

Trophic relationships among Southern Ocean copepods and krill: Some uses and limitations of a stable isotope approach

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Abstract

The use of stable isotopes to study food webs has increased rapidly, but there are still some uncertainties in their application. We examined the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of Antarctic euphausiids and copepods from the Polar Front, Lazarev Sea, and Marguerite Bay against their foodweb baseline of particulate organic matter (POM). Interpretations of trophic level were helped by comparison with other approaches and by calibration experiments with *Euphausia superba* fed known diets. Results for well-known mesozooplankters (e.g., *Calanoides acutus* and *Metridia gerlachei*) were internally consistent and corresponded to those derived from independent methods. This gave confidence in the isotope approach for copepods and probably larval euphausiids. Among the dominant yet poorly known species, it suggested mainly herbivory for *Rhincalanus gigas* but omnivory for *Calanus simillimus* and furcilia larvae of *Thysanoessa* spp. and *Euphausia frigida*. The $\delta^{15}\text{N}$ values of adult copepods were up to 3‰ higher than those of early copepodites, pointing to ontogenetic shifts in diet. In the Lazarev Sea in autumn, the isotopic signals of *E. superba* larvae suggested pelagic, mainly herbivorous, feeding rather than feeding within the ice. In contrast to the mesozooplankton, some anomalous results for postlarval krill species indicated problems with this method for micronekton. The experiments showed that postlarval *E. superba* did not equilibrate with a new diet within 30 d. We suggest that the slower turnover of these larger species, partly in combination with their ability to migrate, has confounded trophic effects with those of a temporally/spatially changing food-web baseline. Interpretations of food sources of micronekton could be helped by analyzing their molts or fecal pellets, which responded faster to a new diet.

Fundamental to an understanding of biochemical fluxes and ecosystem functioning is knowledge of trophic relationships among species (e.g., Perissinotto et al. 2000). Tradi-

tional approaches to pelagic food web studies include gut content analysis and feeding experiments. These methods are able to resolve the broad food web structure, yet each has its drawbacks (Michener and Schell 1994). For gut content analysis, identification of food items can be difficult, soft-bodied or rapidly digested food items are underrepresented, and it allows inferences only on ingestion, not on assimilation (e.g., Boyd et al. 1984). Furthermore, it provides only snap-shot information on recently ingested food (Båmstedt et al. 2000). Incubations give insights into feeding rates and selectivity but can suffer from artefacts. In captivity, zooplankters and their prey can behave unnaturally (Boyd et al. 1984), and it is not always simple to simulate the natural food assemblage in experiments.

More recently, biomarkers have been used as an alternative tool to study food webs. Food sources can have distinct biochemical compositions (e.g., of fatty acids or stable isotopes) that become incorporated into their consumers. Bio-

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markers integrate the diet over a longer time than gut contents, a period that depends on the rates of ingestion, accumulation, tissue turnover and growth (Fry and Arnold 1982; Tieszen et al. 1983).

In food web studies, carbon and nitrogen isotopes have usually been measured. For carbon there appears to be, on average, a slight enrichment in the rarer ^{13}C over ^{12}C in the animal relative to its diet (0.5‰–1‰), whereas that of ^{15}N over ^{14}N is greater (3‰–4‰; Michener and Schell 1994). The more conservative transfer of carbon isotopic compositions can be useful in tracing two food sources with clear differences in $\delta^{13}\text{C}$ values, whereas nitrogen isotope ratios are most frequently used as trophic position indicators (Peterson and Fry 1987).

Although isotopic analysis is rapidly gaining in popularity, there are some confounding factors. Nitrogen isotope fractionation is not constant but can vary according to species (e.g., DeNiro and Epstein 1981), nutritional stress (Hobson 1993), food source (Fantle et al. 1999; Gorokhova and Hansson 1999), and dietary nitrogen content (Adams and Sterner 2000). Also, some ecosystems have multiple organic inputs and consumers often have more than two food sources, which cannot always be discerned by using one or two isotope tracers. Even for a single primary carbon source such as phytoplankton, the isotope ratio can change with species composition, metabolic pathway of photosynthesis, season, and geographical region (Michener and Schell 1994). This spatial or temporal variability in the baseline of the food web hinders comparisons between local and migrating consumers as well as those differing in turnover and growth rate (Fry et al. 1983; Simenstad and Wissmar 1985).

Despite these problems, stable isotope analysis can be a powerful approach and has been applied successfully in the field. Trophic structures determined from $\delta^{15}\text{N}$ data have in some cases agreed well with those from gut content analyses (Hansson et al. 1997), laboratory feeding experiments (Dittel et al. 2000), and fishery production models (Fry 1988). Other studies have provided information about the trophic importance of certain carbon sources such as marsh-derived material (Fantle et al. 1999) or methanotrophic bacteria (Kiyashko et al. 2001) within their specific ecosystems. Stable isotopes have also been used successfully to define the habitat usage of migratory species (Schell et al. 1989; Lesage et al. 2001).

Southern Ocean food webs have seldom been studied with stable isotopes (e.g., Wada et al. 1987; Rau 1991a; Burns et al. 1998; Hodum and Hobson 2000). The results are inconsistent. For a broad range of consumers, Wada et al. (1987) found a tight correlation between isotope abundance and independently estimated trophic level, whereas Rau et al. (1991a) did not. This suggests the need for a better understanding of the uses and limitations of the stable isotope approach to study Southern Ocean food webs.

In this study, we examined regional, seasonal, and ontogenetic differences in the isotope ratios of zooplankton. The results are compared with those from previous gut content analysis, feeding experiments, and fatty acid analysis. A month-long incubation experiment was done to follow changes in the isotope ratios of larval and postlarval *Euphausia superba* while feeding on ice biota or copepods.

This “calibration” experiment helped interpretation of the in situ results and provided information on the timescales of isotopic change in various body organs and metabolic products. The present study aimed to provide indications on the trophic position of Southern Ocean zooplankton and to identify potential problems in the application of the stable isotope method.

Materials and Methods

Field sampling—Polar Front, Weddell Gyre, and Lazarev Sea, March–May 1999: Zooplankton were collected along a transect from the Polar Front ($\sim 49^\circ\text{S}$, 20°E) via the Weddell Gyre ($\sim 60^\circ\text{S}$, 10°E) to the southwestern Lazarev Sea ($\sim 69^\circ\text{S}$, 5°W) and on the return voyage. Samples were taken from the top 150 m with nighttime vertical tows of a Bongo net (0.5 m diameter, 350 μm mesh size, 5 liter closed cod end). Taxonomic groups and development stages were sorted immediately under a stereomicroscope and transferred to filtered seawater, enabling them to evacuate their guts. After ~ 24 h, animals were filtered onto a mesh and stored at -80°C . Juvenile and adult krill were collected on four consecutive nights in the Lazarev Sea. Most were used in experiments, but 10–20 postlarvae were reserved every night for initial isotope values or lipid composition (Stübing et al. unpubl. data). The fecal pellets produced by these batches of freshly caught krill during gut evacuation were also filtered onto a mesh and stored at -80°C . A conductivity-temperature-depth-rosette system was used to obtain water samples from the upper mixed layer at 10 m depth. Suspended particles were collected by gentle vacuum filtration onto precombusted GF/F filters and frozen (-80°C). In the Lazarev Sea, lumps of brown discolored multiyear sea ice were collected, diluted with filtered seawater, melted in the dark at $\sim 1^\circ\text{C}$, and concentrated over a 20- μm mesh. Large particles were sieved out through 200- μm gauze, and the suspension was filtered onto precombusted GF/F filters.

Marguerite Bay, February–March 2000: Zooplankton was collected at the Rothera Time Series monitoring station ($67^\circ 30'\text{S}$, 70°W , ~ 2 km from the Rothera Base) by towing a hand net (0.4 m diameter, 200 μm mesh size) vertically from 250 m to the surface. Taxonomic groups were separated and transferred to filtered seawater for 24 h before freezing at -80°C . On 2 March, surface water was taken by hand, filtered onto precombusted GF/F filters, and also frozen.

South Georgia, January–February 1996: North of South Georgia ($54^\circ 30'\text{S}$, 37°W), juvenile and adult *E. superba* were caught by oblique hauls of a rectangular midwater trawl (RMT 8, 4.5 mm mesh size, nominal opening 8 m^2) deployed to a depth of 250 m (Cripps et al. 1999). Krill were immediately stored at -80°C . Cripps et al. (1999) sampled 14 stations and pooled the krill from each station for a representation of the local population. We analyzed three–four juveniles from six of their stations: A2, A3, B2, C2, C3, and C6 (fig. 1. in Cripps et al. 1999).

Experiments with E. superba—*E. superba* caught in the southwestern Lazarev Sea between 16 and 20 April 1999

were used for incubations on board ship. These were carried out in darkness in a cold room (0–2°C) for either 20 or 30 d. Two batches of ~200 furcilia larvae (stage III) were incubated in 18-liter aquaria, and three batches of ~50 mixed juvenile and adult krill were incubated in aerated 170-liter tanks. Larvae were fed either with melted sea ice biota 20–200 μm in size (see above) or starved in 0.45 μm filtered seawater. For juveniles and adults, the treatments were starvation, a diet of ice biota or a diet of copepods. Every 48 h, animals were transferred to a new batch of food or filtered seawater. The previous incubation water was then sieved through 55 μm mesh to collect molts and fecal pellets. These were frozen at –80°C and used for stable isotope measurements when the biomass was sufficient.

The concentrated ice biota suspension was maintained in dim light at 1°C. Every second day, a subsample was filtered on a precombusted GF/F filter for isotope analysis. Copepods were picked daily from field catches (Bongo net, 350 μm mesh size), excluding damaged animals and large carnivorous species such as *Euchaeta* spp. A subsample of each newly added copepod assemblage and all copepods remaining after 2 d of krill predation were frozen. In the laboratory, copepods were identified, enumerated, and their isotopic composition was measured to calculate the daily ration and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the diet. At the end of the experiment, the remaining furcilia larvae, juveniles, and adults were allowed to clear their guts and deep frozen. Animals were either used for lipid and fatty acid analysis (Stübing et al. unpubl. data) or for stable isotope measurements (present study).

Sample preparation—GF/F filters were dried at 60°C for 24 h and packed into tin capsules. Euphausiid larvae and copepods were thawed, rinsed briefly with deionized water, and transferred into preweighed tin capsules. For each species or development stage, three–four replicates of 0.5–2 mg dry mass were analyzed. Usually, 1–40 individuals were pooled for each replicate depending on their weight. Samples were dried at 60°C for 24–48 h, and dry mass was determined using a Sartorius ultra-microbalance. Juvenile and adult euphausiids were dissected into the hepatopancreas region, the third abdominal segment, and the remaining body, while thawing. In this way, body fractions containing different tissue were separated, according to the method of Mayzaud et al. (1998). Each part was freeze-dried, weighed, and ground in an agate mortar. Two subsamples of the resulting powder were analyzed. Fecal pellets were picked individually into a series of petri dishes of deionized water to clear them from food remains, concentrated onto a GF/F filter, and dried at 60°C. To obtain purified chitin, molts were deproteinized with 2.5M NaOH according to the method of Whistler and BeMiller (1962), washed with ethanol:water mixtures, and demineralized with HCL as described by DeNiro and Epstein (1978). None of the filters or the zooplankton samples were acidified, because the carbonate content was considered to be minor and this procedure may alter $\delta^{15}\text{N}$ values (e.g., Pinnegar and Polunin 1999).

Isotope analysis—Carbon and nitrogen stable isotope ratios were analyzed using a CHN analyzer (ThermoFinnigan

CE 1108) interfaced with a mass spectrometer (Finnigan Delta S) via a Conflow II open split interface. Calibration for the total carbon and nitrogen determination was done daily with an Acetanilide standard. Isotope ratios were expressed as δ values: $\delta X (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where X is ^{13}C or ^{15}N and R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. PeeDee Belemnite and atmospheric nitrogen were used as the standards for carbon and nitrogen, respectively. A laboratory working standard (Pepton, Merck) was run for every sixth sample. The Peptone standard indicated an analytical error associated with the isotope measurements of less than $\pm 0.2\text{‰}$ for both isotopes. Samples of the ground krill tissue were reanalyzed if the difference between the two replicates was $>0.5\text{‰}$ for nitrogen and 0.8‰ for carbon.

Lipids have not been extracted from the samples and all $\delta^{13}\text{C}$ values given in tables and figures are original data, uncorrected for variable lipid content. However, it has been shown, first, that lipids are depleted in $\delta^{13}\text{C}$ relative to protein (by ~6‰; McConnaughey and McRoy 1979) or muscle tissue (by ~3‰; Tieszen et al. 1983) and, second, that the lipid content of a sample can be predicted accurately from its C:N ratio (e.g., Lesage 1999). For *E. superba*, we found a close relationship between average lipid content (Stübing et al. unpubl. data) and C:N ratio (present study) of lipid content (%) = $8.5301 (\text{C:N ratio}) - 23.099$ ($n = 5$, $R^2 = 0.993$, $p < 0.001$).

Therefore, we corrected $\delta^{13}\text{C}$ values when species differed markedly in their C:N ratios using the equation above and a 6‰ difference in $\delta^{13}\text{C}$ values between lipids and rest animal (McConnaughey and McRoy 1979). These results are mentioned in the text. Generally, the application of a lipid correction resulted in a small change in $\delta^{13}\text{C}$ compared with uncorrected values. For species with the extreme lowest and highest C:N ratios (4.3 and 9.8), the different lipid content explains only a difference of 3‰ in their original $\delta^{13}\text{C}$ values.

Statistics—Means of the stable isotope ratios were analyzed with a multiple range test (Student–Newman–Keuls test). For correlation analysis between body nitrogen content and $\delta^{15}\text{N}$ ratio, linear correlation coefficients (r^2) were calculated.

Results

Regional and seasonal differences in the stable isotope ratios of particulate organic matter (POM) and zooplankton—During March–May 1999, there were marked regional differences in the stable isotope ratio of POM from the Polar Front to the Lazarev Sea (Fig. 1). The $\delta^{15}\text{N}$ ratios were lowest in the Weddell Gyre, ~3‰ higher at 65°S and 4‰–6‰ higher at both the Polar Front and in the Lazarev Sea. In contrast, $\delta^{13}\text{C}$ ratios were highest in the Polar Front and 3‰–5‰ lower at the stations further south. Zooplankton reflected this regional pattern of the POM (Fig. 1, Table 1), having significantly lower $\delta^{15}\text{N}$ ratios in the Weddell Gyre and often higher $\delta^{13}\text{C}$ ratios in the Polar Front (Table 2). However, regional changes in $\delta^{15}\text{N}$ ratios were larger for the POM than for most of the zooplankton species, which resulted in a slightly higher trophic offset in the Weddell Gyre and at 65°S

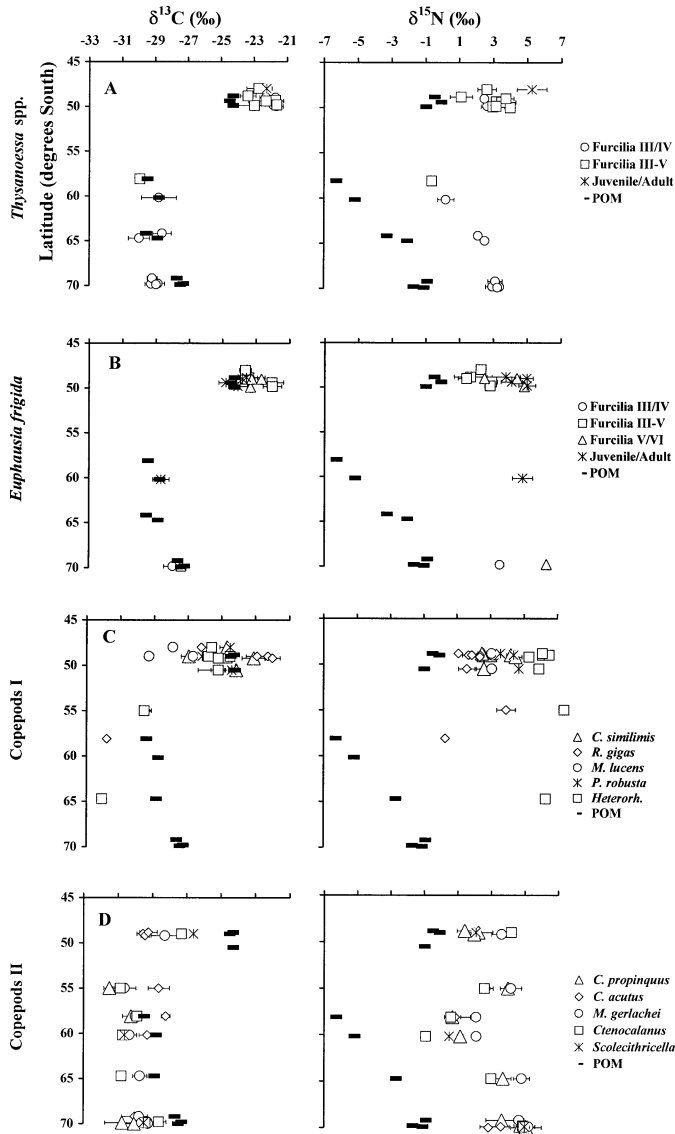


Fig. 1. Regional and species differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (‰, ± 1 SD) in autumn 1999 (data for the POM were supplied by K.-U. Richter). Symbols represent the mean value on a single sampling date, where usually between two and six replicate measurements were made (see also Table 1). $\delta^{13}\text{C}$ values are not lipid corrected. (A) *Thysanoessa* spp.; (B) *E. frigida*; (C) copepods I (subantarctic species); and (D) copepods II (high Antarctic species).

compared with the Lazarev Sea and Polar Front. Having accounted for different lipid content (see Materials and Methods section), copepod species living mainly in the south (Fig. 1D) had fairly constant $\delta^{13}\text{C}$ values, whereas those of the two euphausiid species and the copepod *Rhincalanus gigas* (Fig. 1A–C) decreased markedly from the Polar Front to the south. Regional differences in the $\delta^{13}\text{C}$ ratios of the zooplankters often exceeded those of the POM.

The Lazarev Sea and Marguerite Bay were both sampled in late summer/early autumn. Although the Lazarev Sea was characterized by very low phytoplankton abundances ($\sim 0.5 \mu\text{g}$ chlorophyll *a* L^{-1}), diatoms had been blooming in the Marguerite Bay for several months ($7\text{--}10 \mu\text{g}$ Chl *a* L^{-1} , A.

Clarke, unpubl. data). Correspondingly, the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios of POM in the Lazarev Sea were $\sim 5\text{--}6\text{‰}$ lower than those in Marguerite Bay (Fig. 2). Zooplankton again reflected these differences in the baseline of the food web, showing clearly higher stable isotope ratios in Marguerite Bay (Table 2, Fig. 2). For *E. superba* furcilia larvae and the copepods *Metridia gerlachei* and *Calanoides acutus* the $\delta^{15}\text{N}$ values differed by $4\text{--}5\text{‰}$ and the $\delta^{13}\text{C}$ by $3\text{--}5\text{‰}$ within the two locations.

Differences in stable isotope ratios among zooplankton species—In addition to regional differences in stable isotope values, there were also consistent differences between species within each region (Table 1). Among the copepods characteristic of the Polar Front, *R. gigas* had the lowest $\delta^{15}\text{N}$ value, which indicated the first trophic level ($\sim 3\text{‰}$ above the average $\delta^{15}\text{N}$ of the POM). *Metridia lucens* and *Calanus simillimus* had intermediate $\delta^{15}\text{N}$ values, and *Pleuromamma robusta* and *Heterorhabdus* spp. had the highest (Table 1). The difference between *R. gigas* and *Heterorhabdus* spp. was $\sim 4\text{‰}$, equivalent to one trophic level. In contrast to $\delta^{15}\text{N}$, the $\delta^{13}\text{C}$ values of these species tended not to differ markedly, even after correction for their variable lipid contents.

Of the copepods found south of the Polar Front, *M. gerlachei* often had the highest $\delta^{15}\text{N}$, although the difference from *Calanus propinquus* was not always significant (Table 1). Average $\delta^{15}\text{N}$ values of *M. gerlachei* were $6\text{--}8\text{‰}$ above that of the POM, indicating about two trophic transfers. *Ctenocalanus* spp. and *C. acutus* usually had lower $\delta^{15}\text{N}$ values, whereas the difference between *C. acutus* and *M. gerlachei* was $\sim 1.5\text{‰}$ (Table 1). The latter holds true also for Marguerite Bay (Table 3). Average $\delta^{13}\text{C}$ ratios here were again similar among copepod species.

Stable nitrogen isotope ratios of euphausiid development stages were usually within the range of values found for copepods (Table 1). In the Polar Front, furcilia larvae of *Euphausia frigida*, *Thysanoessa* spp., and *Euphausia triacantha* had $\delta^{15}\text{N}$ values similar to those of *M. lucens* and *C. simillimus* ($3\text{--}4\text{‰}$ above the $\delta^{15}\text{N}$ of the POM), whereas their postlarvae had values similar to those of *P. robusta* or *Heterorhabdus* spp. ($5\text{--}6\text{‰}$ above the $\delta^{15}\text{N}$ of the POM). The $\delta^{13}\text{C}$ values of the euphausiids were slightly higher than those of copepods typically found at the Polar Front, which can be attributed partly to their low lipid contents (indicated by low C:N ratios, Table 1).

At stations further south, $\delta^{15}\text{N}$ of *Thysanoessa* spp. and *E. frigida* furcilia larvae were as low as those of *Ctenocalanus* spp. or *C. acutus* ($5\text{--}6\text{‰}$ above the $\delta^{15}\text{N}$ of the POM). However, juveniles of *E. frigida* from the Weddell Gyre had a $\delta^{15}\text{N}$ clearly higher than that of the copepods but similar to those of individuals collected in the Polar Front (Fig. 1B). South of the Polar Front, the $\delta^{13}\text{C}$ values of *Thysanoessa* spp. and *E. frigida* usually did not differ from those of the copepods.

Furcilia larvae and juveniles of *E. superba* from the Lazarev Sea had lower $\delta^{15}\text{N}$ values than other euphausiid and copepod species, whereas values of the adults were similar (Table 1). Likewise, in Marguerite Bay, larval *E. superba* had lower $\delta^{15}\text{N}$ ratios than the copepods, although here the

Table 1. Autumn 1999 survey. Stable isotope values, carbon content and C:N ratios of copepods and euphausiids within different regions. N gives the total number of measurements of 1–40 individuals depending on size. Species are presented in increasing order of their $\delta^{15}\text{N}$ values. Nonsignificant differences between mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values within a region are indicated by the same letter, either single or out of a group of letters (Homogenous groups, $p < 0.05$, Student-Newman-Keuls multiple range test).

Species/stage	Sampling dates (1999)	<i>n</i>	Mean C content ($\mu\text{g ind.}^{-1}$)	Mean C:N	Mean $\delta^{15}\text{N}$ (‰)	Homog. groups	Mean $\delta^{13}\text{C}$ (‰)	Homog. groups
Polar Front								
POM	29 Mar; 6 Apr; 3 May	3			−0.8		−24.7	
<i>Rhincalanus gigas</i> CIII	28, 30 Apr; 2, 3, 5, 6 May	16	41	7.0	2.0	a	−23.4	def
<i>Calanus propinquus</i> CV	28, 30 Apr; 2 May	9	240	8.1	2.0	a	−29.4	a
<i>Euphausia frigida</i> FIII–V	29 Mar; 4, 6 Apr; 4, 5 May	31	368	4.6	2.5	ab	−22.5	fg
<i>Thysanoessa</i> spp. FIII/IV	26 Mar; 1 May	8	120	4.4	2.6	ab	−21.8	g
<i>Thysanoessa</i> spp. FII–V	27, 29 Mar; 3, 4, 6 Apr; 3, 4, 5 May	103	344	4.3	2.9	ab	−22.6	fg
<i>Euphausia triacantha</i> FIV/V	29 Mar; 6 Apr; 4, 5 May	16	604	4.9	3.2	abc	−22.2	fg
<i>Metridia lucens</i> CV	28 Apr; 2, 3, 6 May	8	16	7.4	3.2	abc	−27.5	b
<i>Calanus simillimus</i> CV	28, 30 Apr; 2, 3, 5, 6 May	23	63	6.7	3.2	abc	−24.6	cd
<i>Euphausia frigida</i> FV/VI	6 Apr; 1, 4 May	4	1,820	4.6	3.6	bcd	−23.1	efg
<i>Metridia gerlachei</i> CV	30 Apr	5	16	7.8	3.6	bcd	−28.3	b
<i>Euphausia frigida</i> juv./ad.	29 Mar; 4, 6 Apr; 4 May	15	10,340	5.3	4.2	cd	−24.2	cde
<i>Pleuromamma robusta</i> CV	2, 3, 6 May	6	16	6.4	4.5	de	−24.5	cd
<i>Thysanoessa</i> spp. juv.	5 May	3	12,150	4.8	5.3	ef	−22.2	fg
<i>Heterorhabdus</i> spp. CIII–V	30 Apr; 2, 3, 5, 6 May	6	32	7.3	6.1	f	−25.1	c
55° South								
<i>Calanoides acutus</i> CIV	27 Apr	4	24	9.5	2.7	a	−31.5	a
<i>Calanus propinquus</i> CV	27 Apr	4	240	6.8	4.0	b	−28.6	b
<i>Metridia gerlachei</i> CV	27 Apr	3	18	8.1	4.2	b	−30.6	a
<i>Rhincalanus gigas</i> CIII	27 Apr	2	106	6.3	4.3	b	−29.37	b
Weddell Gyre								
POM	24, 25 Apr	2			−6.0		−29.5	
<i>Ctenocalanus</i> sp. CV	24, 25 Apr	2	3	6.4	−0.1	a	−30.3	a
<i>Thysanoessa</i> spp. FIII–V	24, 25 Apr	24	320	4.4	−0.0	a	−29.2	a
<i>Calanoides acutus</i> CIV	25 Apr	2	22	8.3	0.9	b	−30.2	a
<i>Calanus propinquus</i> CV	24, 25 Apr	8	313	7.8	0.9	b	−28.7	a
<i>Metridia gerlachei</i> CV	24, 25 Apr	8	18	6.7	2.2	c	−30.1	a
<i>Euphausia frigida</i> juv.	24 Apr	8	5,500	5.6	4.8	d	−28.7	a
65° South								
POM	11, 22 Apr	2			−3.0		−29.5	
<i>Thysanoessa</i> spp. FIII/IV	11, 22 Apr	7	217	5.5	2.3	a	−29.4	a
<i>Ctenocalanus</i> sp. CV	22 Apr	2	3	8.5	3.0	b	−30.8	a
<i>Calanus propinquus</i> CV	22 Apr	4	380	8.7	3.7	c	−30.0	a
<i>Metridia gerlachei</i> CV	22 Apr	4	32	7.5	4.8	d	−29.7	a
Lazarev Sea								
POM	16, 18, 20 Apr	3			−1.5		−27.8	
<i>Euphausia superba</i> juv.	16, 18, 20 Apr	23	28,000	6.8	2.1	a	−31.2	a
<i>Euphausia superba</i> FIII	16, 17, 18, 20 Apr	16	132	4.6	2.1	a	−27.5	c
<i>Thysanoessa</i> spp. FIII/IV	16, 17, 18, 20 Apr	15	250	6.7	3.2	b	−29.1	b
<i>Calanoides acutus</i> CIV	20, 21 Apr	4	20	8.0	3.4	b	−30.6	a
<i>Euphausia frigida</i> FIII/IV	15 Apr	6	197	5.1	3.4	b	−28.0	c
<i>Euphausia superba</i> ad.	16, 17, 18, 20 Apr	20	120,000	8.0	3.6	b	−31.3	a
<i>Ctenocalanus</i> sp. CV	16, 20 Apr	4	7	9.1	4.4	c	−29.2	b
<i>Calanus propinquus</i> CV	16, 18, 20, 21 Apr	19	240	7.8	4.6	c	−29.4	b
<i>Metridia gerlachei</i> CV	16, 18, 20, 21 Apr	16	40	7.0	5.0	c	−29.4	b

adults had $\delta^{15}\text{N}$ ratios even lower than those of juveniles or furcilia (Table 3).

Differences in stable isotope ratios among development stages—The relationship between zooplankton $\delta^{15}\text{N}$ and

their nitrogen mass was investigated throughout the survey period (Table 4). For some species (e.g., *M. gerlachei*), the relationship was variable between regions and sampling dates, whereas for others (e.g., *C. propinquus*) it was fairly constant (Table 4). Overall, the $\delta^{15}\text{N}$ values of *C. propinquus*

Table 2. Autumn 1999 survey. Results of the Student-Newman-Keuls multiple range test ($p < 0.05$) on regional differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of euphausiid and copepod species. Nonsignificant differences between regions are indicated by the same letter.

Region	<i>Thysanoessa</i> spp. FIII–V		<i>Metridia gerlachei</i> CV		<i>Calanus propinquus</i> CV		<i>Calanoides acutus</i> CIV		<i>Ctenocalanus</i> spp. CV		<i>Rhincalanus gigas</i> CIII	
	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
Polar Front	bc	b	b	c	b	ab	—	—	—	—	a	b
55° South	—	—	c	a	c	b	b	a	—	—	b	a
Weddell Gyre	a	a	a	ab	a	b	a	a	a	a	—	—
65° South	b	a	d	b	c	a	—	—	b	a	—	—
Lazarev Sea	c	a	d	b	c	ab	b	a	b	b	—	—

increased sharply with copepodite stage, averaging $\sim 3\%$ from C III to adult (Table 4). Smaller increases (1% – 2%) from early copepodid to adult were seen for *R. gigas*, *C. simillimus*, and *P. robusta*. *M. gerlachei* increased by $\sim 1\%$ from CIV to adult in the Lazarev Sea but decreased by $\sim 0.6\%$ in the Weddell Gyre (Table 4). Among the furcilia, juvenile, and adult stages of euphausiids, the $\delta^{15}\text{N}$ either did not increase with nitrogen mass or did so more slowly than in most of the copepods (Table 4). However, for *E. frigida* and *Thysanoessa* spp., the total change in $\delta^{15}\text{N}$ from early furcilia to juvenile/adult was in the same range as found for the copepods (1% – 3% , Table 1).

Diet and turnover rates of E. superba in autumn: in situ and experimental results—According to the nitrogen isotope

measurements, *E. superba* furcilia and juveniles had a similar diet in the Lazarev Sea in autumn 1999, whereas adults were about half a trophic level higher (Fig. 2A). Being 3% – 5% lighter than the animals, pelagic POM was likely to have been a major food source for *E. superba*, whereas other potential food—large copepod stages, euphausiid larvae, and ice biota—had similar or even higher $\delta^{15}\text{N}$ values (Fig. 2A). Even after correction for their lipid content, none of the zooplankton reflected the high $\delta^{13}\text{C}$ of the ice biota. The harpacticoid *Drescheriella glacialis*, which were found directly in the ice and is known to feed primarily on ice algae (Dahms et al. 1990), had clearly higher $\delta^{15}\text{N}$ ($\sim 5.5\%$) and $\delta^{13}\text{C}$ values (about -25%) than any of the *E. superba* development stages.

In Marguerite Bay in autumn 2000, the $\delta^{15}\text{N}$ values of *E. superba* were all lower than those of the copepods but were 1% – 2% higher than the $\delta^{15}\text{N}$ of the POM (Fig. 2B). In common with the results from the Lazarev Sea, this implies a feeding history dominated by the ingestion of POM rather than copepods.

In the incubation experiments, *E. superba* were fed solely with ice biota or copepods, which had similar or even higher isotope values ($\delta^{15}\text{N}$ of $3.7 \pm 0.5\%$, $\delta^{13}\text{C}$ of $-21.9 \pm 0.5\%$ for ice biota and $\delta^{15}\text{N}$ of $3.0 \pm 1.1\%$, $\delta^{13}\text{C}$ of $-27.0 \pm 2.8\%$ for copepods). In response, the isotope values of the krill tended to increase as well (Fig. 3). These findings provide supporting evidence that, in the months prior to capture of the Lazarev Sea krill, neither ice biota nor copepods were their main food source.

Feeding rates during the incubation were quantified only for postlarvae feeding on copepods, whose mean daily C ration was $1.3 \pm 0.6\%$ ($n = 11$). This low ingestion rate in autumn corresponds with results from a parallel study (Atkinson et al. 2002). Other indications of feeding in these experiments are shorter molting intervals than in the starvation treatment (17–23 compared with 30–32 d) and the production of fecal pellets. Given the low feeding rate of the postlarvae, the only significant isotopic change seen after 20 d was in the $\delta^{13}\text{C}$ ratio of the furcilia larvae (Student-Newman-Keuls test; $p < 0.05$, Fig. 3). Juveniles had significantly increased in their $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios after 30 d. A long response time was also implied by the starvation treatments, where no significant isotopic changes occurred within 20–30 d.

These changes in the isotopic composition can be used to estimate turnover rates. Under the assumption of a trophic

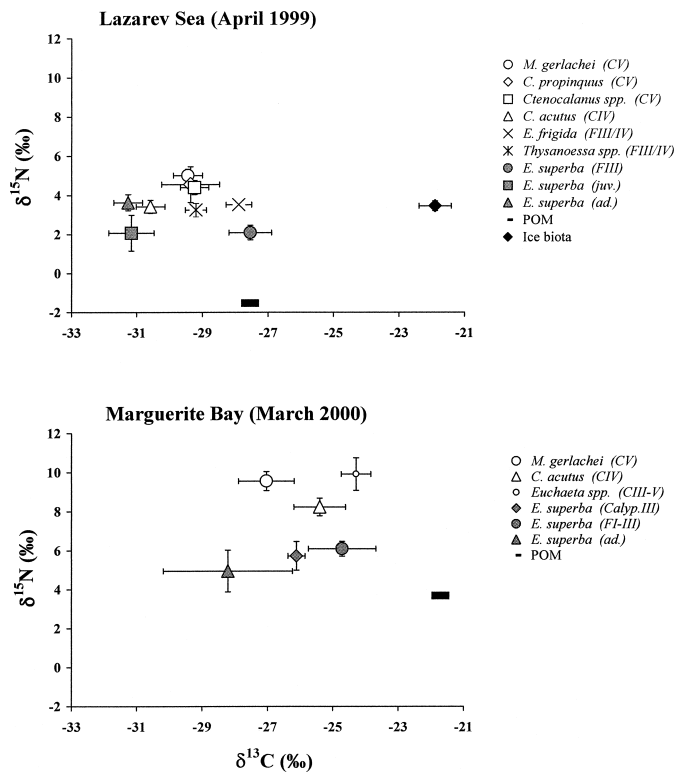


Fig. 2. Stable isotope values (‰ , ± 1 SD) of POM, ice biota, and zooplankton species at two different locations/seasons. Symbols represent the mean value of all measurements conducted in that area.

Table 3. Marguerite Bay, late summer 2000. Summary of stable isotope values, carbon content, and C:N ratios of euphausiid and copepod species. Further information as in Table 1.

Marguerite Bay	Sampling dates (2000)	n	Mean C content ($\mu\text{g ind.}^{-1}$)	Mean C:N	Mean $\delta^{15}\text{N}$ (‰)	Homog. groups	Mean $\delta^{13}\text{C}$ (‰)	Homog. groups
POM	2 Mar	2			3.7		-22.0	
<i>Euphausia superba</i> adults	17 Mar	6	115,000	6.9	4.9	a	-28.2	a
<i>Euphausia superba</i> CIII	28 Feb	4	36	4.7	5.7	b	-26.1	bc
<i>Euphausia superba</i> FI-III	28 Feb; 2, 3, 6, 17, 18, 19 Mar	31	161	4.8	6.1	b	-24.7	d
<i>Calanoides acutus</i> CIV	28 Feb; 2, 18 Mar	13	169	9.8	8.2	c	-25.4	cd
<i>Metridia gerlachei</i> CV	28 Feb; 2, 6 Mar	9	112	6.6	9.6	d	-27.0	b
<i>Euchaeta</i> spp. CIII-V	28 Feb; 2 Mar	8	328	5.4	9.9	d	-24.3	d

offset of 3.5‰ for N and 0.5‰ for C and no significant growth during the study period, the observed shift in the isotopic ratio compared with the equilibrium value equates to the percentage tissue turnover (Frazer et al. 1997). For juveniles feeding on ice biota, the calculated turnover rates were 54% of N and 18% of C over a period of 30 d. In the experiment with copepod food, the turnover was lower for N (24%) but higher for C (36%). This results in a daily turnover of roughly 1%–2% in N and 0.5%–1% in C, which is in line with the calculated ingestion rate of $\sim 1.3\%$ body C d^{-1} . For furcilia larvae and adults, the daily turnover rates did not exceed 1%. Thus, in the transition to winter it takes at least 2 months of feeding to reach isotopic equilibrium with a new diet.

Because specific tissues or metabolic products might respond faster than the whole animal, additional measurements were made on the hepatopancreas region, abdominal segment 3, shed molts, and fecal pellets of juvenile and adult *E. superba*. Generally, the hepatopancreas region had lower $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values than the whole individual, whereas the abdominal segment had higher isotopic enrichments. However, these differences did not change significantly within any of the treatments (Student–Newmann–Keuls test; $p < 0.05$). This implies that the turnover rates of the tissues did not differ markedly.

As was found for the whole krill, the $\delta^{15}\text{N}$ values of their molts increased when feeding on copepods or ice biota (Fig. 4). However, the change was greater for molts than for whole animals: $\sim 3\%$ – 5% compared with $< 1\%$. If, in a steady state, the isotopic difference between the whole animal and exoskeleton is constant (Montoya 1994), then the $\delta^{15}\text{N}$ ratio of the molts had reached isotopic equilibrium with the new diet within the incubation period of ~ 20 d. Thus, compared with the whole animal, molts can give information about more recent food sources. Under the assumption of nitrogen isotopic differences of 11‰–14‰ between molts and diet on the basis of these experiments, the $\delta^{15}\text{N}$ values of molts of freshly caught krill (about -14%) still point to POM as their main food source over the last few weeks. In contrast to the $\delta^{15}\text{N}$ values, the carbon isotope signals of the molts did not show any clear response to different food sources or incubation times (Fig. 4).

As would be expected, fecal pellets of *E. superba* responded even faster to isotopic changes in the diet. There was a significant positive correlation between the isotope

value of fecal pellets and the copepods ingested within the preceding 1–2 d (Fig. 5; $\delta^{15}\text{N}$, $r^2 = 0.674$; $\delta^{13}\text{C}$, $r^2 = 0.908$, $p < 0.01$). The $\delta^{15}\text{N}$ ratios of the fecal pellets were consistently lower than those of the ingested copepods (by $2.1 \pm 0.6\%$), whereas the $\delta^{13}\text{C}$ ratios were first higher and then lower (by $0.6 \pm 1.6\%$). The later is probably caused by a different lipid composition of southern copepod species, offered during the first half of the experiment, and those from the Polar Front, fed during the second half. An increase in the C:N ratio of the pellets but not in the diet suggests that a smaller fraction of the lipids from Polar Front copepods had been assimilated. Pellets of freshly caught juveniles and adults on four consecutive nights in April 1999 in the Lazarev Sea had $\delta^{15}\text{N}$ values of -0.5% , 4.1% , 3.7% , and 1% . Applying the result from our experiments, that krill fecal pellets have lower $\delta^{15}\text{N}$ values than the ingested food, these values imply that the recent diet of krill was variable but contained ^{15}N -enriched sources such as copepods. Ice biota was unlikely to be a major part of the diet, because $\delta^{13}\text{C}$ values of the fecal pellets were low, about -28% .

The trophic position of E. superba from South Georgia: a comparison of stable isotope and fatty acid analyses—Juvenile *E. superba* were collected from regions to the north of South Georgia during the summer of 1996. Cripps et al. (1999) allocated the krill stations to three types—A, B, and C—on the basis of multivariate analysis of their fatty acid compositions. These suggested herbivory at the A and B station groups and carnivory or starvation at the distinct C stations. However, isotopic analysis of krill from these stations showed a different picture (Fig. 6). Both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios were lowest at Sta. C2 but were highest at C3 and B2 (Student–Newman–Keuls multiple range test, $p < 0.05$). Variations in krill body size (56 ± 16 mg C ind.^{-1}) and lipid content (C:N ratio, 6.1 ± 0.6) were minor between stations and could not explain these differing isotope ratios.

Discussion

The $\delta^{15}\text{N}$ values of the POM baseline varied greatly, by up to 10‰, across our study sites. However, the major mesozooplankton tended to respond to this. For example mean $\delta^{15}\text{N}$ values of the copepods *M. gerlachei* and *C. acutus* varied by 7‰–8‰, in a manner generally in step with the POM. This coherence lends some credence to the use of the stable

Table 4. Autumn 1999 survey. Relationship between $\delta^{15}\text{N}$ values and nitrogen content of copepods and euphausiids. Different development stages were analyzed separately. Data have been summarized if there were several sampling dates within a region (Σ). ** ($p < 0.01$); * ($p < 0.05$); n = total number of measurements.

Species/stage	Date/region	n	Range of nitrogen mass ($\mu\text{g N ind.}^{-1}$)	Correlation with $\delta^{15}\text{N}$ (r^2)	Slope ($\%_{\text{N}}/10 \mu\text{g N}$)	
<i>Calanus propinquus</i> (CIII-adult)	20 Apr	9	2–39	0.534*	0.35	
	21 Apr	16	2–88	0.434**	0.31	
	Σ Lazarev Sea			0.484**	0.33	
	22 Apr	13	1–87	0.041	—	
	24 Apr	12	2–85	0.665**	0.31	
	25 Apr	13	1–100	0.659**	0.27	
	Σ Weddell Gyre			0.608**	0.29	
	27 Apr	11	1–65	0.424*	0.11	
	(CIII-CV)	28 Apr	8	4–43	0.144	—
		30 Apr	9	7–45	0.683*	0.47
Σ Polar Front				0.283*	0.21	
<i>Calanoides acutus</i> (CIII–adult)	27 Apr	19	1–18	0.724**	2.0	
<i>Metridia gerlachei</i> (CIV–adult)	20 Apr	16	3–17	0.353*	0.86	
	21 Apr	10	3–16	0.435*	0.80	
(CIV–CV)	Σ Lazarev Sea			0.303**	0.76	
	22 Apr	10	1–13	0.374	—	
	24 Apr	13	2–4	0.463*	–1.9	
	25 Apr	11	2–4	0.711**	–5.7	
	Σ Weddell Gyre			0.383**	–2.9	
	30 Apr	7	1–6	0.262	—	
<i>Rhincalanus gigas</i> (CII–adult)	27 Apr	16	3–90	0.274*	0.15	
	28 Apr	25	3–92	0.283**	0.12	
	30 Apr	14	2–80	0.604**	0.24	
	5 May	14	3–84	0.641**	0.21	
	Σ Polar Front			0.460**	0.18	
<i>Calanus simillimus</i> (CIII–adult)	28 Apr	6	1–21	0.554	—	
	30 Apr	10	1–16	0.470*	1.4	
	2 May	16	3–18	0.771**	1.5	
	3 May	18	5–16	0.279*	0.60	
	5 May	14	4–20	0.447**	0.61	
	6 May	11	3–15	0.001	—	
	Σ Polar Front			0.241**	1.0	
	5, 6 May	11	1–7	0.554**	1.6	
<i>Pleuromamma robusta</i> (CIV/V) <i>Thysanoessa</i> spp. (FIII–V)	27 Mar	8	60–100	0.159	—	
	29 Mar	21	35–210	0.438**	0.1	
	3 Apr	21	15–230	0.287*	0.06	
	4 Apr	17	15–260	0.007	—	
	6 Apr	8	30–150	0.744**	0.1	
	4 May	11	30–180	0.786**	0.07	
	5 May	15	30–190	0.025	—	
	Σ Polar Front			0.09**	0.06	
	24 Apr	17	30–165	0.544**	0.1	
	25 Apr	8	85–125	0.031	—	
	Σ Weddell Gyre			0.014	—	
	(FIII/IV)	Σ 65° South	8	30–50	0.001	—
	Σ Lazarev Sea			0.284*	0.2	
<i>Euphausia frigida</i> (FIII–V)	29 Mar	13	50–155	0.025	—	
	6 Apr	8	25–190	0.429	—	
	Σ Polar Front			0.003	—	
(Juvenile/Adult)	Polar Front	15	700–4,000	0.362*	0.005	
<i>Euphausia triacantha</i> (FIII/IV)	25 Apr	8	860–1,350	0.528*	0.026	
	29 Mar	8	70–200	0.516*	0.049	
<i>Euphausia superba</i> (FIII)	Σ Lazarev Sea	16	20–65	0.123	—	
	(Juvenile)	23	2,100–7,600	0.015	—	
	(Adult)	20	12,000–27,000	0.049	—	

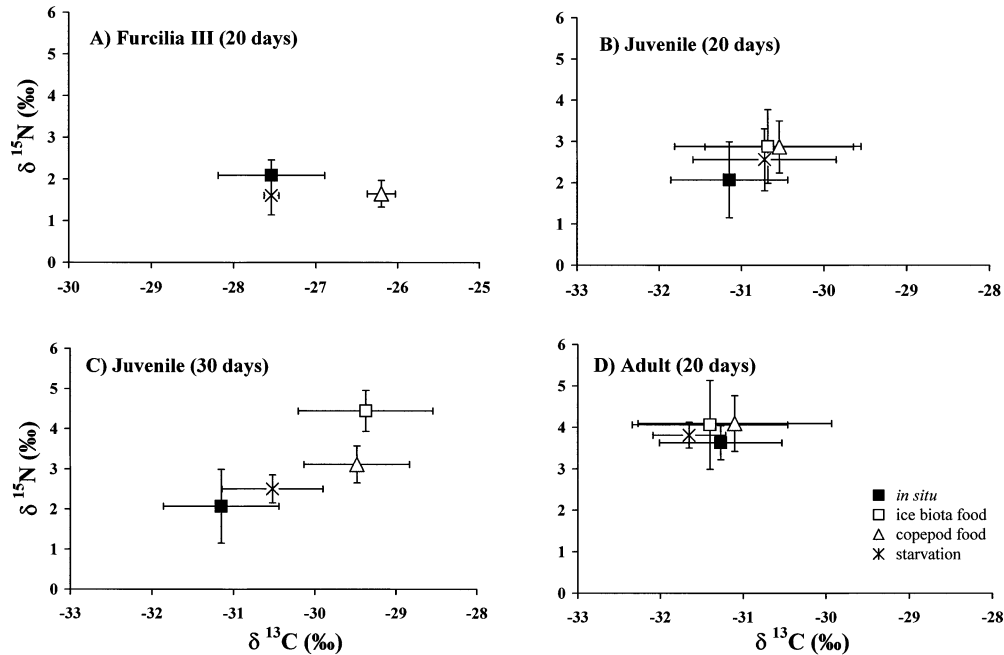


Fig. 3. Long-term incubation experiment. Stable isotope values of *E. superba* (‰, ± 1 SD) collected in the Lazarev Sea during autumn 2000 and after incubation in different food regimes for 20 or 30 d. The number of replicate measurements for furcilia was 4–6, each being of three individuals, whereas, for juveniles and adults, 6–8 replicate individuals were measured. (A) Furcilia, (B, C) juveniles, and (D) adults.

isotope approach for mesozooplankton. Further support is the fact that well-known copepod species had relative and absolute $\delta^{15}\text{N}$ values in line with findings from mouthpart morphology (Vervoort 1957), gut content analyses (Hopkins et al. 1993a,b), and feeding incubations (Atkinson 1995).

For postlarval krill, however, the offset of their $\delta^{15}\text{N}$ relative to POM was at times unrealistically small or large. In contrast to the large and coherent variations among POM and copepods, the mean $\delta^{15}\text{N}$ values of adult *E. superba* varied only by 2.5‰ (including summer data from South Georgia, the South Shetland Islands, and Marguerite Bay, not presented herein). Thus, although mesozooplankton were sensitive to fluctuations in their baseline, postlarval krill integrated them. Below we discuss (1) inferences from stable isotopes on the trophic relationships of mesozooplankton, (2) problems that may have arisen for larger/slower-growing species, and (3) some possible refinements of the method for such species.

Uses of the stable isotope approach: trophic ecology of Southern Ocean mesozooplankton—Because of the balance of sampling in the Southern Ocean, Antarctic copepods are much better known than the inhabitants of the Sub-Antarctic water ring. Among the former, the known carnivores, *Euchaeta* spp. and *Heterorhabdus* spp., had the highest $\delta^{15}\text{N}$ values, whereas suspension feeders like *C. acutus* had low values. Acknowledged omnivores, *C. propinquus* and *M. gerlachei*, were intermediate in position. Of the lower latitude copepods, *R. gigas* and *C. simillimus* dominate the biomass, but their diets are debated (Graeve et al. 1994; Atkinson 1996). In the present study, *R. gigas* had the lowest $\delta^{15}\text{N}$ of all copepods sampled at the Polar Front, $\sim 3\%$ above the

POM baseline, which implies feeding on algae. Higher $\delta^{15}\text{N}$ values of *C. simillimus* suggest omnivory. We are unaware of any food data for larvae of *Thysanoessa* spp. and *E. frigida*. However, their $\delta^{15}\text{N}$ values were higher than those of the mainly herbivorous *E. superba* larvae (Quetin et al. 1994; Meyer et al. 2002), which indicates a more omnivorous diet.

A major benefit of the stable isotope approach is that it can be used for small zooplankton, either early larvae or small species. Although this fraction can dominate community consumption, it remains poorly studied, given that feeding experiments and gut content analyses are hard to conduct (Schnack et al. 1985). Our study shows that, for most copepod species, $\delta^{15}\text{N}$ values increase significantly with body size. Early copepodites of *C. propinquus*, for instance, were $\sim 3\%$ (roughly one trophic level) lighter than adults. This suggests fundamental changes in trophic status with ontogeny and underlines the problem of generalizing trophic relationships from analyzed adults.

A second benefit of isotopes is in providing insights into food sources that may otherwise be indistinguishable. In the Southern Ocean, an example is the question regarding ice biota as an alternative food source for *E. superba* during seasons of low phytoplankton abundance (Frazer 1996; Quetin et al. 1996). Sea ice diatoms are the same taxa as found in the water column, limiting their morphological separation during gut analysis (e.g., Meyer et al. 2002). However, an isotopic separation is possible, because ice algae are typically enriched in ^{13}C and ^{15}N (Wada et al. 1987; Fischer 1991; Rau et al. 1991b). In support, we found higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios in melted sea ice relative to POM and in an

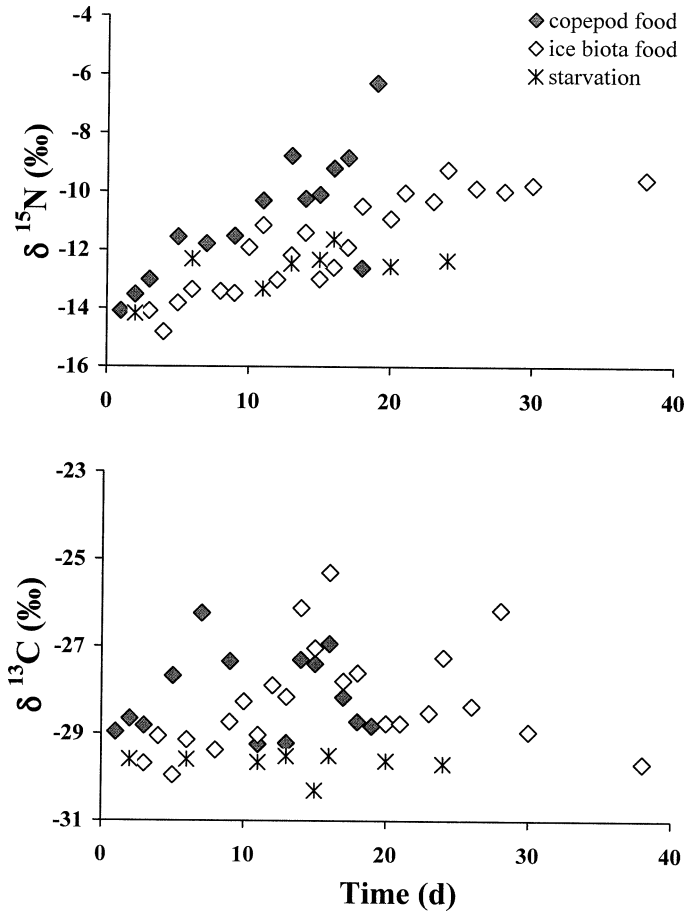


Fig. 4. Long-term incubation experiment. Changes in the stable isotope values of *E. superba* (juvenile/adult) molts during incubation in different food regimes. All molts of juveniles and adults shed between two sampling dates were pooled for a single analysis. After 20 d, most of the krill had been frozen, and molts were often too scarce for further measurements.

ice-dwelling harpacticoid copepod relative to pelagic copepods.

Ice cover was extensive in this autumn Lazarev Sea study, but several strands of evidence suggest that the zooplankton were not exploiting the ice biota. First, the $\delta^{13}\text{C}$ values of all zooplankters and the fecal pellets of postlarval *E. superba* were much lower than those of the ice biota. Second, $\delta^{13}\text{C}$ values of larval and postlarval *E. superba* increased while feeding on ice biota in the lab, which suggests a switch from their main *in situ* food source. However, it is possible that species fed at the ice-water interface, where nutrient concentrations and, thus the isotope signals of the algae, were more similar to those in the pelagial. Nevertheless, extensive feeding actually within the ice seems unlikely. Given the great practical difficulties in observing the under-ice habitat (Daly and Macauley 1991; Quetin et al. 1996), insights from isotopes can add support to direct observation.

Limitations of the stable isotope approach—Our results for the trophic position of postlarval krill seem reasonable for animals sampled in the Lazarev Sea and in the Polar

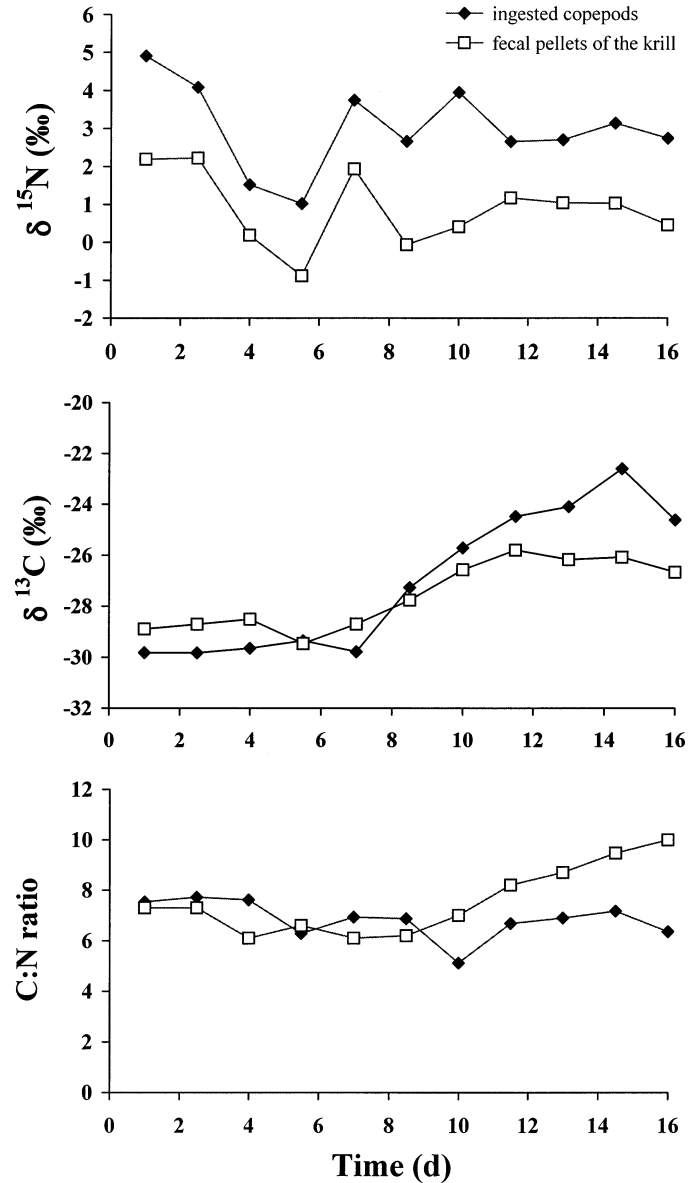


Fig. 5. Long-term incubation experiment. Stable isotope and C:N values of *E. superba* (juvenile/adult) fecal pellets in relation to their copepod food. For each sampling date one filter of concentrated fecal pellets was measured. The mean isotope signal of the ingested copepods was calculated from results of the feeding experiments (Schmidt unpubl. data).

Front, but not for Marguerite Bay or the Weddell Gyre. In Marguerite Bay, the $\delta^{15}\text{N}$ of adult *E. superba* was lower than that of larvae and only $\sim 1\text{‰}$ above the POM baseline. However, the baseline value was markedly high here (Fig. 2). In contrast to the Lazarev Sea where the phytoplankton concentration was very low, Marguerite Bay was sampled near the end of a 3-month phytoplankton bloom, when nitrate availability was decreasing (A. Clarke, unpubl. data). Thus, the high baseline here would be explained by isotopic fractionation accompanying uptake of nitrate. We suggest that the turnover/growth rates of the mesozooplankton were high enough to keep their $\delta^{15}\text{N}$ in line with the baseline increase

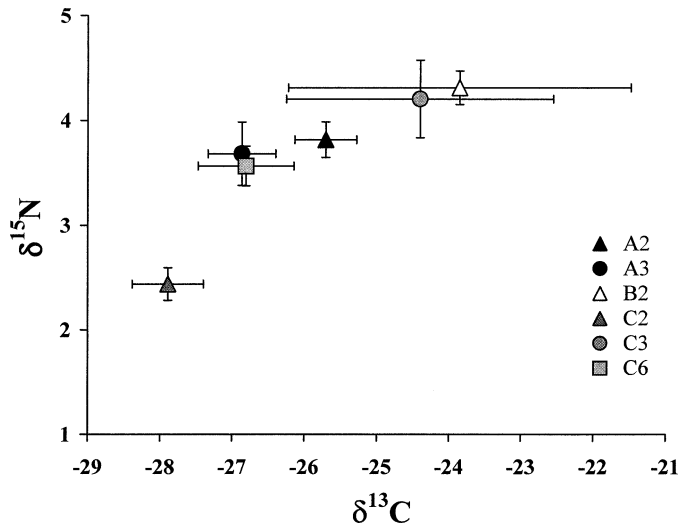


Fig. 6. Stable isotope values of *E. superba* juveniles ($\text{‰} \pm 1$ SD, $n = 4$) collected at six stations around South Georgia in summer 1996. Sampling dates and station positions are in Cripps et al. (1999). Station prefixes A, B, and C were designated by Cripps et al. (1999) from fatty acid analysis and refer to three distinguishable fatty acid compositions. Station positions: A2, 49.81°S, 40.07°W; A3, 53.24°S, 39.58°W; B2, 53.84°S, 38.96°W; C2, 54.01°S, 35.43°W; C3, 53.85°S, 38.46°W; and C6, 53.17°S, 39.37°W.

but those of adult krill were not. Copepods can grow faster than krill (Quetin et al. 1994; Shreeve and Ward 1998), and we found that postlarval *E. superba* had a slow, 2–3-month, N turnover time in autumn, even though offered food concentrations were high.

Another example of the unrealistic results for postlarval krill comes from the transect in autumn 1999. Here, the POM in the Weddell Gyre had lower $\delta^{15}\text{N}$ values than those in the Polar Front or Lazarev Sea. Common copepod species reflected this pattern, whereas postlarval *E. frigida* had the same $\delta^{15}\text{N}$ values in both regions. The latter resulted in an unrealistically large offset from the baseline in the Weddell Gyre, even though the species is known to be omnivorous (Hopkins et al. 1993a). We suggest that postlarval *E. frigida* migrated or were advected to the Weddell Gyre area, but its isotopic composition still reflected a source region with a higher baseline signal.

These characteristics of postlarval euphausiids, namely their slower turnover/growth rates and ability to migrate, might confound trophic effects with those of a temporally/spatially changing baseline. The stable isotope composition is especially vulnerable to such aliasing effects, compared, for example, with fatty acid markers, because it integrates over long timescales. To reach isotopic equilibrium with a new diet, animals must either replace most of their body carbon/nitrogen or increase greatly in mass. In contrast, specific fatty acids can be preferentially assimilated and stored, rapidly changing the total composition. This might explain why the trophic position of South Georgia krill suggested by their $\delta^{15}\text{N}$ values (Fig. 6) did not fit results of their fatty acid composition (Cripps et al. 1999). A recent change in the diet would be revealed by the former but not by the latter.

A slow turnover/growth rate of higher trophic levels and

the ability to migrate will only limit the use of the stable isotope approach if the food web baseline signal varies. However, other Southern Ocean studies, as well as our own, have shown regional/seasonal differences in the $\delta^{15}\text{N}$ of the POM (Wada et al. 1987; Biggs et al. 1989; Rau et al. 1991b; Frazer 1996). The $\delta^{15}\text{N}$ of phytoplankton-dominated POM depends on the isotopic composition of the primary nitrogen source and its fractionation during uptake and assimilation (e.g., Montoya and McCarthy 1995). These are influenced by seasonally and regionally variable factors such as the composition of the phytoplankton community, availability of nitrogen sources and iron, light intensity and temperature (e.g., Mariotti et al. 1981; Waser et al. 1998).

The problems of these changes in the $\delta^{15}\text{N}$ values of the food-web baseline have been emphasized in other, non-Antarctic systems. In Lake Tanganyika, O'Reilly and Hecky (2001) found significant temporal variation in $\delta^{15}\text{N}$ of the zooplankton, but not in fish, in response to the isotopic signal of the primary producers. In the Baltic Sea, Rolff (2000) described a trimodal seasonal cycle in $\delta^{15}\text{N}$ of phytoplankton, which was reflected in all plankton size classes but with different time lags. Thus, our results add to a growing appreciation that, in isotopic analysis of food webs, varying temporal integration of consumers need to be considered.

Some possible refinements—There is obviously no cutoff in animal size/growth or turnover rate after which the $\delta^{15}\text{N}$ -indicated trophic position is prone to artefacts. The robustness of interpretations also depends on the magnitude and the time- and space scales of baseline fluctuations within the system under study. Thus, our field-measured $\delta^{15}\text{N}$ values of larval euphausiids seemed reasonable, even though turnover rates of *E. superba* furcilia in the laboratory were low in autumn/winter (Frazer et al. 1997; this study).

Smaller species and fast growing larvae will generally have higher metabolic activity and faster incorporations of new N and C (e.g., Ikeda 1974; Fry and Arnold 1982). Therefore, isotope analyses of micronekton, outside of its main growth period, should be done on tissues or metabolic products with higher turnover rates than the whole animal. Those $\delta^{15}\text{N}$ values are more likely to reflect the recent food web baseline and are better comparable to values of smaller species with a faster total turnover. In a similar way, baleen or blood serum have been studied in mammals (e.g., Schell et al. 1989; Lesage 2001).

Our experiments attempted to find suitable tissue/metabolic products of postlarval *E. superba*, differentiating among the hepatopancreas region, abdominal muscle, molts, and fecal pellets. The $\delta^{15}\text{N}$ values of the first two tissues did not respond faster to a new diet than those of the whole animal. In contrast, fecal pellets and molts equilibrated with the food much more quickly, supporting results of Gorokhova and Hansson (1999) on mysids. Fecal pellets reflected very recent feeding, whereas molts integrated over ~3 weeks. Applying these results to molts and fecal pellets of freshly caught krill from the Lazarev Sea in autumn, we suggest that animals were mainly herbivorous in the weeks before capture but were feeding omnivorously within the preceding 1–2 d. The latter parallels gut content analysis from the same location (Atkinson et al. 2002).

In contrast to other studies, finding especially high $\delta^{15}\text{N}$ values in feces (Montoya 1994; Gorokhova and Hansson 1999), $\delta^{15}\text{N}$ of krill fecal pellets were always lower than the ingested copepods (Fig. 5). Thus, in common with approaches using the whole animal, it will be absolutely essential to do further laboratory calibrations of tissue-specific fractionation and turnover rates to avoid erroneous conclusions.

Seasonal and regional variability in the $\delta^{15}\text{N}$ of the POM baseline seems to be a common phenomenon not only in the Southern Ocean. This will be a serious problem when comparing consumers which integrate the baseline signal over different periods—such as small/fast-growing copepods and larger/slow-growing krill. To avoid erroneous conclusions, differences in the integration time have to be reduced either by sampling repeatedly over the season to smooth out fluctuations or by analyzing components of larger species with a faster turnover than the whole body. The former is most useful for a general indication of their trophic positions in a food web, while the latter can give specific information—for example, on responses to a phytoplankton bloom or to seasonal food shortage. Which tissue or metabolic product to choose depends on the time scale of interest and on the practicability with the animal under study. Testing and developing stable isotope approaches for specific tissues or metabolic products will be a way forward to get more detailed insights in the feeding ecology of micronekton.

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