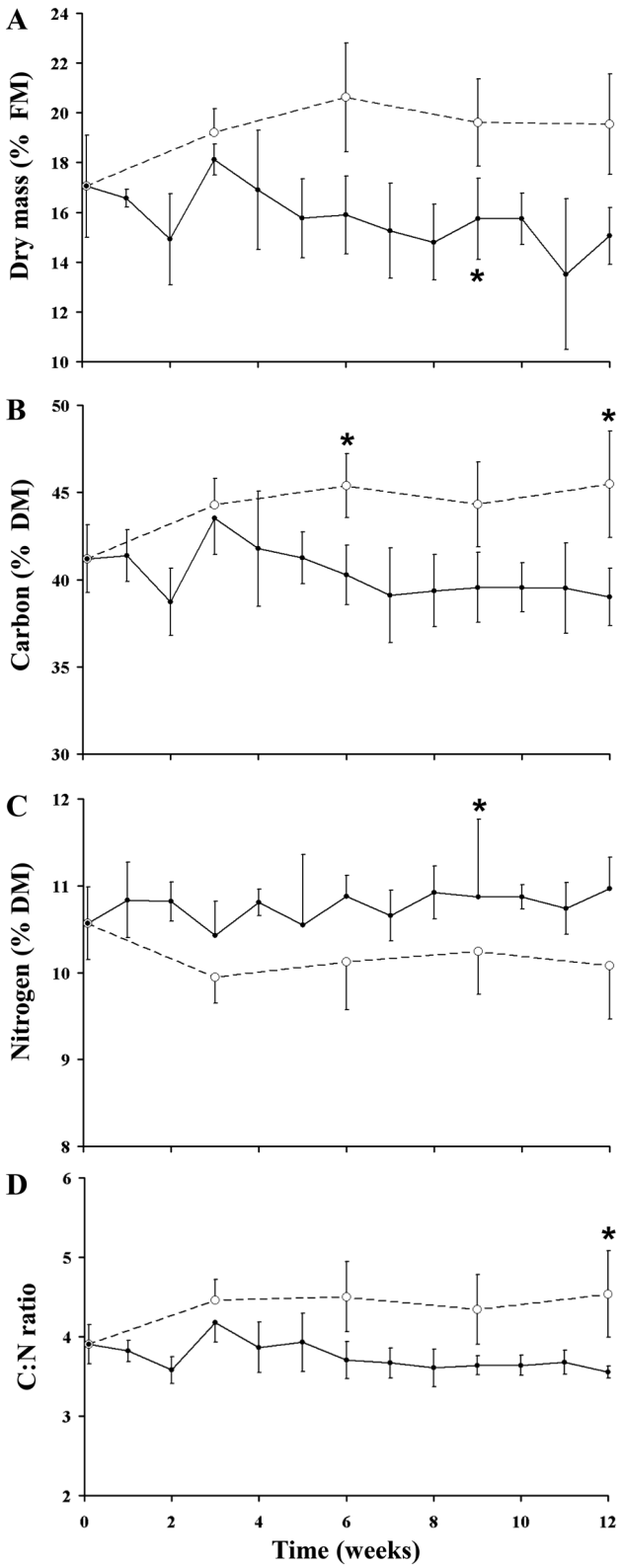
In fed krill, the C content increased continuously during the first 6 weeks, and throughout the following weeks, it remained at a level approximately 10 % higher compared with the start throughout the following weeks, ending with a significantly higher C content (+10 %) after 12 weeks (Fig. 2b). C content for starved krill was approximately 5 % lower than initial values (Fig. 2b). The N content in fed whole animals dropped by approximately 5 % during the first 3 weeks, where it remained until the end of the experiment (Fig. 2c). In starved krill, N content fluctuated around the initial value during the course of the experiment and was 4 % higher at the end (Fig. 2c). Throughout the experiment, the resulting C:N ratios increased by 16 % in fed whole krill, whereas it declined by 9 % in starved krill (Fig. 2d).

In fed whole krill, TL contents increased steadily throughout experimentation and were 71 % higher after 12 weeks compared with initial values (Fig. 3a). In starved krill, TL levels declined and were 41 % lower at the end of the treatment (Fig. 3a). Protein concentrations of fed krill remained constant throughout the course of the experiment, whereas those of starved krill fluctuated and reached a 6 % higher level than in the beginning (Fig. 3b). Glycogen content declined slightly during 12 weeks in fed animals and ended 7 % below the initial content (Fig. 3c). In starved krill, glycogen content decreased steeply by 65 % in the first week of starvation, after which it steadily increased again and reached 70 % of starting concentration.

Lipid composition of body parts

TL contents in abdomen declined throughout the starvation experiment by 44 %, whereas the abdomen of fed krill had a 24 % higher lipid content 12 weeks after the start (Fig. 4a). In the hepatopancreas and the haemolymph, TL contents usually showed a higher variability. Lipids in the hepatopancreas declined continuously in starved krill and reached a 42 % lower level after 12 weeks (Fig. 4b). In fed krill, TL contents of the hepatopancreas declined by 13 % (Fig. 4b). In starved krill, lipid contents in the haemolymph (not presented) showed a stronger decline to a level 80 % below the initial value (from 48.1 ± 8.1 % to 9.9 ± 4.2 % DM) after 10 weeks. In contrast, lipid levels rose about one-third above initial levels (to 68.2 ± 5.4 % DM) in fed animals after 12 weeks.

The relative composition of lipid classes (% of total lipids, %TL) in the abdomen shifted during 12 weeks of starvation (Table 2): phospholipids (PLs) rose slightly from 70 to 81 %, whereas triacylglycerol (TAG) declined strongly from 22 to 4 %. In contrast, in fed animals, PL levels decreased slightly from 70 to 68 % after 12 weeks, while TAG increased from 22 to 25 %. Sterol content rose from 3 to 10 %, and free fatty acids (FFA) increased from 1 to 3 % during 12 weeks of starvation. (such low FFA levels indicate minimum degradation and autolysis of the samples during storage.) In fed specimens, levels of both classes remained largely unchanged during the same period. As a result of the above changes, TAG : ST ratios declined drastically after 12 weeks in starved krill from an initial 7.8 to 0.6, whereas in fed krill, the ratio increased to 12.3 (Table 2).



◀**Fig. 2** Changes in the elemental composition of whole fed (*empty circle*) and starved (*full circle*) adult krill during chronic starvation: **a** dry mass (DM), **b** body carbon (C), **c** body nitrogen (N) and **d** C:N ratio of krill. Values are mean ± S.D., $n = 4$–8. *Indicates significant difference to values at t_0 using Kruskal–Wallis ANOVA with post hoc Dunn test

Fatty acid profiles (of total fatty acids) were analysed for the abdomen, hepatopancreas and haemolymph of adult krill during 12 weeks of starvation, as well as for the corresponding fed controls (Table 3). Principal fatty acids included 16:0, 18:1(n-9), 20:5(n-3) and 22:6(n-3), which comprised more than 50 % of the 15 major FA. Fatty acid compositions of the three components did not change dramatically during the experiment, except for a considerable increase of 22:6(n-3) after 12 weeks of starvation. The percentages of SFA, MUFA and PUFA, respectively, of total FA in the abdomen of krill remained largely unchanged during 12 weeks of starvation, whereas in fed krill, SFA and MUFA percentages declined slightly, while PUFA levels increased. In the hepatopancreas, SFA content decreased significantly from 24.5 to 17.6 % during 12 weeks of starvation, whereas a significant increase from 50.9 to 59.6 % was observed in PUFA content. MUFA content decreased slightly in starved krill. During the same period, percentages of all three FA groups showed only small changes in fed krill with decreases in SFA and increases in PUFA. Similarly, little changes (<2 %) were detected in SFA, MUFA and PUFA percentages in the haemolymph of starved and fed krill after 12 weeks.

Metabolic activity

Mean initial respiration rate was 0.46 ± 0.08 µL O_2 mg^{-1} DM^{-1} h^{-1}, which clearly decreased in starved krill during the observed period to reach a significantly lower rate (reduction by 53 %) after 12 weeks (Fig. 5a). Most of the decline occurred during the first 6 weeks and then became stable. Respiration rates in the fed control group fell only slightly and were always higher than in starved krill. After 12 weeks, respiration remained at 93 % of the initial rate.

The excretion rate at the start was 0.025 ± 0.007 µg NH_4^+ mg^{-1} DM^{-1} h^{-1}. The rate in starved krill was significantly elevated towards the end of the experiment, reaching a 28 % higher rate after 12 weeks (Fig. 5b). Excretion in fed animals remained relatively stable and was 9 % higher after the 12-week experimental period. The resulting atomic oxygen to nitrogen ratio (O:N) was initially 31.8 ± 9.0 and declined consistently throughout the starvation period to a level 63 % below the initial ratio after 12 weeks (Fig. 5c). In fed krill, the ratio remained relatively stable and only declined slightly by 11 % towards the end.

Activities of the Krebs cycle enzymes CS and MDH in the abdominal segment were measured starting 1 week after initiation of experiment (Table 4). CS activity (specific and per FM) declined consistently throughout

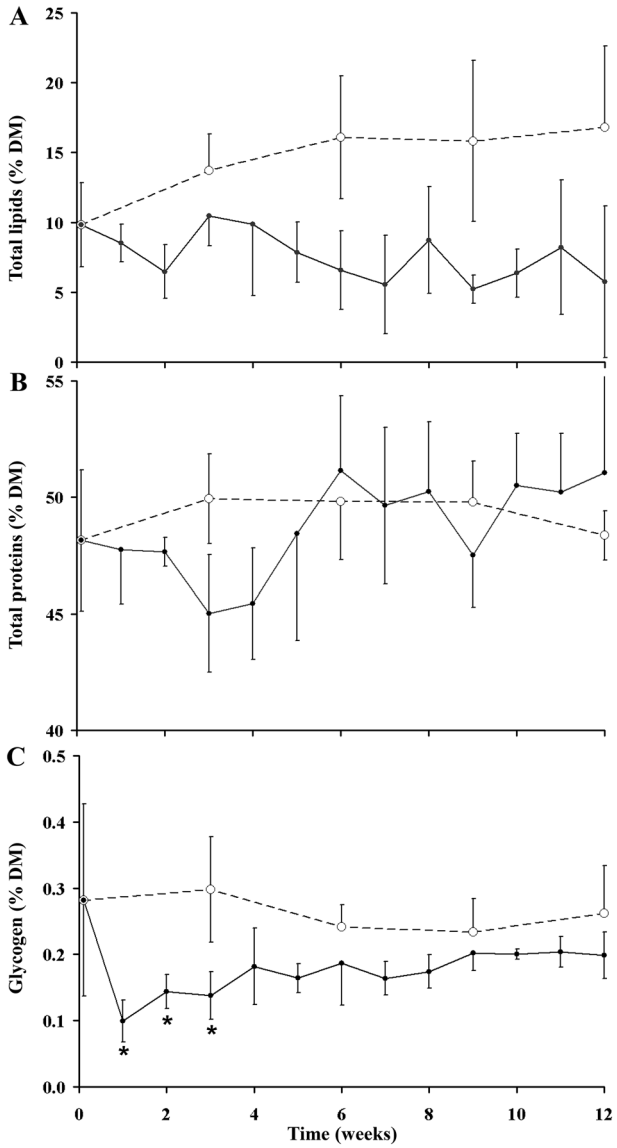


Fig. 3 Biochemical composition of whole fed (*empty circle*) and starved (*full circle*) adult krill during chronic starvation: **a** total lipids, **b** total protein and **c** glycogen. Values are mean ± S.D., $n = 4$–8. *Indicates significant difference to values at t_0 using Kruskal–Wallis ANOVA with post hoc Dunn test

starvation and, after 11 weeks, reached a level 40 % lower than after 1 week of the experiment. In contrast, CS activities remained high in fed animals after 3 and 9 weeks, respectively, higher than the respective activities for starved krill. After 9 weeks, CS activity was 18 % lower than after the first week. The decline of MDH activity in starved krill was less pronounced than that of CS activity and, after 11 weeks, reached a level 26 % below the activity in the first week. In fed krill, MDH activity per FM was unchanged after three and 9 weeks, respectively, whereas specific activity was stable, but at a lower level than after 1 week of starvation. Activities of both Krebs cycle enzymes correlated positively with each other (Fig. 6a) and with respiration (Fig. 6b) during starvation.

Activities of enzymes from the lipolytic and glycolytic pathways

Maximum activities of metabolic enzymes, mainly from lipid metabolism, were measured in extracts from whole adult krill in one-week intervals (Table 5). 3-hydroxyacyl-CoA dehydrogenase (HOAD) activity fluctuated throughout the experimental period in starved krill and reached lower levels at the end (32 % specific activity, 43 % activity per FM). In contrast, HOAD activity of fed krill remained higher than in starved specimens during most of the 12 weeks and was 10 % lower at the end of the experimental period. GAPDH activity fluctuated around a relatively stable level in starved krill and was only slightly lower (7, 20 %) after 12 weeks. It remained unchanged in fed krill. The resulting GAPDH : HOAD ratio showed no trend in either fed or starved krill, but was always lower in fed than in starved krill at the respective sampling times. Lipase activity was declining slightly in both starved (30, 41 %) and fed krill (24, 27 % of initial values), whereas activity of total esterases (including lipases) declined significantly in starved krill by 51 and 60 %. In fed krill, total esterase activity remained stable during the same period. Abdominal enzyme activities were tested after 9 weeks of starvation to yield values that are not influenced by activities of digestive esterase and lipase (Table 6): esterase and lipase activities were similar in both starved and fed krill. Esterase activity was much lower in the abdomen (Table 6) than in whole krill (Table 5), whereas lipase activity was much higher. Abdominal activities of both HOAD and GAPDH were substantially lower in starved krill and the GAPDH : HOAD ratio fell by 13 %.

Discussion

Physiological state of krill at start of experiment

Physiological condition of krill varies strongly with the enormous seasonal changes in their environment (Meyer et al. 2010). Careful assessment of initial body condition of krill is therefore a prerequisite for a meaningful interpretation of possible changes during starvation. Adult krill for the present study were caught several months before experimentation, during Antarctic spring, and fed for several months under laboratory conditions. Six weeks prior to the start of the experiment, they were conditioned to a constant light regime resembling that of Antarctic spring/autumn (LD 12:12) and continuous food supply. The resulting physiological condition (i.e. starting condition)

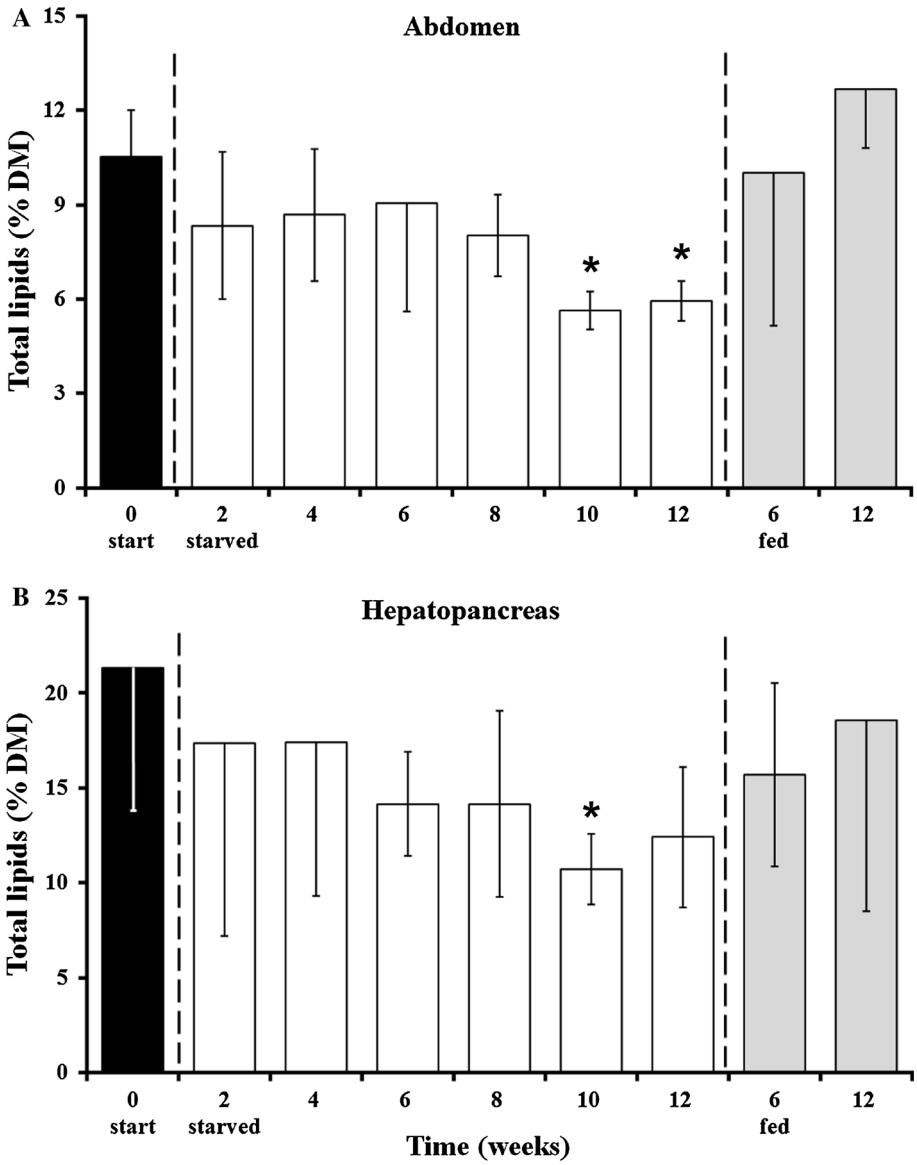


Fig. 4 Changes in total lipid content in body parts of adult krill during chronic starvation: **a** abdomen and **b** hepatopancreas of fed (*grey* columns) and starved (white) krill. Values are mean ± S.D., $n = 4$. *Indicates significant difference to values at t_0 using Kruskal–Wallis ANOVA with post hoc Dunn test

came closest to that previously measured in krill collected during late spring (Auerswald et al. 2009; Meyer et al. 2010), the season that allows krill to start feeding ad libitum on the massive algal blooms and replenish their energy reserves that were depleted during the winter period.

The initial level of TL, the most important storage metabolite in krill, was approximately 10 % of DM, well above the 5 % deemed necessary for membrane functioning and, hence, survival (Hagen et al. 1996; 2001), but far below maximum levels of around 45 % measured from field caught krill in autumn and just before winter (Falk-Petersen et al. 2000; Atkinson et al. 2002). The relatively low lipid content is also reflected in the C:N ratio of about 4, much lower than that of 7 measured in the field at the end of the feeding period (Atkinson et al. 2002). There is also a distinctly different composition of lipids than found previously (Auerswald et al. 2009): most likely caused by a higher accumulation of the depot lipid class triacylglycerol (TAG: 22 % of TL in abdomen) during the long feeding period prior to the experiment; phospholipid percentage was relatively lower (~70 %) than usually found in spring and this also caused a much higher TAG : ST ratio of about eight. The O:N ratio of 32 indicated that lipid compounds, either via digestion or from body reserves, were preferentially catabolised. Above a value of 24, lipids are preferred to proteins as an energy metabolite to fuel biological processes (Ikeda and Mitchell 1982; Mayzaud and Conover 1988; Ikeda et al. 2000).

The first inter-moult period (IMP) observed in the experiment (approximately 26 days) could also serve as an indicator of physiological state at the beginning of the experiment. It is as short as in krill caught during late

Table 2 Lipid classes (% of total lipid, %TL) of abdomen of adult *E. superba* during the course of a long-term starvation experiment, including fed controls

		Starved						Fed	
	0 week $n = 4$	2 weeks $n = 4$	4 weeks $n = 4$	6 weeks $n = 3$	8 weeks $n = 4$	10 weeks $n = 3$	12 weeks $n = 3$	6 weeks $n = 4$	12 weeks $n = 3$
Abdomen									
TL (%DM)	10.5 ± 1.5	8.3 ± 2.3	8.7 ± 2.1	9.1 ± 3.4	8.0 ± 1.3	5.6 ± 0.6*	6.0 ± 0.6*	10.0 ± 4.9	12.7 ± 1.9
PL	70.0 ± 2.2	71.4 ± 4.2	68.6 ± 4.0	77.5 ± 9.2	72.8 ± 3.9	79.9 ± 2.5*	81.4 ± 1.9*	74.5 ± 10.8	67.2 ± 2.0
TAG	21.7 ± 1.8	17.0 ± 5.5	19.3 ± 5.1	12.4 ± 10.3	14.4 ± 3.9	3.5 ± 4.7*	4.4 ± 3.8*	15.9 ± 13.1	24.6 ± 2.7
ST	2.9 ± 0.6	4.5 ± 1.7	4.6 ± 1.4	4.6 ± 2.3	5.0 ± 0.9*	9.7 ± 3.0*	9.5 ± 3.5*	4.9 ± 4.2	2.0 ± 0.3
FFA	1.3 ± 0.5	2.5 ± 0.6	3.0 ± 0.5*	1.9 ± 1.2	3.0 ± 1.1*	4.8 ± 1.0*	2.9 ± 0.4	1.6 ± 1.5	1.0 ± 0.3
Others	4.2 ± 0.8	4.6 ± 0.8	4.5 ± 1.3	3.6 ± 2.7	4.8 ± 1.7	2.1 ± 1.5	1.9 ± 1.5	3.1 ± 2.7	5.1 ± 1.5
TAG : ST ratio	7.8 ± 1.9	4.6 ± 3.0	4.7 ± 2.6	3.8 ± 3.6	3.1 ± 1.3*	0.5 ± 0.8*	0.6 ± 0.5*	7.9 ± 8.3	12.3 ± 2.8

Data are presented as mean ± S.D., $n = 4$. *DM* dry mass, *PL* phospholipids, *TAG* triacylglycerols, *ST* sterols, *FFA* free fatty acids

* Indicates significant difference to values at t_0 using Kruskal–Wallis ANOVA with post hoc Dunn test

spring (Meyer et al. 2010) or summer (Tarling et al. 2006). In the field, short IMPs occur during periods of high food availability, i.e. late spring and summer (Buchholz 1991; Meyer 2012). It can therefore be assumed that this short IMP indicates fast growth and high energy uptake.

Metabolic activity, as expressed by individual respiration rates, was high at 0.46 µL O_2 mg^{-1} DM^{-1} h^{-1} and so were in situ enzyme activities of both MDH (323 mU mg^{-1} protein) and CS (140 mU mg^{-1} protein) early in the experiment. Metabolism was therefore fully active and, despite the relatively low lipid reserves (which is not unusual at this time of the year), krill was in an excellent overall physiological state, when the experiment started.

Physical and metabolic changes during starvation

Prolonged starvation has consequences for the size of krill: animals killed at the end of the 12-week starvation period were 52 % lighter than those measured at the beginning of the experiment. Fed krill also reduced their mass but to a much smaller extent (34 %), possibly due to sub-optimal feeding conditions. Since reduction in body size in crustaceans is only possible through moulting, these values indicate that shrinkage must have occurred. A similar trend during starvation of 211 days has been observed by Ikeda and Dixon (1982) and in another 207-day starvation experiment by O'Brien et al. (2011). Shrinkage at moult would contribute to preservation of body functions with fewer reserves available. It has been proposed, for that reason, as a possible overwintering mechanism in Antarctic krill (Ikeda and Dixon 1982, Meyer et al. 2010). The reduction in the size of the hepatopancreas also confirms this trend: its relative mass declined by 40 % during starvation and its absolute size (per individual) declined by 65 %. Hence, in addition to the adjustment to the decreasing body reserves, which was also observed during the seasonal decline of food availability (Meyer et al. 2010), the hepatopancreas shrinks to adjust to the reduced body size (Virtue et al. 1993). This relative and absolute reduction in hepatopancreas size also took place in fed krill, but this was not observed in a previous study with krill also feeding at a constant 12:12 light regime (Teschke et al. 2007). In the latter study, however, krill were kept as groups in large 100-l tanks and may have been able to feed more efficiently than those kept individually in this study.

As soon as starvation commenced, krill metabolism slowed down in the present experiment. The slowdown effect is most evident from the course of the respiration rate. The decline was steepest in the first week of starvation and resembled results of previous short-term starvation experiments (Atkinson et al. 2002; Auerswald et al. 2009), although in the present study krill was in a better condition prior to experimentation (see above). Such a swift reduction in metabolism is more typical of animals that do not store large reserves of energy metabolites such as the copepod *Temora longicornis* (Kreibich et al. 2008). The decline continued more or less to the end of experimentation, when it reached 50 % of the initial value. When calculated per individual, i.e. taking shrinkage (see above) into account, oxygen consumption was even lower with approximately 20 % of the initial rate. Such a low level was also exhibited by animals that had starved for 211 days and shrunk (Ikeda and Dixon 1982). Reduction in metabolic activity is regarded as the most efficient overwintering mechanism (Quetin et al. 1994; Meyer 2012). In contrast, respiration rates in the control group remained approximately 0.2 µL O_2 g^{-1} DM h^{-1} higher than in starved krill, although fed krill also showed a moderate

Table 3 Major fatty acids (≥1 % of total fatty acids) of abdomen, hepatopancreas and haemolymph of adult *E. superba* during the course of a long-term starvation experiment, including fed controls

		Starved						Fed	
	0 week $n = 4$	2 weeks $n = 4$	4 weeks $n = 4$	6 weeks $n = 4$	8 weeks $n = 4$	10 weeks $n = 4$	12 weeks $n = 4$	6 weeks $n = 4$	12 weeks $n = 3$
Abdomen									
14:0	4.8 ± 0.4	4.6 ± 1.1	4.9 ± 1.0	3.9 ± 2.1	4.0 ± 1.0	5.7 ± 0.5	3.6 ± 2.4	2.9 ± 1.9	2.0 ± 1.1
16:0	19.6 ± 0.6	20.2 ± 0.4	20.0 ± 0.5	20.4 ± 0.9	20.5 ± 0.5	19.9 ± 0.8	21.0 ± 2.2	19.6 ± 0.4	20.2 ± 0.8
16:1(n-7)	6.7 ± 0.2	5.9 ± 0.9	6.2 ± 1.2	5.1 ± 2.3	5.3 ± 1.0	6.7 ± 0.8	4.7 ± 2.0	4.0 ± 2.1	3.0 ± 1.6
18:0	1.0 ± 0.1	1.0 ± 0.0	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.3	0.9 ± 0.1	0.9 ± 0.1
18:1(n-9)	11.7 ± 0.5	12.1 ± 0.3	12.8 ± 0.3	12.7 ± 0.6	13.1 ± 0.4	13.1 ± 0.2	13.4 ± 0.7	13.1 ± 0.5	12.8 ± 0.1
18:1(n-7)	5.5 ± 0.3	5.6 ± 0.3	5.8 ± 0.4	6.0 ± 0.4	5.8 ± 0.1	5.3 ± 0.2	5.7 ± 0.9	5.8 ± 0.4	6.2 ± 0.3
18:2(n-6)	6.8 ± 0.1	6.5 ± 0.2	6.6 ± 0.3	6.6 ± 0.3	6.8 ± 0.2	6.8 ± 0.3	6.6 ± 0.6	6.6 ± 0.2	6.4 ± 0.5
18:3(n-3)	4.3 ± 0.2	3.6 ± 0.4	3.5 ± 0.2	3.3 ± 0.8	3.2 ± 0.4	4.5 ± 0.5	3.3 ± 1.2	3.4 ± 1.3	2.7 ± 0.8
18:4(n-3)	1.5 ± 0.5	0.9 ± 0.2	0.8 ± 0.2	0.7 ± 0.5	0.7 ± 0.1	1.5 ± 0.3	0.8 ± 0.7	0.8 ± 0.7	0.3 ± 0.3
20:1(n-7)	0.7 ± 0.0	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.0	0.9 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	0.5 ± 0.1
20:4(n-6)	2.1 ± 0.2	2.3 ± 0.4	2.3 ± 0.3	3.0 ± 1.3	2.7 ± 0.4	1.8 ± 0.2	2.7 ± 1.5	3.3 ± 1.2	3.7 ± 0.5
20:5(n-3)	13.9 ± 0.4	14.6 ± 0.8	13.6 ± 1.1	14.8 ± 2.5	14.0 ± 0.6	12.0 ± 0.3	12.9 ± 2.3	15.1 ± 2.0	16.1 ± 1.0
22:x (PUFA)	1.7 ± 0.2	2.0 ± 0.2	1.6 ± 0.2	1.8 ± 0.3	1.7 ± 0.4	1.7 ± 0.1	1.8 ± 0.1	1.9 ± 0.2	1.8 ± 0.2
22:5(n-3)	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.1	0.3 ± 0.4	0.6 ± 0.1	0.8 ± 0.0	0.6 ± 0.4	0.2 ± 0.4	0.2 ± 0.3
22:6(n-3)	13.3 ± 0.2	15.4 ± 2.1	14.6 ± 1.5	16.3 ± 4.6	15.7 ± 1.7	12.8 ± 0.8	16.7 ± 6.0	19.3 ± 5.3	20.5 ± 3.5
FA <1.0 %	5.8 ± 0.8	3.8 ± 1.6	4.9 ± 1.2	3.6 ± 2.9	4.3 ± 1.2	5.5 ± 1.2	4.4 ± 2.7	2.6 ± 3.0	2.8 ± 2.1
Σ SFA	26.8 ± 0.7	26.9 ± 1.3	26.9 ± 1.3	26.1 ± 3.1	26.5 ± 1.2	28.1 ± 0.4	26.7 ± 5.2	24.1 ± 2.4	23.6 ± 1.8*
Σ MUFA	25.7 ± 0.8	25.3 ± 1.4	27.2 ± 1.2	25.4 ± 3.2	26.0 ± 1.5	27.6 ± 0.5	25.6 ± 1.8	24.0 ± 2.6	23.1 ± 2.1*
Σ PUFA	39.4 ± 0.8	40.5 ± 2.9	38.3 ± 2.4	41.1 ± 6.9	39.9 ± 2.6	36.3 ± 0.3	40.3 ± 7.5	44.8 ± 5.9	46.5 ± 4.3*
	$n = 4$	$n = 4$	$n = 4$	$n = 3$	$n = 3$	$n = 4$	$n = 4$	$n = 4$	$n = 3$
Hepatopancreas									
14:0	4.5 ± 2.2	1.1 ± 0.4	1.6 ± 0.5	1.1 ± 0.7	1.3 ± 0.1	0.8 ± 0.5	0.4 ± 0.2	3.3 ± 1.6	2.4 ± 0.5
16:0	17.1 ± 1.0	17.8 ± 0.5	18.2 ± 0.3	17.3 ± 0.7	18.1 ± 0.3	17.0 ± 0.6	15.3 ± 2.1	17.5 ± 0.7	18.2 ± 0.3
16:1(n-7)	7.2 ± 2.4	3.0 ± 0.6	3.1 ± 0.9	2.6 ± 1.2	2.9 ± 0.3	1.9 ± 1.0	1.3 ± 0.8	5.0 ± 1.7	4.0 ± 0.6
18:0	1.3 ± 0.4	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.0	1.4 ± 1.0	1.2 ± 0.6	1.0 ± 0.3	1.2 ± 0.1	1.3 ± 0.2
18:1(n-9)	9.8 ± 1.4	8.7 ± 0.7	9.9 ± 0.5	9.8 ± 0.5	9.9 ± 0.3	10.0 ± 0.9	9.8 ± 0.6	10.8 ± 0.6	11.2 ± 0.5
18:1(n-7)	5.4 ± 1.5	8.3 ± 0.2	9.1 ± 0.9	9.0 ± 1.2	8.2 ± 0.2	9.1 ± 0.9	10.3 ± 1.4	6.3 ± 0.9	6.8 ± 0.5
18:2(n-6)	6.6 ± 0.6	6.3 ± 0.4	6.6 ± 0.3	7.0 ± 0.6	7.3 ± 0.3	6.4 ± 0.7	6.3 ± 1.0	6.9 ± 0.4	7.3 ± 0.2
18:3(n-3)	4.3 ± 0.5	4.3 ± 0.2	4.4 ± 0.5	4.2 ± 1.1	4.1 ± 0.3	3.1 ± 0.9	3.0 ± 1.5	5.4 ± 0.2	3.9 ± 0.1
18:4(n-3)	2.3 ± 1.4	0.5 ± 0.1	0.7 ± 0.2	0.4 ± 0.3	0.7 ± 0.4	0.2 ± 0.2	0.4 ± 0.3	2.1 ± 0.9	1.0 ± 0.2
20:1(n-7)	0.9 ± 0.3	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.2 ± 0.3	0.6 ± 0.2	0.4 ± 0.1	0.8 ± 0.3	0.7 ± 0.1
20:4(n-6)	1.8 ± 0.6	3.0 ± 0.3	2.8 ± 0.1	3.3 ± 0.8	3.1 ± 0.2	4.2 ± 1.5	4.8 ± 1.5	2.6 ± 0.9	2.0 ± 0.2
20:5(n-3)	16.3 ± 3.6	23.1 ± 1.2	21.0 ± 1.0	23.1 ± 2.4	22.5 ± 0.8	22.8 ± 1.4	22.9 ± 1.4	15.3 ± 2.3	17.2 ± 0.8
22:x (PUFA)	2.1 ± 0.3	1.4 ± 0.2	1.0 ± 0.1	0.8 ± 0.5	0.9 ± 0.1	0.8 ± 0.2	1.0 ± 0.2	1.8 ± 0.1	1.9 ± 0.2
22:5(n-3)	1.0 ± 0.1	0.7 ± 0.1	0.5 ± 0.3	0.5 ± 0.4	0.3 ± 0.3	0.4 ± 0.3	0.3 ± 0.3	1.0 ± 0.0	1.0 ± 0.1
22:6(n-3)	12.6 ± 2.9	15.9 ± 1.2	15.3 ± 0.9	15.6 ± 1.3	15.0 ± 0.5	15.8 ± 1.0	17.5 ± 1.5	14.6 ± 2.0	16.5 ± 0.5
FA <1.0 %	6.8 ± 3.4	4.6 ± 1.4	4.5 ± 2.0	4.0 ± 1.5	4.2 ± 1.8	5.7 ± 2.5	5.3 ± 3.2	5.6 ± 1.7	4.6 ± 1.0
Σ SFA	24.5 ± 2.1	20.4 ± 0.4	21.3 ± 0.4	19.9 ± 1.1	21.6 ± 0.9	20.0 ± 1.1	17.6 ± 1.9*	23.3 ± 1.5	22.7 ± 0.6
Σ MUFA	24.6 ± 2.4	21.5 ± 1.7	23.6 ± 1.3	22.6 ± 1.8	22.2 ± 0.6	22.8 ± 0.7	22.9 ± 2.0	24.2 ± 2.0	24.0 ± 0.9
Σ PUFA	50.9 ± 4.4	58.0 ± 1.6	55.1 ± 1.6	57.5 ± 1.9	56.2 ± 0.5	57.1 ± 1.7	59.6 ± 1.5	52.5 ± 3.5	53.2 ± 1.3

Table 3 continued

	$n = 4$	$n = 4$	$n = 4$	$n = 4$	$n = 4$	$n = 3$	$n = 1$	$n = 3$	$n = 4$
Haemolymph									
14:0	4.6 ± 1.9	7.2 ± 0.6	6.8 ± 0.8	5.4 ± 2.8	6.0 ± 0.6	3.8 ± 0.6	4.9	7.0 ± 0.8	6.5 ± 0.2
16:0	18.7 ± 0.7	19.4 ± 0.6	19.6 ± 0.7	21.0 ± 0.6	20.5 ± 1.3	19.6 ± 1.2	20.2	19.0 ± 0.5	20.0 ± 0.7
16:1(n-7)	9.1 ± 0.7	9.9 ± 0.5	10.2 ± 1.1	7.9 ± 2.5	8.9 ± 0.7	7.2 ± 0.8	8.2	8.5 ± 0.9	7.9 ± 0.2
18:0	1.7 ± 0.5	0.9 ± 0.6	0.8 ± 0.6	1.8 ± 1.2	1.2 ± 0.2	1.5 ± 0.3	1.1	1.1 ± 0.2	1.2 ± 0.2
18:1(n-9)	14.6 ± 1.3	14.2 ± 0.6	15.0 ± 0.7	13.5 ± 0.7	14.3 ± 1.3	14.8 ± 0.6	14.7	14.7 ± 1.3	14.6 ± 0.4
18:1(n-7)	5.6 ± 0.3	5.3 ± 0.2	5.8 ± 0.5	5.9 ± 0.4	5.3 ± 0.5	5.4 ± 0.4	6.1	5.2 ± 0.2	5.3 ± 0.3
18:2(n-6)	7.6 ± 0.4	7.2 ± 0.1	7.4 ± 0.3	7.3 ± 0.2	7.6 ± 0.3	7.9 ± 0.2	7.4	7.0 ± 0.3	7.6 ± 0.1
18:3(n-3)	5.3 ± 0.1	4.6 ± 0.1	4.4 ± 0.1	4.4 ± 0.5	4.2 ± 0.4	4.5 ± 0.8	4.4	5.5 ± 0.2	4.9 ± 0.2
18:4(n-3)	2.1 ± 0.5	1.6 ± 0.1	1.4 ± 0.1	1.2 ± 0.4	1.2 ± 0.1	1.4 ± 0.7	0.9	2.2 ± 0.3	1.8 ± 0.3
20:1(n-7)	1.3 ± 0.2	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.0	0.9 ± 0.1	1.0 ± 0.1
20:4(n-6)	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.2 ± 0.4	1.1 ± 0.2	1.3 ± 0.2	1.1	1.1 ± 0.3	0.9 ± 0.1
20:5(n-3)	10.7 ± 1.0	10.5 ± 0.7	10.2 ± 0.8	12.4 ± 3.1	10.9 ± 0.7	11.3 ± 1.1	10.3	10.3 ± 0.4	10.1 ± 0.4
22:x (PUFA)	1.2 ± 0.2	1.2 ± 0.1	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.2	1.0 ± 0.2	0.8	1.1 ± 0.1	1.3 ± 0.1
22:5(n-3)	0.8 ± 0.0	0.9 ± 0.0	0.6 ± 0.4	0.6 ± 0.4	0.8 ± 0.1	0.9 ± 0.1	0.8	0.8 ± 0.0	0.9 ± 0.1
22:6(n-3)	7.2 ± 0.5	7.1 ± 0.5	7.0 ± 0.9	7.4 ± 0.6	6.8 ± 0.9	7.4 ± 1.5	6.2	8.2 ± 0.5	8.6 ± 0.4
FA <1.0 %	8.4 ± 2.1	8.1 ± 1.4	7.7 ± 1.3	8.0 ± 3.7	7.2 ± 1.2	8.5 ± 2.3	7.5	7.3 ± 1.5	7.3 ± 0.9
Σ SFA	26.8 ± 2.2	29.2 ± 0.4	28.8 ± 1.7	29.7 ± 1.2	29.2 ± 1.3	26.4 ± 2.0	27.5	28.8 ± 0.4	29.4 ± 0.8*
Σ MUFA	33.1 ± 1.6	33.0 ± 1.3	34.7 ± 1.5	30.7 ± 2.7	32.0 ± 2.0	31.1 ± 1.4	32.6	31.6 ± 0.7	31.3 ± 0.7
Σ PUFA	40.1 ± 1.4	7.8 ± 1.4	36.6 ± 2.5	39.6 ± 3.7	38.9 ± 3.3	42.5 ± 2.7	40.0	39.6 ± 0.5	39.3 ± 1.5

Values are given as mean ± S.D. ($n = 1$–4). *Σ SFA* total saturated fatty acids, *Σ MUFA* monounsaturated fatty acids, *Σ PUFA* polyunsaturated fatty acids, *FA* fatty acids, *22:x (PUFA)* PUFA with unidentified number of double bonds

* Indicates significant difference to t_0 using Kruskal–Wallis ANOVA followed by post hoc Dunn test

decline in the middle of the experimental period. Throughout starvation, activities of CS and MDH, two marker enzymes of the Krebs cycle, declined continuously and in good correlation with respiration (in contrast with fed krill), thus representing another indicator that reflects metabolic slowdown. The latter also led to a prolonged IMP in starved krill, whereas in fed krill, this trend was absent. The low mortality rates of starved krill, which were similar to those of fed krill, indicate that metabolic depression is a successful survival strategy.

Starved krill lost relatively little carbon (5.3 % of initial C) during the long starvation period, whereas carbon increased concomitantly by approximately 11 % in fed krill. However, if shrinkage at moult (see above) is accounted for, it becomes clear that starved krill (and even fed krill) lost a large amount of their initial total C: starved krill lost about 60 % C compared with only 17 % in fed krill. For the full 84-day starvation period, starved krill lost 0.25 mg C or 0.7 % per day, whereas fed krill lost only 0.07 mg C or 0.2 % per day. Similar C losses of 0.7 % during starvation of adults were found at the end of a short-term experiment (18 d, Auerswald et al. 2009), whereas lower losses of 0.18 % C were recorded during 18 days of starvation by Atkinson et al. (2002). No shrinkage occurred in the latter two studies. Carbon loss during starvation of adults is low compared with larval krill, which show a loss of ~2 % per day (Meyer and Oettl 2005).

Starvation experiments can reveal what substrates are used and in what sequence the different energy stores are depleted. Furthermore, the extent to which each compound class contributes to meet the energy demand during such a period can be estimated. In crustacean muscles, glycogen and lipids are generally mobilised first, when energy is required, but proteins can also be catabolised (Vinagre and da Silva 1992; Ceccaldi 2006).

Of all metabolites measured in the present study, glycogen reserves declined most rapidly during the first starvation week and were restored thereafter from other storage metabolites. Glycogen is readily available for muscle activity and other body functions and is therefore depleted, before substantial amounts of other energy metabolites are used up. The restoration of these depots allowed fast movement of krill and tail-flip escape reactions even after 12 weeks of starvation.

The decline in TL levels, the most important energy reserve, sets in more slowly and is more protracted than that of glycogen. Lipids are consumed throughout the experiment, but this is sometimes only evident when

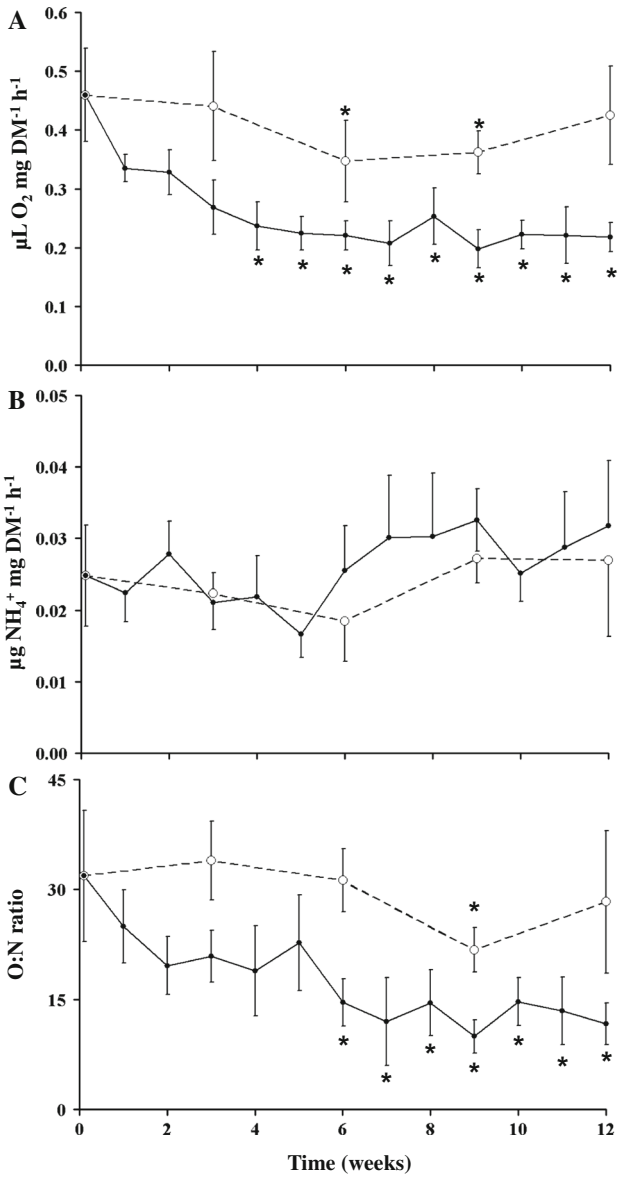


Fig. 5 Metabolic activity of adult krill during chronic starvation: **a** respiration measured as oxygen consumption, **b** excretion measured as ammonium production and **c** O:N ratio of fed (*empty circle*) and starved (*full circle*) krill. Values are mean ± S.D., $n = 5$–6. *Indicates significant difference to values at t_0 using Kruskal–Wallis ANOVA with post hoc Dunn test

contrasted with the continuously increasing lipid levels in fed krill and when shrinkage is considered. Such a slow decline in lipid reserves is similar to that measured previously in adult krill from late winter/spring (Auerswald et al. 2009). The declining trend is strongest in the haemolymph, possibly reflecting the slowing flow from depleted reserves in hepatopancreas and abdominal muscles. The TL content of starved whole krill fell from an initial level of 10 % DM by approximately 40 % to levels close to the proposed minimum level of 5 % DM (Hagen et al. 2001). Fed krill continued to accumulate reserves, leading to a 17 % higher lipid content at the end of experimentation compared with initial values. These results demonstrate that TL levels reflect feeding conditions of krill well, as already shown during numerous field studies (see Meyer 2012). It has to be considered, however, that the absolute amount can be disguised by previous shrinkage to retain a minimum, sub-lethal level.

In polar euphausiids, the composition of total lipids is dependent on its overall content (Stübing and Hagen 2003). In the present study, this is obvious from the drastic shift in the composition of lipid classes in the abdomen of starved krill concomitant with the decline in TL, which was dominated by the decline in relative TAG levels (about 80 %). In contrast, TAG levels increased substantially in fed krill following the upward trend in TL. This underlines the function of TAG as the major storage lipid in *E. superba* (Hagen et al. 1996, 2001). Accordingly, all other lipid classes increased relatively. Whereas TAG declined absolutely and relatively in starved krill, PLs increased relatively. However, due to the absolute decline of TL of about 43 %, even PLs declined drastically by approximately 47 %. These trends show that TAG and PL are the lipid classes that contribute most to meet the energy demand during starvation. In contrast with almost all other animals, PLs, namely phosphatidylcholine (PC) (Hagen et al. 1996), occur in the free form in polar euphausiids, i.e. are not bound to membranes (Stübing 2004) and are therefore available for consumption. The TAG : ST ratio declined in accordance with the above changes. TAG percentage as well as TAG : ST ratios therefore sufficiently describe the deteriorating physiological condition of krill. The TAG : ST ratio was also successfully used to evaluate the condition of *Homarus* larvae (Harding and Frazer 1999).

FA composition in different compartments of krill developed differentially: composition was stable in the abdomen of starved krill, whereas in fed krill, polyunsaturated fatty acids (PUFA) increased relatively to other FA groups. In the haemolymph, no changes where measured in starved krill or fed krill, despite a drop of TL content over time in starved krill. FA composition in haemolymph most likely only reflects specific needs in particular body tissues at a certain time. The only substantial changes took place in the hepatopancreas of starved krill. Most of this shift happened already early in the starvation period. The shares of monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs) decreased swiftly, whereas in fed krill, these FA groups remained unchanged. PUFAs, in turn, rose strongly in starved krill, resulting in an increase in SFA : PUFA from 2.1 to 3.4 after 12 weeks of starvation. The decline in SFA content in starved krill is almost exclusively due to the disappearance of 14:0 (myristic acid), whereas the PUFA increase is mainly the result of a relative increase in eicosapentaenoic acid (EPA; 20:5(n-3))

Table 4 Maximum activities of abdominal Krebs cycle enzymes (citrate synthase CS and malate dehydrogenase MDH) of starved and fed adult *E. superba* during the long-term experiment

Duration (weeks)	CS		MDH	
	Starved	Fed	Starved	Fed
1	139.8 ± 10.7 (7.5 ± 0.6)		373.2 ± 50.7 (19.9 ± 0.6)	
3	115.0 ± 19.5 (6.6 + 0.6)	128.3 ± 19.9 (8.6 ± 1.2)	364.1 ± 106.5 (18.6 ± 1.3)	292.1 ± 25.5 (19.7 ± 2.4)
5	124.6 ± 21.6 (6.0 ± 1.0)		351.8 ± 78.8 (17.7 ± 2.3)	
7	111.3 ± 28.9 (5.3 ± 0.6)		321.8 ± 47.4 (16.7 ± 2.0)	
9	87.7 ± 6.9* (5.0 ± 0.4)	115.1 ± 5.1 (7.8 ± 1.0)	330.6 ± 39.5 (17.0 ± 2.3)*	298.9 ± 23.5 (20.2 ± 1.1)
11	84.3 ± 5.1 (4.7 ± 0.8)*		274.6 ± 50.1 (16.1 ± 1.7)	

Values are given as mean ± S.D. ($n = 4$). Specific enzyme activity is expressed as mU mg^{-1} protein with U g^{-1} fresh mass in brackets. Enzyme activity of 1 mU is defined as 1 nmol substrate used per minute

* Indicates significant difference to values at t_1 using Kruskal–Wallis ANOVA followed by Dunn test

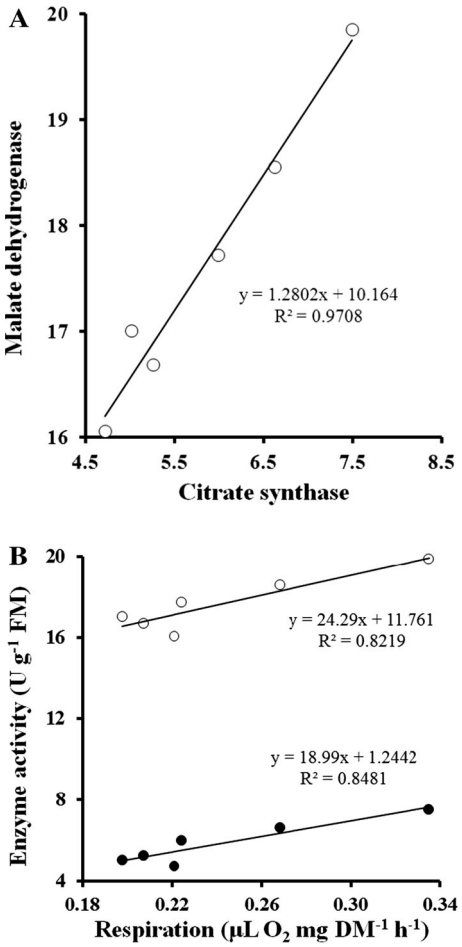


Fig. 6 Correlation of abdominal activities (U g^{-1} FM) of Krebs cycle enzymes MDH and CS (**a**) and correlation of respiration (measured as oxygen consumption) with corresponding abdominal MDH (*empty circles*) and CS (*full circles*) activities (**b**) in adult krill starved for up to 11 weeks. Each *data point* represents the mean of four individual enzyme activities (see Table 4) and 5–6 respiration measurements (Fig. 5a). All correlations are significant ($p < 0.05$)

and docosahexaenoic acid (DHA; 22:6(n-3), usually major components of biomembranes. The decrease of 14:0 is evident in the haemolymph in the first weeks of starvation, possibly for preferential consumption.

Previously, proteins were thought to be the principal energy source in crustaceans (New 1976), but recent research has revealed that crustacean species differ widely in this regard (Sánchez-Paz et al. 2006). In krill, body proteins play a minor role as energy reserve, but can be catabolised during critical situations such as the winter season (Torres et al. 1994). During starvation, the O:N ratio correlates with the availability of energy reserves and the use of body protein (Mayzaud and Conover 1988). The decreasing O:N ratio in this study, from 32 to 14, indicates a shift from intense lipid utilisation towards more pronounced protein catabolism. However, this is mainly caused by the drastic lowering of oxygen consumption and only to a lesser extent by increasing ammonium excretion during starvation. The shift towards protein catabolism after 5 weeks coincides with the depletion of lipid reserves, which reach a low level after this period, and also with very low TAG levels. The C:N ratio is close to 4 at the start and declines only slightly, due to a slight decrease in C and a minor increase in N. Similar results were reported by Stübing et al. (2003). Together with a dropping O:N ratio, this is an additional indication of a shift towards protein catabolism.

Overall, metabolic changes revealed that storage metabolites were depleted in the following succession: (1) lipids, mainly TAG, and glycogen (immediately after onset of starvation), (2) lipids dominate with increasing contribution of proteins (from week 1 to week 4–5, when parity is reached), and (3) proteins dominate with declining contribution of lipids (from week 5 onwards), until depot lipids are largely depleted and proteins are used almost exclusively (from week 6–7 onwards).

Table 5 Maximum activities of metabolic enzymes in whole starved and fed adult *E. superba* during a 12-week experiment

Duration (weeks)	Lipase		Total esterases		HOAD		GAPDH		GAPDH : HOAD	
	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved	Fed
0	5.9 ± 3.4 (0.29 ± 0.17)		173.7 ± 47.8 (8.5 ± 2.4)		0.41 ± 0.37 (0.021 ± 0.02)		41.2 ± 5.5 (2.0 ± 0.4)		168.1 ± 96.9	
1	5.6 ± 2.9 (0.26 ± 0.13)		79.1 ± 23.2* (3.6 ± 1.0)		0.48 ± 0.13 (0.022 ± 0.01)		42.8 ± 9.3 (2.0 ± 0.5)		91.2 ± 10.6	
2	5.5 ± 1.8 (0.28 ± 0.08)		103.1 ± 22.4* (4.0 ± 1.5)		0.20 ± 0.06 (0.007 ± 0.00)		36.0 ± 5.4 (1.4 ± 0.4)		203. 2 ± 92.8	
3	4.1 ± 1.7 (0.16 ± 0.09)	6.2 ± 3.1 (0.31 ± 0.15)	76.1 ± 22.4* (3.8 ± 1.1)	133.0 ± 21.6 (6.9 ± 0.8)	0.31 ± 0.16 (0.015 + 0.01)	0.66 ± 0.19 (0.034 ± 0.01)	33.1 ± 5.1 (1.6 ± 0.2)	41.3 ± 8.3 (2.2 + 0.5)	130.3 ± 68.7	69.2 ± 32.7
4	3.8 ± 1.4 (0.19 ± 0.10)		83.5 ± 8.3* (3.9 ± 0.4)		0.26 ± 0.23 (0.014 ± 0.01)		39.7 ± 8.1 (1.9 ± 0.5)		232.0 ± 140.3	
5	2.6 ± 0.2 (0.12 ± 0.01)		93.1 ± 27.6* (4.3 ± 1.4)		0.40 ± 0.07 (0.018 ± 0.00)		37.4 ± 6.3 (1.7 ± 0.3)		96.7 ± 32.4	
6	4.6 ± 2.6 (0.21 ± 0.08)	5.8 ± 1.4 (0.31 ± 0.09)	81.1 ± 14.4* (4.0 ± 0.7)	125.7 ± 15.5 (6.7 ± 0.8)	0.55 ± 0.50 (0.029 ± 0.03)	0.73 ± 0.18 (0.036 ± 0.01)	33.2 ± 5.3 (1.6 + 0.4)	42.1 ± 8.3 (2.2 ± 0.4)	94.3 ± 54.6	60.0 ± 16.9
7	4.0 ± 0.7 (0.15 + 0.04)		114.0 ± 12.5* (4.2 ± 1.1)		0.32 ± 0.21 (0.011 ± 0.01)		38.2 ± 12.1 (1.3 ± 0.3)		153. 5 ± 69.8	
8	5.3 ± 3.5 (0.12 ± 0.01)		107.8 ± 28.7* (3.2 ± 1.0)		0.42 ± 0.55 (0.019 ± 0.03)		31.7 ± 16.3 (1.1 + 0.7)*		163.8 ± 122.9	
9	3.8 ± 0.5 (0.14 ± 0.04)	4.3 ± 2.6 (0.21 ± 0.13)	96.8 ± 3.5* (3.5 ± 0.9)	156.6 ± 22.5 (7.8 ± 1.6)	0.32 ± 0.18 (0.013 ± 0.01)	0.55 ± 0.34 (0.027 ± 0.02)	44.0 ± 5.2 (1.6 ± 0.4)	42.5 ± 4.3 (2.1 ± 0.2)	190.3 ± 139.2	131.8 ± 126.6
10	4.8 ± 0.3 (0.21 ± 0.03)		95.8 ± 14.7* (4.1 ± 0.3)		0.33 ± 0.31 (0.014 ± 0.01)		43.8 ± 3.5 (1.9 ± 0.1)		220.0 ± 133.5	
11	4.2 ± 0.6 (0.15 ± 0.04)		96.7 ± 21.3* (3.4 ± 0.5)*		0.20 ± 0.13 (0.008 ± 0.01)		45.6 ± 16.1 (1.6 ± 0.6)		284.3 ± 164.7	
12	4.1 ± 0.8 (0.17 ± 0.05)	4.3 ± 1.2 (0.22 ± 0.07)	104.0 ± 20.0* (4.3 ± 1.0)	169.1 ± 35.1 (8.5 ± 1.6)	0.28 ± 0.13 (0.012 ± 0.01)	0.37 ± 0.17 (0.019 ± 0.01)	38.4 ± 7.3 (1.6 ± 0.1)	43.8 ± 5.3 (2.2 ± 0.1)	195.2 ± 183.4	138.5 ± 60.6

Values are given as mean ± S.D. (n = 4–8) of specific enzyme activity (mU mg^{-1} protein; U g^{-1} fresh mass in brackets). For definition of enzyme activity, see Table 3

* Indicates significant difference to values at t_0 using Kruskal–Wallis ANOVA followed by post hoc Dunn test

Table 6 Maximum activities of abdominal metabolic enzymes of adult krill after a 9-week starvation experiment

	Starved	Fed
Esterase	27.7 ± 6.5 (1.61 ± 0.15)	27.3 ± 25.6 (1.79 ± 1.56)
Lipase	20.0 ± 18.5 (1.23 ± 1.21)	18.7 ± 8.6 (1.26 ± 0.58)
HOAD	1.49 ± 0.18 (0.09 ± 0.01)	2.03 ± 0.17* (0.14 ± 0.01)*
GAPDH	39.2 ± 5.6 (2.29 ± 0.14)	46.3 ± 6.3 (3.13 ± 0.35)*
GAPDH : HOAD	26.5 ± 3.8	23.0 ± 4.1

Values are given as mean ± S.D. ($n = 4$–8) of specific enzyme activity (mU mg^{-1} protein; U g^{-1} fresh mass in brackets). For definition of enzyme activity, see Table 3

* Indicates significant difference between starved and fed krill using Student's t test ($p < 0.05$)

Activities of catabolic enzymes

"Constant-proportion enzymes" (Beenakkers et al. 1984) have been proven to detect metabolic shifts in insects during starvation (Auerswald and Gäde 2000). In krill, they reflected increases in glycolytic and lipolytic fluxes relative to the proteolytic flux during short starvation (Auerswald et al. 2009). In the present research, activities of enzymes from lipolytic and glycolytic pathways, including those of two "constant-proportion enzymes", were quantified in whole krill specimens. Moreover, activities of some enzymes from the abdominal muscle were compared with those in the hepatopancreas.

In the present experiments, the trend of enzyme activities in lipid metabolism (total esterases, lipases and HOAD) is generally downward during prolonged starvation compared with initial activities. In contrast, activities in fed krill showed generally higher levels than in starved krill.

The total activity of esterases (mostly non-specific hydrolases, but also including lipases) displayed the most substantial change in activity of all enzymes measured during starvation. A sharp decline in esterase activity took place already in the first week of starvation and remained at this level (40 % of initial) for the remaining 11 weeks. Such a strong decline in activity was absent in fed krill and can probably be explained by a rapid reduction in the activity of digestive esterases, assuming that esterase activity is concentrated in the hepatopancreas. This is underlined by the results from the abdominal muscle, where digestive enzymes are absent and in which esterase activity was almost identical with that of fed krill after 9 weeks of starvation.

The activity of lipases, a specific lipid-hydrolysing subclass of esterases, represented only a small fraction of total esterase activity in whole krill (3–4 %), in contrast with the abdominal muscle (~70 %). After the onset of starvation, lipase activity in whole krill remained stable for 2 weeks, before declining to a level of approximately 45–75 % of initial levels, at which it remained stable until the end of the starvation period. This trend is consistent with the declining importance of lipids for energy provision and the increase in the importance of proteins (see above). In fed krill, lipase activity remained stable for longer and only declined towards the end of the experiment. In abdominal muscle, lipase activity was about fourfold that in whole animals and also represented a much higher portion of total esterase activity compared with whole animals. This clearly indicates its involvement in catabolism of storage lipids, whereas the total esterase activities in whole animals possibly reflect esterase activity during digestion.

The ratio resulting from activities of the constant-proportion enzymes GAPDH and HOAD fluctuated in starved whole specimens, whereas it dropped substantially in fed krill and remained consistently lower than in starved krill at comparable time points. This reflects the ability of fed krill to accumulate lipid reserves, whereas starved krill consistently made use of stored lipids in the absence of food. The decline in enzyme activities of the lipid metabolism seems therefore linked to the general metabolic slowdown and the shift towards protein metabolism observed during the same time. These trends suggest an association with deteriorating condition, but more systematic research is required for the proper use of enzyme activities, especially those from constant-proportion groups, in the evaluation of krill condition.

Light regime versus food availability

Winter season in the Antarctic region is characterised by reduced day length and low light intensity and, as a result, scarcity of algal food (Atkinson et al. 2002; Vernet et al. 2012). There is very little information, however, that provides an explanation as to what extent either environmental conditions contribute to the behavioural and physiological changes that take place in overwintering krill.

Light regime seems to set in motion a range of behavioural and physiological changes in krill: light simulations of prolonged photoperiods force animals into a state of

increased maturity, feeding and metabolic activity, whereas shortened photoperiods initiate regression of these parameters (Teschke et al. 2007, 2008; Seear et al. 2009; Brown et al. 2011). These changes seem to be independent of food availability and are thought to represent inherent adaptations to seasonal cycles that are governed by an endogenous timekeeping system (Teschke et al. 2011; Meyer 2012). The seasonal cycle of the photoperiod may therefore act as an important *Zeitgeber* cue to link these rhythms to the natural year.

The study of Teschke et al. (2007) implies that darkness first induces a reduction in feeding activity, in contrast with constant light (polar day, summer) or light–dark (changing seasons). As a result, metabolic rate is swiftly reduced. In the long term, hepatopancreas size is reduced and metabolism shifts towards protein catabolism.

In the present study, many of these changes also happened during starvation at constant light–dark conditions of LD 12:12, such as the dramatic reduction in metabolic activity, decrease in hepatopancreas size and a shift towards protein consumption at a later stage. The reduction in respiration rate set in very abruptly as soon as food supply stopped and glycogen reserves in the muscles were rapidly depleted to ensure mobility (present study; Auerswald et al. 2009). Importantly, starved krill reduced their body mass by approximately 50 % (present study), whereas that of fed krill kept in complete darkness (polar night) maintained their initial body mass (Teschke et al. 2007). Also, at polar night, TL levels in fed krill remained remarkably constant, in contrast with starved krill, in which not only the TL content dropped but also the absolute amount of lipids was reduced massively due to shrinkage. The time course of relative carbon content seems similar in both studies, but there was a dramatic absolute C loss of 60 % due to shrinkage in starving krill that did not occur in krill in winter mode.

When comparing changes in both experiments, it is important to look at the respective control treatments as well. In the present study, some changes observed in starved krill were also present in fed krill but to a much lesser extent. This applies, for example, to body size (shrinkage also occurred in fed krill) and respiration in terms of oxygen consumption and Krebs cycle enzymes.

There are some possible explanations for these observations: the most likely are insufficient food quality and inability of krill to make use of the offered food (individual vs. group rearing, see above). It is also possible that krill's natural winter mode (see above) cannot completely be overruled by the artificial light regime. The present study started in March, i.e. in the transition period from summer to winter.

Conclusions

Comprehensive morphometric and metabolic analyses revealed specific adaptive responses of adult Antarctic krill to long-term starvation during a constant spring/autumn light regime, which lead to a number of drastic physical and physiological changes. In particular, metabolic rates and lipid reserves declined concomitant with enzyme activities of the lipolytic pathway. Shrinkage occurred to adjust body size to the reduced biomass and energy reserves. Our study emphasises the importance of the control (fed) group to understand the cause for the observed changes. Potential explanations may be that (a) food quality and quantity offered (ability to feed sufficiently) were sub-optimal (as also suggested in Ikeda and Dixon 1982), (b) that feeding in the control group was limited by keeping the krill specimens individually and/or c) that the proposed inherent timing mechanism cannot be completely overruled by a consistent food supply.

To conclusively distinguish between the effect of light regime and that of food supply, more complex, two-factor experiments have to be conducted, in which different light regimes and different levels of food supply are combined at the same time and in which krill can feed freely. Moreover, such experiments should be performed at different times of the year to take into account the potential seasonal effects of the hypothesised inherent timing mechanism.

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