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Seasonal variation in biochemical indicators of physiological status in *Euphausia superba* from Port Foster, Deception Island, Antarctica

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Abstract

Seasonal changes in biochemical indicators of physiological status were analyzed in abdominal muscle of the Antarctic krill, *Euphausia superba*, collected from Port Foster, Deception Island, an active volcano located in the Shetland Island chain west of the Antarctic Peninsula. Krill were collected with a 10 m² MOCNESS trawl during four cruises (November 1999, February, May, November 2000). RNA:DNA mirrored the chlorophyll *a* concentration, with the highest values found during seasons of abundant phytoplankton. Activities of the glycolytic enzyme lactate dehydrogenase (LDH) and the mitochondrial enzyme citrate synthase (CS) were significantly higher in male krill when compared to females of similar size, indicating that their burst and aerobic swimming performance may be higher than females throughout the year. RNA:DNA ratio and enzyme activities were highly elevated in summer as compared to the earliest spring sampling period. Krill showed significant seasonal changes in LDH activity, with lowest values in spring and highest values in summer (females) or autumn (males). Krill showed significant seasonal changes in CS activity with highest values in summer. Protein and % water varied significantly among seasons for both males and females. Lower CS activity and RNA:DNA ratio suggest krill exhibit reduced metabolism during the winter when phytoplankton production is reduced, perhaps enhancing survival. Lower enzyme activities in female krill in early spring suggest they may achieve greater metabolic suppression during overwintering.

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1. Introduction

Extreme seasonal changes in light levels, day length, ice cover, and primary production in the Southern Ocean profoundly affect food availability for Antarctic zooplankton. The Antarctic krill,

Euphausia superba, is one of the most important links between primary producers and higher trophic levels in the Antarctic pelagic ecosystem (Ainley et al., 1991). Antarctic krill have very high metabolic demands (Opalinski, 1991; Clarke and Morris, 1983; Kils, 1981) and thus should be especially vulnerable to strong seasonal shifts in food resources. Indeed, Quetin and Ross (1991) used the instantaneous growth rate (IGR) technique and observed high rates of growth (1.75–4.4%

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increase in body length per molt) in adult krill in late summer and autumn and shrinkage of -0.16% to -2.03% body length per molt in winter. Growth rates determined by IGR are similar to those determined in laboratory feeding experiments (Ikeda and Dixon, 1982).

Krill growth rates appear exquisitely sensitive to food availability, responding rapidly as phytoplankton blooms develop and dropping precipitously when phytoplankton concentrations decline. Ross et al. (2000) reported that the growth rates of juvenile krill increased with increased phytoplankton availability and were influenced not only by absolute abundance but also by species composition of the phytoplankton. Maximal growth rates were seen only when chlorophyll *a* concentrations exceeded 3.5 mg m^{-3} , a concentration considered to be near the lower end of bloom conditions. Nicol et al. (1992) observed that juvenile krill with high IGR began to shrink after only 10 days of food limitation. Under extreme food limitation, however, krill may achieve maximum shrinkage rates within days (Ikeda and Dixon, 1982).

Several biochemical measures, including the ratio of muscle RNA to DNA and concentrations of several enzymes of aerobic and anaerobic metabolism, have been strongly correlated with nutritional status in larval and juvenile fishes (Ferron and Leggett, 1994; Lowery and Somero, 1990). Krill demonstrate strong swimming capabilities (Kils, 1981) and achieve a large body size, including a substantial amount of abdominal muscle utilized during the tail flip that produces burst swimming (O'Brien, 1987). These characteristics suggest that krill may show biochemical changes in the abdominal muscle similar to those exhibited by fishes in response to changes in food availability.

Some invertebrate studies have shown links between food resources and biochemical indicators and indicated their promise for examining seasonal changes in nutritional status and metabolism of Antarctic krill. Particularly important are studies that show a link between ecological availability of preferred foods and tissue biochemical indicators. For instance, increased RNA:DNA ratios in intertidal mussels were associated with the

occurrence of nearshore phytoplankton blooms (Dahlhoff and Menge, 1996). Herbivorous copepods showed increased levels of citrate synthase (CS), an enzyme of aerobic metabolism, and increased oxygen consumption during phytoplankton blooms associated with the receding ice edge in the Weddell Sea (Geiger et al., 2001; Kawall et al., 2001). Other species of copepods showed elevated lactate dehydrogenase (LDH), an enzyme indicative of the anaerobic metabolic potential. Meyer et al. (2002) found that CS activity in adult krill abdominal muscle correlated with whole-animal respiration rates and varied substantially between summer and autumn.

Examination of patterns of changes in enzyme concentrations and RNA:DNA ratio can yield insight into the relative contributions of aerobic and anaerobic metabolic pathways and rates of protein synthesis to the organism's metabolic demands. Each of these measures shows a different sensitivity and rate of response to changes in food availability, with RNA:DNA being the most sensitive to short-term variation. More persistent trends in food abundance are necessary to achieve elevation or decline of the enzymes of metabolism. Thus, physiological parameters measured from krill collected at a single time period can be correlated with phytoplankton abundance measured concurrently, but also can be evaluated as representing an integrated response to long-term (e.g., seasonal) trends in food availability.

Previous studies have considered the overwintering response of krill in association with the extensive seasonal expansion and contraction of the Antarctic pack ice. However, this study addresses seasonal changes in biochemical indicators in Antarctic krill found in Port Foster, Deception Island, a locale where no consistent ice cover developed over the study period (Smith et al., 2003a).

Ross and Quetin (1986) suggested that krill may require chlorophyll *a* levels above $1\text{--}5 \mu\text{g chl } a \text{ l}^{-1}$ for reproduction, if the energy for reproduction comes primarily from summer feeding. Therefore, krill populations in coastal habitats with elevated productivity or krill associated with phytoplankton blooms at receding ice edges may be more

likely to spawn and may exhibit higher fecundity than oceanic populations of krill. Investigating the seasonal changes in the physiological condition of krill captured in Port Foster, a coastal, shallow, partially enclosed bay may provide insight into whether krill found in shallow coastal waters are as physiologically robust as those associated with receding ice edge blooms.

This study was conducted as part of a comprehensive, multidisciplinary research program focused on the ecology of Deception Island, Antarctica (Smith et al., 2003a). Krill were collected between November 1999 and November 2000 to address whether there were changes in the concentration of metabolic enzymes or RNA:DNA ratio in krill abdominal muscle over an annual cycle.

2. Materials and methods

2.1. Study site: Deception Island, Antarctica

Deception Island is located at 62°59'S, 60°34'W at the southwest end of the South Shetland Island chain. It is an active volcano (most recent eruption 1970) that contains a 7-km-wide flooded caldera called Port Foster. Port Foster supports pelagic and benthic communities that are representative of the Antarctic coastal zone (e.g., Everson, 1987; Gallardo, 1987; Kaufmann et al., 2003). *E. superba* is a conspicuous component of the pelagic community in Port Foster and was collected during four seasonal sampling cruises in 1999–2000. These cruises were conducted as part of the Erupt Program to document ecosystem changes in the enclosed bay within Deception Island (see Smith et al. 2003a for a more complete description of the Erupt Program).

Water temperatures in Port Foster ranged from -1.0°C to 2.5°C at the surface and -1.6°C to 0.5°C at 150 m (Sturz et al., 2003). Deception Island exhibits a climate similar to that of coastal continental Antarctica. (See Smith et al. (2003b) for more information about weather patterns at Deception Island and conditions within Port Foster.)

2.2. Collection of *E. superba*

This study included samples collected on four cruises aboard the R.V. *Laurence M. Gould* between November 1999 and November 2000. Antarctic krill, *E. superba*, were collected using a 10-m² MOCNESS trawl with a 4-mm mesh net in the body and 505- μm mesh in the cod end. Six trawls were made per cruise: three nighttime trawls, flanking local midnight (2300–2330, 0000–0030, and 0100–0130 h) and three daytime trawls, flanking local noon (1100–1130, 1200–1230, and 1300–1330 h). A separate trawl was taken at discrete 50 m depth strata (0–50, 50–100, and 100–150 m depth). Sampling dates were 1–7 November 1999, 11–17, 28, February 2000, 23–31 May 2000, and 21–26 November 2000. Trawls were made in the deepest portion of Port Foster, around 160 m (see Fig. 2 in Smith et al., 2003a), and collections were taken in three depth strata: 0–50, 50–100, and 100–150 m.

Upon retrieval of the MOCNESS trawl, 100 live *E. superba* were selected from those similar in standard length to the mid-range of all krill collected. These krill immediately were wrapped in aluminum foil (10 krill per packet), flash frozen in liquid nitrogen, and placed in a -80°C freezer aboard the ship. Samples were returned to the United States on dry ice and kept at -80°C until biochemical assays were performed.

Krill used for biochemical analyses were sorted according to gender, and wet mass and standard length (tip of rostrum to tip of telson) were measured. Male krill were identified by the presence of a petasma. Krill without a petasma were designated as adult female, since krill collected for this study correspond to length ranges of krill identified as more than 3 years old in Pakhomov (2000). To avoid size specific effects on biochemical parameters, krill used for all analyses reported in this study were selected from the mid-range of collected sizes. Muscle was sampled from the middle portion of the abdominal section after removal of the carapace. Krill were kept on ice during processing and homogenates were utilized for biochemical assays immediately. Separate individuals were processed for RNA:DNA, protein, and enzyme quantifications.

Separate analysis of krill from different trawl times or trawl depths within a cruise was not conducted.

Gut fullness was estimated with a subjective scale from 1 to 5, with 1 indicating an empty gut and 5 reflecting maximum observed fullness, based on intensity of color, distention and relative volume of the gut. Gut contents from a sample of krill assigned values of 1 were inspected under a dissecting microscope, but no organisms or body parts were observed (data not shown).

2.3. Nucleic acid quantification

Concentrations of RNA and DNA in abdominal muscle were determined by a fluorimetric method adapted from [Bentle et al. \(1981\)](#). Sample and reagent volumes were adjusted so that measurements could be accomplished in a HTS 7000 Perkin Elmer fluorimetric microplate reader. Individuals used in this assay remained frozen during removal of muscle samples. Muscle samples were homogenized on ice in 2 M NaCl (20 mg/400 μ l) with a glass homogenizer. Homogenates remained on ice until added to a buffer containing protease K and ethidium bromide, an initial solution that eliminates endogenous nucleases.

2.4. Enzyme assays and protein determination

LDH activity of abdominal muscle was determined according to the method of [Yancey and Somero \(1978\)](#). Abdominal muscle samples were homogenized in an 80 mM imidazole–HCl buffer (pH 7.0 at 20°C) for LDH activity assays. LDH activity was measured as μ mol pyruvate converted $\text{min}^{-1} \text{g muscle}^{-1}$, hereafter referred to as units g^{-1} .

Abdominal muscle was homogenized in a 50 mM imidazole–HCl buffer (pH 8.0 at 10°C) for determination of CS activity. CS was quantified according to the method of [Somero and Childress \(1980\)](#) as μ mol oxaloacetate converted $\text{min}^{-1} \text{g muscle}^{-1}$, hereafter referred to as units g^{-1} .

LDH and CS activities were quantified from abdominal muscle samples taken from the same individuals. All homogenates were held on ice until used in the enzyme assays. Protease degradation of

LDH and CS in the homogenates appeared minimal, since activities recorded from selected homogenates assayed at the beginning and end of the assay period were similar.

A preliminary investigation of the effect of temperature on LDH and CS activity showed that these enzymes in krill were not denatured by temperatures less than 15°C (data not shown). All enzyme activities reported here were measured at 8°C in temperature-controlled cuvettes in a UviKon spectrophotometer. The assay temperature was selected because activity measurements at 8°C yielded higher rates than at the in situ water temperature, yet this temperature was not high enough to risk denaturation of the enzymes. All enzyme activities are reported as mean international units (μ mol substrate converted min^{-1}) $\text{g muscle}^{-1} \pm$ standard error.

Total protein was determined with the bicinchoninic acid assay of [Smith et al. \(1985\)](#) after homogenization of abdominal muscle in 50 mM imidazole–HCl buffer (pH 8.0, 10°C). Water content of abdominal muscle was determined by drying tissue at 60°C for 24 h then dividing the mass of water lost by the mass of wet tissue and multiplying by 100.

2.5. Statistical analysis

A Kruskal–Wallis nonparametric analysis of variance was used to perform among-cruise comparisons of standard length, gut indices, enzyme activities, RNA:DNA, protein content, % water, and nucleic acid concentration. The Mann–Whitney U test was used to determine differences between females and males within a cruise. Results were considered significantly different at a p value of <0.05 . All statistical analyses were performed with Statistica for Windows v. 5.5.

3. Results

3.1. Krill size and gut index

On average, male krill used for biochemical analysis were larger than females, both in mass and length ([Table 1](#)). In addition, krill collected

Table 1

Mass (g), standard length (mm), gut index (1–5), total protein (mg protein g muscle⁻¹), and % water [mean ± standard error (*n*)] for krill used for biochemical analysis

Cruise	Mass	Standard length	Gut index	Protein	% Water
November 1999					
Male	0.51 ± 0.04 (27) ψ	45 ± 0.9 (27) ψ	2 ± 0.1 (27)	111.1 ± 3.72 (7)	83.61 ± 0.46 (7)
Female	0.36 ± 0.01 (35) ψ	41 ± 0.4 (35) ψ	2 ± 0.1 (35)	99.0 ± 4.83 (10)	80.37 ± 1.68 (8)
February 2000					
Male	1.31 ± 0.04 (26) ψ	53 ± 0.4 (26) ψ	3 ± 0.3 (26)	100.7 ± 2.73 (13)	76.43 ± 0.44 (8)
Female	1.19 ± 0.04 (18) ψ	51 ± 0.7 (18) ψ	3 ± 0.3 (18)	98.1 ± 3.10 (13)	76.46 ± 0.35 (10)
May 2000					
Male	1.42 ± 0.04 (49) ψ	56 ± 0.4 (49) ψ	1 ± 0.0 (49)	121.2 ± 3.13 (10)	76.51 ± 0.33 (10)
Female	1.10 ± 0.03 (57) ψ	52 ± 0.5 (57) ψ	1 ± 0.0 (57)	130.2 ± 3.67 (8)	77.09 ± 0.22 (9)
November 2000					
Male	1.14 ± 0.04 (36)	53 ± 0.5 (36)	3 ± 0.2 (36)	109.5 ± 3.71 (7)	81.08 ± 1.00 (7)
Female	1.08 ± 0.04 (36)	53 ± 0.6 (36)	3 ± 0.2 (36)	96.9 ± 10.6 (10)	78.95 ± 0.60 (9)

ψ indicates significant differences between males and females ($p < 0.05$). Significant differences were found for all parameters among all seasons for both males and females ($p < 0.05$).

during November 1999 were significantly smaller (mass and standard length) than krill collected on the other cruises, having an average weight of 0.44 ± 0.03 g for females and males combined ($n = 62$). By contrast, krill collected during May 2000 were the largest and had a mean weight of 1.26 ± 0.04 g ($n = 106$).

Gut indices for the same krill were lowest for animals collected during May 2000, late in austral autumn (Table 1). Male and female krill averaged 1.0 ± 0.0 ($n = 106$). Significantly higher values were observed for krill in late spring (Nov 2000) and midsummer (Feb 2000) samples.

3.2. RNA:DNA ratios

Female RNA:DNA ratios were highest for krill collected during February 2000 and November 2000 (Fig. 1). The lowest values for females were measured for krill collected during November 1999 and May 2000. The highest values for male RNA:DNA were measured for krill collected in November 2000 and the lowest values during November 1999. Significant differences were evident in RNA:DNA ratios for females ($p < 0.001$) and males ($p < 0.001$) among seasons. Significant differences were exhibited between RNA:DNA

ratios for females and males from November 1999 ($p < 0.05$), February 2000 ($p = 0.017$) and November 2000 ($p = 0.022$) krill.

Muscle RNA and DNA content varied significantly among seasons for both males and females (Table 2). Krill from November 1999 had a significantly higher muscle DNA content ($\mu\text{g DNA g muscle}^{-1}$) compared to krill from other seasons, and krill from May 2000 had significantly lower muscle RNA content ($\mu\text{g RNA g muscle}^{-1}$) compared to other sampling times. There was a significant difference in DNA content between males and females only in November 1999. However, there was a significant difference in RNA content between males and females in May 2000 and November 2000.

3.3. CS enzyme activities

Females showed the highest values of CS activity, 9.76 ± 0.49 units g^{-1} , during February 2000 (Fig. 2). The lowest value, 2.79 ± 0.60 units g^{-1} , was measured for females collected during November 1999. Male CS activity was also highest during February 2000 at 12.03 ± 0.87 units g^{-1} . The lowest value for males, 6.00 ± 0.83 units g^{-1} , was recorded in May 2000. Significant

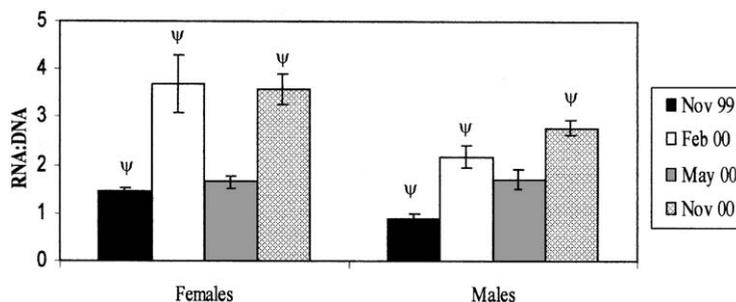


Fig. 1. RNA:DNA ratios for females and males for all seasons. ψ indicates significant differences between males and females ($p < 0.05$). Significant differences were found for RNA:DNA ratios among all seasons for both males and females ($p < 0.05$).

Table 2

RNA and DNA from abdominal muscle for males and females from all seasons

Cruise	$\mu\text{g RNA g muscle}^{-1}$	$\mu\text{g DNA g muscle}^{-1}$
November 1999		
Male	529.56 ± 45.35 (9)	632.14 ± 78.50 (9) ψ
Female	622.37 ± 66.08 (11)	429.9 ± 43.21 (11) ψ
February 2000		
Male	472.38 ± 50.88 (10)	216.35 ± 17.89 (10)
Female	689.04 ± 156.12 (10)	185.43 ± 23.05 (10)
May 2000		
Male	279.72 ± 15.32 (14) ψ	174.14 ± 14.47 (14)
Female	222.89 ± 44.19 (10) ψ	142.28 ± 9.82 (15)
November 2000		
Male	730.79 ± 44.19 (10) ψ	272.17 ± 15.02 (10)
Female	561.04 ± 59.28 (9) ψ	165.09 ± 22.84 (9)

ψ indicates significant differences between males and females ($p < 0.05$). Significant differences were found for all parameters among all seasons for both males and females ($p < 0.05$).

differences in CS activities were apparent among seasons for both females ($p = 0.019$) and males ($p = 0.0007$). Within a time period, significant differences between CS activity of females and males were observed only during November 1999 ($p = 0.044$).

3.4. LDH enzyme activities

The highest LDH activity in females, 23.27 ± 1.83 units g^{-1} , was measured in krill

collected during February 2000. By contrast, the lowest values for females were found in krill from November 1999 (12.08 ± 0.42 units g^{-1}) and November 2000 (11.99 ± 0.65 units g^{-1}). Significant differences were seen in LDH activities for females ($p = 0.0001$) and males ($p = 0.0004$) among seasons. Significant differences were found between LDH activity of females and males from November 1999 ($p < 0.05$), May 2000 ($p < 0.05$) and November 2000 ($p < 0.05$) (Fig. 3).

3.5. Total protein and percent water content

Significant differences were found for abdominal muscle protein content and % water among seasons for both males and females (Table 1). Krill collected in May 2000 had the highest protein content, 121.2 ± 3.13 (males) and 130.15 ± 3.67 mg protein g muscle^{-1} (females). The highest values for % water were measured in November 1999 for both males, $83.61 \pm 0.46\%$, and females, $80.37 \pm 1.68\%$. Significant differences existed between water content of males and females in November 1999 ($p = 0.028$) and May 2000 ($p = 0.045$).

4. Discussion

Metabolic indicators have been used in assessing physiological status for larval fishes (e.g., Lowery and Somero, 1990; Fiedler et al., 1998) and some marine invertebrates (Dahlhoff and Menge, 1996; Vetter et al., 1997; Geiger et al., 2001; Meyer et al.,

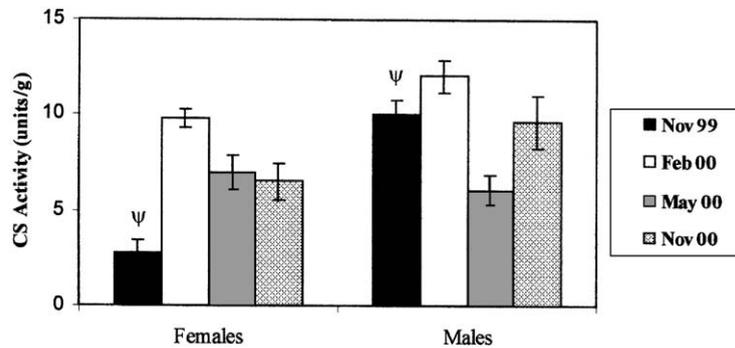


Fig. 2. CS activities (means \pm standard error) for female and male krill from November 1999 to November 2000. ψ indicates significant differences between males and females ($p < 0.05$). Significant differences were found for CS activities among all seasons for both males and females ($p < 0.05$).

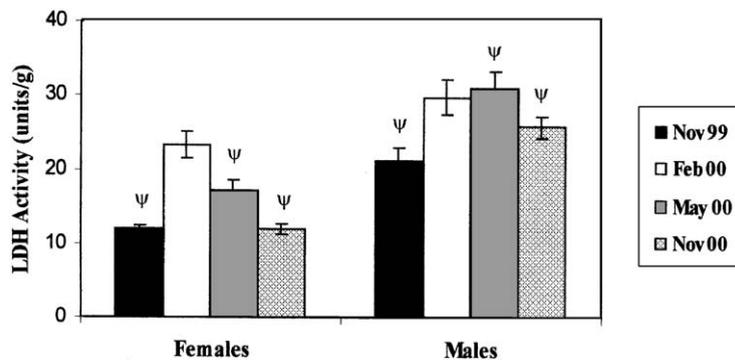


Fig. 3. LDH activities (means \pm standard error) for female and male krill from November 1999 to November 2000. ψ indicates significant differences between males and females ($p < 0.05$). Significant differences were found for LDH activities among all seasons for both males and females ($p < 0.05$).

2002). Strong links between nutritional status and metabolic indicators have been shown through laboratory manipulations of food ration and have also been observed in ecologically relevant settings. Since Antarctic krill have very high metabolic demands (Kils, 1981; Clarke and Morris, 1983; Opalinski, 1991), it is logical to expect that they would be exceptionally sensitive to seasonal changes in food resources. A close link between changes in phytoplankton availability and growth rates has been demonstrated with the IGR technique (Ross et al., 2000; Quetin and Ross, 1991; Ikeda and Dixon, 1982) and is supported by the data presented here from krill collected during the Erupt study.

Metabolic suppression has been proposed as an important potential strategy for surviving long

winters with scarce resources (Quetin and Ross, 1991). Evaluating biochemical indicators of metabolism can shed light on the relative significance of this strategy for krill overwintering at Port Foster, Deception Island and on the response of these krill to changes in phytoplankton through an annual cycle.

Phytoplankton blooms usually occur twice annually in this region, once in late November and again later in the summer (Laws, 1985), and this pattern was observed in Port Foster (Sturz et al., 2003). Low chlorophyll *a* concentrations were seen in early November followed by a phytoplankton bloom in late November (early spring), another bloom or continued high abundance in February (summer), and finally a decline as winter approached in May 2000 (autumn). Even

the lowest chlorophyll *a* concentrations observed in Port Foster in May 2000 were more than 20 times higher than autumn values in the southwestern Lazarev Sea (Meyer et al., 2002). Ice cover, which affects most pelagic species in Antarctica, was not present during the sampling periods within this study (Smith et al., 2003b).

Localized high concentrations of phytoplankton have been proposed as a crucial factor influencing the reproductive potential of krill populations (Ross and Quetin, 1986). Krill in bays where productivity levels between blooms may remain higher than oceanic regions therefore could play an important role in regional krill population growth. Biochemical indicators may provide insights into the relative robustness of krill collected from bays and potential comparisons to krill collected in ice-edge-associated blooms.

The effect of the first phytoplankton bloom in late November was apparent in the differences detected between krill collected when chlorophyll *a* levels were quite low in early November 1999 and those from late November 2000 when chlorophyll *a* levels were 25 times higher. Krill collected during the phytoplankton bloom of late November 2000 were feeding heavily (based on gut contents) and showed high RNA:DNA ratios. Similarly, krill captured in February 2000 had relatively full guts and high RNA:DNA ratios during a period characterized by elevated levels of chlorophyll *a* in surface waters. We saw no evidence of carnivory or detritus feeding in krill that showed the lowest gut index during May 2000 when the lowest chlorophyll *a* concentration was observed.

Reid (2001) stated that variation in the growth rates of krill most likely was due to interactions between temperature and food availability. Over an ecologically realistic range of temperatures, Antarctic krill oxygen consumption appears to be relatively temperature insensitive, with a Q_{10} near 1.0 (Opalinski, 1991). However, studies have shown adult krill respiration rates to be highly correlated with food availability (Atkinson et al., 2002; Meyer et al., 2002).

Compared to other biochemical indicators measured in the current study, RNA:DNA ratios showed the strongest association with food availability. RNA:DNA ratios for males and females

appear to mirror values of chlorophyll *a* abundance for all cruises, with the highest RNA:DNA ratios seen during sampling periods with the highest levels of chlorophyll *a*. This correlation is consistent with the rapid increases observed by Dahlhoff and Menge (1996) in RNA:DNA ratios for mussels responding to local phytoplankton blooms. Although high RNA:DNA ratios in krill from Port Foster were measured when sea surface temperature was at its highest (2.5°C), these increased ratios were similar to those seen during the chlorophyll *a* bloom in November 1999 when maximum water temperature was -0.2°C, suggesting that food availability rather than temperature was the more important determinant.

High RNA:DNA ratios suggest that krill rapidly respond to food availability with an increase in protein synthesis. Stimulation of abdominal muscle protein synthesis could facilitate an increased potential for power output by muscle used for burst swimming and for overall body growth. Elevated protein synthesis would place a high metabolic demand on krill that ultimately should be reflected in increased concentrations of metabolic enzymes as well as muscle protein concentrations. Indeed, CS activity was at a maximum in February 2000 when RNA:DNA ratios were high.

Geiger et al. (2001) found no significant changes in RNA:DNA for five species of calanoid copepods examined in relation to phytoplankton blooms associated with the receding ice edge in the northwestern Weddell Sea. They suggested that high food resources in conjunction with low Antarctic temperatures already had resulted in elevation of the copepods' RNA:DNA before their initial collection, implying that the ratio may be highly sensitive to immediate changes in food supply. Results reported here from krill collected during Erupt may show a tighter coupling of chlorophyll *a* concentration and RNA:DNA due to the seasonal sampling strategy or perhaps due to a specific focus on abdominal muscle tissue in large adult krill. Biochemical measurements performed by Geiger et al. (2001) involved whole copepods, and tissue-specific variations in RNA:DNA could have been obscured. Dahlhoff and Menge (1996) reported that biochemical

indicators in certain tissues were closely linked to environmental changes in food availability, while other tissues showed little correlation with the availability of food.

In addition to ratios varying as a function of food availability, RNA:DNA levels in *E. superba* appeared to vary according to gender with female krill showing a larger RNA:DNA ratio during seasons with the highest phytoplankton concentration. It is possible that females experience a higher seasonal fluctuation in protein synthesis rates as a result of the demands of reproductive efforts. Female krill allocate 13.6% of ingested energy to reproduction but only 3.4% to growth and molting (Ross and Quetin, 1986). At all seasons, female krill examined in the Erupt study were smaller than males in terms of both length and mass (Table 1), parameters substantially linked to total muscle mass and therefore total muscle metabolic demand. By virtue of their smaller size alone, females should have lower metabolic rates (Opalinski, 1991; Quetin and Ross, 1991) with less energy expended on muscle maintenance.

The largest krill measured in Port Foster were collected during May 2000 in austral autumn. It is possible that these krill exhibited accumulated growth stimulated by a summer spent feeding on high concentrations of phytoplankton. The smallest krill were collected in November 1999, possibly reflecting the consequences of a winter season characterized by food scarcity. Based on shrinkage during laboratory starvation experiments, Ikeda and Dixon (1982) proposed that krill shrink during the winter in response to limited food availability in the Southern Ocean, yielding an adult population in which krill of the same length may be different ages.

McGaffin et al. (2002) observed that shrunken adult krill (experimentally food limited) had nearly twice the density of muscle nuclei as adults (freshly caught) that had not shrunk. Krill from Erupt 2 (November 1999) exhibited DNA g muscle⁻¹ substantially higher than that of krill from any other sampling period. In fact, the DNA concentration was approximately twice that of other seasons, consistent with the doubled density of nuclei observed by McGaffin et al. (2002) in

experimentally shrunken krill. Coupled with their significantly smaller size, the high DNA concentration in November 1999 krill suggests that shrinkage in krill may occur by loss of cell volume, not cell number, as has been proposed for experimentally shrunken krill (McGaffin et al., 2002). Length differences between krill from Erupt 2 and those from other cruises were not nearly so conspicuous as mass differences (Table 1). Krill from Erupt 2 were unlikely to be juveniles, based on the presence of mature male reproductive structures and high muscle DNA concentrations, consistent with a shrunken state.

Other muscle parameters such as protein concentration and activities of CS and LDH were also low and water content was high in krill from early November 1999. These changes are consistent with utilization of muscle as an energy store and decreased muscle volume over winter. Changes in all other muscle parameters were less dramatic than the increase in muscle DNA concentration from krill collected at the end of winter, suggesting that relative proportions of cytoplasmic components are maintained as muscle cells shrink.

Since CS and LDH activities show mass specific changes (Somero and Childress, 1980; Berges et al., 1990), a subset of krill from a similar size range (38–60 mm standard length) was chosen to examine seasonal differences. Within this size range and within a single season, the slope of enzyme activity plotted against mass was not significantly different from zero (data not shown).

CS is a mitochondrial enzyme important to aerobic metabolism, and its concentration has been linked positively to metabolic rate in several Antarctic species (Torres and Somero, 1988; Meyer et al., 2002). Significant differences in CS activity among krill from different cruises suggest that Antarctic krill showed seasonal variation in metabolism, with reduced rates apparent during the autumn. Changes in CS activity from summer to autumn in krill collected at Deception Island were similar to those seen by Meyer et al. (2002) in adult krill collected in the southwestern Lazarev Sea. At all seasons, females from Deception Island showed lower CS activity, suggesting that their abdominal muscle metabolic demand may be consistently lower than that of males. Lower

muscle metabolic demand in female krill is consistent with lower metabolic rates observed in individual females by [Opalinski \(1991\)](#). CS values in females were more closely matched to general seasonal changes in food availability than were values in males. Females collected during early spring (November 1999) showed the lowest CS, suggesting that they achieved a greater overall metabolic depression than males during the previous winter.

[Geiger et al. \(2001\)](#) reported that CS in herbivorous copepods from the northwestern Weddell Sea changed in response to phytoplankton availability and was elevated in the same species that showed elevated oxygen consumption in response to phytoplankton blooms ([Kawall et al., 2001](#)). [Atkinson et al. \(2002\)](#) observed reduced feeding and respiration rates in Antarctic krill from the southwestern Lazarev Sea at the beginning of winter and concluded that experimental alterations of food availability also induced changes in respiration. [Meyer et al. \(2002\)](#) found that respiration rates of adult krill from the southwestern Lazarev Sea decreased from summer to autumn and CS decreased concurrently. Larval krill showed similar respiration rates during summer and autumn, with CS activity linked to size and stage rather than season. The low values of CS in females during the early spring sampling period at Deception Island are consistent with expected lower respiration retained after winter conditions of low temperature and food scarcity.

LDH is an enzyme important in maintaining redox balance during anaerobic metabolism, and its concentration often is correlated positively with burst swimming capabilities. LDH activity was lowest in early November 1999, perhaps reflecting a loss of enzyme activity in response to scarce food resources during the previous winter. LDH activity in fish is sensitive to feeding and activity levels, showing a decrease with starvation ([Lowery and Somero, 1990](#)) or inactivity. Male krill showed an increase in LDH activity from November 1999 to late autumn (May 2000), perhaps reflecting a long-term increase in food availability throughout the year and summer feeding on abundant phytoplankton. LDH activity was lower in late November 2000 compared to summer and autumn values,

but was higher than in early November 1999. Higher values in November 2000 compared to November 1999 could be related to the larger size of krill and the positive allometry of LDH ([Somero and Childress, 1980](#)) and/or to higher food availability during November 2000.

Summer increases in day length allow increased predation by visual predators such as chinstrap penguins, which are common at Deception Island ([Kendall et al., 2003](#)), and it is likely that krill perform vigorous tail flip escape responses to elude small, selective predators like penguins ([O'Brien, 1987](#)). Increased LDH activity at the end of summer would be consistent with enhanced burst swimming capability stimulated by repeated and vigorous tail flips. Krill swimming behavior in response to predators targeting the school should involve a coordinated escape response ([O'Brien, 1987](#)) that includes some localized tail flips but generally involves more consistent swimming patterns that might contribute to an elevated aerobic metabolism and higher CS as the summer progresses.

In contrast to the close correlation of chlorophyll *a* concentration and RNA:DNA ratios, enzyme concentrations appeared to lag behind changes in food abundance. This pattern can be seen in CS and LDH activity for both males and females. Higher protein levels were not evident until after a full season of increased food resources, but at a collection time coincident with decreased phytoplankton abundance. Enzyme activity appeared to increase throughout the summer as krill fed on generally elevated concentrations of phytoplankton and to decrease as krill start to utilize internal energy stores in response to a lack of food in autumn. However, RNA:DNA appeared to respond rapidly to phytoplankton blooms and decline when chlorophyll *a* levels decreased.

Males maintained higher enzyme activities than females, indicating that their burst and aerobic swimming performance may be higher than that of females throughout the year. Females showed lower mass-specific CS and LDH activities in general, even during seasons of phytoplankton abundance. Lower enzyme activity was particularly noticeable in the November 1999 samples.

These data support the hypothesis that females achieve greater metabolic suppression than males during overwintering. The consistently smaller size of females compared to males in early spring through autumn may provide an indication that females were allocating energy for reproduction.

Even the lowest phytoplankton concentrations observed by Sturz et al. (2003) in Port Foster were highly elevated compared to those of the autumnal southwestern Lazarov Sea (Meyer et al., 2002). If the phytoplankton levels seen in May 2000 and November 1999 generally characterized the lowest food availability, then krill from Port Foster may experience chlorophyll a levels considered crucial for successful reproduction (Ross and Quetin, 1986) early in the year and perhaps sustained long enough to support repeated bouts of reproduction. The robust condition of krill at Port Foster suggests that they may play an important role in krill population growth in this region.

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