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FEATURE ARTICLE

Seasonal variation in body composition, metabolic activity, feeding, and growth of adult krill *Euphausia superba* in the Lazarev Sea

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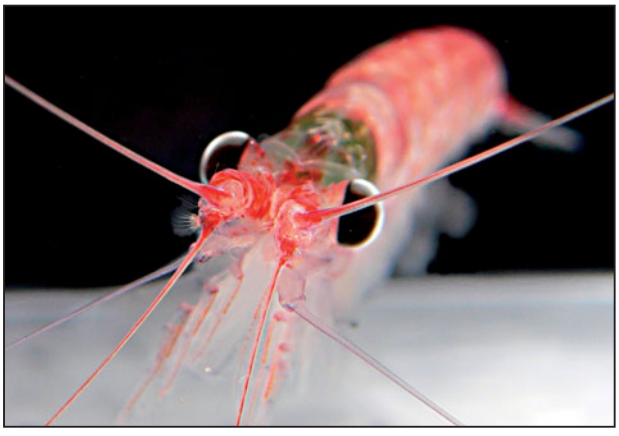
ABSTRACT: We investigated physiological parameters (elemental and biochemical composition, metabolic rates, feeding activity and growth) of adult Antarctic krill in the Lazarev Sea in late spring (December), mid autumn (April) and mid winter (July and August) to evaluate proposed hypotheses of overwintering mechanisms. Our major observations are: (1) respiration rates were reduced by 30 to 50% in autumn and winter, compared to values in late spring; (2) feeding activity was reduced by 80 to 86% in autumn and winter, compared to late spring, at similar food concentrations; (3) feeding was omnivorous during winter; (4) with each moult, krill grew by 0.5 to 3.8% in length; (5) body lipids and, to a small extent, body proteins were consumed during winter. Adult *Euphausia superba* thus adopt metabolic slowdown and omnivorous feeding activity at low rates to survive the winter season in the Lazarev Sea. By mid autumn, metabolic activity is reduced, most likely being influenced by the Antarctic light regime, which is accompanied by a reduction in feeding activity and growth. Although at a low level, the feeding activity during winter seems to provide an important energy input.

KEY WORDS: Antarctic krill · Seasonal condition · Overwintering



Antarctic krill *Euphausia superba*

Photo: Carsten Pape

INTRODUCTION

Antarctic krill (hereafter krill) is a key organism in the Southern Ocean and has been studied for over 80 yr. Despite this long history of research, owing to krill's vast distribution range and the inaccessibility of much of this, knowledge is still patchy and important scientific questions remain unanswered. In addition to

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the extent of sea ice coverage, overwintering success is regarded as a major factor that dictates physiological condition, survival, recruitment, and population size of krill in the Southern Ocean (Loeb et al. 1997, Atkinson et al. 2004). The biological mechanisms that facilitate the overwintering of krill are, however, still unclear.

Proposed overwintering mechanisms for adult krill fall into 2 categories: (1) non-feeding mechanisms, and (2) switching from algae in the water column to alternative food sources. Non-feeding strategies consist of the reduction in metabolic rates (Kawaguchi et al. 1986, Quetin & Ross 1991, Torres et al. 1994a), shrinkage in size (Ikeda & Dixon 1982, Quetin & Ross 1991) and the utilisation of body lipids (Quetin & Ross 1991, Hagen et al. 2001). Winter studies on krill have shown that alternative food sources might be zooplankton (Huntley et al. 1994) and seabed detritus (Kawaguchi et al. 1986). The reduction of metabolic and feeding rates to 60 and 20%, respectively, of summer values has already been demonstrated in the Lazarev Sea in late autumn (Atkinson et al. 2002). The reasons for this metabolic slowdown are not completely understood, but recent research has indicated that Antarctic light regime, rather than food supply, might influence these seasonal physiological changes (Teschke et al. 2007).

All these adaptations to the winter season have been observed at different times and places, making it difficult to judge their relative significance. Even within one region, the Antarctic Peninsula, conflicting observations on krill overwintering have been made. For example, Quetin & Ross (1991) reported that krill undergo lipid utilisation, shrinkage and a reduction in metabolic rates, whereas Huntley et al. (1994) found them feeding and excreting at summer rates. Such contradictory findings may point to methodological inconsistencies but could, on the other hand, be indications for an exceptional behavioural and physiological flexibility. The existence of 2 different overwintering mechanisms seems very unlikely, however, as both strategies (i.e. reduced metabolism and business as usual with high feeding activity and growth, as in summer) require specific adaptations on an organismic, cellular and molecular level that preclude each other. Much of our knowledge comes from a few regions, such as the intensively studied Antarctic Peninsula (Hofmann et al. 2004), and neither the local environment (e.g. along the Antarctic Peninsula) nor the response of krill to it should be extrapolated easily to a wider area. Thus, one prerequisite for a generalisation of krill overwintering is a better geographic coverage.

This was attempted as part of the Lazarev Sea Krill Study (LAKRIS), the German contribution to the Southern Ocean Global Ocean Ecosystem Dynamics (SO-GLOBEC) program. The Lazarev Sea (Fig. 1) is the eastern part of a broad region of high krill abundance, which extends from the Antarctic Peninsula to the

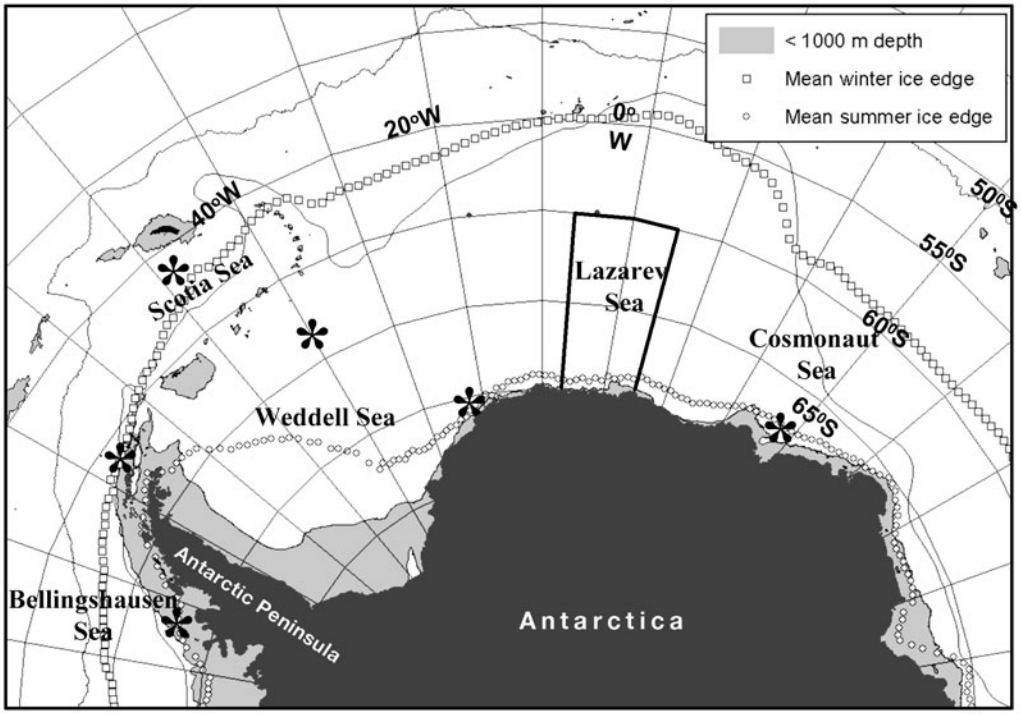

Fig. 1. Lazarev Sea study area in relation to other winter studies in the Southern Ocean (✻) with mean summer and winter ice edges (based on NOAA ice data from 1979 to 2006). Irregular grey lines (from north to south): Antarctic Polar Front and Southern Boundary of the Antarctic Circumpolar Current

Greenwich Meridian (e.g. Marr 1962, Atkinson et al. 2008). This area shows the widest latitudinal range of krill throughout their entire circumpolar distribution, stretching from 50° S to the Antarctic continent at 70° S. The continental shelf is narrow and the majority of krill live in the oceanic region, where water depths exceed 4000 m. Only a few historical data on krill from this area exist, focusing mainly on distribution and abundance (e.g. Makarov & Sysoyeva 1985), and only a few studies provide data on their physiology (Atkinson et al. 2002, Schmidt et al. 2003, Stübing & Hagen 2003).

The present study covered different seasons—i.e. late spring and the beginning of summer (December), mid autumn (April) and mid winter (July and August)—in the Lazarev Sea, and a wide range of analytical methods were applied. In addition to biotic and abiotic environmental conditions, we analysed (1) morphometrics, elemental composition, nutritional status and growth rates, (2) feeding activity and digestive gland (DG) indices, and (3) respiration and excretion rates and activity of metabolic enzymes. Concurrent with this suite of measurements on adults, we conducted a parallel study to determine overwintering mechanisms of larvae, reported in a separate paper (Meyer et al. 2009). The comprehensive data set we obtained from this investigation enabled us to draw a clearer picture of the behavioural and physiological responses that enable adult krill to survive the Antarctic winter.

MATERIAL AND METHODS

Surveys and environmental conditions. Three expeditions in austral late spring and beginning of summer (ANTXXIII-2, 19 November 2005 to 12 January 2006), mid autumn (ANTXXI-4, 27 March to 6 May 2004), and winter (ANTXXIII-6, 11 June to 27 August 2006) were carried out on board RV 'Polarstern' along 4 (late spring and beginning of summer, and mid autumn) and 3 (winter) parallel meridional transects which extended from 60° S to the Antarctic continent at 70° S. The station grids for all 3 cruises are given in Fig. 2a–c. Highlighted stations mark the positions at which krill were caught for most of the presented analyses. During the late spring and beginning of summer survey, sampling of krill took place in the study area in December, hereafter referred to as late spring, whereas sampling took place in April during the autumn expedition and in July until mid-August during the winter expedition (Fig. 2a–c).

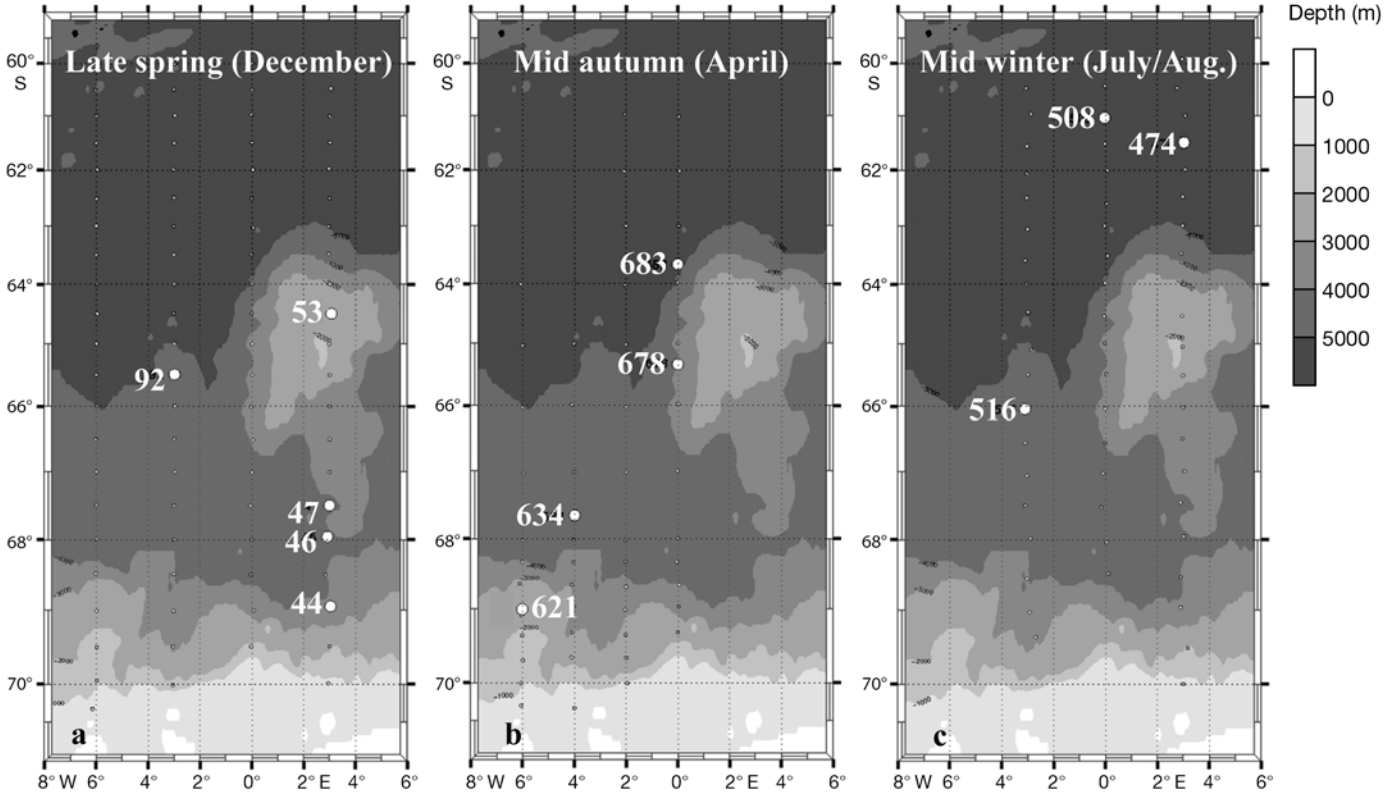

Fig. 2. Station grid (small dots) and stations in the Lazarev Sea at which krill were caught (large white dots) in late spring (December), mid autumn (April) and winter (July to mid August)

In the different seasons, the chlorophyll *a* (chl *a*) concentration and water temperature in the upper 30 m of the water column were as follows: During the late spring expedition, ice melting was in progress and phytoplankton blooms started to develop in December (Fig. 3). Chl *a* concentration was highly variable, ranging from 0.2 to 4.8 mg m^{-3} in 5 to 30 m depth with 1.0 mg m^{-3} on average. In autumn, sea ice formation had already started and the ice edge was located at 68° S. The chl *a* concentration ranged from 0.06 to 0.5 mg m^{-3}. During the winter cruise, the whole study area from 60 to 70° S, was covered by sea ice and chl *a* concentrations in the water column were very low, ranging from 0.01 to 0.04 mg m^{-3}. The mean seawater temperature was –1.5 ± 0.3°C in late spring, –1.4 ± 0.4°C during the autumn survey, and –1.8 ± 0.1°C during winter.

Krill sampling and processing. Krill were collected using a RMT8+1 (Rectangular Midwater Trawl) in the top 50 m of the water column. The net was equipped with a 20 l (RMT8) and 10 l (RMT1) closed cod end to collect krill in suitable condition for ecophysiological experiments and analyses.

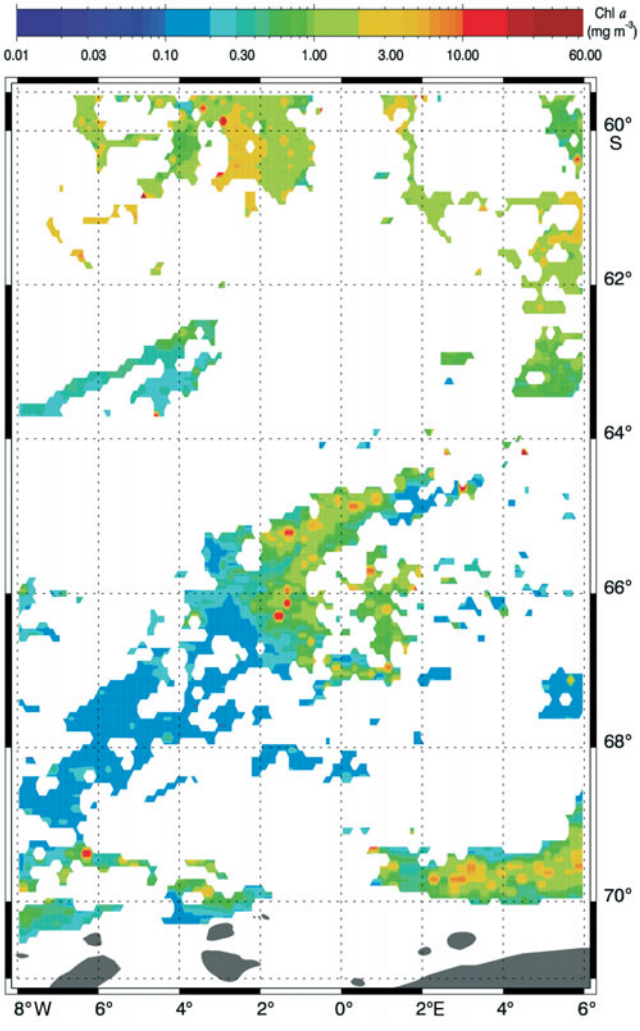

Fig. 3. Monthly mean SeaWiFS (Sea-viewing Wide Field-of-view Sensor) chlorophyll *a* values (mg m^{-3}) in December 2005. White: no data

Immediately after the haul, one fraction of subadult and adult krill (staged after Siegel 1987) was used to measure metabolic rates (oxygen uptake, ammonium production), growth rates (only late spring and winter), and feeding activity. From the remaining animals, body length and size and colour of the DG (only late spring and winter) were recorded before snap-freezing in liquid nitrogen and storage at –80°C for analysis of dry mass, elemental (carbon, nitrogen) and biochemical composition (total body lipid and protein), as well as activity of metabolic enzymes (CS: citrate synthase, MDH: malate dehydrogenase and HOAD: 3-hydroxyacyl-CoA dehydrogenase).

The total length of krill (mm) was measured from the front of the eye to the tip of the telson. The carapace length was measured from the tip of the rostrum to the posterior notch of the carapace, whereas the DG was measured through the carapace along its longest axis (Nicol et al. 2004).

Analysis of dry mass and body composition (carbon, nitrogen, protein and lipid content). After lyophilisation for 24 h, individual krill were weighed and ground to a powder in liquid nitrogen. For analyses of carbon (C) and nitrogen (N), aliquots of 0.2 to 0.5 mg from each krill homogenate was analysed as described elsewhere (Meyer et al. 2002a, 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 (2mg) was incubated in 1 ml 1 N NaOH for 2 h at 60°C, then diluted to 0.5 N NaOH and centrifuged at 2000 *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 is appropriate for determination of the total body protein content in krill. The method of Bradford (1976) underestimates the protein level in krill up to 3-fold. Lipids were extracted from 10 to 15 mg of krill homogenate in dichloromethane/methanol (2:1 v/v) and total lipid content was determined gravimetrically (Hagen 2000).

Measurements of metabolic rates. The rates of oxygen consumption and ammonium excretion 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, 4 bottles containing krill and 3 control bottles without krill were used. All experimental bottles were incubated at 0°C for 12 to 24 h in a water bath located in a constant temperature room. After incubation, subsampling was carried out by rapidly in-

serting 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 Falken tubes for analysis of ammonium content. For oxygen and ammonium determination, 3 replicate subsamples 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). In all experiments, individual krill were freely swimming in the incubation bottles and the decrease in oxygen concentration was <10%, which is not believed to effect 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 the activities of metabolic enzymes. CS and MDH were measured to estimate metabolic activity, and HOAD was measured to estimate the metabolic flux of catabolism of storage lipids. Both CS and MDH catalyze reactions in the citric acid cycle, serving as proxies for aerobic potential. MDH, however, also plays other roles, such as shunting electrons between the cytosol and mitochrondrion, and might therefore better represent overall metabolism. HOAD is a key enzyme of ß-oxidation of fatty acids and therefore a good proxy of the utilisation of body lipids (Auerswald & Gäde 1999). A detailed description of the rationale of this selection, as well as the function and position of these enzymes in the respective metabolic pathways, is given in Auerswald et al. (2009).

Tissue preparation. The fifth abdominal segment (AS) of frozen animals was dissected carefully for determination of the activities of the metabolic enzymes CS and MDH, whereas the hepatopancreas region (HR) was used for the determination of HOAD. In adult krill, this region is important for lipid storage, where lipid turnover is high. All dissections took place on a cooling element to avoid thawing. The HR and AS were homogenised in a pre-weighed and pre-cooled glass potter (Tissue grind pestle, Kontes Glass Company, US) in ice-cold deionised water at a concentration of 100 mg fresh weight (f_w) ml^{-1}, which corresponds to a dilution of 1:10. Aliquots of 50 to 150 µl of the homogenised tissues were transferred into new reaction tubes, dipped in liquid nitrogen and stored at –80°C until analysis of enzyme activities within 1 wk.

Enzyme assays. Before measuring the activity of the metabolic enzymes, the frozen samples were defrosted gently on ice and centrifuged at 5000 *g* and 4°C for 10 min. The supernatants were used to determine the enzyme activity and the corresponding protein content for calculation of specific enzyme activity (per g protein, g_{prot}). The protein content was measured with the BIO-RAD Protein assay, based on the method of Bradford (1976), for comparison with the majority of enzyme studies and due to the sensitivity of this method for soluble proteins at low concentrations.

MDH (EC 1.1.1.37): In a semi-microcuvette, 810 µl reaction buffer (0.1 M potassium phosphate, pH 7.0), 30 µl nicotinamide adenine dinucleotide, reduced form (NADH, 7 mM in Milli-Q Sigma N8129), and 30 µl sample extract (diluted 1:50 with the reaction buffer, see above) were mixed. After 5 min of pre-incubation at 25°C, the reaction was initiated by adding 30 µl oxaloacetate (12 mM in Milli-Q). The change in absorbance at 340 nm was recorded for 5 min. The conversion of 1 µM substrate per g_{prot} and min to U g_{prot}^{-1} min^{-1} was carried out using the extinction coefficient of $\varepsilon_{340} = 6.22\ mM^{-1}\ cm^{-1}$.

CS (EC 4.1.3.7.): CS activity was determined according to Stitt (1984) using the modifications described by Meyer et al. (2002b). To a semi-microcuvette filled with 780 µl 0.5 M Tris/HCl-buffer (pH 8, supplemented with 0.1 M KCl and 1 mM EDTA), we added 30 µl DTNB (5,5′-dithiobis(2-nitrobenzoic acid), 6 mmol l^{-1} in buffer, Sigma D8130), 20 µl acetyl-CoA (acetyl-coenzyme A tri-lithium salt, 6 mM, Roche Diagnostics 101907) and 30 µl sample. After 5 min of incubation at 30°C, the reaction was initiated by adding 20 µl oxaloacetic acid (12 mM, Sigma O 4126) and monitored continuously at 412 nm for 3 to 5 min. The molecular turnover was expressed as U g_{prot}^{-1} min^{-1} using the extinction coefficient of $\varepsilon_{412} = 13.6\ mM^{-1}\ cm^{-1}$.

HOAD (EC 1.1.1.35): The activity of HOAD was determined according to Auerswald and Gäde (1999). In brief: to a semi-microcuvette, 810 µl TRA (triethanolamine)/HCl buffer (107 mM, pH 7, supplemented with 5.3 mM EDTA) and 30 µl sample were added and mixed. The reaction was initiated with 30 µl acetoacetyl-CoA (Sigma A-1625) and monitored photometrically at 340 nm and 25°C for 5 min. The activity of HOAD was calculated as described for MDH (see above).

Activation energy of the metabolic enzymes. The activation energy (E_a) of MDH, CS and HOAD was calculated in order to transform their activity assayed at the different temperatures in the laboratory to the *in situ* enzyme activity at field temperature. E_a can be determined from temperature (T in K) and the corresponding enzyme activity (rate constant = k) by graphing ln k versus $1/T$. We measured the activity of MDH, CS and HOAD in a sample at different temperatures (30, 20, 10, 2°C) and determined E_a from the slope of the regression line between ln k and $1/T \times 10^3$ (K) for each enzyme (Table 1). The relationship between slope and E_a is: slope = $-E_a/R$, where R is the gas constant (8.31 J mol^{-1} K^{-1}). The enzyme activities were corrected for the *in situ* temperature by applying the E_a

Table 1. Regression of the relationship between $1/T \times 10^3$ (K) vs. ln(enzyme activity) and calculated activation energy (E_a) of malatdehydrogenase (MDH), citrate synthase (CS), and 3-hydroxyacyl-CoA dehydrogenase (HOAD)

Enzymes	Regression	r^2	E_a
MDH	$y = -7262.6x + 28.3$	0.997	60.38
CS	$y = -1570.2x + 6.3$	0.996	13.05
HOAD	$y = -9395.3x + 32.7$	0.996	78.12

determined for each enzyme (Table 1) to the Arrhenius equation:

$$ME_{situ} = ME_{incubation} \times \exp[E_a \times (T_{incubation} - T_{situ}) \times R^{-1}] \quad (1)$$

where $ME_{incubation}$ is enzyme activity at $T_{incubation}$ (assay temperature in K) and ME_{situ} is enzyme activity at T_{situ} (field temperature in K).

Feeding experiments. The experiments were performed in the same way as described in detail previously (Atkinson et al. 2002). In brief, to determine the feeding activity in the different seasons, 4 krill were incubated in 60 l containers filled with surface seawater enriched with various concentrations of algae obtained from melted brown discoloured chunks of multiyear ice (Table 2). In each season, 4 containers with krill and 3 control containers without krill were used. The animals were acclimated for 24 h in the same food concentrations prior to the start of the feeding experiment and the different food concentrations for each experiment were determined in terms of chl *a*.

At the start of each experiment and after 24 h incubation, 3 samples of 250 ml were taken for chl *a* analysis from each container, as described elsewhere (Atkinson et al. 2002), and analysed according to Meyer et al. (2002a). In the feeding experiments, no significant changes in chl *a* concentration were detected in controls, so we calculated the clearance rates of the total phytoplankton biomass in each tank as follows (Båmstedt et al. 2000):

$$F = \ln(C_c/C_k)V/m_k t \quad (2)$$

where F is the clearance rate (ml mg^{-1} body C h^{-1}), C_c is the initial concentration in the tanks, C_k is the final concentration in the tanks, V is the volume of the container (ml), m_k is the body mass (mg C) of the krill, and t is the experimental duration (h).

Ingestion rates were calculated as the product of the clearance rate of the phytoplankton biomass (ml mg^{-1} body C h^{-1}) and its initial C concentration (mg ml^{-1}) and then expressed as a daily C ration (% body C d^{-1}) under the assumption that the krill feeding rates reflect the daily average rate. Due to the low chl *a* concentration of the surface seawater used to prepare the algal mixture for the feeding experiments (late spring: ≤0.1 mg m^{-3}, autumn: ≤0.08 mg m^{-3}, winter: ≤0.02 mg m^{-3}), the algae in the incubation container was derived mainly from the melted sea ice. The multiyear ice used for our feeding experiments had a very similar species composition in the different seasons. The algal mixture was dominated by diatoms of the taxon *Fragilariopsis* spp., followed by large pennate diatoms, small centric diatoms, and *Chaetoceros* spp. The melted sea ice had chl *a* concentrations ranging from 80 to 450 mg m^{-3} and the C:chl *a* ratios of these algae are reported to be 31 (Palmisano & Sulivan 1983), 53 (Kang et al. 2001), 48 to 61 (Meyer et al. 2002a), 20 to 40 (Lizotte 2003), or 55 (Garrison et al. 2005). Therefore, in the different seasons, the measured chl *a* concentrations were converted into C equivalents using a C:chl *a* ratio of 50.

Table 2. Concentration of algal mixture and mean individual dry mass (DM) of krill used in the feeding experiments

	Algal mixture (µg chl *a* l^{-1})	Individual DM (mg)
Late spring	0.11	148.7
	0.63	153.1
	1.59	145.1
	3.60	110.9
	6.69	142.1
	7.99	121.2
	9.80	128.5
Autumn	0.08	155.9
	0.56	265.4
	4.20	249.5
	5.10	299.3
	6.22	282.0
	6.38	179.6
	8.40	255.2
	11.96	195.6
	12.50	168.5
Winter	0.55	200.7
	0.68	188.5
	1.05	193.9
	1.30	202.0
	2.85	266.7
	9.00	192.4
	13.12	132.7
	14.59	195.4
	16.65	173.1
	19.48	109.4

Determination of growth rate and inter-moult period. Growth rates were measured following the Instantaneous Growth Rate (IGR) method outlined by Quetin & Ross (1991) and modified by Nicol et al. (1992). Freshly caught krill (100 to 129 ind.) were randomly selected and individually placed in 2 l plastic jars filled with freshly collected surface seawater. The number of incubated individuals is comparable with previous growth rate studies using the IGR method (e.g. Nicol et al. 1992, Arnold et al. 2004). They were incubated in the jars for 4 to 5 d in the dark at 0°C in a constant tem-

perature room because the measured growth in the first 5 d post capture still reflects the field situation (Buchholz 1991, Nicol et al. 1992). The jars were checked twice daily for moults and dead individuals. If the krill had moulted, the individual and the exuvia were removed and the length of the right uropod was measured (when damaged the left uropod was used). The daily moulting frequency f was calculated as:

$$f = N_m/(N_i d) \quad (3)$$

where d is the duration of the experiment in d, N_m is the number of krill that moulted in this time, and N_i is the total number of krill incubated at the start, minus the animals that died during the experiment. The intermoult period (IMP) is the inverse of the moulting frequency. The Growth Increment (GI) on moulting (% growth IMP^{-1}) was defined as the percentage difference between the length of the right uropod (when damaged the left uropod was used) on the newly moulted animal (U_a) and the moult (U_m):

$$GI = [(U_a - U_m)/U_m]\ 100 \quad (4)$$

The change in body length (BL) over the IMP was determined from a linear regression of BL of uropod length U (mm) of the post-moult krill. Length measurements from all experiments in both years were pooled due to insignificant differences between regressions of seasons:

$$BL = 6.89U - 0.50,\ r^2 = 0.96,\ n = 71,\ p < 0.001 \quad (5)$$

The growth in mm d^{-1} was then calculated as the difference between the premoult and postmoult BL divided by the IMP in d (Daly 2004).

Statistical analysis. Prior to statistical analyses, data were tested for normality. Non-normal data were square-root transformed to achieve a normal distribution (Zar 1999). For testing of significant differences between data groups, a 1-way ANOVA (Model I) was calculated and the Holm-Sidak post-hoc test applied for multiple comparisons. These tests were performed by SigmaStat 3.0 (SPSS). A Type I linear regression was used for all correlations presented, and the difference between regression lines were tested according to Zar (1999) using GraphPad Prism 4. The significance level for all tests was set at $p < 0.05$.

RESULTS

Krill distribution and population structure in the various seasons

During the late spring and beginning of summer survey, krill were caught in open waters in the polynya as well as in ice-covered areas. The main krill concentrations occurred in the northern part between 61° and 64°S as well as south of 67°S, extending as a tongue across the southern slopes of Maude Rise. Spatial distribution of krill size groups was not uniform across the survey area. The length composition was dominated by a large fraction of 1 yr old juvenile krill of 17 to 21 mm modal size. More than 50% of the population consisted of this size and age group, which was located at stations in the central and northern areas. South of 67° S, we predominately found medium- to large-sized immature and adult krill, ranging mainly between 27 and 37 mm. The length frequency distribution showed a gap in size classes around 30 mm, the expected modal size of the 2 yr old krill at this time of the year. Larger krill between 30 and 45 mm were mostly adults and were only observed at some scattered stations in the southern and northern parts of the area. However, almost none of the specimens in the entire population were >50 mm, although krill of this size regularly occur in the Scotia Sea region. Despite the great dominance of pre-spawning maturity stages, we also found animals in advanced spawning conditions, indicating the onset of the spawning season.

In autumn 2004, major krill concentrations occurred in the northern part between 61° and 64° S. Another patch of relatively high krill concentration was found across the survey area just outside the marginal ice zone. The autumn population was clearly dominated by the modal size class of 36 mm. This age class (Age class 2) usually consists of immature specimens that have not yet participated in the spawning process. The juvenile Age class 1, with a mean length of approximately 26 to 28 mm, was almost completely missing during our study. Medium-sized krill, from ca. 28 to 43 mm, were mainly located further offshore. Most of the krill between 61° and 68°S were juveniles or immature stages with a modal length of 34 mm. South of the ice edge (68° S), the size composition of krill changed markedly. Here, the length composition had a peak around 34 mm but mainly centred around 47 mm. During the survey, most females were already in post-spawning condition, so it can be concluded that spawning ended before late March.

During winter 2006, the net sampling programme took place during a period of complete sea-ice coverage of the survey area. The main krill concentrations occurred in the north-western part between 60° and 66° S. The poorest catches were obtained south of 67° S. The krill population in winter 2006 was dominated by a large fraction of 1 and 2 yr old krill (modal size classes around 25 and 35 mm). The smallest size class (26 to 27 mm), which represented mainly Age class 1, was found in the more southern and western areas. In the central part of our study region, we found a mixture of 1 and 2 yr old krill, while in the east, krill had a

Table 3. *Euphausia superba*. Body length (BL), dry mass (DM), carbon (C), nitrogen (N) and biochemical body composition of freshly caught krill in different seasons; data are mean ± SD (range); n: number of samples. Superscripted letters indicate a significant difference from A: late spring, B: autumn, C: winter

Measurement	Late spring	Autumn	Winter
BL (mm)	33 ± 3 (30–39)	45 ± 5 (35–52)$^{A, C}$	35 ± 4 (30–45)
DM (mg)	51.3 ± 12.9 (35.2–72.3)	174.7 ± 24.7 (77.5–314.2)$^{A, C}$	74.7 ± 24.7 (36.7–128.5)
%C (mg DM)	39.8 ± 1.4 (37.4–42.7)$^{B, C}$	52.2 ± 3.5 (46.4–65.2)$^{A, C}$	49.8 ± 1.7 (45.5–53.2)$^{A, B}$
%N (mg DM)	10.8 ± 0.4 (9.9–11.5)$^{B, C}$	8.3 ± 0.9 (6.6–10.6)$^{A, C}$	9.1 ± 0.5 (8.0–10.2)$^{A, B}$
C:N	3.7 ± 0.2 (3.4–4.0)$^{B, C}$	6.4 ± 0.9 (4.7–8.8)$^{A, C}$	5.5 ± 0.4 (4.5–6.7)$^{A, B}$
%Lipid (mg DM)	5.2 ± 0.7 (4.0–6.2)$^{B, C}$	31.9 ± 5.8 (20.6–41.7)	29.6 ± 3.4 (19.5–33.6)
%Protein (mg DM)	46.0 ± 2.7 (40.9–50.3)$^{B, C}$	38.3 ± 4.6 (28.7–51.7)	38.4 ± 2.8 (31.4–44.0)
n	32	45	24

modal size of ca. 35 mm, which would reflect the dominance of Age class 2 krill. The maturity stage composition showed a clear dominance of subadult (immature) stages. Some males and females were found in the adult resting stage.

During the sampling of krill for the physiological investigations, the specific subareas with their different krill composition characteristics, described above for each season (Fig. 2), were taken into consideration. In late spring, the size range of adult krill used for the experiments and analyses was relatively small, ranging from 30 to 39 mm, whereas autumn krill were 45 mm on average (Table 3). During winter, all adult krill sampled were immature with a mean length of 35 mm.

Body composition

The observed seasonal differences in elemental body composition appear to reflect the seasonal build up of extensive lipid stores at the onset of winter (Hagen et al. 2001), because they are C-rich. The C content was lowest in late spring (40% of dry mass) with higher values in autumn (52% DM) and winter (50% DM, Table 3). The N content, however, was relatively stable throughout the year at approximately 10% DM (Table 3). As a result of the higher C content in autumn and winter, the C:N ratios are almost double that in late spring (Table 3). The lipid levels followed the same pattern as the body C content, with highest values in autumn (32% DM) and winter krill (30% DM), and lowest in late spring krill (5% DM, Table 3). Conversely, protein content was highest in late spring (46% DM; 38% DM in both autumn and winter), due to the high lipid content in mid autumn and winter (Table 3).

The relationship between BL and DM is similar for all seasons (Table 4). The same is true for the relationship between DM and N as well as that between N and protein, whereas the relationship between DM and C calculated from late spring krill is different from that from autumn and winter krill (Table 4). Body C levels correlate positively with the lipid content, but the relationship between them is different in late spring compared with autumn and winter (Table 4). When plotting C:N ratios against the relative lipid content, it becomes clear that there are major differences between seasons: while there is a relationship in autumn and winter, there is no correlation at all in late spring (Table 4).

Metabolic rates and the activity of the metabolic enzymes CS, MDH and HOAD

Similarly sized adult krill in the Lazarev Sea showed significant differences in their respiration rates between seasons (Table 5). The oxygen consumption rate of autumn krill was 50% and that of winter krill only 30% of that of late spring krill. Correspondingly, the ammonium excretion rates were highest in krill from late spring. In autumn and winter the rates were 55

Table 4. *Euphausia superba*. Seasonal relationship of body length (BL) and dry mass (DM), DM and carbon (C), DM and nitrogen (N), C and lipid, N and protein, and C:N ratio and % body lipid (mg DM) of adult krill from the Lazarev Sea. The unit of BL is mm and that of the other parameters is mg. Data from different seasons were pooled when no significant differences were found; nc: no correlation; n = number of data points

Relationship	Season	Equation	r^2	n
BL to DM	All seasons	$DM = 4.0 \times 10^{-4}\ BL^{3.41}$	0.94	105
DM to C	Late spring	C = 0.29DM + 4.56	0.78	32
	Autumn and winter	C = 0.55DM – 3.56	0.98	69
DM to N	All seasons	N = 0.08DM + 1.08	0.95	101
C to Lipid	Late spring	Lipid = 0.11C + 0.39	0.76	32
	Autumn and winter	Lipid = 0.66C – 3.56	0.92	69
N to Protein	All seasons	Protein = 4.78N – 2.58	0.96	101
C:N to % Lipid	Late spring	nc		32
	Autumn and winter	% Lipid = 4.90C:N + 1.30	0.73	69

Table 5. *Euphausia superba*. Oxygen consumption rate per individual and per mg dry mass (DM) of krill, ammonium excretion rate and atomic O:N ratio; data are mean ± SD (range). Subscripted letters indicate a significant difference from A: late spring, B: autumn, C: winter. n: no. of experiments

	Oxygen consumption ($\mu l\ O_2\ ind.^{-1}\ h^{-1}$)	Oxygen consumption ($\mu l\ O_2\ mg^{-1}\ DM\ h^{-1}$)	Excretion ($\mu g\ N\ ind.^{-1}\ h^{-1}$)	O:N ratio	DM (mg)	n
Late spring	$91.5 \pm 19.0^{B, C}$ (76.6–116.6)	$0.71 \pm 0.16^{B, C}$ (0.53–1.13)	$3.7 \pm 1.2^{B, C}$ (1.4–5.4)	$28 \pm 12^{B, C}$ (15.7–54.9)	123.2 ± 29.1 (63.2–153.1)	12
Autumn	$50.3 \pm 15.2^{A, C}$ (24.7–68.6)	$0.37 \pm 0.04^{A, C}$ (0.31–0.44)	0.3 ± 0.1 (0.1–0.5)	$15 \pm 4^{A, C}$ (11.1–21.4)	137.2 ± 40.9 (77.8–193.6)	13
Winter	$26.3 \pm 13.1^{A, B}$ (9.3–40.3)	$0.19 \pm 0.04^{A, B}$ (0.13–0.28)	0.5 ± 0.3 (0.1–0.8)	$66 \pm 26^{A, B}$ (22.9–109.4)	140.4 ± 64.6 (50.0–195.4)	18

and 29%, respectively, of late spring rates. The resulting O:N ratio differed significantly between seasons. The highest ratios were found in winter (66), with less than half that value in late spring (28) and only a quarter in autumn (15).

The activities of CS and MDH (Table 6) as proxies for metabolic activity mirror the pattern of the oxygen uptake rates (see previous paragraph). Both enzymes showed their highest activity in krill from late spring, with significantly lower activities in the other seasons. In autumn and winter, respectively, the CS and MDH activities in krill were 47 and 39% (CS), and 77 and 66% (MDH) of those in late spring krill. The enzyme HOAD, an indicator for lipid breakdown (turnover), showed significantly different activities in krill from the late spring and autumn surveys compared with winter krill. In winter, the activities were approximately 400% higher than those in late spring and autumn (Table 6).

Feeding activity

The functional response of krill to increasing food supply was significantly different between seasons (Fig. 4, Table 7). Late spring krill displayed a clear functional response with increasing food concentration, with a maximum daily C ration (DR) of 10% body C d^{-1} at a food concentration of approximately 600 µg C l^{-1}. In autumn and winter, when krill were exposed to similar food concentrations as in late spring (Table 1), they showed only 20 and 14% of the equivalent maximum rates determined in late spring (Fig. 4).

The size of the DGs of freshly caught krill reflects the large difference in feeding activity of krill between late spring and winter (Figs. 5 & 6, Table 7). Krill of similar size (in terms of carapace length) had less than half the DG size in winter (2.9 ± 0.5 mm, n = 75) compared to late spring (7.3 ± 0.9 mm, n = 100, Figs. 5 & 6). During winter in the Lazarev Sea, the majority of the DGs of freshly caught krill showed no colouration or were milky white (Fig. 6c) or pale yellow (Fig. 6d), whereas those of late spring krill were mainly yellow (Fig. 6a) or black-green (Fig. 6b), suggesting that different food sources were used in different seasons.

Table 6. *Euphausia superba*. Seasonal differences in the activity of the metabolic enzymes malatdehydrogenase (MDH), citrate synthase (CS), and 3-hydroxyacyl-CoA-dehydrogenase (HOAD); data are mean ± SD; n: number of replicate samples. Metabolic enzyme activities are expressed as U g_{prot}^{-1} (1 U = 1 µmol substrate used per min). Subscripted letters indicate a significant difference from A: late spring, B: autumn, C: winter

Season	CS	MDH	HOAD	n
Late spring	$95 \pm 31^{B, C}$	$370 \pm 79^{B, C}$	0.12 ± 0.08	8
Autumn	45 ± 14	284 ± 98	0.10 ± 0.05	15
Winter	37 ± 11	245 ± 34	$0.50 \pm 0.36^{A, B}$	10

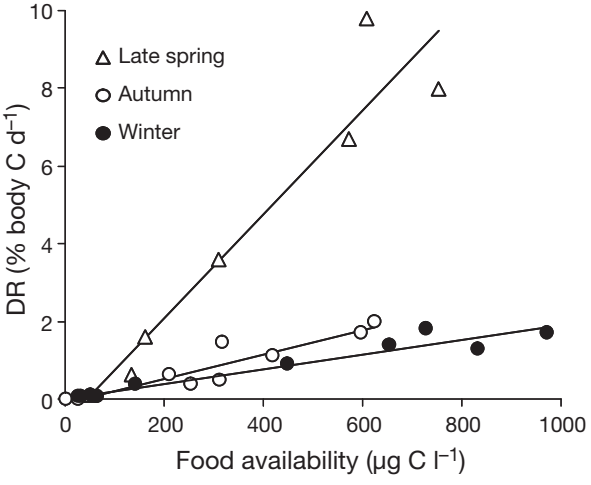

Fig. 4. *Euphausia superba*. Seasonal daily C ration (DR) as a function of food availability. Data points represent individual experiments. The seasonal linear regressions are shown in Table 7

Table 7. *Euphausia superba*. Seasonal linear regressions of daily C ration (DR) in % body C d^{-1} and food availability (FA) in mg C m^{-3}, carapace length (CL) and size of digestive glands (DG) in mm, and body dry mass (DM) in mg and oxygen uptake rates (OR) of individual krill in µl O_2 $ind.^{-1}$ h^{-1}

Relationship	Season	Equation	r^2	n	p
DR to FA	Late spring	$y = 0.013x - 0.623$	0.91	7	<0.01
	Autumn	$y = 0.003x - 0.104$	0.86	9	<0.01
	Winter	$y = 0.002x + 0.032$	0.93	10	<0.001
CL to DG	Late spring	$y = 0.47x + 1.26$	0.68	100	<0.001
	Winter	$y = 0.28x$	0.70	75	<0.001
DM to OR	Late spring	$y = 0.74x$	0.97	15	<0.001
	Autumn	$y = 0.37x$	0.94	15	<0.001
	Winter	$y = 0.19x$	0.91	20	<0.001

Growth

The growth rates of krill from both late spring and winter followed the overall pattern of metabolic activity for these seasons. Growth was positive in both seasons, but significantly lower in winter than in late spring (Table 8). The IMP of late spring krill was approximately half of that of winter krill (Table 8). The variations in growth rates in both seasons are given in Fig. 7a–d. In late spring, growth rate ranged from 0.03 to 0.10 mm d^{-1} and GIs from 4 to 9% IMP^{-1}. In winter, the growth rates ranged from 0.0003 to 0.0024 mm d^{-1} and GIs from 0.5 to 3.8% IMP^{-1}. The chl *a* concentration in the upper 30 m of the water column was highly variable in late spring, ranging from 0.2 to 2.2 mg m^{-3} (Table 8), resulting in a high variability of growth rates between stations (Fig. 7a,b). In winter, the chl *a* concentrations were more or less uniform at an extremely low level, ranging in mean from 0.01 to 0.04 mg m^{-3}. Consequently, there was no significant variation in growth rates between stations (Fig. 7c,d).

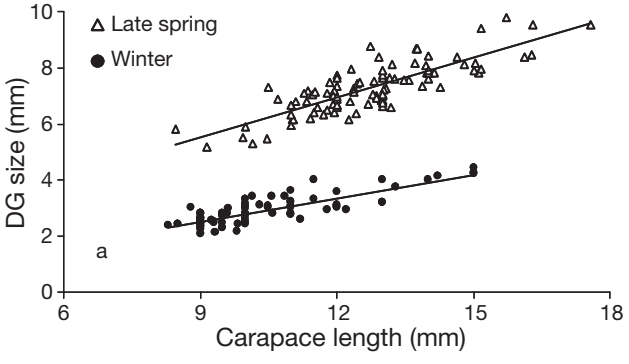

Fig. 5. *Euphausia superba*. Relationship of carapace length and size of digestive glands (DG) of krill from 3 stations in late spring and 2 stations in winter. The linear regressions are shown in Table 7

DISCUSSION

The present study aimed to analyse physiological parameters of Antarctic krill in different seasons in the Lazarev Sea with a comprehensive suite of methods in order to better understand their overwintering in the Southern Ocean. The major conclusions from our results are: (1) Krill strongly reduced oxygen uptake rates to 50% in autumn and only 30% in winter of those measured in late spring. (2) The feeding activity of adult krill was only 20% in autumn and 14% in winter of late spring rates at similar food concentrations. (3) In winter, krill displayed a flexible (omnivorous) feeding activity. (4) Adult krill showed very low positive growth during winter and no shrinkage. (5) In addition to the low feeding activity, adult krill used mainly body lipids and, to a moderate extent, body protein for energy provision during winter. Food intake (at low levels) during winter seems to be an important energy input for krill to survive the winter season.

Below, we discuss our findings with regard to proposed overwintering mechanisms for adult krill, with reference to previous studies of krill overwintering in other regions of the Southern Ocean (Table 9, Fig. 1).

Adaptation of physiological functions for overwintering

Unlike water temperature, which remains within a narrow range throughout the year, photoperiod and light intensity are environmental parameters with strong seasonality. The scarce amount of daylight at this high latitude and the extensive sea ice coverage in winter rigorously limit primary production and hence the krill food resources. The low food supply during winter is thought to induce reduced feeding rates and subsequent starvation which, in turn, causes the slowdown of physiological functions such as metabolism and growth, or even shrinkage (Quetin & Ross 1991, Ettershank 1983).

Oxygen uptake rates

In the Lazarev Sea, metabolic rates were 50% in autumn and 30% in winter of rates measured in late spring (Table 5). The majority of published data on oxygen uptake rates are from the late spring and summer season, and data from autumn and winter are rare. Our weight-specific respiration rates measured in krill from late spring (0.5 to 1.1 µl O_2 mg^{-1} DM h^{-1}, Table 5) are in a comparable range with previous results (Kils 1978: 0.9 to 1.1, Rakusa-Suszczewski & Opalinski

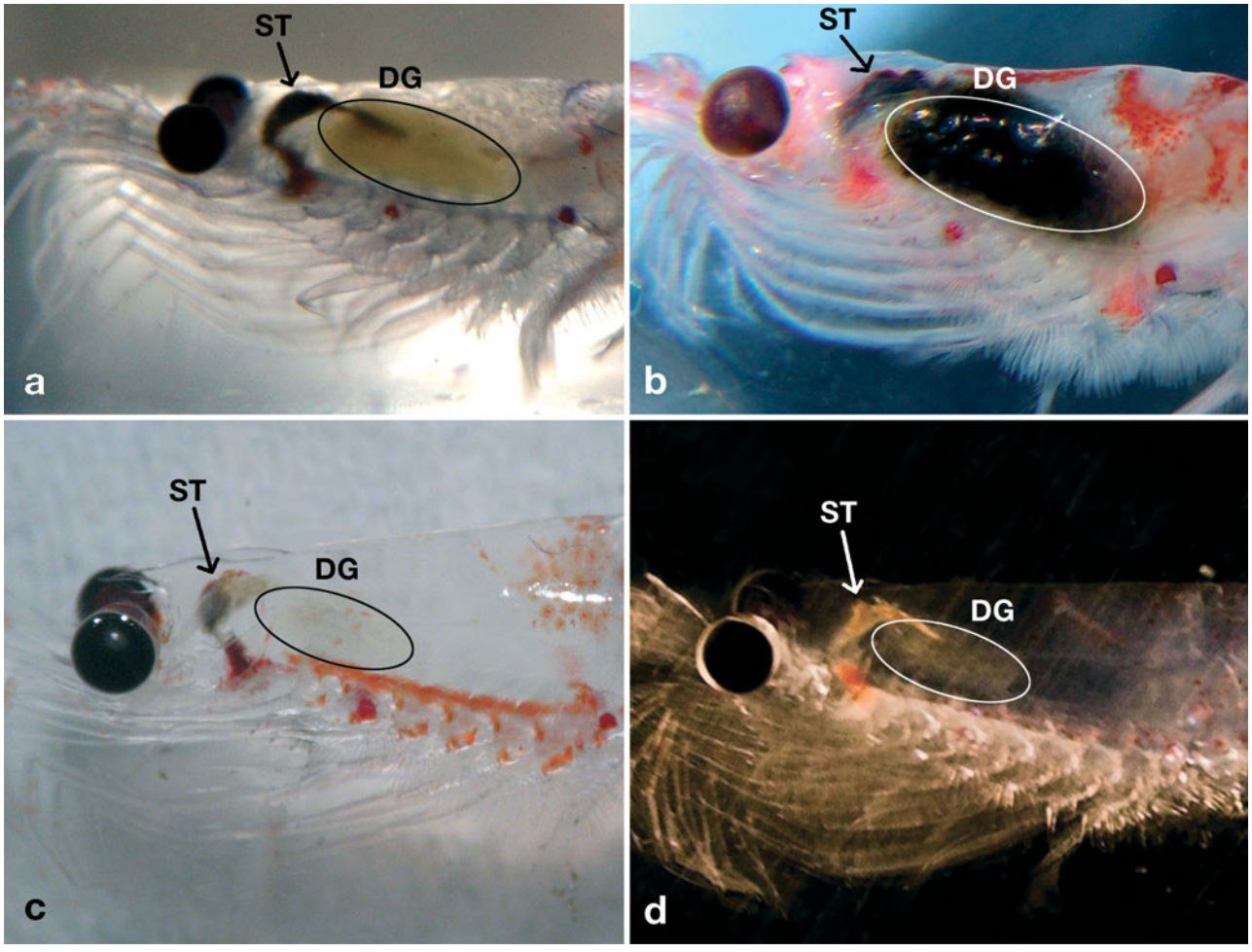

Fig. 6. *Euphausia superba*. Carapace with digestive gland (DG) and stomach (ST) of freshly caught adult krill; (a) yellow and (b) green-black DG in late spring, and (c) milky white and (d) pale yellow DG in winter. *Photos (a) and (d) by A. Hayden and H. Flores, respectively*

Table 8. *Euphausia superba*. Mean growth and intermoult period (IMP) given as growth increment (GI: % change in uropod length on moulting) and daily growth rate. The range of data in both seasons is given in Fig. 7; n: number of moulted individuals. Chl *a* concentrations and environmental temperature (EVT) are from the upper 30 m of the water column. Krill length is the mean in each experiment

Stn	Krill length (mm)	EVT (°C)	Chl *a* (mg m^{-3})	n	IMP (d^{-1})	Growth GI (%)	Growth (mm d^{-1})
Late spring							
44	37	−1.71	0.19	10	50	4.9	0.0737
46	36	−1.57	0.27	9	42	6.4	0.0556
47	30	−1.58	0.24	10	43	7.5	0.0713
92	41	−0.84	2.17	12	34	7.7	0.1036
Winter							
474	35	−1.84	0.04	8	102	2.4	0.0013
508	33	−1.86	0.04	8	77	2.3	0.0014
516	33	−1.77	0.03	14	55	1.5	0.0014

1978: 0.4 to 0.7, Ikeda & Mitchell 1982 and Ikeda & Bruce 1986: 0.4 to 0.6, Hirche 1983: 0.6, Kawaguchi et al. 1986: 0.4 to 0.7, Torres et al. 1994a: 0.6 to 0.7 µl O_2 mg^{-1} DM h^{-1}). In autumn, oxygen uptake rates similar to ours (0.3 to 0.4 µl O_2 mg DM h^{-1}) were reported by Kawaguchi et al. (1986) from Lütow-Holm Bay (0.2 to 0.6 µl O_2 mg^{-1} DM h^{-1}) and Atkinson et al. (2002) from the Lazarev Sea (0.3 to 0.4 µl O_2 mg^{-1} DM h^{-1}). The winter values reported from the Western Antarctic Peninsula (WAP, Quetin & Ross 1991), Southern Scotia-Northern Weddell Sea region (Torres et al. 1994a), and Lützow-Holm Bay (Kawaguchi et al. 1986), are in line with our data, namely 30% of summer rates.

Feeding activity

The metabolic slow-down in autumn and winter was accompanied by low feeding activity: Maximum feed-

ing activity on natural food assemblages was 20% in autumn and 14% in winter of rates measured in late spring (Fig. 4) and summer (Atkinson & Snÿder 1997). Similarly low feeding activity in autumn was observed in a previous study in the Lazarev Sea (Atkinson et al. 2002). In addition, seasonal feeding studies at the WAP determined a feeding activity in winter of only 2% of that observed in summer (Quetin & Ross 1991). Stomach, gut and DG fullness and size can be used as indicators for feeding activity in the field (Morris et al. 1983, Morris & Priddle 1984, Nicol et al. 2004). In the Lazarev Sea, the DG size of winter krill was less than half that of late spring krill, pointing to a low feeding activity in the field (Nicol et al. 2004). This is consistent with overwintering studies which found low stomach and/or gut contents in krill (Table 9: Morris & Priddle 1984, Kawaguchi et al. 1986, Lancraft et al. 1991, Daly & Macaulay 1991, Hopkins et al. 1993, Nishino & Kawamura 1994).

Individual growth

The low feeding activity in the Lazarev Sea during winter was accompanied by low individual growth rates in krill (Fig. 7). Shrinkage of krill has also been documented in the Bransfield Strait during winter (Quetin & Ross 1991), and other authors have hypothesised that krill do not grow in winter (Ikeda 1985, Ikeda & Thomas 1987). Ikeda & Dixon (1982) were the first to propose, from laboratory experiments, that shrinkage in response to starvation is a possible overwintering mechanism for krill. Previous studies measuring growth using traditional length-frequency analysis also reported similar results to ours, i.e. nearly zero or low growth during winter (Mackintosh 1972, Stepnik 1982, Morris & Priddle 1984, Kawaguchi et al. 1986, Siegel 1987, Buchholz et al. 1989) as well as shrinkage (Ettershank 1983). However, determining shrinkage of krill with length-frequency analysis on a population level is problematic. Different size structures of krill within a population might occur due to selective size mortality (predation) and/or selective size immigration/emigration (exchange of water masses). In our winter study, individual growth rates were clearly positive and, at each moult, the krill increased in length by 0.5 to 3.8%. The average daily growth rates, however, were very low due to the longer IMP in winter compared to late spring values (Table 8). Although published data suggest that postlarval krill might be able to shrink in the field during winter, our results indicate that such behaviour may not be the rule but rather the exception

Table 9. *Euphausia superba.* Studies which examined overwintering mechanisms in the Southern Ocean

Region	Results	Study period	Source
Northern Weddell Drift, Scotia Sea, Eastwind drift (Pacific sector)	Zero to low growth	June–August 1925–1927, 1923–1939, 1950–1951	Mackintosh (1972)
Admiralty Bay (King George Island, South Shetland Islands)	Low growth	May–July 1979	Stepnik (1982)
Off South Georgia	Low feeding and growth activity	August–September 1983	Morris & Priddle (1984)
Lütz-Holm Bay (Ongul Islands)	Low feeding activity (on sediments) Reduced oxygen consumption rates	May–November 1984	Kawaguchi et al. (1986)
Western Antarctic Peninsula (Bransfield Strait, north of South Shetland Islands)	Shrinkage Lipid utilisation Reduced oxygen consumption rates Low feeding activity	March and April 1985, August and September 1985 January and July 1987	Quetin & Ross (1991)
Southern Scotia Sea/Northern Weddell Sea region	Low feeding activity Reduced oxygen consumption rates Lipid utilisation	March 1986, June–August 1988	Daly & Macaulay (1991) Torres et al. (1994a), Torres et al. (1994b)
Southern Scotia Sea	Low feeding activity Omnivory	June–August 1988	Lancraft et al. (1991), Hopkins et al. (1993)
Western Antarctic Peninsula (Gerlache Strait, Crystal Sound)	Intense feeding on zooplankton organisms	December 1991–January 1992, July–August 1992	Huntley et al. (1994)
South Georgia area	Low feeding activity Carnivory	July–August 1992	Nishino & Kawamura (1994)
Northern Antarctic Peninsula, Eastern and western Weddell Sea and Lazarev Sea	Lipid utilisation	October and November 1983, January and February 1985, July and August 1986, October and November 1986, April and May 1992	Hagen et al. (2001)
Lazarev Sea	Reduced oxygen consumption rates Very low growth rates Low feeding activity Lipid utilisation	April 2004, December 2005, July and August 2006	Present study

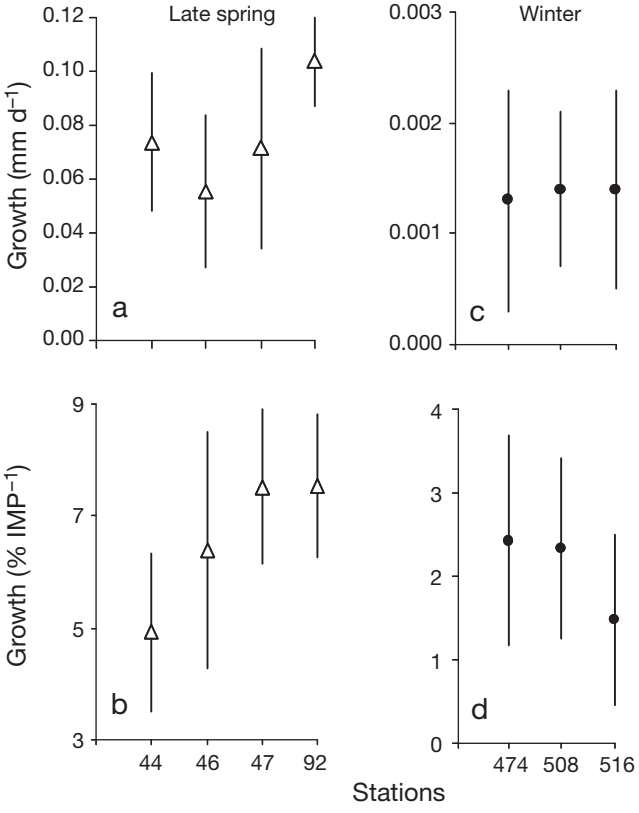

Fig. 7. *Euphausia superba*. (a,b) Late spring and (c,d) winter (a,c) growth rate and (b,d) growth increment (GI) in % per intermoult period (IMP), (mean ± SE). Note different scales for late spring and winter

during the dark season. Shrinkage of krill in the field might be a result of prolonged starvation and consequently severe physical conditions.

Compared with results from previous growth rate studies using the IGR method, the growth rates of krill from the Lazarev Sea in December are moderate (0.076 mm d^{-1} on average), in contrast to those measured overall in the Scotia Sea in January and February, where growth rates reached 0.32 mm d^{-1}, with mean values of 0.1 mm d^{-1} (Atkinson et al. 2006). On the other hand, growth rates similar to those in the Lazarev Sea in late spring were found around South Georgia (Arnold et al. 2004) and off east Antarctica (Nicol et al. 2000) during summer. In winter in the Lazarev Sea, the IMP was nearly twice as long as in late spring, which is consistent with previous studies (Quetin & Ross 1991, Buchholz et al. 1989). However, the IMP in late spring (December) was much longer than that reported from studies made in January and February (e.g. Buchholz et al. 1989, Arnold 2004). As outlined in Buchholz (1991, 2003), growth and IMP are functions of energy intake, temperature, length and maturity. The use of mainly subadult krill of similar length in our growth experiments during both seasons ensured that growth variability due to length and sexual maturation, as compared to other studies, can be neglected (Buchholz 2003). The same is true for temperature, which was very similar in both seasons (late spring: −1.5°C, winter: −1.8°C). In the Lazarev Sea, phytoplankton blooms just started to develop in December, so that lipid storage of krill was still low after the winter depletion. These circumstances might explain the moderate growth rates and long IMPs in the Lazarev Sea in December compared to previous growth studies performed in January and February (see above).

Influence of the Antarctic light regime on physiological functions

It is still under debate whether the low food supply is the only reason for the low metabolic rate in krill during winter or whether there is a more fundamental seasonal transition in the krill's physiology induced by environmental cues (Torres et al. 1994b). First indications for such a cue were reported by Kawaguchi et al. (1986). The authors reported a decline of physiological functions (feeding and metabolic activity) in krill from April and May to August and September and a slow but steady increase thereafter. This correlated exclusively with solar radiation and not food availability. Most recently, more clarity was brought to this topic from controlled experiments in the laboratory. Feeding, metabolic activity (Teschke et al. 2007) and gene expression (Seear et al. 2009) of krill were affected by the Antarctic light regime. Simulated summer conditions (24 h light) caused high feeding and metabolic rates as well as increased expression of genes involved in functions such as metabolism, motor activity, protein binding and various other cellular activities. In contrast, simulated winter conditions (no light) led to the opposite results. In the Lazarev Sea, krill showed significant differences in feeding activity between seasons. In contrast to late spring, krill were unable to respond to high food concentrations in autumn and winter, despite exposure to abundant food for up to 2 wk (Fig. 4). Together, laboratory and field findings indicate that reduced feeding and metabolic activity of krill during Antarctic winter are not caused directly by the scarcity of food at this time of the year, but represent an inherent adaptational overwintering mechanism influenced by the Antarctic light regime. However, the effects of latitudinal differences on metabolic and feeding activity of krill during winter due to differences in daylight duration are unknown. It also remains unclear whether the observed effects on physiological functions of krill from the field are the result of differences in photoperiods or light intensity. During laboratory experiments carried out by Teschke et al.

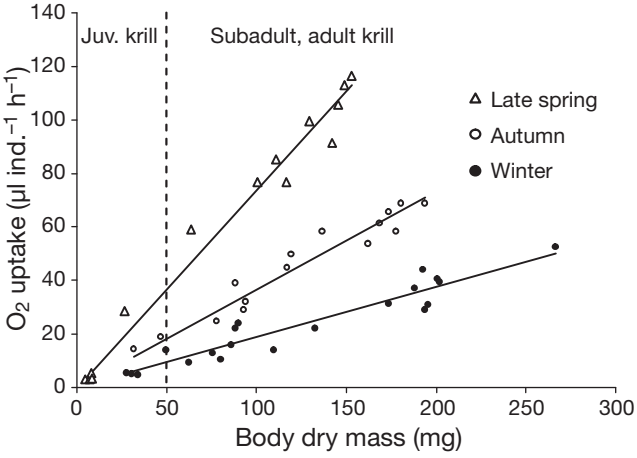

Fig. 8. *Euphausia superba*. Relationship of body dry mass and oxygen uptake rates of individual krill in different seasons. The linear regressions are shown in Table 7. Juvenile, subadult and adult krill were defined after Siegel (1987)

(2007), the light regimes were adjusted to different photoperiods and light intensities, in order to simulate the seasonal Antarctic light regime most accurately. These open questions warrant future research. It appears that the influence of the light regime may vary during krill ontogenesis. Adults showed significant differences in metabolic rates between seasons (Fig. 8, Table 7), whereas the seasonal metabolic differences are less pronounced in juvenile krill (Fig. 8). Recent investigations have demonstrated that metabolic activity in larval krill depends exclusively on food supply and not on light (Meyer et al. 2009). It is also unknown whether the shift takes place abruptly with the final larval moult or whether there is a subtle transition during juvenile stage.

Energy provision during winter

Although physiological functions are reduced to a minimum, energy must be provided in order for the organism to function, albeit at low rates, for several months during the absence of autotrophic food in the water column. There seem to be 2 adaptations to accomplish this: (1) accumulation of large lipid reserves during summer until the onset of winter for winter consumption and (2) an omnivorous feeding activity at low rates during winter.

Lipid and protein utilisation

It is now well known that Antarctic krill accumulate large reserves of body lipids during the course of summer when availability of phytoplankton is high, and consume these reserves during the winter season (Hagen et al. 2001). Our study confirms this for the Lazarev Sea. The lipid content showed strong seasonality (Table 3), with highest lipid levels in April (32% DM^{-1}) and lowest values in December (5% DM^{-1}). Furthermore, the metabolic enzyme HOAD, an indicator for lipid breakdown (turnover), showed significantly higher activities in winter krill than in those sampled in late spring and autumn (Table 6). In addition, the low O:N ratio (15) of krill in autumn (April) indicated that they had not yet resorted to burning these reserves (Atkinson et al. 2002). On the other hand, the high O:N ratio (66) in winter krill (July and August) demonstrated full usage of lipid reserves (Ikeda et al. 2000). Krill from the eastern and western Weddell Sea and the Lazarev Sea showed average lipid depletion from their initial values in April and May (mid to late autumn) to October and November (mid to late spring) by 70% (Hagen et al. 2001). In the WAP and the Southern Scotia-Northern Weddell Sea region, lipid levels in krill declined 40 to 50% from early to mid autumn (March and April) until late winter (August and September, Quetin & Ross 1991, Torres et al. 1994b). It appears that the rate of lipid depletion in krill during winter is approximately 10% mo^{-1} of their initial lipid levels at the onset of winter.

In our study, the mid autumn and late spring season was not researched in consecutive seasons, so we are cautious in interpreting the lipid levels of krill in April 2004 and December 2005 as a seasonal sequence. In the Lazarev Sea, the remaining lipid content of 5% DM^{-1} in December 2005 is very low and on the border of what is deemed essential for the functioning of biomembranes and hence survival (Hagen et al. 2001). Such low reserves in krill were documented previously in late October to mid November (Hagen et al. 2001). The accumulation of energy reserves during the feeding season until the onset of winter depends on the quantity and quality of phytoplankton during the preceding summer and autumn (Hagen et al. 2001). In the Lazarev Sea, food supply in terms of chl *a*, varied considerably from year to year. The mean March chl *a* concentrations were 0.4, 0.2, and 0.8 mg m^{-3} in 2004, 2005 and 2006, respectively (Fig. 9). The low body lipid content of krill in December 2005 might therefore be the result of low initial lipid storage in krill at the onset of winter in April and May 2005. It is most likely that this lipid level (April and May 2005) was lower than that measured in April 2004 due to the lack of favourable feeding conditions during summer 2005 (Fig. 9b). Low lipid reserves at the beginning of winter and any delay in the availability of adequate food in spring would affect the onset and success of the spawning season, which then sets up the reproductive success of the entire year class (e.g. Siegel & Loeb

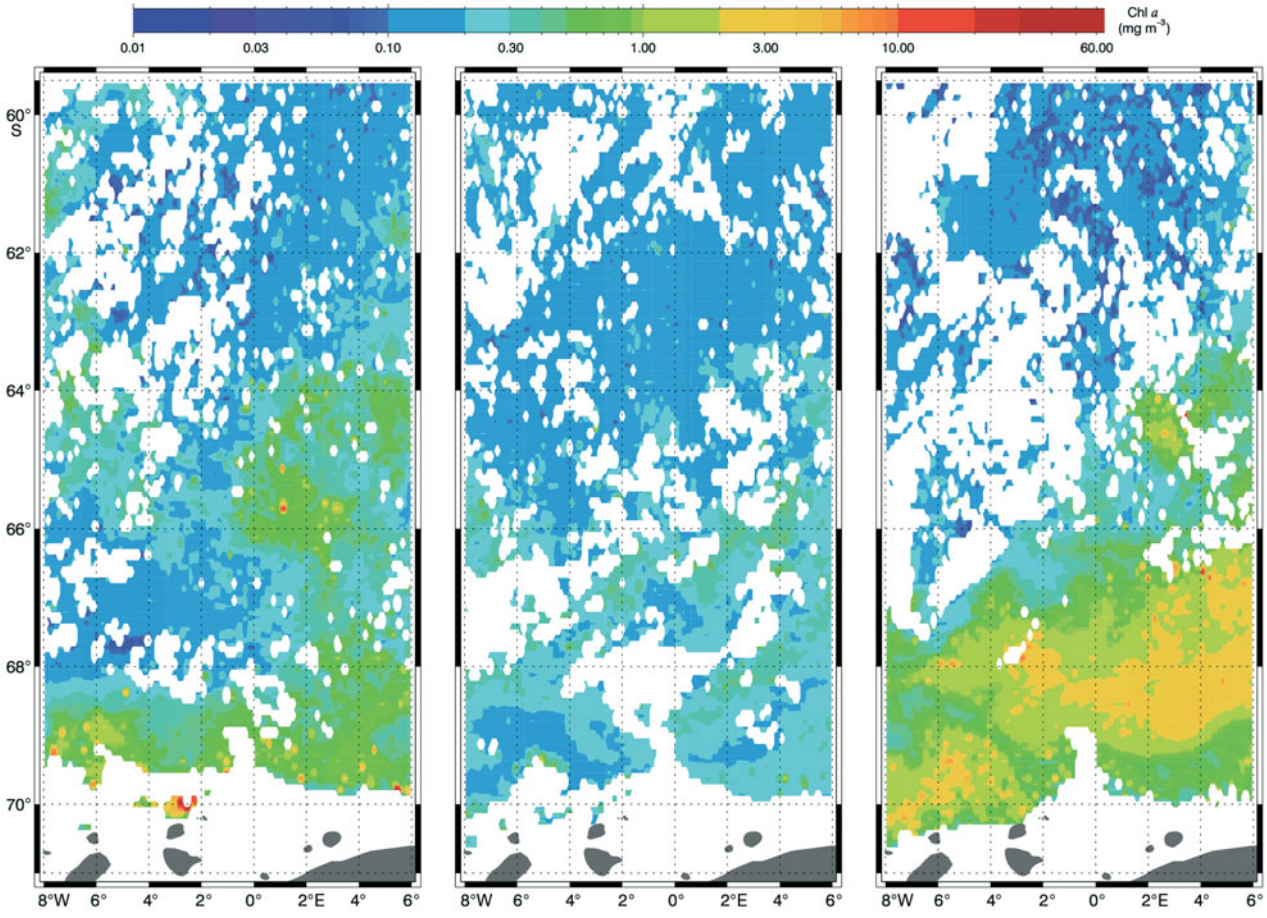

Fig. 9. Monthly mean SeaWiFS chlorophyll *a* (mg m^{-3}) in March of (a) 2004, (b) 2005, and (c) 2006. White: no data

1995). This highlights the importance of favourable feeding conditions during summer to accumulate sufficient energy reserves for a successful overwintering period of krill.

Compared with lipids, proteins play a minor role as an energy reserve in overwintering krill. Krill from the Southern Scotia-Northern Weddell Sea region depleted protein levels from March to August by 16% (Torres et al. 1994b). Such utilisation is minor (ca. 3% mo^{-1}) compared to the lipid depletion in krill during winter. In the Lazarev Sea, similarly sized krill had a mean individual protein content of 30 mg DM^{-1} in winter (July 2006) and 24 mg DM^{-1} in late spring (December 2005), suggesting a negligible utilisation of body protein during winter. The moderate use of body protein in krill during winter is also confirmed by the relationship between N and protein. In our multi-seasonal study, this relationship did not differ significantly and a slope of 4.8 (Table 4) indicates that most of the N is bound in the muscle protein fraction (Anger 2001). A significantly increased utilisation of body protein and/or digestion of heterotrophic diet would result in an increase of N and a decrease of muscle protein content, measurable as a shallower slope of the N:protein relationship and increasing excretion rates. This was demonstrated recently for larval krill (Meyer et al. 2009). Neither was found in adult krill, suggesting that the use of body protein and/or a heterotrophic diet during winter was minor compared with the use of stored lipids during the study period in the Lazarev Sea.

Food sources during winter

Most authors addressing the overwintering of krill mention the possibility of switching to alternative food sources other than phytoplankton in the water column, such as zooplankton, phytodetritus in shallow regions and ice algae. When comparing results from various feeding studies during winter (summarised in Table 10), however, it becomes apparent that krill display more of an opportunistic feeding behaviour rather than a preference for a specific food source during winter. Several studies have demonstrated that colouration of the DG (Kawaguchi et al. 1999, Nicol et al. 2004) and the stomach (Kawaguchi et al. 1986) is related to the ingested

Table 10. *Euphausia superba.* Studies of feeding during the Antarctic winter. The stomach and gut contents are listed in order of their abundance

Measurement	Results	Source
Field studies		
Stomach and gut content	Phytoplankton: diatoms, dinoflagellates Protozoan: tintinnids Metazoans: *Oithona*, other copepods Euphausiid debris: moults	Lancraft et al. (1991), Hopkins et al. (1993)
	Phytoplankton: diatoms, dinoflagellates, silicoflagellates Protozoa: foraminifera, tintinnids, radiolarians, naked ciliates Metazoa: copepods	Nishino & Kawamura (1994)
Stomach colour and gut content	Light green	Daly & Macaulay (1991)
Colour of digestive gland	Yellowish brown or ochre	Kawaguchi et al. (1986)
	Transparent, milky white or pale yellow	Present study
Laboratory studies		
Feeding rates	High feeding activity on *Oithona* spp., *Oncaea* spp.	Huntley et al. (1994)

food source. Phytoplankton diet is indicated by a black-green, yellow and/or greenish DG (Kawaguchi et al. 1999), phytodetritus by a brownish ochre stomach and gut (Kawaguchi et al. 1986), and a milky-white DG is an indicator of a zooplankton diet (Atkinson et al. 2002). In the Lazarev Sea, the small size of the DG in winter compared with late spring (Figs. 5 & 6) and its colouration ranging from colourless to milky white (Fig. 6c) or pale yellow (Fig. 6d) suggested that krill had either not been feeding or had ingested a heterotrophic and autotrophic diet at low rates.

The majority of krill feeding studies performed in winter found low feeding activity (Table 9). However, an investigation by Huntley et al. (1994) reported a high feeding activity of krill on small zooplankton organisms (*Oithona* spp, *Oncaea* spp.). Another study implies in its title that a winter study was performed and a high feeding activity of krill on ice algae was observed (Marschall 1988). However, this observation was made between the middle and end of October (Hempel 1987). In the light of the preceding discussion, at this time of the year, the high feeding activity of krill on ice algae is not surprising. Feeding activity, growth and metabolic rate start increasing at the onset of spring despite low food availability (Mackintosh 1972, Stepnik 1982, Morris & Priddle 1984, Kawaguchi et al. 1986). During the middle to end of October, it is most likely that the krill had already returned to their active physiological mode due to changes in the Antarctic light regime.

A compromise strategy used by krill for overwintering

Torres et al. (1994b) were the first to propose a compromise strategy for overwintering krill. Our study has demonstrated that the Antarctic light regime (and not food supply) seems to be the single most important ecological cue for commencement of physiological adaptation to winter. During this adaptation, metabolic activity is reduced to a minimum and reflects low feeding and low growth (or even shrinkage). Overwintering success, however, depends on the sufficient accumulation of energy reserves (mainly lipids) during the preceding productive season. However, the flexible (omnivorous) feeding activity, at low rates, during winter contributes to a valuable energy input. Therefore, during studies investigating seasonal feeding activity of krill based on stomach content analyses, a rough estimate of the energy content of the ingested food should be made rather than just an inventory of the food items.

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