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The effect of the receding ice edge on the condition of copepods in the northwestern Weddell Sea: results from biochemical assays

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Abstract

We compared six biochemical measures of nutritional condition: citrate synthase activity (CS), malate and lactate dehydrogenase activity (MDH and LDH), RNA:DNA ratio, and percent body protein and lipid. Adult females of five species of calanoid copepod (*Calanoides acutus*, *Calanus propinquus*, *Metridia gerlachei*, *Rhincalanus gigas* and *Paraeuchaeta antarctica*) were collected in the marginal ice zone of the northwestern Weddell Sea at the time of the annual phytoplankton bloom that occurs in association with the receding ice edge during austral spring. Three zones within the marginal ice zone were sampled: heavy-ice-cover pre-bloom, ice-edge bloom and low-ice-cover post-bloom. Lipid generally increased greatly from ice-covered to open water zones, and its importance in the life of polar copepods cannot be overstated. Increases in protein from ice-covered to open water were also observed, but were of less significance. Each species exhibited significant changes in at least one enzyme activity level. Citrate synthase activity in *C. acutus*, *C. propinquus* and *R. gigas*, all herbivores, increased between pre- and post-bloom stations. *C. propinquus* and *M. gerlachei*, which feed during winter, had large increases in LDH activity between pre- and post-bloom stations. *Rhincalanus gigas* and *P. antarctica*, the two largest species studied, showed variations in MDH activity, with peak enzyme activity occurring in post-bloom stations. RNA:DNA ratio did not change in any species. The effects of size, shipboard handling and freezer storage were easily corrected statistically, and did not alter any conclusions. The patterns observed in copepod nutrition at the Antarctic ice edge were consistent with existing models of life history for each species. The observations reported here, in conjunction with previously reported data, suggested that measurement of metabolic enzyme activity, especially in concert with lipid, enables estimation of nutritional condition in adult copepods. Additional studies comparing metabolic activity and ecology of common species should yield more information on the ecology of rarer species.

Introduction

In the wake of the receding Antarctic ice edge during austral spring, there is a large increase in primary productivity (Smith & Nelson, 1985; Bianchi et al., 1992), which may last up to 60 days. The bloom exhibits an average chlorophyll biomass of 2.7–3.5 mg m^{-3} (Laubscher et al., 1993), with peaks of 7.5–11.5 mg chl a m^{-3} (Lancelot et al., 1993). After the peak of the bloom further primary production is present at low levels for an additional one to three months (Gleitz et al., 1994). Garrison & Buck (1989) reported chlorophyll a values exceeding 1.0 mg m^{-3} as late as the 16 March in the vicinity of the ice edge. In the same study, biomass of protozooplankton (bacteria, heterotrophic flagellates, ciliates, tintinnids and sarcodines) exceeded 18 mg C m^{-3} in the upper 50 m.

The increase in productivity associated with the ice-edge bloom yields an increase in food supply for most copepods. Copepods usually represent more than 50% of the mesozooplankton biomass in oceanic waters of the Antarctic (Atkinson & Peck, 1988), and are nearly always numerically dominant. Three dominant species, *Calanoides acutus* (Giesbrecht), *Calanus propinquus* Brady and *Metridia gerlachei* Giesbrecht, typically comprise 50% of the biomass in surface wa-

ters of the Weddell Sea (Hopkins & Torres, 1988). Grazing pressure from copepods probably drives the changes in species composition of phytoplankton during the spring and summer season (Socal et al., 1997; Pakhomov et al., 1997). They are an important part of the pelagic community in the Southern Ocean, playing a role similar to that of their counterparts in cold waters throughout the globe.

Food supply is traditionally considered to be limiting for most species during the austral winter, and many species may enter into a period of dormancy or diapause to ameliorate the effects of starvation, followed by a rapid increase in condition once food is available. Two species thought to follow this strategy are primarily herbivorous: *C. acutus* (Schnack-Schiel et al., 1991) and *Rhincalanus gigas* Brady (Atkinson, 1991; Kattner et al., 1994). Some species may switch diets and continue feeding throughout winter, as is suspected of *C. propinquus* (Hopkins & Torres, 1989) and *M. gerlachei* (Schnack, 1985; Båmstedt & Tande, 1988). Still others are primarily carnivorous, such as *Paraeuchaeta antarctica* (Giesbrecht) (Hopkins et al., 1993), and would presumably be more independent of seasonal fluctuations. Variations in food supply will have physiological consequences, which vary depending on their severity and a species' ability to adapt to the changes.

Ferron & Leggett (1994) compared six broad classes of indices of nutritional condition in fish larvae: morphological, histological, RNA:DNA ratio (the ratio of ribo-deoxyribonucleic acids), proximate chemical composition (lipid), digestive enzymes and metabolic enzymes. All have been much more extensively studied in fish larvae than in other plankton. Lipid and digestive enzymes were generally found unreliable due to interference from gut contents. Histological and morphological indices were found to have low sensitivity. RNA:DNA, and metabolic enzymes seem to show promising results, combining good sensitivity and reliability. RNA:DNA is an indicator of protein synthesis rates; DNA content per cell is relatively constant (except during division), while RNA will vary depending on the demand for protein synthesis. Citrate synthase (CS) is the entry point to the Kreb's cycle, and is an indicator of aerobic potential. Lactate dehydrogenase (LDH), the terminal enzyme in anaerobic glycolysis, obviously controls an organism's anaerobic potential. Malate dehydrogenase (MDH) is important in the Kreb's cycle, NAD^+/NADH transfers across the mitochondrial membrane, gluconeogenesis and both lipid and amino acid processing pathways. It is thus widely used throughout a cell's metabolic machinery (Voet & Voet, 1995). With each enzyme, increases should indicate an increase in the ability to generate extra ATP, meaning increased scope for growth.

Each of the five dominant species of copepod used for this study deals with the Antarctic's large seasonal swings in productivity using their own unique strategy. The Antarctic ecosystem supplies a natural laboratory that can be used to study the changes in biochemical systems in these species. In this study, we show changes in metabolic (CS, LDH & MDH) and anabolic (RNA:DNA) biochemistry as well as basic measures of body composition (mass; protein, %P; lipid). The objectives of this study were: 1. to compare the reaction times of biochemical measures of condition in Antarctic copepods, and attempt to show that metabolic enzymes and nucleic acids are more responsive than basic measures of proximate chemical composition to changes in ambient food supply; and 2. to use all available measures of condition to assess current theories regarding the life histories of five species of copepods.

Materials and methods

Sample collection and processing

Five species of copepods were collected in sufficient quantities to address the study objectives: *C. acutus, R. gigas*, *C. propinquus, M. gerlachei* and *P. antarctica*. Only adult females were used in the current study. Sampling was conducted aboard the R/V Polar Duke during November and December 1993. A series of stations formed two transects, one west to east along the ice edge, and a second north to south into the pack ice (Fig. 1). The west to east transect was used to locate phytoplankton biomass peaks that coincided with the receding ice edge. The north to south transect was used to map the north–south extent of the bloom. It was initiated far enough north of the ice edge to be seaward of the bloom influence, as indicated by low chlorophyll and productivity. The transect then traversed the bloom area, and proceeded into the pack ice until a decrease in chlorophyll was again detected (Burghart et al., 1999). This was assumed to represent pre-bloom conditions. Five days of intense sampling were completed in each of the three regions, which were interpreted on the basis of chlorophyll biomass and productivity as representing pre-bloom, bloom and post-bloom conditions.

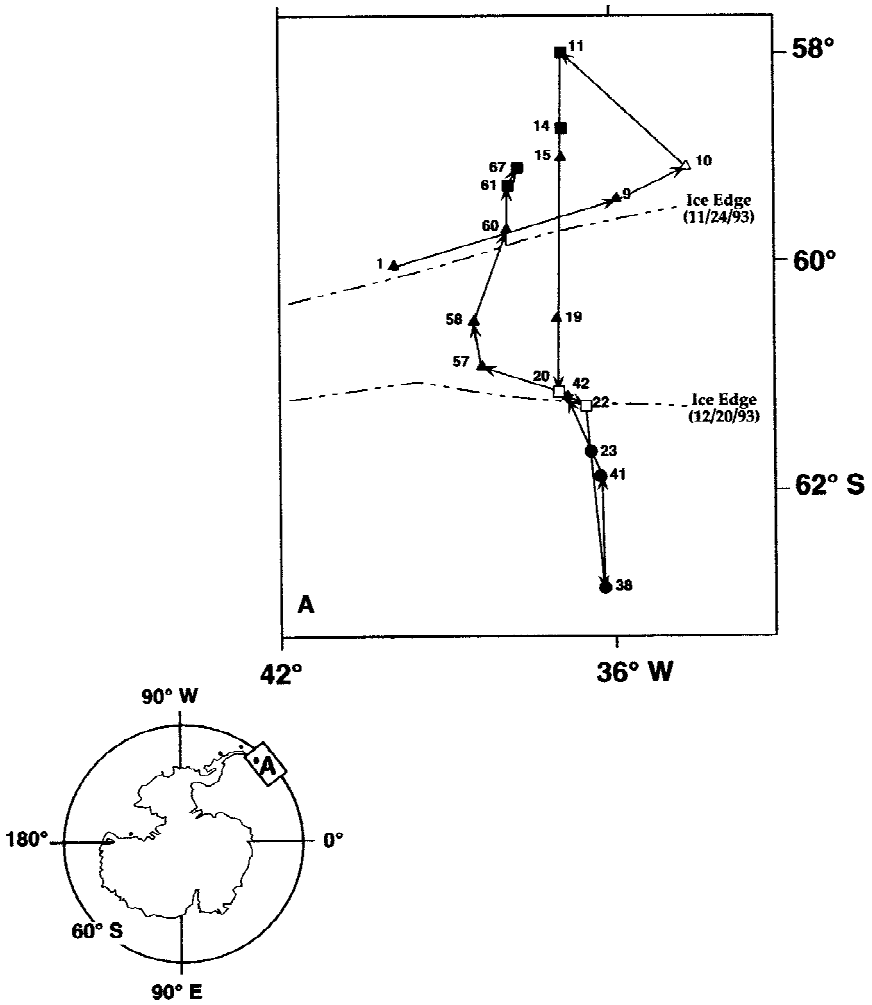

Figure 1. Location of sample region relative to Antarctic Peninsula (lower right). Inset (A): cruise track. Ice-covered stations (solid circles), 23–41; ice-edge stations (solid triangles), 42–60; open water stations (solid squares); 61–67.

Samples were collected using primarily a 1 m^2 vertically deployed (0–200 m) plummet net (163 μm). Additional samples were collected from tucker trawls (10 m^2 mouth area, 3 mm mesh in the main body of the net, 750 μm cod end) deployed obliquely from 0 to 1000 m (often) or deeper (less often). The contents of the cod end were diluted with 5–15 l of coarsely filtered surface water, and then transported to a shipboard lab where sorting was conducted. Ice-packs were used to maintain the temperature of the cod end bucket. Individual copepods were then chosen under a dissecting scope. Only adult females with no apparent damage to antennae or other appendages were chosen. Females with attached eggs or spermatophores were generally not used. However, in some of the *P. antarctica* samples, which were limited, spermatophores or eggs were present, but were removed before the females were used in assays.

Oxygen uptake rates were determined on individuals of each of the five dominant Antarctic species (Kawall et al., 2001). Oxygen consumption was measured by sorting out uninjured individuals from the cod end, rinsing them in filtered seawater, and then transferring the animals to 5 ml syringes. The needle end of the syringe had been removed and fitted with a Clark style oxygen electrode, which recorded PO_2 once every minute. These experiments lasted 10 h. Copepods not used in respiration experiments were frozen directly after sorting. Prior to freezing, all of the copepods were rinsed in filtered seawater, and then

in 280 mm sucrose to minimize osmotic shock. They were then blotted dry, placed in microcentrifuge tubes, and frozen in liquid N_2. Specimens were stored on board ship at –50 °C, transferred to St. Petersburg, Florida, on dry ice, and then placed in a cryogenic deep freeze, –80 °C, for long-term storage. Additional samples of each species from the trawl and plummet net catches from each zone were frozen directly after sorting, i.e. with no respiratory determinations.

Six biochemical indices of condition were used. Enzyme activities were determined by slight modifications of Sudgen & Newsholme (1975) for citrate synthase (CS) (Childress & Somero, 1979). Malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) activities were determined using the technique described in Walsh et al. (1989). All enzyme assays were run at 10 °C. Percent protein (%P) was determined according to Lowry et al. (1951). The ratio of ribonucleic – deoxyribonucleic acid (RNA:DNA) was determined by a sequential degradation fluorimetric technique modified from Bentle et al. (1981). The main modifications were as follows: an increased temperature, 37 °C, was used in all incubations, NaCl was added to the incubation after homogenization, and RNase was added before DNase. Lipid was determined by a modification of Bligh & Dyer (1959) suggested by Reisenbichler & Bailey (1991) using a reduced volume for all steps, but maintaining the solvent proportions designated. For details, see Geiger (1999).

For enzyme assays, %P, and RNA:DNA, each copepod was homogenized in 190 μl of ice-cold deionized water (or in a 25-fold dilution, whichever provided more homogenate; 1:24 w/v). Three aliquots were made from the homogenate: 80 μl for RNA:DNA, 40 μl for %P, and the remainder for metabolic enzymes. The RNA:DNA aliquot was combined with 120 μl 0.2 M NaCl, and the %P aliquot was combined with 40 μl H_2O. The aliquots were then frozen for one to several days at a temperature of –50 °C. Metabolic enzyme activity was assayed on the day the sample was homogenized. The citrate synthase assay required about 40 μl of homogenate, leaving about 20 μl for MDH and LDH assays. Ten μl of homogenate was used for each MDH and LDH assay, but these samples were occasionally insufficient to provide detectable enzyme activity. Thus, most copepods were analyzed for RNA:DNA, protein, CS, MDH and LDH. An additional group of animals was homogenized for lipid analysis only, because this technique requires larger quantities of homogenate. Each individual in the lipid analysis was homogenized at 25-fold (1:24 w/v) dilution (or 150 μl, whichever was larger) of deionized water, and 100 μl was used in the assay. Because of the small sizes of copepods, no replicates were possible for most animals in most assays.

Statistical analyses

The primary objective of the study was to compare the metabolic enzyme and nucleic acid assays with more traditional methods of analyzing nutritional condition. In order to do this, variation in the values for each species was examined between zones of the study area: pre-bloom, bloom and post-bloom, with two initial controls for artifact. Artifacts were eliminated from the analysis when they were not found to contribute a significant portion of the variability, i.e. groups were pooled. If the artifact did contribute to the overall variability, it was retained in the analysis for that species.

First, effects of freezer storage on each index were evaluated by regression analysis in order to determine if keeping tissue samples frozen for periods of up to 48 months resulted in any detectable changes. For indices that varied over time, freezer storage was retained as a covariate for further analysis. Second, both shipboard treatments of specimens, respired and non-respired animals, were compared either by *t*-test or the Mann-Whitney U test. In the cases where a respiration effect was apparent, this factor was retained in the analysis as a categorical factor. Since all specimens used for lipid analysis were quickly sorted and there was no variation in either of these first two factors, they were not applicable. Finally, the index was compared to length by correlation analysis. If the index was found to vary with size, analysis of covariance (ANCOVA) was performed, with the index as the dependent, and zone as independent variable (as well as freezer and respiration treatment if necessary), and size as covariate. If no variation with size was found, zonal variation was compared by ANOVA, if the assumptions of normal distribution and homogeneity of variances were met by the data. If the necessary assumptions were not met, transformation of the data in order to meet the assumptions of parametric statistics was attempted; if transformation was not successful, zonal variation was compared by a Kruskal-Wallis test. When any test indicated that significant variation occurred, the appropriate post-hoc analyses were conducted to determine where the variation occurred.

Results

The bloom associated with the receding ice-edge was extensive, with a marked increase in chlorophyll persistent along the entire ice edge front. Chlorophyll biomass in the top 40 m was 15–17 mg chl per m^2 in ice-covered stations (pre-bloom), peaked at 113 mg chl m^2 in the ice-edge bloom, and dropped to a mean of 69 mg chl m^2 in open water (post-bloom) (Burghart et al., 1999). The conditions indicated that our experimental condition of high chlorophyll in the bloom, with lower values (spatially and temporally) on either side of the bloom, was realized.

Freezer storage was retained as a covariate for the following significance analyses (Table 1).

Freezer storage affected both wet mass and MDH activity in *C. acutus*. Freezer storage affected wet mass, CS activity and MDH activity in *C. propinquus*. Freezer storage affected only RNA:DNA in *M. gerlachei*. Even though the months of freezer storage explained only about 11% of the variation, it was significant and was retained as a covariate for further analyses of RNA:DNA. Freezer storage did not affect any measure of condition in *R. gigas*. Freezer storage affected both LDH and RNA:DNA in *P. antarctica*.

Shipboard respiration treatment affected both wet mass and RNA:DNA in *C. acutus* (Table 1). In *C. propinquus*, it affected wet mass, CS, MDH and RNA:DNA, and it affected protein and RNA:DNA in *M. gerlachei*. Only wet mass was affected by shipboard respiration treatments in *R. gigas*. Shipboard respiration treatment affected both LDH and %P in *P. antarctica*.

Mass and % lipid varied with length in *C. acutus* (Table 1). Only mass varied with length in *C. propinquus* and *P. antarctica*. Wet mass, %P, and lipid varied with length in *M. gerlachei*. Wet mass, %P, and lipid varied with length in *R. gigas*.

Wet mass did not vary between zones in any species. As stated above, length accounted for most of the variability in wet mass.

Citrate synthase activity increased significantly at the ice edge, in open water, or both, in three of five species, but not *M. gerlachei* or *P. antarctica* (Table 2). In *C. acutus*, CS was higher in open-water stations than in edge and ice-covered stations; ice edge and ice-covered stations were similar to one another. In *C. propinquus* and *R. gigas*, CS was higher in both open-water and ice-edge stations than ice-covered stations.

Malate dehydrogenase activity varied significantly in two of five species (Table 3). In *R. gigas,* MDH was highest at open-water stations, but ice-edge and ice stations were similar to each other. In *P. antarctica*, MDH was higher at open-water than ice-edge stations, but other comparisons were not significant.

Lactate dehydrogenase activity in *C. propinquus* was lowest at ice-covered stations, increased at ice-edge stations, and was highest at open-water stations (Table 4). LDH in *M. gerlachei* was highest in open water, intermediate at ice-edge stations, and lowest at ice-covered stations.

Protein varied in three of five species (Table 5). Protein in *C. acutus* was higher at the open-water stations than in ice-covered stations, but ice-edge stations were intermediate and not significantly different from either. Protein in *C. propinquus* was highest at ice-edge stations, but %P at both ice-edge and ice-covered stations was similar to open-water stations. Protein in *M. gerlachei* was highest at the ice-edge stations, and ice-covered and open water stations were both lower but not different from each other.

RNA:DNA did not vary between zones in any species (Table 6). The artifacts of freezer storage, shipboard treatment and size of individuals caused most of the explained variability.

Lipid varied in four of the five species studied (Table 7). Lipid in *C. acutus* captured in open-water stations was higher than ice-covered stations, but other comparisons were not significant. Lipid in *C. propinquus* did not vary between zones. Lipid in *M. gerlachei* was higher at open water than ice-covered stations, other comparisons were not significant. Lipid in *R. gigas* was lowest at ice-covered stations, and higher at ice-edge and open water stations, which were similar to each other. Lipid in *P. antarctica* was higher at open water than ice-edge stations, other comparisons were not significant.

Discussion

Metabolic enzymes have received little attention in copepods. For example, LDH activity was not found in measurable levels in Clarke & Walsh (1993), while large, deep-sea copepods in the families Augaptilidae and Heterorhabdidae showed very high levels of LDH (Thuesen et al., 1998). We found measurable levels of LDH, CS and MDH in all five species of copepods studied. In our study, CS varied most strongly in the three most herbivorous species, *C. acutus*, *C. propinquus* and *R. gigas*. MDH varied most strongly in the two largest species, *R. gigas* and *P. antarctica*

Table 1. Results of the analyses of the effects of artifacts on nutritional condition indices. The first line of each species grouping shows the effects of freezer storage time (regression analysis). The second line shows the effects of shipboard respiration (*t*-test). The third line shows the effects of changes in copepod length (correlation analysis). Significance level indicated by $P < 0.05$, *; $P < 0.01$, **; $P < 0.001$. ***. 'Spp' = species column: Ca, *Calanoides acutus*; Cp, *Calanus propinquus*; Mg, *Metridia gerlachei*; Rg, *Rhincalanus gigas*; Pa, *Paraeuchaeta antarctica*. Coefficient of determination indicated by 'r^2'; correlation coefficient, '*r*'; 'Z' indicates Mann-Whitney U-test was performed; 't^Y' indicates a log transformation was used; '*t*' indicates an inverse transformation was used

Spp	Wet mass	CS	MDH	LDH	% P	RNA:DNA	Lipid
Ca	r^2= 0.090**	r^2 = 0.002	r^2 = 0.076*	r^2 = 0.014	r^2 = 0.002	r^2 = 0.326	–
	t = 2.106*	t = –0.44	Z = -1.620	t = –0.719	t =–0.453	t' = 4.566***	–
	r = 0.80*	r = –0.14	r = 0.12	r = 0.10	r = –0.17	r = –0.06	r = 0.54
Cp	r^2 = 0.039*	r^2 = 0.002*	r^2 = 0.102***	r^2 = 0.023	r^2 = 0.003	r^2 = 0.011	–
	t'=18.902***	t^Y = 9.029***	t = -3.119**	t^Y = 1.284	t' = 0.770	Z = 2.039*	–
	r = 0.67*	r = 0.04	r = 0.01	r = –0.23	r = –0.06	r = 0.19	r = –0.07
Mg	r^2 = 0.007	r^2 = 0.000	r^2 = 0.015	r^2 = 0.042	r^2 = 0.016	r^2 = 0.253***	–
	t = 0.906	t = 0.787	t = –1.219	t^Y = 1.757	t = –2.209*	Z = 4.033***	–
	r = 0.47*	r = –0.21	r = 0.14	r = 0.12	r = –0.33*	r = –0.25	r = –0.54*
Rg	r^2 = 0.005	r^2 = 0.013	r^2 = 0.128	r^2 = 0.002	r^2 = 0.005	r^2 = 0.016	–
	t = 0.906*	t = 0.088	t = –1.060	t = –1.145	t = 1.110	t = 1.291	–
	r = 0.82*	r = –0.11	r 0.02	r = –0.02	r = –0.39*	r = 0.09	r = 0.62^Y*
Pa	r^2 = 0.006	r^2 = 0.059	r^2 = 0.028	r^2 = 0.245**	r^2 = 0.044	r^2 = 0.086*	–
	Z = 0.852	t = 1.191	t = –1.569	t^Y = 4.077***	t = –2.363*	Z = 1.277	–
	r = 0.84*	r = –0.09	r = –0.36	r = 0.13	r = 0.15	r = 0.26	r = –0.16

Table 2. ANOVA results for citrate synthase activity (μmol substrate converted min^{-1} mg^{-1}) across three zones; ice, edge and open water. Means (+/– std. dev.) from raw data. Species abbreviations as follows: Ca, *Calanoides acutus*; Cp, *Calanus propinquus*; Mg, *Metridia gerlachei*, Rg, *Rhincalanus gigas*; Pa, *Paraeuchaeta antarctica*. F value, ANOVA test statistic (mean square effect / mean square error); significance values, $P < 0.05$, *; $P < 0.01$, **; $P < 0.001$,***; sample size, n. Log-transformed data indicated by yes or no (Y/–). Additional factors (Af) included: months of freezer storage prior to analysis, S, and length, L as covariates; respiration treatment, R as an additional grouping factor (the ANOVA results for Af are not shown). In cases where significant variation occurred, common groups are joined by similar superscripts (^{a} or ^{b})

Af	*F* value	*n*	Ice	Edge	Open	
Ca	–	3.772*	80	2.73 (2.763)^a	2.60 (1.368)^a	4.87 (4.614)^b
Cp	S, R, Y	4.691**	90	2.34 (0.895)^a	3.79 (2.889)^b	4.41 (3.474)^b
Mg	–	2.375	88	2.54 (1.173)	5.60 (6.215)	4.82 (7.583)
Rg	–	3.785*	79	0.80 (0.394)^a	1.91 (2.749)^b	1.95 (1.165)^b
Pa	S	0.073	39	1.68 (1.008)	1.42 (0.613)	1.27 (0.431)

Table 3. ANOVA results for MDH activity (μmol substrate converted min^{-1} mg^{-1}) across three zones. All abbreviations as in Table 2

	Af	*F* value	*n*	Ice	Edge	Open
Ca	S	0.321	68	13.3 (28.45)	14.6 (14.83)	12.3 (11.75)
Cp	S, R	1.042	85	9.1 (7.14)	16.3 (9.81)	16.9 (11.56)
Mg	–	0.879	74	15.4(17.90)	35.1 (52.78)	55.1 (164.29)
Rg	Y	8.824***	69	4.4 (3.21)^{a}	9.2 (15.35)^{a}	28.9 (55.92)^{b}
Pa	Y	3.550*	37	6.1 (2.30)^{a,b}	3.9 (1.61)^{a}	8.3 (5.08)^{b}

Table 4. ANOVA results for LDH activity (μmol substrate converted min^{-1} mg^{-1}) across three zones. All abbreviations as in Table 2

	Af	*F* value	*n*	Ice	Edge	Open
Ca	Y	2.502	48	4.6 (4.91)	10.1(13.78)	114 (414.6)
Cp	Y	6.203**	67	3.6 (3.19)^{a}	9.4(14.02)^{b}	14.9 (17.65)^{b}
Mg	Y	6.506**	43	6.8 (6.60)^{a}	9.5 (8.95)^{b}	45.6 (53.11)^{c}
Rg	–	0.528	61	5.8 (4.99)	26.2 (61.14)	20.0 (59.44)
Pa	S, R, Y	1.536	22	8.3 (6.24)	1.4 (0.75)	3.5 (3.62)

while LDH also varied significantly in two species, *M. gerlachei* and *C. propinquus*, that are capable of using omnivorous feeding to remain active in winter.

Nucleic acids did not vary significantly with zone in any species, even though increased growth and productivity clearly means that all five species must synthesize protein during the spring bloom. Ota & Landry (1984) concluded that RNA:DNA was too sensitive to temperature for use as an indicator of condition in copepods, but temperature did not vary in the cross-ice transect studied here. The RNA:DNA ratios reported by us, varying from 3.3 to 18.0, were higher than those observed for the more temperate *Calanus finmarchicus* in the Gulf of Maine, at a temperature of 12 °C (range: 0.5–7; Goolish et al., 1984). The RNA:DNA ratio can be expected to increase by about 50% over a 15 °C decline in temperature (Brightman et al., 1997). The combination of high food supply and the lower temperature in the Antarctic suggests that RNA:DNA had responded before we initiated sampling in late November. Thus, the high values of the copepods we observed may be due to good condition at all stations. Overall, the results of the current study suggest that RNA:DNA is not a good choice for assessing nutritional condition in adult copepods. In other life stages, especially copepodites and nauplii, RNA:DNA may be a more useful indicator (Wagner et al., 1998).

Body composition clearly changed across the zones. Both protein and lipid increased during the progression and passage of the bloom in four of the five species, while wet mass did not. These results suggest recovery from a period of famine. Lipid values available for Antarctic copepods vary from 0.4 to 13.3% of the wet mass, regardless of season (Clarke, 1984; Reinhardt & Van Vleet, 1986; Hagen, 1988; Donnelly et al., 1994). In the present study, the measured values were somewhat higher (3.2–18.9% WM). Recent studies showed lower protein levels (3–5% of wet mass) (Donnelly et al., 1994; estimates inferred from Hagen, 1995) than those we observed (4.2–11.7% of wet mass). The only exception to this pattern was found for *C. propinquus*, in which lipid and protein were intermediate between fall and winter values reported by Donnelly et al. (1994), and similar to those reported by Kattner et al. (1994) for winter. In the remaining four species, both lipid and protein were higher than previous reports.

Both proximate chemical composition (Buckley & Lough, 1987; Davis & Alatalo, 1992; Durbin & Durbin 1992; Christou & Verriopoulos, 1993) and enzyme activities (Berges et al., 1990; Childress & Somero, 1990) have been shown to vary with size. In this study, length affected only one measure of condition consistently, and that was wet mass. Body composition measures, protein and lipid, varied with

Table 5. ANOVA results for % protein across three zones (mg protein per mg wet mass × 100). All abbreviations as in Table 2

	Af	*F* value	*n*	Ice	Edge	Open
Ca	–	3.545*	80	7.4 (2.80)^{a}	8.6 (2.97)^{a,b}	9.6 (3.13)^{b}
Cp	–	5.281**	94	8.8 (2.43)^{a}	11.1 (3.34)^{b}	10.9 (2.48)^{a,b}
Mg	L, R	10.354***	85	7.1 (2.87)^{a}	11.4 (3.18)^{b}	8.0 (3.75)^{a}
Rg	–	0.488	77	4.2 (1.07)	5.7 (2.47)	6.3 (1.65)
Pa	L, R	3.168	37	11.7 (5.27)	8.8 (1.71)	8.5 (2.18)

Table 6. ANOVA results for RNA:DNA ratio across three zones. All abbreviations as in Table 2

	Af	*F* value	*n*	Ice	Edge	Open
Ca	R, Y	0.063	81	10.7 (7.50)	15.2 (8.74)	18.0 (13.59)
Cp	R	0.805	91	12.0 (4.71)	10.6 (5.52)	9.26 (5.13)
Mg	S, R, Y	1.556	87	17.5 (14.26)	13.3 (6.86)	12.3 (6.05)
Rg	–	1.597	77	4.5 (2.67)	4.9 (2.49)	5.2 (2.24)
Pa	S	2.40	37	3.3 (1.88)	5.8 (2.29)	4.6 (2.35)

changes in length in two out of five and three out of five species, respectively. The protocol of using only adult females undoubtedly affected this result; when the full size range of a species is used, size-related differences are more easily discerned. The four biochemical measures (CS, LDH, MDH and RNA:DNA) did not vary with size at all, underscoring one of the benefits of the indices. Comparisons between populations, and sometimes even between species, are possible.

In any measure of condition, variability related to sample processing should not be confused with trends in the actual data. In our study, variability due to processing artifacts, shipboard respiration and freezer storage time, was easily accounted for statistically. Retaining these factors in the statistical analyses, when warranted, allowed us to deal with any variability they generated, so that it did not obscure the trends shown by the data as a whole. Freezer storage time had little effect on any measure of condition except in one species, *C. propinquus* (Table 1). In contrast, more differences were observed between respired and non-respired copepods: 11 of 30 comparisons were significant.

The marginal ice zone examined in the present study, and the zonal occupations used to study it, offered a compressed temporal view of the spring bloom common to polar climates (Burghart et al., 1999). The local currents were generally from the southwest (under hard ice) towards the northeast. Thus, any planktonic organisms present would be unlikely to have encountered an abundance of food prior to our arrival at ice-covered stations. However, some variability in past feeding history cannot be ruled out, especially in open water, post-bloom stations.

Calanoides acutus has been described as largely herbivorous, feeding intensely during spring and summer, followed by a fall-winter dormancy. *Calanoides acutus* individuals fast in winter (Bathmann et al., 1993; Pasternak, 1995) and then mate in late winter, during August and September, before beginning spring feeding (Marín, 1988a). The adult females collected for the present study during the height of the spring bloom showed obvious signs of recovery from fasting. Three of seven measures of condition (CS, % P and lipid) increased significantly in bloom and open-water stations. Kawall et al. (2001) measured similar increases in oxygen uptake rates at the same stations. Our results suggest that females had mated in August/September, and were either preparing to spawn or were actually spawning. This is in agreement with previous studies on the life cycle strategy of *C. acutus* (Andrews, 1966; Marín, 1988b; Marín & Schnack-Schiel, 1993; Lopez et al., 1993; Burghart, 1999).

Table 7. ANOVA results for % lipid (mg lipid per mg wet mass ∗ 100) across three zones. All abbreviations as in Table 2. 'H' indicates a Kruskal-Wallis test was run for Pa; values listed are median and interquartile ranges

	Af	*F* value	*n*	Ice	Edge	Open
Ca	L, Y	4.500*	15	6.7 (2.37)^{a}	11.8 (5.13)^{a,b}	18.9 (7.66)^{b}
Cp	–	0.708	15	4.3 (2.32)	5.0 (1.04)	4.1 (0.82)
Mg	Y	4.548*	15	9.0 (1.04)^{a}	9.9 (1.33)^{a,b}	12.7 (3.28)^{b}
Rg	L, Y	4.147*	15	3.2 (7.16)^{a}	7.8 (8.34)^{b}	13.8 (7.98)^{b}
Pa^{H}	–	6.634**	24	8.6 (3.41)^{a,b}	6.4 (3.30)^{a}	14.2 (5.74)^{b}

Calanus propinquus may feed at relatively stable levels all year long (Bathmann et al., 1993; Delgado et al., 1998) or feeding may be reduced in winter (Pasternak, 1995). The species opportunistically shifts its diet from strict or preferential herbivory to a diet of dinoflagellates and ciliates (Hopkins et al., 1993; Atkinson et al., 1996). The flexibility provided in an omnivorous diet likely allows flexibility in reproductive strategies; when a good food source is available, excess energy may be used for egg production. Spawning has been observed prior to the bloom, enabling nauplii to utilize the peak production (Burghart, 1999), but egg production usually peaks in late December (Kurbjeweit, 1997) with early copepodites found between March and May (Marín, 1988a; Marín & Schnack-Schiel, 1993). Activity probably increases in spring, as indicated by the rise in metabolic enzyme activity and oxygen consumption (Kawall et al., 2001) that also peaked in ice-edge bloom stations, but stockpiling of lipid reserves may not be as urgent a need as in more strict herbivores. The high variability in lipid at ice-covered stations may be indicative of two groups with asynchronous life histories (adult overwinterers and recently molted adults which overwintered as CV's) (Bathmann et al., 1993; Hopkins et al., 1993), although we could not discern two distinct modes.

Metridia gerlachei was actively feeding and migrating daily during the spring bloom (Lopez & Huntley, 1995) and clearly fed through the winter (Hopkins et al., 1993; Pasternak, 1995), with a diet of dinoflagellates and ciliates, but at times preferentially feeding on diatoms (Atkinson et al., 1996). This species would be expected to be less reliant on the early spring bloom of diatoms than are more strict herbivores. However, *M. gerlachei* has a protracted spawning season, and must rely on short-term food supply for continued egg production (Huntley & Escritor, 1992; Schnack-Schiel & Hagen, 1995; Kurbjeweit, 1997). Calbet & Irigoien (1997) suggested that large lipid stores were accumulated early in the summer (which we also observed), and that these lipid stores shielded *M. gerlachei* from short-term fluctuations in food supply to enable continuous, low-level egg production through the season. Large increases in LDH activity (anaerobic potential) may be related to increased metabolic activity associated with mating at the ice-edge, followed by a spawn of eggs in open waters (Kurbjeweit, 1997).

Rhincalanus gigas below the Antarctic Convergence is at the southernmost limit of its distribution and requires two (Marín, 1988a, 1988b) to three (Bathmann et al., 1993) years to complete its life cycle. At lower latitudes, from the Antarctic Circumpolar Current and northward, reproduction is most likely annual (Ommaney, 1936 in Bathmann, 1993). *Rhincalanus gigas* clearly showed evidence of a rapid recovery from a period of reduced feeding (Marín, 1988a; Pasternak, 1995), especially through rapid increases in lipid (Ward et al., 1996; this study). Peaks in enzyme activity (CS and MDH) and in oxygen consumption (Kawall et al., 2001) in open water stations indicate a large rise in metabolic activity. Our results suggest that spawning was imminent, agreeing with previous reports of early copepedites found from December through May (Marín, 1988b; Marín & Schnack-Schiel, 1993).

Paraeuchaeta antarctica showed few clear trends in condition, with large variability in all indices. The high variability may be related to its seasonal ontogeny, which is more protracted than the other four species studied; reproductively active females have been observed in all seasons (Littlepage, 1964, in Hagen, 1995; Clarke, 1984; Øresland, 1995). We collected females with attached spermatophores and with eggs in ice stations. The drop in condition at edge stations may indicate either spawning, where stored

energy is used for increased egg production, or increased respiration of recently molted CVs into CVI adults, which would also have temporarily reduced energy stores.

The use of metabolic enzymes can enhance the understanding of physiological processes in copepods. In each species studied, at least one biochemical measure of nutritional condition varied significantly, and correlated with the better-established measures of proximate chemical composition. A benefit that was not discussed yet is the use of much smaller amounts of tissue than more traditional assays. Some measures of proximate chemical composition, such as percent water and ash, require pooled samples of several animals to measure. To measure lipid accurately requires most of the tissue from a single copepod in small species. In contrast, several biochemical indicators can be measured on a single individual. Secondly, the amplitude of change was usually high in biochemical measures relative to proximate composition. The one biochemical measure that yielded disappointing results was RNA:DNA, which appeared more sensitive to artifactual effects (freezer storage, shipboard effects) than other biochemical measures. The observation that the response of the seven measures chosen was similar in species which have similar life history strategies suggests that biochemical measures might be useful in elucidating life histories for rarer species, where sample size is limited.

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References

Andrews, K. J. H., 1966. The distribution and life-history of *Calanoides acutus* (Giesbrecht). Discovery Rep. 34: 117–162.

Atkinson, A., 1991. Life cycles of *Calanoides acutus*, *Calanus simillimus* and *Rhincalanus gigas* (Copepoda: Calanoida) within the Scotia Sea. Mar. Biol. 109: 79–91.

Atkinson, A. & J. M. Peck, 1988. A summer-winter comparison of zooplankton in the oceanic area around South Georgia. Polar Biol. 8: 463–473.

Atkinson, A., P. Ward & E. J. Murphy, 1996. Diel periodicity of Subantarctic copepods: relationships between vertical migration, gut fullness and gut evacuation rate. J. Plankton Res. 18: 1387–1405.

Båmstedt, U. & K. S. Tande, 1988. Physiological responses of *Calanus finmarchicus* and *Metridia longa* (Copepoda: Calanoida) during the winter-spring transition. Mar. Biol. 99: 31–38.

Bathmann, U. V., R. R. Makarov, V. A. Spiridonov & G. Rohardt, 1993. Winter distribution and overwintering strategies of the Antarctic copepod species *Calanoides acutus*, *Rhincalanus gigas* and *Calanus propinquus* (Crustacea, Calanoida) in the Weddell Sea. Polar Biol. 13: 333–346.

Bentle, L. A., S. Dutta & J. Metcoff, 1981. The sequential determination of DNA and RNA. Anal. Biochem. 116: 5–16.

Berges, J. A., J. C. Roff & J. S. Ballantyne, 1990. Relationship between body size, growth rate and maximal enzyme activities in the brine shrimp, *Artemia franciscana*. Biol. Bull. 179: 287–296.

Bianchi, F., A. Boldrin, F. Cioce, G. Dieckmann, H. Kuosa, A.-M. Larsson, E.-M. Nöthig, P.-I. Sehlstedt, G. Socal & E. E. Syvertsen, 1992. Phytoplankton distribution in relation to sea ice, hydrography and nutrients in the northwestern Weddell Sea in early spring 1988 during EPOS. Polar Biol. 12: 225–235.

Bligh, E. G. & W. J. Dyer, 1959. A rapid method of total lipid extraction. Can. J. Biochem. Physiol. 37: 911–937.

Brightman, R. I., J. J. Torres, J. Donnelly & M. E Clarke, 1997. Energetics of larval red drum, *Sciaenops ocellatus*. Part II: Growth and biochemical indicators. Fish. Bull. U. S. A. 95: 431–444.

Buckley, L. J. & R. G. Lough, 1987. Recent growth, biochemical composition and prey field of larval haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) on Georges Bank. Can. J. Fish. aquat. Sci. 44: 14–25.

Burghart, S. E., T. L. Hopkins, G. A. Vargo & J. J. Torres, 1999. Effects of a rapidly receding ice edge on the abundance, age structure and feeding of three dominant calanoid copepods in the Weddell Sea, Antarctica. Polar Biol. 22: 279–288.

Calbet, A. & X. Irigoien, 1997. Egg and faecal pellet production rates of the marine copepod *Metridia gerlachei* northwest of the Antarctic Peninsula. Polar Biol. 18: 273–279.

Childress, J. J. & G. N. Somero, 1979. Depth-related enzymic activities in muscle, brain and heart of deep-living pelagic marine teleosts. Mar. Biol. 52: 273–283.

Childress, J. J. & G. N. Somero, 1990. Metabolic scaling: a new perspective based on scaling of glycolytic enzyme activities. Am. Zool. 30: 161–173.

Christou, E. D. & G. C. Verriopoulos, 1993. Length, weight and condition factor of *Acartia clausi* (Copepoda) in the eastern Mediterranean. J. mar. biol. Ass. U. K. 73: 343–353.

Clarke, A., 1984. The lipid content and composition of some antarctic macrozooplankton. Bull. brit. Antarct. Surv. 63: 57–70.

Clarke, M. E. & P. J. Walsh, 1993. Effect of nutritional status on citrate synthase activity in *Acartia tonsa* and *Temora longicornis*. Limnol. Oceanogr. 38: 414–418.

Davis, C. S. & P. Alatalo, 1992. Effects of constant and intermittent food supply on life-history parameters in a marine copepod. Limnol. Oceanogr. 37: 1618–1639.

Delgado, L. E., R. Jaña & V. H. Marín, 1998. Testing hypotheses on life-cycle models for Antarctic calanoid copepods, using qualitative, winter, zooplankton samples. Polar Biol. 20: 74–76.

Donnelly, J. J., J. J. Torres, T. L. Hopkins & T. M. Lancraft. 1994. Chemical composition of Antarctic zooplankton during austral fall and winter. Polar Biol. 14: 171–183.

Durbin, E. G. & A. G. Durbin, 1992. Effects of temperature and food abundance on grazing and short-term weight change in the marine copepod *Acartia hudsonica*. Limnol. Oceanogr. 37: 361–378.

Ferron, A & W. C. Leggett, 1994. An appraisal of condition measures for marine fish larvae. Adv. mar. Biol. 30: 217–303.

Garrison, D. L. & K. R. Buck, 1989. Protozooplankton in the Weddell Sea, Antarctica: abundance and distribution in the ice-edge zone. Polar Biol. 9: 341–351.

Geiger, S. P., 1999. A comparative assessment of biochemical measures of nutritional condition in pelagic organisms: oceanic frontal systems as natural laboratories. Ph.D. Dissertation. Univ. of South Florida, Tampa: 150 pp.

Gleitz, M., U. V. Bathmann & K. Lochte, 1994. Build-up and decline of summer phytoplankton biomass in the eastern Weddell Sea, Antarctica. Polar Biol. 14: 413–422.

Goolish, E. M., M. G. Barron & I. R. Adelman, 1984. Thermoacclimatory response of nucleic acid and protein content of carp muscle tissue: influence of growth rate and relationship to glycine uptake by scales. Can. J. Zool. 62: 2164–2170.

Hagen, W., 1995. On the lipid biochemistry of polar copepods: compositional differences in the Antarctic calanoids *Euchaeta antarctica* and *Euchirella rostromagna*. Mar. Biol. 123: 451–457.

Hagen, W., 1988. Zur Bedeutang der Lipide im antarktischen Zooplankton. Ber Polarforsch 49: 1–129 (in German) (English version, 1989: on the significance of lipids in Antarctic zooplankton. Can Transl. Fish. Aq. Sciences 5458: 1–149.)

Hopkins, T. L., D. G. Ainley, J. J. Torres & T. M. Lancraft, 1993. Trophic structure in open waters of the marginal ice zone in the Scotia-Weddell confluence region during spring (1983). Polar Biol. 13: 289–397.

Hopkins, T. L. & J. J. Torres, 1988. The zooplankton community in the vicinity of the ice edge, western Weddell Sea, March 1986. Polar Biol. 9: 79–87.

Hopkins, T. L. & J. J. Torres, 1989. Midwater food web in the vicinity of a marginal ice zone in the western Weddell Sea. Deep-Sea Res. 36: 543–560.

Huntley, M. E. & F. Escritor, 1992. Ecology of *Metridia gerlachei* Giesbrecht in the western Bransfield Strait, Antarctica. Deep Sea Res. 39: 1027–1055.

Kattner, G., M. Graeve & W. Hagen, 1994. Ontogenetic and seasonal changes in lipid and fatty acid/alcohol compositions of the dominant Antarctic copepods *Calanus propinquus*, *Calanoides acutus* and *Rhincalanus gigas*. Mar. Biol. 118: 637–644.

Kawall, H. G., J. J. Torres & S. P. Geiger, 2001. The effects of the ice-edge bloom and season on the metabolism of copepods in the Weddell Sea. Hydrobiol./Dev. Hydrobiol. 453/454: 67–77.

Kurbjeweit, F., 1997. Reproduction of *Calanus propinquus* and *Metridia gerlachei* in spring and summer in the Weddell Sea, Antarctica. In Battaglia B., J. Valencia & D. W. H. Walton (eds), Antarctic Communities: Species, Structure and Survival. Cambridge Univ. Press, Cambridge: 112–117.

Lancelot, C., S. Mathot, C. Veth & H. De Baar, 1993. Factors controlling phytoplankton ice-edge blooms in the marginal ice-zone of the northwestern Weddell Sea during sea ice retreat 1988: field observations and mathematical modeling. Polar Biol. 13: 377–387.

Laubscher, R. K., R. Perissinotto & C. D. McQuaid, 1993. Phytoplankton production and biomass at frontal zones in the Atlantic sector of the Southern Ocean. Polar Biol. 13: 471–481.

Littlepage, J. L., 1964. Seasonal variation in lipid content of two Antarctic marine Crustacea. In Carrick R., M. W. Holdgate, & J. Prevost (eds), Biologie Antarctique. 1st SCAR Symp., Hermann, Paris: 463–470 (not seen, cited in Hagen, 1996).

Lopez, M. D. G. & M. E. Huntley, 1995. Feeding and diel vertical migration cycles of *Metridia gerlachei* (Giesbrecht) in coastal waters of the Antarctic Peninsula. Polar Biol. 15: 21–30.

Lopez, M. D. G., M. E. Huntley & J. T. Lovette, 1993. *Calanoides acutus* in Gerlache Strait, Antarctica. I. Distribution of late copepodite stages and reproduction during spring. Mar. Ecol. Prog. Ser. 100: 153–165.

Lowry, O. H., N. J. Rosebrough, A. L. Farr & R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

Marín, V., 1988a. Independent life cycle: an alternative to the asynchronism hypotheses for Antarctic calanoid copepods. Hydrobiologia 167: 161–168.

Marín, V., 1988b. Qualitative models of the life cycles of *Calanoides acutus*, *Calanus propinquus* and *Rhincalanus gigas*. Polar Biol. 8: 439–446.

Marín, V. H. & S. B. Schnack-Schiel, 1993. The occurrence of *Rhincalanus gigas*, *Calanoides acutus* and *Calanus propinquus* (Copepoda: Calanoida) in late May in the area of the Antarctic Peninsula. Polar Biol. 13: 35–40.

Ommaney, F. D., 1936. *Rhincalanus gigas* (Brady), a copepod of the southern macroplankton. Discovery Rep. 13: 277–384 (not seen, cited in Bathmann, 1993).

Øresland, V., 1995. Winter population structure and feeding of the chaetognath *Eukrohnia hamata* and the copepod *Euchaeta antarctica* in Gerlache Strait, Antarctic Peninsula. Mar. Ecol. Prog. Ser. 119: 77–86.

Ota, A. Y. & M. R. Landry, 1984. Nucleic acids as growth rate indicators for early developmental stages of *Calanus pacificus* Brodsky. J. exp. mar. Biol. Ecol. 80: 147–160.

Pakhomov, E. A., H. M. Verheye, A. Atkinson, R. K. Laubscher & J. Taunton-Clark, 1997. Structure and grazing impact of the mesozooplankton community during late summer 1994 near South Georgia, Antarctica. Polar Biol. 18: 180–192.

Pasternak, A. F., 1995. Gut contents and diel feeding rhythm in dominant copepods in theice-covered Weddell Sea, March, 1992. Polar Biol. 15: 583–586.

Reinhardt, S. B. & E. S. Van Vleet, 1986. Lipid composition of twenty-two species of Antarctic midwater zooplankton and fish. Mar. Biol. 91: 149–159.

Reisenbichler, K. R. & T. G. Bailey, 1991. Microextraction of total lipid from mesopelagic animals. Deep Sea Res. 38: 1331–1339.

Schnack, S. B., 1985. Feeding by *Euphausia superba* and copepod species in response to varying concentrations of phytoplankton. In Siegfried W. R., P. R. Condy & R. M. Laws (eds), Antarctic Nutrient Cycles and Food Webs. Springer-Verlag, Berlin: 311–323.

Schnack-Schiel, S. B. & W. Hagen, 1995. Life-cycle strategies of *Calanoides acutus*, *Calanus propinquus* and *Metridia gerlachei* (Copepoda: Calanoida) in the Bellingshausen Sea, Antarctica. ICES J. mar. Sci. 52: 541–548.

Schnack-Schiel, S. B., W. Hagen & E. Mizdalski, 1991. Seasonal comparison of *Calanoides acutus* and *Calanus propinquus* (Copepoda: Calanoida) in the southeastern Weddell Sea, Antarctica. 1991. Mar. Ecol. Prog. Ser. 70: 17–27.

Smith, W. O., Jr. & D. M. Nelson, 1985. Phytoplankton bloom produced by a receding ice edge in the Ross Sea: spatial coherence with the density field. Science 227: 163–166.

Socal, G., E. M. Nöthig, F. Bianchi, A. Boldrin, S. Methot & S. Rabitti, 1997. Phytoplankton and particulate matter at the Wed-

dell / Scotia Confluence (47° W) in summer 1989, as a final step of a temporal succession (EPOS project). Polar Biol. 18: 1–9.

Sudgen, P. H. & E. A. Newsholme, 1975. Activities of citrate synthase, NAD^+-linked and $NADP^+$-linked isocitrate dehydrogenases, glutamate dehydrogenase, aspartate aminotransferase and alanine aminotransferase in nervous tissues from vertebrates and invertebrates. Biochem. J. 150: 105–111.

Thuesen, E. V., C. B. Miller & J. J Childress, 1998. An ecophysiological interpretation of oxygen consumption rates and enzymatic activities of deep-sea copepods. Mar. Ecol. Prog. Ser. 168: 95–107

Voet, D. & J. G. Voet, 1995. Biochemistry. 2nd edn. John Wiley & Sons, inc. New York.

Wagner, M., E. Durbin & L. Buckley, 1998. RNA:DNA ratios as indicators of nutritional condition in the copepod *Calanus finmarchicus*. Mar. Ecol. Prog. Ser. 162: 173–181.

Walsh, P. J., C. Bedolla & T. P. Mommsen, 1989. Scaling and sex-related differences in toadfish (*Opsanus beta*) sonic muscle enzyme activities. Bull. mar. Sci. 45: 68–75.

Ward, P., R. S. Shreeve & G. C. Cripps, 1996. *Rhincalanus gigas* and *Calanus simillimus*: lipid storage patterns of two species of copepod in the seasonally ice-free zone of the Southern Ocean. J. Plankton Res. 18: 1439–1454.