

Limnol. Oceanogr., 47(4), 2002, 943–952

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Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter—I. Furcilia III larvae

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Abstract

Physiological condition and feeding behavior of furcilia larvae were investigated in autumn (April 1999) in the southwestern Lazarev Sea prior to the critical overwintering period. Furcilia stage III (FIII) larvae were most abundant, so only these were used for all analyses (dry mass [DM], elemental and biochemical composition, gut content) and experiments (metabolic and ingestion rates, selective feeding behavior). Chlorophyll *a* (Chl *a*) concentrations in the mixed layer were $<0.1 \mu\text{g L}^{-1}$. Respiration rates of freshly caught FIII larvae were between 0.4 and $1.2 \mu\text{l O}_2 \text{ mg}^{-1} \text{ DM h}^{-1}$, similar to larvae fed for 7 d on high food concentrations ($4 \mu\text{g Chl } a \text{ L}^{-1}$). Excretion rates ranged between 0.01 and $0.02 \mu\text{g NH}_4 \text{ mg}^{-1} \text{ DM h}^{-1}$. Their atomic O:N ratio of 72 indicated that lipids were the main metabolic substrate of FIII larvae in the field. The daily C ration ranged from 0.4% at the lowest food concentration of $3 \mu\text{g C L}^{-1}$ to 28% at the highest enriched food concentration of $216 \mu\text{g C L}^{-1}$, whereas clearance rates decreased with increasing food concentrations. In natural seawater, $115 \text{ ml mg}^{-1} \text{ C h}^{-1}$, and in natural seawater enriched with ice biota, $24 \text{ ml mg}^{-1} \text{ C h}^{-1}$, the clearance rates on specific phytoplankton taxa revealed no significant difference across a food size range of 12–220 μm . The study suggests that during periods of low food supply in the water column, larvae have to exploit ice biota to cover their metabolic demands.

Antarctic krill (*Euphausia superba*, hereafter “krill”) is a key species in the Antarctic ecosystem, being a major food item for a large number of top predators such as whales, seals, and sea birds. Krill are very successful in the extreme environment of the Southern Ocean because they are capable of exploiting a food supply that is both patchy and seasonal (Quetin and Ross 1991). However, various aspects of their biology are still poorly known. Most of the available infor-

mation is from spring and summer and is based on data from juvenile and adult krill. Comparatively little is known about their larval ecology. Most data on krill larvae concern their distribution patterns in the Southern Ocean (e.g., Fraser 1936; Siegel 2000), and for both larvae and adults, very little is known about their overwintering strategies (Daly 1990; Hagen et al. 2001). However, these data are essential for modelling population dynamics and for estimating krill production (Hofmann and Lascara 2000).

The differing biochemical compositions, energy requirements, and behavior of krill larvae, juveniles, and adults during winter suggest that they have fundamentally different overwintering strategies (Quetin et al. 1994). For postlarval krill, four main overwintering strategies are under debate: reduced metabolism, body shrinkage, lipid utilization, and the use of alternative food sources. Kawaguchi et al. (1986) first measured reduced metabolism in krill during winter. Ikeda and Dixon (1982) concluded from a long-term star-

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Acknowledgment

We thank the captain and crew of RV *Polarstern* for their collaboration and the chief scientist V. Smetacek for friendly support during the field work. We also thank two anonymous referees for their constructive comments on the manuscript. This work was supported by funding from the BMBF through project 03PL025A, Seasonal Nutrition Strategy of Antarctic Krill (*Euphausia superba*). This is publication awi-n10226 of the Alfred-Wegener Institute.

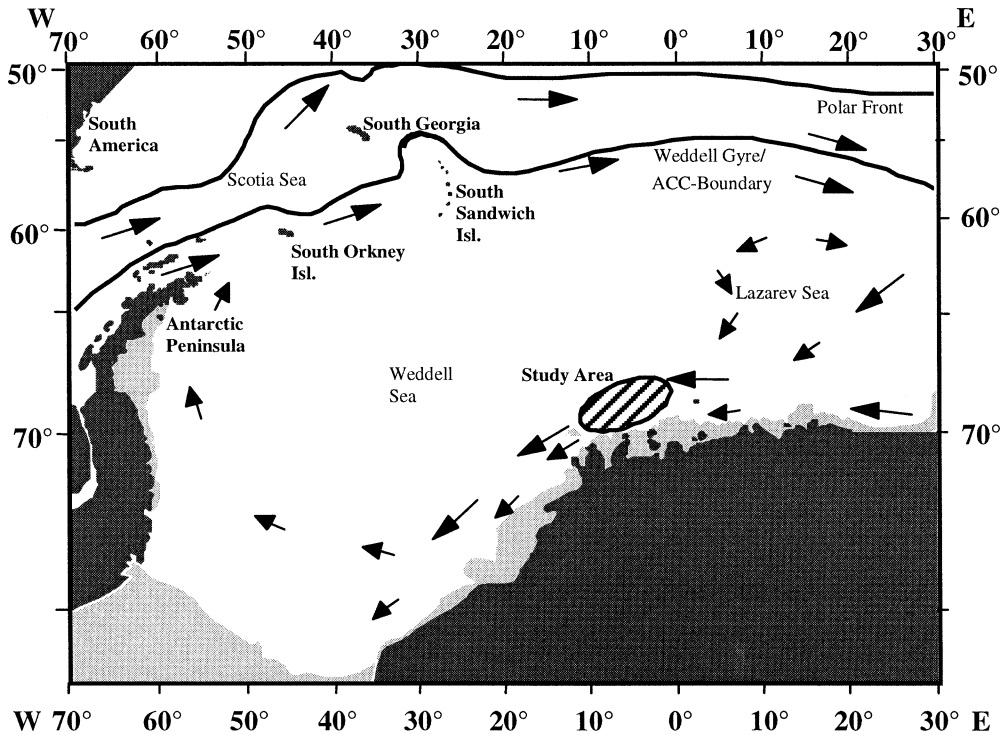


Fig. 1. Investigation area in the SW Lazarev Sea (ACC, Antarctic Circumpolar Current).

vation experiment that body shrinkage is also a possible overwintering mechanism of krill to conserve energy, but it is not clear yet if shrinkage is common in field populations (Nicol et al. 1992). Torres et al. (1994) and Hagen et al. (1996) demonstrated that lipid utilization helps to satisfy energy requirements in winter, and Kawaguchi et al. (1986) reported that krill changed feeding habit from the pelagic to the benthopelagic and ice algae during winter. Also, Hopkins et al. (1993) and others have shown carnivory in adult krill in winter.

Early furcilia of krill usually appear at the end of summer, develop during their first dark season, and moult to juveniles before December. The need for the larvae to feed during winter is suggested by their continuous growth in the field (Daly 1990), their low lipid reserves (Hagen et al. 2001), and thus their inability to tolerate long starvation periods. Larval krill have been shown to be unable to meet their energy requirements during winter if phytoplankton in the water column is the only food source, suggesting that they have to supplement their diet with heterotrophic organisms and sea ice biota (Daly 1990).

The aim of the study was to characterize the physiological condition and feeding behavior of furcilia larvae in the southwestern (SW) Lazarev Sea during autumn, prior to the critical overwintering period. It was part of a larger study that simultaneously looked at the energy budget of larvae, juveniles, and adults. Parallel measurements on juveniles and adults are described in the companion paper (Atkinson et al. 2002). The present study is integrated in the Southern Ocean Global Ocean Ecosystems Dynamics Program (SO GLOBEC). One goal of this program is to examine the factors

that govern krill survivorship and, hence, availability to higher trophic levels.

Methods

Investigation area—The ice-covered area of the Antarctic Coastal Current and the shelf slope were surveyed in the region 69°43'–69°70'S and 4°38'–6°44'W on board RV *Polarstern* during early autumn from 14 to 20 April 1999 (Fig. 1). The upper mixed layer varied between 40 and 220 m in depth, seawater temperature ranged from -1.8 to 0°C , and salinity ranged from 33.9 to 34.6 (Strass et al. 2000). Chlorophyll *a* (Chl *a*) concentrations were between 0.06 and 0.09 $\mu\text{g L}^{-1}$ in the mixed layer in open areas around the ice floes.

Sampling—Larvae of *Euphausia superba* were collected using a 350- μm mesh Bongo net with a 5-liter closed cod end, towed vertically from 150 m to the surface. Furcilia larvae were sorted under the stereomicroscope and identified following Fraser (1936). All samples were dominated by the furcilia III (FIII) larval stage, so only these were used in all analyses and experiments during the 7-d sampling period. One fraction of the freshly caught larvae was immediately frozen on a 200- μm mesh and stored at -80°C for later determination of dry mass, elemental (carbon, nitrogen) and biochemical (protein, lipid, carbohydrate) composition, and gut content in the home institute. The other fraction was used to measure metabolic rates (oxygen uptake and ammonium production) and to determine feeding rates and selective feeding behavior.

Analysis of dry mass and elemental and biochemical composition—Dry mass (DM), carbon (C), and nitrogen (N) were measured using replicates of two FIII larvae for each analysis following the procedure described by Donnelly et al. (1990). The samples were freeze dried, weighed on a Mettler UM3 microbalance, then analyzed in a Carlo Erba CN analyzer.

Total proteins and carbohydrates were measured by homogenizing five FIII larvae in 4 ml of 5% trichloroacetic acid (TCA) on crushed ice using a Branson Sonifer B15 cell disrupter. After centrifugation at 6,000 rpm ($5,000 \times g$) for 10 min, the supernatant was used for measuring carbohydrates according to Holland and Gabbott (1971), using glucose as a standard. The remaining pellet was used for protein analysis after Lowry et al. (1951), with bovine serum albumin as a standard.

Total lipid was measured by extracting 15 freeze-dried FIII larvae with dichloromethane/methanol (2:1 v/v), and the lipid content was determined gravimetrically according to Hagen (2000). Lipid class composition was determined by thin layer chromatography–flame ionization detection (TLC-FID) with an Iatroscan MK V (Iatron Laboratories; Fraser et al. 1985). The response of the FID was calibrated using a mixture of commercial standards with a composition similar to the samples. Because different lipid classes have different FID responses, separate calibration curves were used for each class.

To analyze fatty acid (FA) composition, lipids were hydrolyzed and the FAs converted to their methyl ester derivatives (FAMES) in methanol containing 3% concentrated sulfuric acid at 80°C for 4 h (Kattner and Fricke 1986). FAMES were extracted with hexane, analyzed in a gas chromatograph (HP 6890A), and identified by comparing retention time data with those obtained from standard mixtures.

Gut content analysis—From each sampling day, 10 frozen, freshly caught FIII larvae were used for the gut content analyses. In total, the guts of 60 frozen larvae were isolated, dried overnight on a glass plate, then mounted on an aluminium stub with silver conducting paint and coated with gold/palladium. Scanning electron microscopy of gut content was performed using an ISI DS-130 microscope.

Metabolic rate measurements—Oxygen uptake and ammonium production rates were compared between freshly caught larvae sampled every day during the 7-d period and mass-reared larvae acclimated for 7 d either at high food concentrations (40 larvae in 25 liters of $4 \mu\text{g Chl } a \text{ L}^{-1}$: well-fed larvae) or in filtered seawater (40 larvae in 25 liters of $0.45\text{-}\mu\text{m}$ filtered seawater: starved larvae). The concentrated food was natural seawater enriched with sea ice biota. This was obtained by collecting brown discolored lumps of multiyear ice with the ship's crane. These were melted according to Garrison and Buck (1986). The melted assemblages were then added to one container filled with natural seawater. The incubation water was changed daily and animals were checked for mortality. During the 7-d incubation, no mortality could be observed in either treatment.

For measuring oxygen uptake and ammonium production, 4 FIII larvae were incubated in sealed 100-ml bottles filled

Table 1. Available food concentrations in grazing experiments. Carbon biomass was converted from the abundance and sizes of diatoms, ciliates, and flagellates (*see Table 3*) into carbon equivalents via biovolume.

Treatment	Experiment	Food concentration	
		($\mu\text{g C L}^{-1}$)	($\mu\text{g Chl } a \text{ L}^{-1}$)
Natural seawater	1	2.9	0.081
	2	4.0	0.087
	3	4.7	0.097
	4	2.7	0.067
Natural seawater enriched with ice biota	1	35	0.71
	2	216	3.51
	3	86	1.77
	4	118	1.92

with filtered seawater ($0.45 \mu\text{m}$ pore size) for 15 h at in situ temperature (-1°C). Respiration and excretion rates of larvae were measured after 7 d at high food concentration and in filtered seawater, respectively (*see above*). Each respiration and excretion measurement comprised seven to eight replicates with larvae and five controls without larvae. Dissolved oxygen was determined by Winkler titration using a 716 DMS Titrimo (METROHM). The decrease of oxygen concentration in the experiments was $<10\%$, which is believed not to affect larval respiration (Johnson et al. 1984). Ammonium was measured using a Technicon Autoanalyzer II system (Bran and L ubbe) according to Koroleff (1983). All samples were analyzed in duplicate. Four standards at the beginning and two standards at the end of each run were used. The analytical precision of replicates was about $0.05 \mu\text{mol}$.

Design of feeding experiments—Feeding experiments were conducted using natural seawater from depths of 10 m and natural seawater enriched with various concentrations of melted sea ice biota (Table 1). Natural seawater from 10 m deep was used because Chl *a* concentration in this depth was in the upper range of values measured in the mixed layer during the sampling period ($0.06\text{--}0.09 \mu\text{g Chl } a \text{ L}^{-1}$). The incubation water was collected with a rosette sampler fitted with 24 Niskin bottles of 12 liters fitted with teflon springs. The water was immediately drawn through silicon tubing into two 50-liter containers. For enriched conditions, we added the biota of melted sea ice to one 50-liter container (*see above*). Both food media were transferred to the cold room and left for 1 to 2 h to stabilize.

After mixing at the start of each experiment, one subsample was examined immediately under a microscope to check the condition of the food assemblage, and two replicate subsamples (1 liter natural seawater, 0.5 liter enriched seawater) were siphoned for Chl *a* analysis to measure the concentration of the food source. Those samples were filtered onto Whatman GF/F filters, which were sonicated on ice for 30 s with 10 ml of 90% aqueous acetone and centrifuged ($700 \times g$) for 3 min. The supernatant was used to measure Chl *a* with a Turner 700D fluorometer. In addition, a 250-ml

Table 2. Dry mass (DM), carbon (C), nitrogen (N), and biochemical composition of freshly caught furcilia III larvae.

Measurements	Mean ± SD	Range	n
DM (μg)	375.7 ± 25.9	335–400	61
C (% DM)	35.7 ± 4.1	31.5–38.5	61
N (% DM)	9.7 ± 1.0	8.8–14.8	61
C:N	3.7 ± 0.1	3.6–3.9	61
Lipid (% DM)	15.5 ± 4.3	10.6–25.1	18
Protein (% DM)	34.0 ± 6.9	23.9–49.3	20
Carbohydrate (% DM)	0.9 ± 0.1	0.6–1.1	20

subsample was siphoned and fixed in 1% lugol's solution for cell counting.

For the feeding experiments, each food medium was transferred through silicon tubing to 2.4-liter bottles. Experiments comprised eight replicate bottles with five FIII larvae and four controls without larvae. Bottles were then incubated on a plankton wheel (0.5 rpm) under dim light at in situ temperature for 24 h.

At the end of the experiment, animals were checked for mortality, and subsamples of incubation water were siphoned for cell counts and Chl *a* analyses (see above). The entire bottle contents were filtered onto a submerged 100-μm sieve and preserved in 4% formaldehyde for zooplankton counts.

Analysis of feeding experiments—Cell concentration in the incubations waters was determined by inverted microscopy at ×100 and ×400 magnification after settling volumes of 100 ml (natural seawater) and 20 ml (enriched seawater) in counting chambers. Identification of diatoms, ciliates, and flagellates was based on Thomas et al. (1996). Three replicates were counted from each experiment, and the mean of these were used for further calculations. The standard deviation of the replicates was <10% of the mean. Cell counts and sizes were converted into carbon equivalents via biovolume according to Menden-Deuer and Lessard (2000). Chl *a* was analyzed as described above. The total biomass estimated by cell counts and the Chl *a* concentration in the feeding experiments is given in Table 1. The organisms counted in the feeding experiments are shown in Table 3.

Feeding rate calculations—Clearance rates on the total phytoplankton biomass and on individual cell taxa were calculated according to $F = \ln(C_c/C_k)V/m_k t$, where F is the clearance rate (ml mg⁻¹ body C h⁻¹), C_c is the final concentration in the control, C_k is the final concentration in the incubation bottles grazed by FIII larvae, V is the experimental volume (ml), m_k is the FIII larval body mass (mg C), and t is the experimental duration (h).

Ingestion rates on the total algal biomass were calculated as the product of the clearance rate on the total algal biomass (ml mg⁻¹ body C h⁻¹) and its C concentration in the final control (mg C ml⁻¹). Ingestion rates were then expressed as a daily C ration under the assumption that FIII larvae feeding rates recorded during each incubation reflect the daily average rate. The depletion of autotrophic biomass was between 12 and 17% in all experiments.

Table 3. The contribution of the various counted taxa to the ambient and enriched food sources in the grazing experiments of furcilia III larvae and their minimum and maximum lengths.

Taxon	Contribution to the total biomass (%)		
	Ambient food source	Enriched food source	Length range (μm)
<i>Fragilariopsis</i> spp.	57.7	32.1	32–60
<i>Chaetoceros</i> sp.	4.1	30.2	12–30
<i>Nitzschia</i> sp.	1.8	8.5	60–120
<i>Pseudonitzschia</i> sp.	9.0	0.9	80–120
<i>Entomoneis</i> sp.	2.1	5.0	40–60
Small centric diatoms	3.9	0.8	20–50
Large centric diatoms	0.2	18.1	60–250
Other large diatoms	4.3	1.9	90–180
Dinoflagellates	9.3	1.3	12–30
Ciliates	7.6	0.3	20–45
Organisms present only in the enriched food source			
Nauplii		0.9	122–427
Copepods		0.2	320–1600

Determination of feeding selectivity—Selective feeding behavior was characterized using the chi-square (χ^2) goodness of fit test. The frequency distribution of food types offered was compared to the frequency distribution of food types ingested. Selective feeding was indicated by a significant divergence between the distribution in the offered food and in the diet. The student *t*-test was used to test the significance of differences in clearance rates between the different prey items in the ambient and enriched food source.

Results

Condition of freshly caught FIII larvae—Elemental and biochemical composition: The dry mass, carbon, nitrogen, and the total protein, lipid, and carbohydrate contents of freshly caught FIII larvae are shown in Table 2. Dry mass ranged from 335 to 400 μg ind⁻¹. Carbon and nitrogen contributed a mean of 36 and 10% to the body mass, respectively. Protein, lipid, and carbohydrate contents were 34, 16, and <1% of DM, respectively. The lipids comprised 70 ± 5% phospholipids, 25 ± 5% triacylglycerols, and 5 ± 1% sterols. Accordingly, the fatty acid analyses revealed a signature typical of phospholipids, dominated by the long-chain polyunsaturated fatty acids 20:5(n-3) and 22:6(n-3) with 26 and 17%, respectively, and 16:0 with 19% of total fatty acids (Table 3).

Metabolic rates: Respiration rates of freshly caught FIII larvae ranged from 0.4 to 1.2 μl O₂ mg⁻¹ DM h⁻¹, similar to those of the well-fed larvae (0.9 to 1.5 O₂ mg⁻¹ DM h⁻¹). The lowest rates were measured after 7 d of starvation, with a mean value of 0.5 μl O₂ mg⁻¹ DM h⁻¹ (Fig. 2). Using a respiratory quotient of 0.97 (after Ikeda et al. 2000), the oxygen uptake rates of freshly caught FIII larvae correspond to a C loss of 1.4–4.2% body C d⁻¹, whereas after 7 d of starvation, FIII larvae lost 0.2% body C d⁻¹.

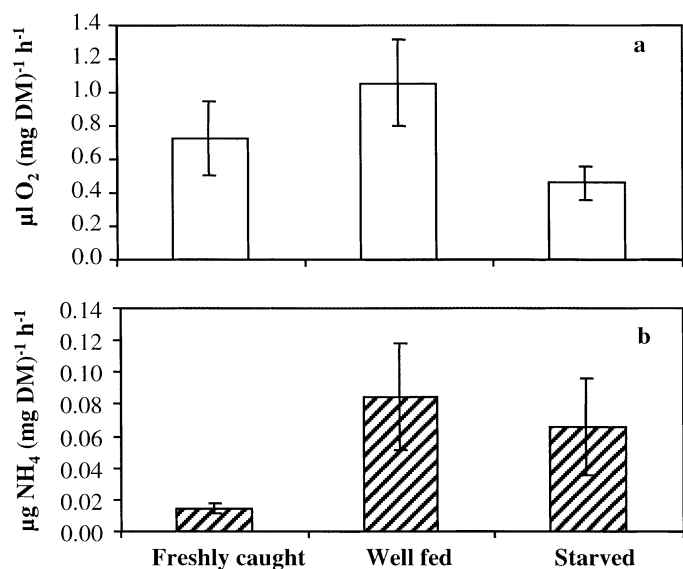


Fig. 2. *Euphausia superba*: furcilia III larvae. (a) Oxygen uptake and (b) ammonium production rates of freshly caught larvae, larvae incubated for 7 d in high food concentrations (well fed) and those that were starved for 7 d. Bars indicate standard deviations of the mean of 16 measurements with freshly caught larvae and 9 measurements each with well-fed and starved larvae.

Ammonium excretion rates were lowest in freshly caught FIII larvae, ranging between 0.01 and 0.02 $\mu\text{g NH}_4$ mg $^{-1}$ DM h $^{-1}$ (Fig. 2). They correspond to a N loss of 0.7–1.3% body N d $^{-1}$. Well-fed and starved FIII larvae showed similar values between 0.02 and 0.14 $\mu\text{g NH}_4$ mg $^{-1}$ DM h $^{-1}$, corresponding to a N loss of 1.3–9.3% body N d $^{-1}$. The atomic O:N ratios, calculated from the mean of the measured respiration and excretion rates for freshly caught, well-fed, and starved FIII larvae, were 72, 18, and 10, respectively.

Food sources used in the field—Gut contents: The gut contents of freshly caught FIII larvae provide a snapshot of information on food ingested in the field. All 60 guts analyzed were full, and diatom frustules were the only recognizable food item (Fig. 3). Fragments of *Fragilariopsis* spp., the dominant diatom species in the ambient phytoplankton (Table 3), could be seen in most of the furcilia guts. However, most of the algal fragments could not be identified, and no zooplankton remains were found in any of the guts analyzed. The food composition of the ambient and enriched food sources showed that no phytoplankton species is exclusively associated with the water column or the ice (Table 3). Hence, it was not possible to deduce the origin of the FIII larval diet in the field from gut content analysis.

Trophic marker fatty acids: No clear pattern from the diatom marker fatty acids 16:1(n-7) and 20:5(n-3) in freshly caught larvae could be detected. The polyunsaturated fatty acid 20:5(n-3) was present in high amounts, whereas 16:1(n-7) played only a minor role (Table 4). The dinoflagellate marker fatty acid 18:4(n-3) and the major components of calanoid copepod lipids, 20:1 and 22:1 fatty acid isomers (Kattner and Hagen 1995) were found in small quantities

(Table 4). However, in four of the samples, the portion of fatty acid isomers from calanoid copepods rose to 4% of total fatty acids. The 18:1(n-9)/18:1(n-7) ratio, the total 20:1 plus 22:1 (Falk-Petersen et al. 2000), and the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) (Cripps and Atkinson 2000) have all been suggested to reflect carnivory. The mean values of these indicators are 1.0, 2.4, and 3.0, respectively (Table 4).

Grazing activity and selectivity: In the natural seawater, the diatom *Fragilariopsis* spp. was the dominant alga comprising 58% of biomass, whereas in the enriched feeding experiment, *Fragilariopsis* spp., *Chaetoceros* sp., and large centric diatoms prevailed with 32, 30, and 18%, respectively (Table 3). In the natural seawater, dinoflagellates and ciliates comprised 17% of C, and zooplankton were too rare to enumerate. In the natural seawater enriched with ice biota, copepod nauplii formed 1% of the total biomass, and the proportion of copepodite stages and adult copepods was too low (0.2%) to calculate reliable clearance rates.

The C ration estimated by cell counts increased gradually at low food concentration (Fig. 4a). At in situ food concentrations (2.7–4.7 $\mu\text{g C L}^{-1}$), the daily ration was between 0.4 and 1.3% body C d $^{-1}$ and increased in the enriched food assemblages from 2 to 28% body C d $^{-1}$, corresponding to a food concentration of 35–216 $\mu\text{g C L}^{-1}$.

In the natural seawater, FIII larvae had high clearance rates of 84–115 ml mg $^{-1}$ C h $^{-1}$ and in enriched food assemblages, lower rates of 24–41 ml mg $^{-1}$ C h $^{-1}$ (Fig. 4b). The clearance rates on specific phytoplankton species in both incubation waters (Fig. 5) showed no significant difference between their contribution to the food mixture and to that of the diet ($P > 0.05$). Moreover, within the food size spectrum in both food assemblages (mean size range 12–220 μm), the larvae showed no significant preference to a specific food size (Fig. 5, $P > 0.05$).

Discussion

During this study in the SW Lazarev Sea in April, Chl *a* concentrations in the water column were low and in the range of winter values found previously at Admiralty Bay, South Shetland Islands (McClatchie 1988), and in the Scotia Sea (Daly 1990). Hence, our data show how furcilia cope with these low concentrations of pelagic phytoplankton prior to the winter season. Freshly caught FIII larvae had oxygen uptake rates similar to those during summer (Ikeda 1981). Small lipid stores and high O:N ratios suggest rapid lipid turnover. The larvae showed high clearance rates at in situ food concentrations and were able to utilize high food concentrations when they were available. The results further suggest that during periods of low food supply in the water column, larvae have to exploit ice algae to cover their metabolic demands. We outline the evidence for this below.

Feeding—Quantifying the diet and feeding intensity of krill larvae is of central importance to understand their survival and development prior to the dark season. Therefore, we used three complementary approaches—namely, feeding

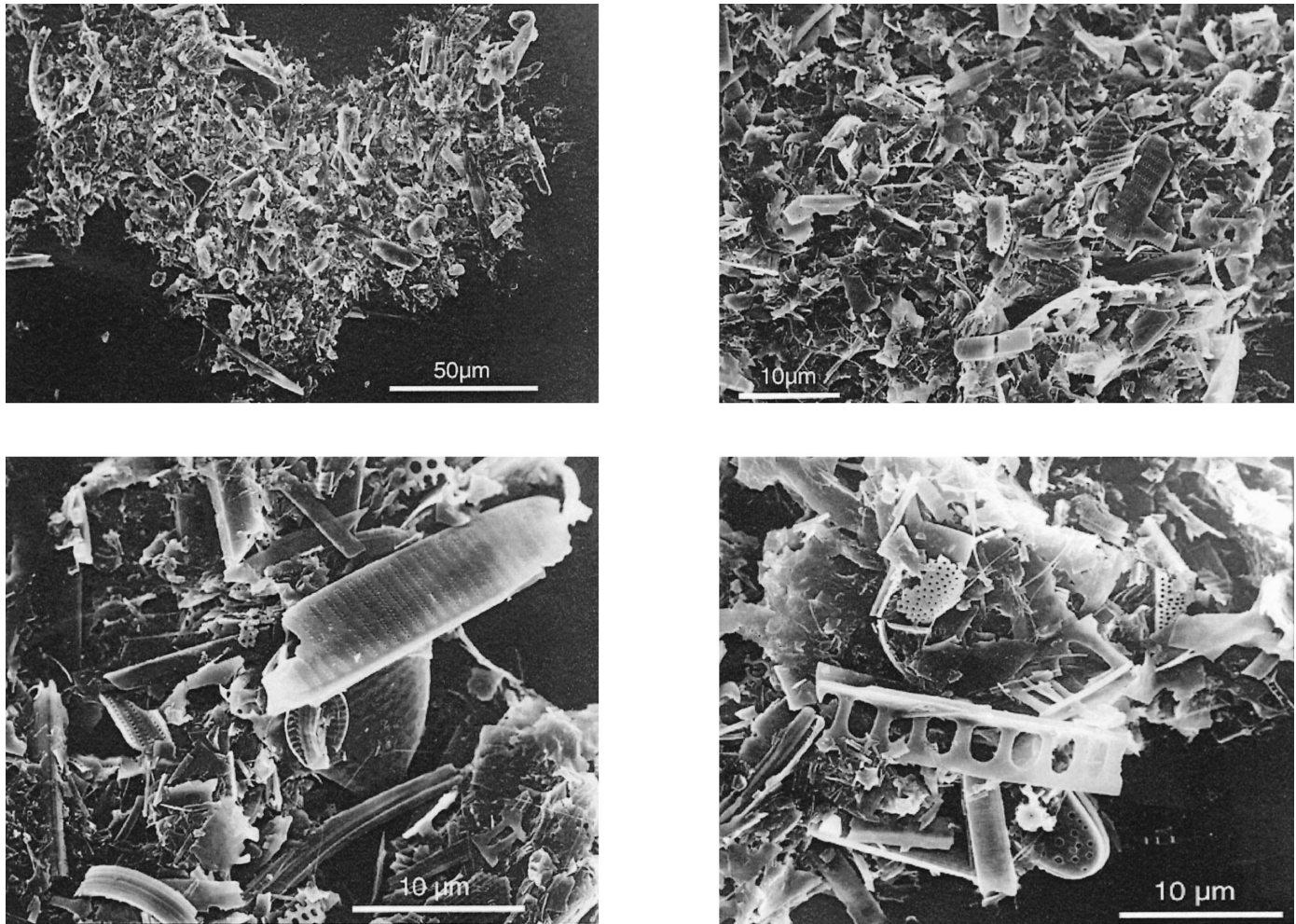


Fig. 3. *Euphausia superba*: furcilia III larvae. Gut contents of furcilia III larvae using scanning electron microscopy. Top left: overview of a gut content; top right, bottom left, and right: detailed pictures of gut contents.

experiments, gut content analyses, and fatty acid trophic markers.

Based on the incubations, ingestion and clearance rates of FIII larvae were in the same range as summer rates of calanoid copepods with similar dry masses, such as *Metridia gerlachei* and *Calanus propinquus* (Schnack 1985). The clearance rates of 8-mm-long furcilia in winter, estimated by Daly (1990) from gut fluorescence analyses, were $228 \text{ ml mg}^{-1} \text{ body C d}^{-1}$, or twice those of our data in ambient food. These are high clearance rates given the cold temperatures, and they contrast with the low rates obtained for juveniles and adults during this study (Atkinson et al. 2002). The juvenile and adults had clearance rates of $\sim 25\%$ of summer values, and they failed to respond even after prolonged exposure to high food concentrations.

Many studies have focused on food selection in adult krill (e.g., Habermann et al. 1993 and reference therein), but there is little information on larvae (Daly 1990). FIII larvae can feed across a large size spectrum, which includes protozoans and nauplii of metazoans. Indeed, the maximum size of potential food is probably even larger than our largest food items (Table 4; Fig. 5). This wide range in food items mirrors

the findings for adults, which adopt several methods (Hamner and Hamner 2000) to capture planktonic food ranging from $\sim 5 \mu\text{m}$ up to large calanoid copepods of several millimeters (e.g., Atkinson and Snýder 1997 and references therein). The larvae had no clear preference toward a specific size or motility of prey. Unselective feeding was also found for juveniles from the same study site, whereas adults fed mainly carnivorously (Atkinson et al. 2002). The unselective feeding behavior over a wide size range might be a response to maximize intake in a suspension of generally suitable food items (Huntley 1981).

Gut content analysis provides a snapshot of information on food recently ingested in the field, whereas fatty acid biomarkers reflect the feeding history over several weeks. Diatoms dominated in the identifiable food items in the gut, in common with the findings of Hopkins and Torres (1989). Although soft-bodied organisms are easily digested and thus not visible in the gut, 83 and 98% of the particles counted in natural and enriched seawater, respectively, were diatoms (Table 3). Therefore, dinoflagellates, ciliates, and micrometazoa probably played only a minor role as food sources. However, the fatty acid composition of freshly caught FIII

Table 4. Mean percentage of total fatty acids of freshly caught furcilia III larvae. Summary data in bold type are fatty acid characteristics used in previous studies as an index of carnivory (Cripps and Atkinson 2000; Falk-Petersen et al. 2000). A total of 23 samples were analyzed.

Fatty acid	Mean	Range
14:0	3.4	2.1–5.6
15:0	0.4	0.3–0.5
16:0	18.7	16.0–22.2
16:1(n-7)	3.5	2.7–4.7
16:1(n-5)	0.2	0.0–0.3
16:2(n-7)	1.0	0.7–1.9
16:3	0.2	0.1–0.4
16:4(n-1)	0.7	0.2–1.2
17:0	0.2	0.1–0.4
18:0	1.1	1.0–1.3
18:1(n-9)	6.0	5.1–7.5
18:1(n-7)	5.9	4.4–7.2
18:2(n-3)	2.4	2.1–2.7
18:3(n-3)	0.6	0.5–0.7
18:4(n-3)	1.8	1.1–2.9
19:0	0.3	0.1–0.5
20:1(n-9)	0.9	0.5–1.8
20:1(n-7)	0.1	0.0–0.2
20:4(n-3)	1.3	1.0–1.9
20:4(n-6)	0.5	0.2–0.7
20:5(n-3)	26.4	21.1–29.2
22:1(n-11)	0.5	0.0–3.9
22:1(n-9)	0.9	0.1–3.9
22:4(n-3)	0.6	0.4–1.0
22:5(n-3)	0.7	0.4–1.0
22:6(n-3)	17.0	15.2–18.6
24:1	0.1	0.0–0.5
Unknown	4.7	1.8–6.7
Total PUFA (% FA)	71.2	66.1–73.9
Total SFA (% FA)	24.1	21.3–26.8
PUFA/SFA ratio	3.0	2.7–3.4
18:1(n-9)/18:1(n-7) ratio	1.0	0.9–1.6
20:1 plus 22:1 (% FA)	2.4	1.0–9.3

FA, fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

larvae did not show a strong diatom signal (16:1[n-7]), pointing to the immediate utilization of dietary lipids rather than energy storage. However, the occurrence of fatty acids typical of copepods (22:1[n-9], 22:1[n-11]) (Kattner and Hagen 1995) in 4 of the 23 samples indicates the potential for carnivorous feeding already in the larval stages of *E. superba*. Cripps and Atkinson (2000) propose that a ratio of polyunsaturated to saturated fatty acids (PUFA/SFA) >1.5 is indicative of carnivory. However, ice algae are also characteristic in containing large amounts of PUFAs, namely 20:5(n-3) (Falk-Petersen et al. 1998). Such trophic indices should be interpreted with caution because there are various factors other than food influencing the fatty acid composition. The high PUFA/SFA ratio of 3 in this study can be explained by the low lipid content of furcilia and the predominance of phospholipids as part of biomembranes. Further experimental studies are required to elucidate the complex pathways of lipid metabolism in euphausiids to improve our

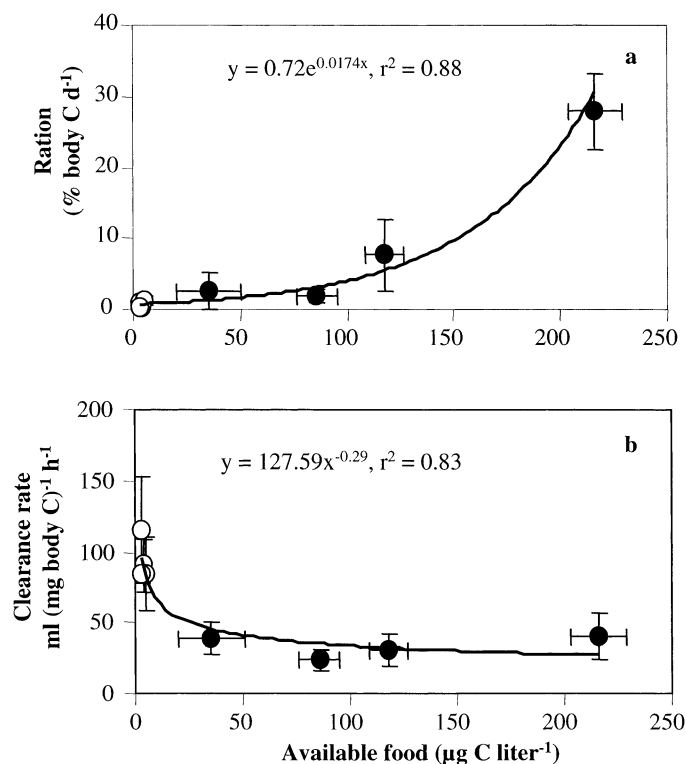


Fig. 4. *Euphausia superba*: furcilia III larvae. (a) Mean daily ration and (b) mean clearance rate versus food concentration. Open circles indicate natural seawater and filled circles natural seawater enriched with ice biota. Bars indicate standard deviation. Eight measurements of each food concentration were carried out with ambient food and six measurements of each concentration with enriched food.

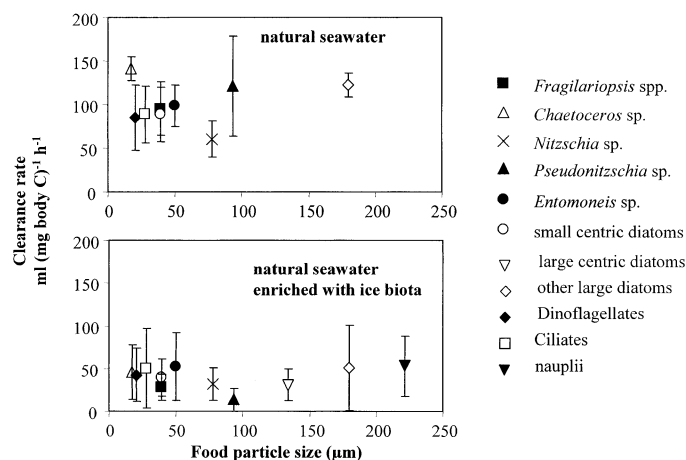


Fig. 5. *Euphausia superba*: furcilia III larvae. Clearance rates on the various prey categories in experiments with natural seawater and natural seawater enriched with ice biota (see Table 3 for length range of food items). Bars indicate standard deviations of the means of all experiments (ambient food source, 32 experiments; enriched food source, 30 experiments).

Table 5. Comparison of FIII dry mass (DM) measured in this study, in other regions, and in the laboratory. Dry mass of FIII larvae from other regions were calculated from the length given in the manuscripts and calculated to DM using the length–weight regression in Daly (1990). The Ikeda (1984) study was of laboratory-grown larvae.

Study area	Sampling time	Body length (mm)	DM (mg)	Data source
Scotia Sea	Jan, Feb	7.8	0.28	Brinton and Townsend (1984)
	Apr	6.4	0.15	
South Orkney Islands	mid-Mar	6.3–8.5	0.14–0.39	Fraser (1936)
Scotia-Weddell Sea	Jun	5.9	0.11	Daly (1990)
			0.50	Ikeda (1984)
Lazarev Sea (this study)	Apr		0.33–0.40	This study

understanding of trophic relationships via biochemical markers.

Metabolism—The dry mass of FIII larvae in our study is in the upper range of values reported from other regions of the Southern Ocean, but lower than those for FIII larvae raised in the laboratory (see Table 5). Their C composition in this study (36% of DM) is lower than the value of 41% of DM for laboratory-reared furcilia (Ikeda 1984), whereas their N contents is the same. Huntley and Brinton (1991) demonstrated that development and growth of krill larvae were correlated with food availability. Therefore, the high DM in this study inferred good food conditions. On the other hand, their low C:N ratio of 3.7 and lipid content of 15% of DM show that the FIII larvae did not have a substantial lipid store (see also Ikeda and Mitchell 1982). In a seasonal study, Hagen et al. (2001) found that the lipid contents of furcilia were highest in autumn ($18.1 \pm 5.4\%$ of DM), which is comparable to our values, before dropping to 7–10% of DM in winter/spring. The major lipid classes in *E. superba* were phospholipids (PLs), including membrane lipids and triacylglycerols, the main storage lipids (Hagen et al. 1996). However in our study, triacylglycerols comprised only ~25% of total lipids, the rest being mainly PLs (70%). Saether et al. (1986) suggested that PLs might also function as depot lipids, and Hagen et al. (1996) identified phosphatidylcholine as the primary polar lipid class accumulated. According to Saether et al. (1986), they are more easily catabolized than neutral lipids. Starvation experiments with furcilia over 3 weeks, concurrent with our study, showed that PLs were utilized in preference to triacylglycerols (D. Stübing and W. Hagen unpubl. data). Hence, PLs may be an additional energy source even in the larvae, although their PL reserves must have been small because of the low lipid levels. Euphausiids appear to be the only organism found so far to use PLs as an energy reserve (Hagen et al. 2001).

The low lipid content of furcilia contrast with that of the adults, which averaged 44% of DM (Atkinson et al. 2002). This suggests that furcilia have a much lower resistance to starvation. Hagen (1988) calculated that the lipid reserves of furcilia may last for about 9–18 d. During our study, after 7 d of starvation, metabolic substrate (as indicated by the O:N ratio) shifted dramatically from a lipid-based metabolism (O:N ratios >50) to one based on protein (O:N ratios <20). Daily C and N losses were high: 1.6% body C d⁻¹ and 4.7% body N d⁻¹. Unlike the adults, which can with-

stand >200 d of starvation (Ikeda and Dixon 1982), the metabolic losses of FIII larvae determined in our study indicate that their lipid reserves are depleted in only 24 d, taking into account that 3–5% of lipids are essential (Hagen et al. 1996). This provides further evidence that winter feeding is essential for the furcilia, but its importance decreases with ontogeny (Quetin et al. 1994; Hofmann and Lascara 2000). Although the lipid stores were low in freshly caught furcilia from this study, their high O:N ratio of 72 suggested rapid lipid turnover.

The metabolic rates of freshly caught FIII larvae equate to a loss of 2.5% body C d⁻¹ and 0.9% body N d⁻¹, which is comparable to summer values (Ikeda 1981). From our feeding experiments, phytoplankton appears to have been the main food source in the natural seawater and the natural seawater enriched with ice biota (Table 3). The low N loss reflects a preferential utilization of dietary N to replenish body protein rather than for general metabolic purposes (Ikeda and Kirkwood 1989). Daly (1990) calculated a winter growth rate of 4.6% body C d⁻¹ for furcilia, so allowing for the respiration loss of 2.5% body C d⁻¹, the larval rations would need to be at least 7% of body C d⁻¹ to achieve these growth rates. This requires a food concentration of 115 μg C L⁻¹ based on Fig. 4a. Le Fèvre et al. (1998) calculated a range of 105–125 μg C L⁻¹ for krill to cover their metabolic demands, which is in line with our calculation. In our study, the values in the ambient incubation water were 3–5 μg C L⁻¹, which is far too low to cover the metabolic needs of the larvae. This is further evidence for their need to feed on other resources, such as ice algae, to survive and to grow during winter.

Survival strategy—Torres et al. (1994) classified three overwintering strategies for Antarctic pelagic species. One extreme is “dormancy,” with large accumulations of lipid reserves, diapause, and cessation of feeding, which is typical for some calanoid copepods. The other extreme is “business as usual,” with continuous feeding and growth, usually found in carnivorous species. The third strategy is a “compromise,” which is a mixture of reduced metabolism (quiescence) and feeding. Our study took place at the transition between summer and winter, and at the time of sampling, juveniles and adults were adopting a compromise strategy (Atkinson et al. 2002), while furcilia seem to be undergoing business as usual. We speculate that they did this by shifting from pelagic to under-ice feeding, responding flexibly to the

seasonal changes in food supply. In our study, carnivorous feeding increased with ontogeny, so the ontogenetic differences in overwintering strategy we found point to spatial partitioning of the food resource, possibly between sea ice biota (larvae) and the water column (postlarvae).

References

- ATKINSON, A., AND R. SNYDER. 1997. Krill-copepod interactions at South Georgia, Antarctica, I. Omnivory by *Euphausia superba*. *Mar. Ecol. Prog. Ser.* **160**: 63–76.
- , B. MEYER, U. V. BATHMANN, D. STÜBING, W. HAGEN, AND K. SCHMIDT. 2002. Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter. II. Juveniles and adults. *Limnol. Oceanogr.* **47**: 953–966.
- BRINTON, E., AND A. W. TOWNSEND. 1984. Regional relationships between development and growth in larvae of Antarctic krill, *Euphausia superba* from field samples. *J. Crustac. Biol. (spec. 1)* **4**: 224–246.
- CRIPPS, G. C., AND A. ATKINSON. 2000. Fatty acid composition as an indicator of carnivory in Antarctic krill, *Euphausia superba*. *Can. J. Fish. Aquat. Sci.* **57**: 31–37.
- DALY, K. L. 1990. Overwintering development, growth and feeding of larval *Euphausia superba* in the Antarctic marginal ice zone. *Limnol. Oceanogr.* **35**: 1564–1576.
- DONNELLY, J., J. J. TORRES, T. L. HOPKINS, AND T. M. LANCRAFT. 1990. Proximate composition of Antarctic mesopelagic fishes. *Mar. Biol.* **106**: 13–23.
- FALK-PETERSEN, S., J. R. SARGENT, J. HENDERSON, E. N. HEGSETH, H. HOP, AND Y. B. OKOLODKOV. 1998. Lipids and fatty acids in ice algae and phytoplankton from the Marginal Ice Zone in the Barents Sea. *Polar Biol.* **20**: 41–47.
- , W. HAGEN, G. KATTNER, A. CLARKE, AND J. SARGENT. 2000. Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. *Can. J. Fish. Aquat. Sci.* **57**: 178–191.
- FRASER, A. J., D. R. TOCHER, AND J. R. SARGENT. 1985. Thin-layer chromatography-flame ionization detection and the quantitation of marine neutral lipids and phospholipids. *J. Exp. Mar. Biol. Ecol.* **88**: 91–99.
- FRASER, F. C. 1936. On the development and distribution of the young stages of krill (*Euphausia superba*). *Disc. Rep.* **14**: 1–192.
- GARRISON, D. L., AND K. R. BUCK. 1986. The biota of Antarctic pack ice in the Weddel Sea and Antarctic Peninsula regions. *Polar Biol.* **10**: 211–219.
- HABERMAN, K. L., R. M. ROSS, AND L. B. QUETIN. 1993. Grazing by the Antarctic krill *Euphausia superba* on *Nitzschia* sp. and *Phaeocystis* sp. monocultures. *Antarct. J. U.S.* **28**: 217–219.
- HAGEN, W. 1988. On the significance of lipids in the Antarctic zooplankton. *Rep. Polar Res.* **49**: 1–129.
- . 2000. Lipids, p. 113–119. *In* R. Harris, P. Wiebe, J. Lenz, H. R. Skjoldal, and M. Huntley [eds.], ICES zooplankton methodological manual. Academic Press.
- , E. S. VAN VLEET, AND G. KATTNER. 1996. Seasonal lipid storage as overwintering strategy of Antarctic krill. *Mar. Ecol. Prog. Ser.* **134**: 85–89.
- , G. KATTNER, A. TERBRÜGGEN, AND E. S. VAN VLEET. 2001. Lipid metabolism of the Antarctic krill *Euphausia superba* and its ecological implications. *Mar. Biol.* **139**: 95–104.
- HAMNER, W. M., AND P. P. HAMNER. 2000. Behavior of Antarctic krill (*Euphausia superba*): Schooling, foraging, and antipredatory behavior. *Can. J. Fish. Aquat. Sci.* **57**: 192–202.
- HOFMANN, E. E., AND C. M. LASCARA. 2000. Modelling the growth dynamics of Antarctic krill *Euphausia superba*. *Mar. Ecol. Prog. Ser.* **194**: 219–231.
- HOLLAND, D. L., AND P. A. GABBOTT. 1971. A micro-analytical scheme for the determination of protein, carbohydrate, lipid and RNA levels in marine invertebrate larvae. *J. Mar. Biol. Ass. U.K.* **51**: 659–668.
- HOPKINS, T. L., AND J. J. TORRES. 1989. Midwater food web in the vicinity of a marginal ice zone in the western Weddel Sea. *Deep-Sea Res.* **36**: 543–560.
- , T. M. LANCRAFT, J. J. TORRES, AND J. DONNELLY. 1993. Community structure and trophic ecology of zooplankton in the Scotia Sea marginal ice zone in winter (1988). *Deep-Sea Res.* **40**: 81–105.
- HUNTLEY, M. E. 1981. Nonselective, nonsaturated feeding by three calanoid copepod species in the Labrador Sea. *Limnol. Oceanogr.* **26**: 831–842.
- , AND E. BRINTON. 1991. Mesoscale variation in growth and early development of *Euphausia superba* Dana in the western Bransfield Strait region. *Deep-Sea Res.* **38**: 1213–1240.
- IKEDA, T. 1981. Metabolic activity of larval stages of Antarctic Krill. *Antarct. J. U.S.* **16**: 161–162.
- . 1984. Sequences in metabolic rates and elemental composition (C, N, P) during the development of *Euphausia superba* Dana and estimated food requirements during its life span. *J. Crustac. Biol. (spec. 1)* **4**: 273–284.
- , AND P. DIXON. 1982. Body shrinkage as a possible overwintering mechanism of the Antarctic krill, *Euphausia superba* Dana. *J. Exp. Mar. Biol. Ecol.* **62**: 143–151.
- , AND R. KIRKWOOD. 1989. Metabolism and body composition of two euphausiids (*Euphausia superba* and *E. crystallorophias*) collected from under the pack ice off Enderby Land, Antarctica. *Mar. Biol.* **100**: 301–308.
- , AND A. W. MITCHELL. 1982. Oxygen uptake, ammonia excretion and phosphate excretion by krill and other Antarctic zooplankton in relation to their body size and chemical composition. *Mar. Biol.* **71**: 283–298.
- , J. J. TORRES, S. HERNÁNDEZ-LEÓN, AND S. P. GEIGER. 2000. Metabolism, p. 455–520. *In* R. Harris, P. Wiebe, J. Lenz, H. R. Skjoldal, and M. Huntley [eds.], ICES zooplankton methodological manual. Academic Press.
- JOHNSON, M. A., M. C., MACAULAY, AND D. C. BIGGS. 1984. Respiration and excretion within a mass aggregation of *Euphausia superba*: Implications for krill distribution. *J. Crustac. Biol.* **4**: 174–184.
- KATTNER, G., AND H. S. G. FRICKE. 1986. Simple gas-liquid chromatography method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. *J. Chromatogr.* **361**: 263–268.
- , AND W. HAGEN. 1995. Polar herbivorous copepods—different pathways in lipid biosynthesis. *ICES J. Mar. Sci.* **52**: 329–335.
- KAWAGUSHI, K., S. ICHIKAWA, AND O. MATSUDA. 1986. The overwintering strategy of Antarctic krill (*Euphausia superba* Dana) under the coastal fast ice off the Ongul Islands in Lutzow-Holm Bay Antarctica. *Mem. Natl. Inst. Polar Res. Tokyo (Special Issue)* **44**: 67–85.
- KOROLEFF, F. 1983. Determination of ammonia, p. 150–157. *In* K. Grasshoff, M. Ehrhardt, and K. Kremling [eds.], *Methods of seawater analysis*. Verlag Chemie, Weinheim.
- LE FÈVRE, J., L. LEGENDRE, AND R. B. RIVKIN. 1998. Fluxes of biogenic carbon in the Southern Ocean: Roles of large microphagous zooplankton. *J. Mar. Syst.* **17**: 325–345.
- LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275.
- MCCLATCHIE, S. 1988. Food-limited growth of *Euphausia superba* in Admiralty Bay, South Shetland Island, Antarctica. *Cont. Shelf Res.* **8**: 329–345.

- MENDEN-DEUER, S., AND E. J. LESSARD. 2000. Carbon to volume relationships for dinoflagellates, diatoms and other protist plankton. *Limnol. Oceanogr.* **45**: 569–579.
- NICOL, S., M. STOLP, T. COCHRAN, P. GEIJSEL, AND J. MARSHALL. 1992. Growth and shrinkage of Antarctic krill *Euphausia superba* from the Indian Ocean sector of the Southern Ocean during summer. *Mar. Ecol. Prog. Ser.* **89**: 175–181.
- QUETIN, L. B., AND R. M. ROSS. 1991. Behavioral and physiological characteristics of the Antarctic krill, *Euphausia superba*. *Am. Zool.* **31**: 49–63.
- , ———, AND A. CLARKE. 1994. Krill energetics: Seasonal and environmental aspects of the physiology of *Euphausia superba*, p. 165–184. *In* S. El-Sayed [ed.], *Southern Ocean ecology: The BIOMASS perspective*. Cambridge Univ. Press.
- SAETHER, O., T. E. ELLINGSEN, AND V. MOHR. 1986. Lipid biochemistry of zooplankton from high latitudes. *Océanis* **7**: 623–632.
- SCHNACK, S. B. 1985. Feeding by *Euphausia superba* and copepod species in response to varying concentrations of phytoplankton, p. 311–323. *In* W. R. Siegfried, P. R. Condy, and R. M. Laws [eds.], *Antarctic nutrient cycles and food webs*. Springer-Verlag.
- SIEGEL, V. 2000. Krill (Euphausiacea) demography and variability in abundance and distribution. *Can. J. Fish. Aquat. Sci.* **57**: 151–167.
- STRASS, V., A. MACRANDER, C. DIETRICH, P. ROTH, S. GONZALES, AND H. LEACH. 2000. CTD stations and water bottle sampling. *Rep. Polar Res.* **364**: 23–30.
- THOMAS, C. R., G. R. HASLE, K. A. STEIDINGER, E. E. SYVERTSEN, AND K. TANGEN. 1996. *Identifying marine diatoms and dinoflagellates*. Academic Press.
- TORRES, J. J., J. DONNELLY, T. L. HOPKINS, T. M. LANCRAFT, A. V. AARSET, AND D. G. AINLEY. 1994. Proximate composition and overwintering strategies of Antarctic micronektonic crustacea. *Mar. Ecol. Prog. Ser.* **113**: 221–232.

Received: 9 August 2001

Accepted: 14 February 2002

Amended: 2 April 2002