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On the ecology of larval krill, Euphausia superba, during winter: krill - sea ice interactions

A Dissertation submitted in partial satisfaction
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in

Biology

by

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PREFACE

This dissertation consists of four related chapters that have been formatted for different scientific outlets. As a consequence, some inconsistencies in style among the different chapters will be encountered by the reader. Each chapter, however, is complete in itself, and contains a general introduction, methods and results sections, and a discussion of the preceding materials. A separate list of literature cited accompanies each chapter.

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My graduate committee contributed, in large part, to my current scientific philosophy, and thus fulfilled a most important professional obligation. I will be forever thankful to Langdon Quetin and Robin Ross for affording me the opportunity to work in the Antarctic, and for allowing me the freedom to explore. This work is a result of their own, and should be viewed as a collaborative effort. Alice Alldredge and Barbara Prézelin helped to broaden my scientific perspective, and both were instrumental in my success here at UCSB. My wife, Charlene, helped me to balance science with the rest of my life, and she deserves more credit than I can give here.

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FIELDS OF STUDY

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Studies in Zooplankton Ecology

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Studies in Ecological Physiology

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Studies in Biological Oceanography

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ABSTRACT

On the ecology of larval krill, Euphausia superba, during winter: krill - sea ice interactions

by
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Antarctic krill, Euphausia superba, is the dominant zooplankter and a keystone species in the Southern Ocean. Krill has a broad circumpolar distribution, with highest concentrations generally restricted to areas affected by seasonal advance and retreat of sea ice. Both adults and larvae have been observed to feed on ice-associated food resources, and larval krill may be obligate consumers of ice-algae during winter. The degree of dependence of larval krill on ice and its associated food resources, however, is based largely on anecdotal information.

Chapters I and II of this work contain the first quantitative characterization of the abundance and distribution of larval krill directly associated with sea ice west of the Antarctic Peninsula during winter, and establish that a large portion of the larval population occupies the ice habitat. Samples of larvae collected by SCUBA divers and with nets are compared with respect to abundance, size and stage

composition in Chapter II. Larval aggregation size and frequency of occurrence are reported to differ in early and late winter. Details of patch characteristics for larvae in the sea ice are given, and several possible explanations for the observed small-scale distribution patterns are discussed.

The use of stable isotopes as a tool to quantify the assimilation of ice-associated biota in the winter diet of larval krill is evaluated in Chapter III. Reported variation in the stable carbon and nitrogen isotope composition of larval krill and suspended particulate organic matter, particularly in early winter, has significant consequences for the interpretation of field data. A complete isotopic characterization of the early life history of krill (egg through juvenile stage) is provided in this section. Use of stable isotopes as tracers to measure carbon and nitrogen turnover in larval krill is discussed in Chapter IV. These are the first coupled measures of carbon and nitrogen turnover, using this approach, for any marine organism, and provide essential information for the interpretation of isotopic data collected from the field. The results of this research will be of interest to krill biologists as well as those studying food web interactions and/or biogeochemical cycling in a broader ecosystem context.

CHAPTER I

Abundance and distribution of larval krill, Euphausia superba, associated with annual sea ice in winter

ABSTRACT

Larval krill, Euphausia superba, associated with annual sea ice were censused visually using SCUBA during three winter cruises to a region west of the Antarctic Peninsula. Sampling during September 1991 and June 1993, was restricted to a small number of stations offshore of Adelaide Island. A more extended, mesoscale survey was conducted in September 1993. Larval krill were observed feeding on ice-associated biota at all sampling locations (N = 36) on each of the three cruises. Mean numbers of larval krill (individuals per m^2) per station for September 1991, June 1993 and September 1993 were 24.60, 2.04 and 16.72, respectively. Larval abundances were significantly greater during late winter (September) sampling periods. A majority of larval krill censused during late winter occurred in large aggregations ($\geq 10^3$ individuals). Large aggregations of larvae were not found in early winter. Eighty percent of larvae were observed under complex habitat provided by over-rafted and/or eroded ice floes, and were generally associated with upward facing ice surfaces. Larval krill were rarely observed on the downward facing surfaces of unilayer floes, though ice-algae was often visible in these areas.

INTRODUCTION

Qualitative observations of larval krill, Euphausia superba, feeding on the undersides of sea ice (e.g., Guzman 1983, Stretch et al. 1988, Marschall 1988 and Daly 1990) are suggestive of an important ecological coupling. Quetin and Ross (1991) suggested that the association is based on the need to feed on the ice-associated community, although sea ice may also provide a refuge from predation. The two scenarios are not mutually exclusive, and it is clear that the intricacies of the linkage are not completely understood.

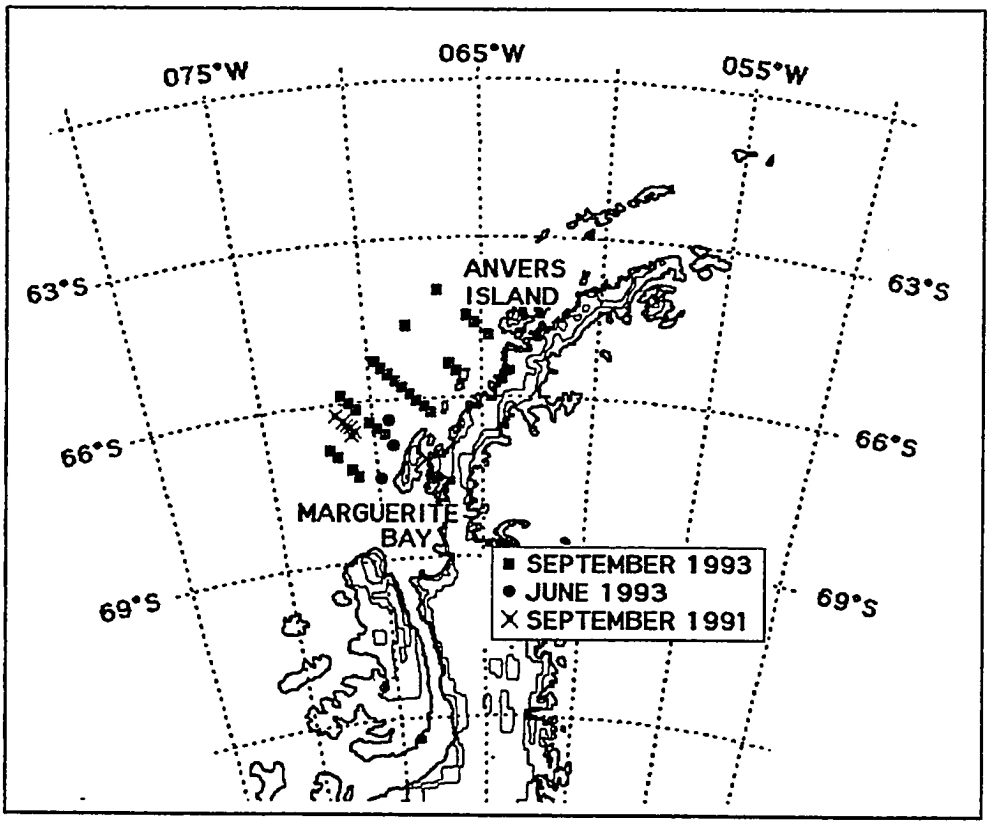
Smetacek and coworkers (1990) suggested "that the bulk of the krill population moves into the ice habitat upon its formation", but quantitative data needed to evaluate this claim are lacking. Direct measures of the abundance of krill associated with sea ice are few (e.g., O'Brien 1987, Marschall 1988 and Hamner et al. 1989), and no systematic surveys of larvae have been reported to date. As part of our ongoing study on the ecology and physiology of larval krill during winter, we have quantified the abundance and distribution of larvae in the sea ice habitat during three cruises in the austral winter.

We intend to combine small scale distributional data and behavioral observations to increase our understanding of how krill larvae exploit the ice habitat. Quantitative census data from early and late winter sampling periods provides additional insight into temporal variablity of krill associated with sea ice. Our results are essential detailed information for formulating hypotheses and further investigating the krill/ice interaction.

METHODS

Larval krill associated with annual sea ice were censused visually with SCUBA during three winter cruises to a region west of the Antarctic Peninsula (Figure 1). During September 1991 and June 1993, sampling was restricted to a relatively small area offshore of Adelaide Island. During September 1993, a mesoscale survey was conducted between Anvers Island and Marguerite Bay. The mesoscale survey of the under-ice habitat for larval krill was conducted within the confines of the Palmer Long-Term Ecological Research (LTER) site (Waters and Smith 1992) and encompassed the sampling areas of the two previous cruises.

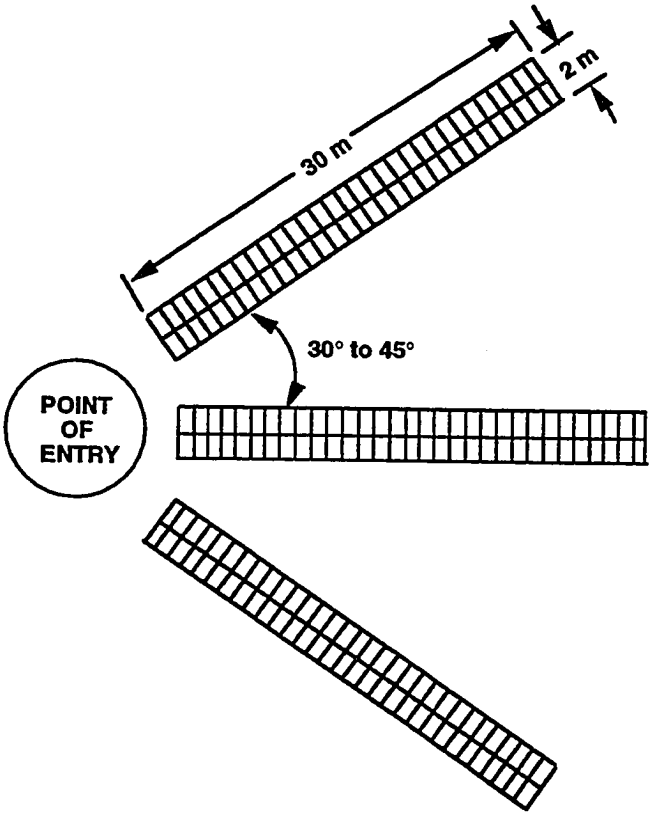
Figure 1. Sampling stations occupied during each of the three winter cruises to the west of the Antarctic Peninsula September 1991, June 1993 and September 1993. Adelaide Island is the large island north of Marguerite Bay.



At each sampling station krill were censused along 2-m wide X 30-m long transects originating at the divers point of entry (Figure 2). Observations were made to a depth of 3 m below the nearest ice surface, though larval krill were rarely observed more than 0.5 m away from any ice surface. Three replicate transects, between 30° and 45° apart during a single 30 to 60 minute dive, were generally completed at each station (32 of 36 total). At the conclusion of each dive additional observations were recorded, e.g., estimated percentage of over-rafted ice in the sampling area and other fauna present.

Estimates of animal abundance in situ are influenced by such variables as visibility, sampling by different individuals and animal behavior. The censuses reported here were completed by a single individual to eliminate between sampler variability. Krill were censused during daylight hours. Visibility was generally greater than 30 m so it was easy to see individual krill to either side of the transect line. Larval krill associated with sea ice generally remain site-attached during a dive and do not exhibit an escape response into the water column when approached slowly by a diver; likewise, larvae do not exhibit directed movements along ice surfaces over any appreciable

Figure 2. Schematic depiction of diver transects at each sampling station. Krill larvae were censused continuously along replicate strip transects (30 m X 2 m).



distance (< 1 m). Even when disturbed by a diver with a collecting net, the integrity of the aggregation is soon re-established, generally at the collection site. For small aggregations, less than 50 individuals, absolute counts were possible. The total number of krill in larger aggregations was estimated by counting the number of volumes in an aggregation equivalent to a sub-volume with a counted number of individuals (10's, 20's, 50's).

Unless otherwise specified, larval abundances were calculated as the number of individuals per m^2 and are reported as the mean of replicate transect counts at each sampling station. A Kruskal-Wallis non-parametric procedure with Chi-square approximation (Sokal and Rolf 1981) was used to compare larval counts among sampling periods as variances were determined to be heterogeneous. For latitudinal comparisons the data from September 1993 were binned into one of three broad categories: (1) transect data collected at or north of 65°S, (2) transect data collected between 65°S and 66°S, and (3) transect data collected at or south of 66°S. Standard ANOVA procedures were used for the analysis.

RESULTS

Larval krill were observed in close association with sea ice at all under-ice stations during each of the three winter cruises where quantitative sampling was conducted (Figure 3). The mean number of larvae (individuals per m^2) per station did not differ between September 1991 and September 1993, but counts during the sampling period in June 1993 (early winter) were significantly less than those from either late winter cruise (Table 1).

Large, distinct patches of larval krill ($\geq$ 1,000 individuals) accounted for less than five percent of our observations, but comprised 50 percent or more of the censused population (Figure 4). Large patches of larval krill were not observed on diver transects in June 1993. Early winter sampling during both years was conducted in the same general geographic area west of Adelaide Island. Within the mesoscale sampling grid, there was no latitudinal gradient in krill abundance or distribution (ANOVA, df = 2, $p \geq 0.05$). No cross-shelf gradients in abundance and distribution of larval krill were apparent (Figure 3A).

Figure 3. Mean number of krill larvae per m^2 at each sampling station for each of the three winter cruises: (A) September 1993, (B) September 1991 and (C) June 1993. All data are plotted with reference to established sampling locations within the Palmer LTER grid (Waters and Smith 1992).

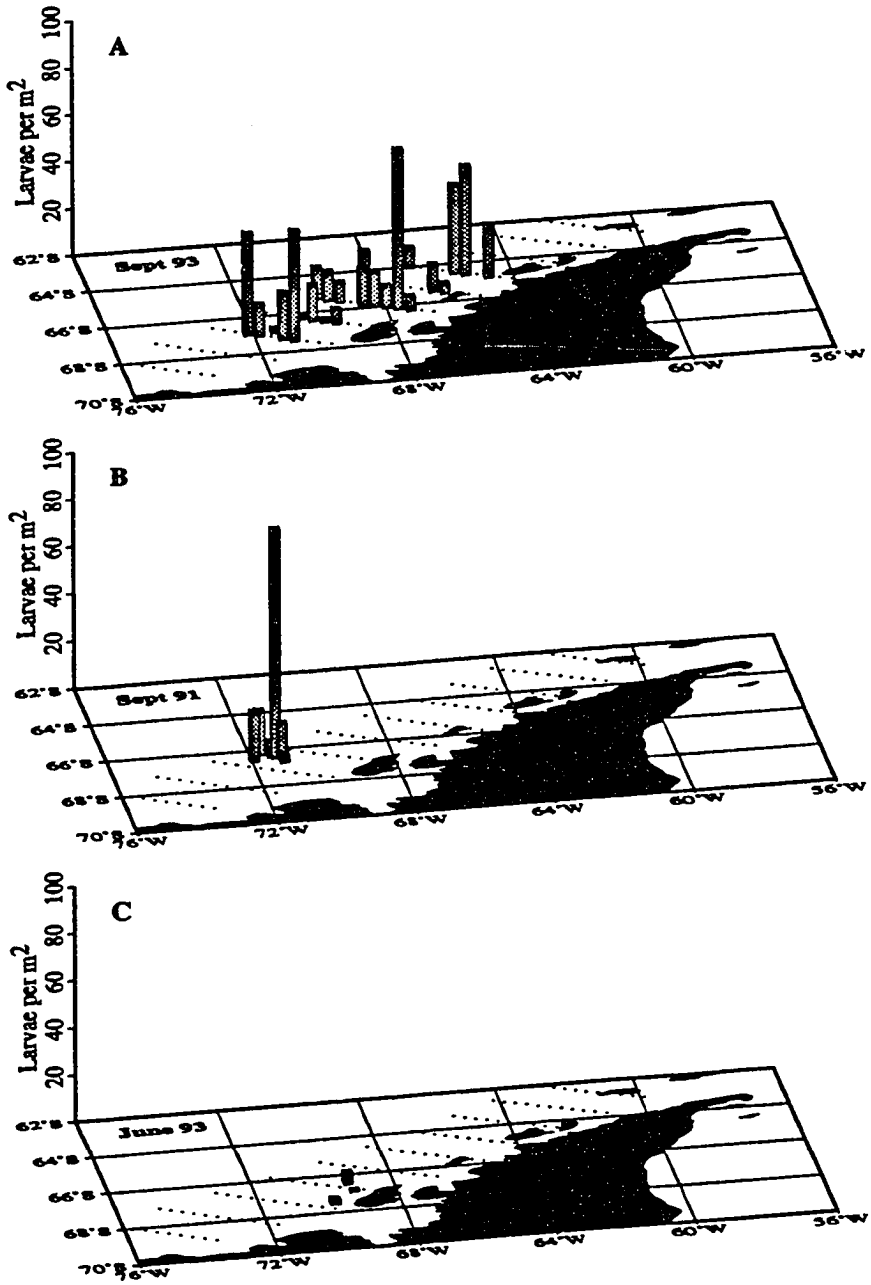


TABLE 1. Mean number of krill larvae per m^2 ($\pm$ standard error) for each of the three winter cruises. Sampling periods are ranked in order of increasing larval abundance (June 1993 < September 1993 = September 1991).[a]

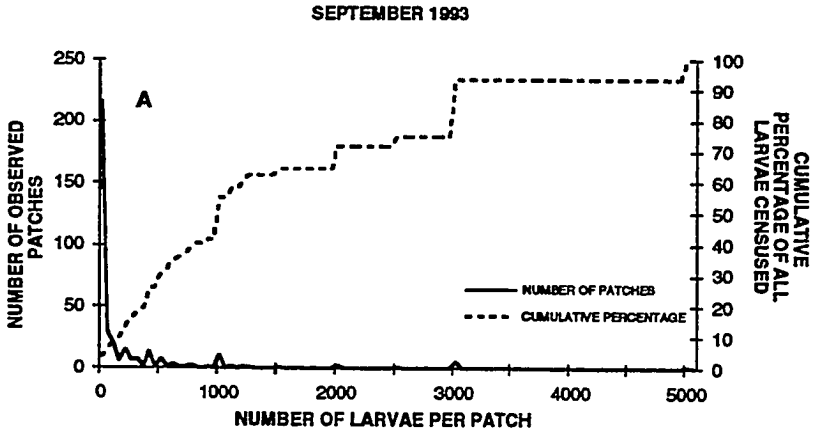
SAMPLING PERIOD	LARVAE per m^2	SAMPLING STATIONS	TOTAL # of TRANSECTS
JUNE 1993	2.04 ($\pm$ 1.32)	3	9
SEPT 1993	16.72 ($\pm$ 3.39)	26	73
SEPT 1991	24.60 ($\pm$ 12.42)	6 [b]	21

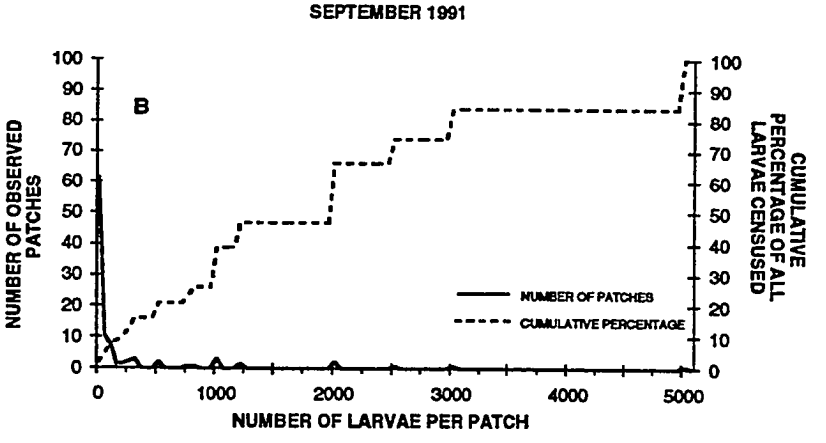
[a] the inequality is significant at the $p \leq 0.08$ level, Kruskal-Wallis test with Chi-square approximation (X^2=4.92, df=2)

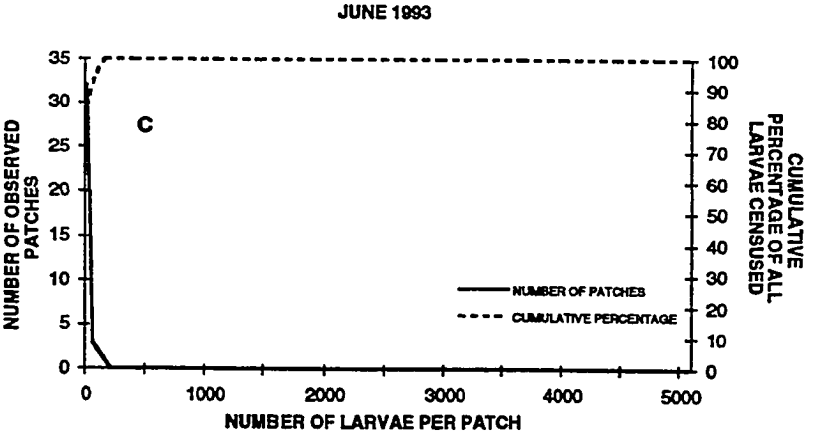
[b] one sampling station was occupied twice during September 1991

Behavioral observations of larvae and the green coloration of their digestive gland indicated that most animals were feeding. However, larvae were not necessarily found in areas of highest plant pigment concentration. Greater than 80 percent of all larval krill were associated with over-rafted and/or eroded ice surfaces where plant pigment was rarely visible to the naked eye. Larval krill were less common on the downward facing surfaces of floes that were not over-rafted, although ice-algae was often visible in these areas.

Figure 4. Observed frequency of krill patches and cumulative abundance of individuals plotted against patch size for each of the three winter cruises: (A) September 1993, (B) September 1991 and (C) June 1993.







DISCUSSION

Though sea ice had just recently formed in the northern portion of the mesoscale sampling grid in September 1993, larvae were observed at all stations with no statistical differences in abundance with latitude. This raises several questions: (1) Do krill larvae inhabiting the water column stay with sea ice once it is formed? (2) Did larvae sampled in the northern part of the LTER grid overwinter in ice-free water or (3) were they advected into the region prior to ice formation?

The distributional range of larval krill under sea ice during winter is not known. Nordhausen (1994) investigated the abundance and distribution of E. superba in ice-covered waters west of the Antarctic Peninsula. Although Nordhausen's study was restricted to the Gerlache Strait and inshore waters of Crystal Sound, no larvae were found during July 1992. This suggests that larval krill may, in general, overwinter in shelf and slope waters of the Antarctic Peninsula as observed and reported here. Comparative sampling in these distinctly different areas during the same year(s) would provide a test of the implicit hypothesis above.

Quantitative sampling of krill larvae is essential to evaluate spawning success and for subsequent predictions of year class strength and recruitment to the adult population. However, few quantitative studies of larval krill abundance and distribution have been conducted during winter in the Southern Ocean, especially in the region covered by sea ice. One major difficulty is that in the complex ice habitat, larvae often occur on upward facing ice surfaces and net sampling is clearly not representative of larval abundance in toto. Quantitative surveys by divers is currently the best way to evaluate larval abundance and distribution in the sea ice habitat.

The mesoscale survey during September 1993 covered a region large enough region to let us estimate the importance of sea ice to larval krill as a population. Larval krill were consistently found under the ice during September 1993, and a simple extrapolation of their mean abundance (16.72 larvae per m^2) yields a total estimate of 1.3 X 10^{12} larvae within the 200 km X 400 km sampling region. If we assume that instantaneous rates of mortality (M) for all age-classes of post-larval krill are equal and $\leq$ 1.0 (cf. Priddle et al. 1988 and references therein, Siegel and Kalinowski 1994), and that recruitment is the same every year, then the larval concentrations in September 1993

are sufficient to generate an adult krill population on the same order of magnitude (Table 2). The projected number of adults within the sampling region would be between 9.7 and 17.1 per m^2, with an M of 1.0 and 0.65, respectively (Table 2). These numbers compare favorably with Smetacek et al.'s (1990) estimation of 10 to 30 adults per m^2 under sea ice at its maximal extent (based on population estimates by El-Sayed (1988)), and, if emigration is zero, suggest larval concentrations in the geographic region immediately west of the Antarctic Penisula are not greater than necessary to maintain an average adult population. This would be counter to a generally held contention that the region west of the Antarctic Peninsula is an important nursery area for larval krill compared to other regions of the Southern Ocean.

TABLE 2. Projected numbers of post-larval krill within the 200 km x 400 km sampling region based on larval abundance estimates from September 1993 [a]. The projected numbers will vary depending on the assumed instantaneous rates of mortality (M). Two different scenarios are given.

age-class (year)	Mean number of krill per m^2	Total number of krill
age-class 1 [a]	16.72	1.3×10^{12}
Projection #1 (M = 1.0 [b])		
age-class 2-7	9.70	7.5×10^{11}
Projection #2 (M = 0.66 [c])		
age-class 2-7	17.10	1.3×10^{12}

[a] The larval population present at the end of winter (September 1993) and the one year old age-class at t_0 are, for the purposes of this exercise, assumed to be the same. It is assumed also that the numbers of krill in age-class 1 is constant from year to year. Rates of immigration and emigration of post-larval krill into and out of the 200 km x 400 km sampling region are assumed to be equal.

[b] M = 1.0 is the average value assumed by Priddle et al. (1988).

[c] M = 0.66 is the lowest value expected by Siegel and Kalinowski (1994).

We recognize that larval numbers observed in September 1993 may not be representative of those in other years, but larval abundances in September 1993 were remarkably similar to those estimated during September 1991 and suggest otherwise. Continued censuses of larval krill associated with sea ice in late winter need to be interpreted relative to the variability of winter ice cover and the spawning success of the previous season to understand the population dynamics of krill in this region.

Larval krill were found to be most concentrated in areas of over-rafted sea ice with upward facing ice surfaces. Although Smetacek et al. (1990) suggested that ice surface area would be greater by about one-third because of ridging of cover, estimates of over-rafting by divers west of the Antarctic Peninsula are generally less ($\leq$ 5%), particularly in early winter. More work is needed to understand the ecology of larval krill relative to habitat complexity. A lack of habitat complexity may explain, in part, patch characteristics and low counts of larval krill during early winter (Figure 4A, Table 1). Little is known about krill's early winter transition from the water column to the ice, but the fact that large patches of larval krill observed during late winter were not common during early winter sampling is consistent with the hypothesis that sea ice

facilitates aggregation and formation of krill swarms (Hamner et al. 1989). Since krill numbers and the frequency of large patches appear to increase over winter in the sea-ice habitat, estimates of larval numbers based on diver observations in early winter may not be valid.

Contrary to several schematic depictions of krill feeding on downward facing ice surfaces during winter (e.g., Garrison et al. 1986 and Smetacek et al. 1990), larvae associated with sea ice are generally oriented with feeding appendages down and scraping the upward facing and eroded ice surfaces they tend to occupy. Since larval krill are generally not found in areas where plant pigment is most concentrated, structural characteristics of sea ice appear to be a primary determinant of krill abundance and distribution during winter. Larval krill appear to have an affinity for areas of over-rafted ice and the refuge it might afford. Unfortunately, there is a paucity of information regarding predator-prey interactions in the annual sea ice zone during winter and direct observations of predation on krill larvae are few. Hamner et al. (1989) observed ctenophores and an amphipod feeding on furcilia and further suggested that larval krill might be a significant component of the diet of fishes and migratory invertebrates, particularly in winter. Several alternative hypotheses can be posited to

explain krill larvae's apparent affinity for areas of over-rafted sea ice: (1) Feeding costs may be less for larvae on upward facing ice surfaces since feeding on downward facing surfaces will entail the additional cost of maintaining contact with the ice surface against a negative buoyancy. (2) Upward facing surfaces may also act as sediment traps which concentrate food resources and/or provide a substrate for prefered food items (O'Brien 1987). The relative importance of food resources and shelter to larval krill, as provided by annual sea ice, merits further investigation.

CONCLUDING REMARKS

This is the first quantitative account of larval krill associated with sea ice during austral winter and observations using SCUBA have proved to be a requisite method for investigating the the krill/ice interaction. The data reported here are essential information for the formulation of hypotheses and further study of this ecologically important linkage.

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CHAPTER II

Abundance, sizes and developmental stages of larval krill, Euphausia superba, in ice-covered seas west of the Antarctic Peninsula

ABSTRACT

Larval krill were sampled west of the Antarctic Peninsula during three winter cruises: September 1991, June 1993, and September 1993. Larval abundance estimates from net catches were compared directly to visual estimates made by divers of larvae occupying the ice habitat at the same sampling stations. Although the mean number of larve per m^2 sampled with nets was greater than observed by divers during each of the three cruises, statistical comparisons were not significant at the $P < 0.05$ level. Larval krill collected by divers were larger than those collected with nets during each of the three cruises. The stage composition of larvae depended also on the collection method; net-collected samples contained a disproportionate number of early furcilia larvae in June 1993 (early winter), and a disproportionate number of juveniles during September 1991 and 1993 (late winter). These results suggest that larval/juvenile krill occupy both the water column and sea ice habitat during the austral winter, and that there are often differences in the sizes and developmental stages of the two groups. For larval krill occupying the sea ice habitat, aggregations were larger and more numerous during late winter than in early winter. In addition, larvae within aggregations occupied structurally complex microhabitats,

provided by over-rafted ice floes, more often than they occupied smooth, downward facing ice-surfaces where ice was not over-rafted.

INTRODUCTION

Many investigators have reported observations of larval krill associated with annual sea ice in the Southern Ocean (e.g., Garrison et al. 1986, Kottmeier and Sullivan 1987, Stretch et al. 1988, Hamner et al. 1989, Daly 1990, Quetin and Ross 1988, 1991, Daly and Macaulay 1991). It is generally thought that ice-algae supports survival and growth of larval krill during austral winter when phytoplankton availability in the water column is low. However, the energetic role of ice-associated food resources in the winter diet of larval krill has not been adequately quantified (see Quetin et al. 1994), and the intricacies of the krill/ice interaction, as noted by Frazer et al. (in press), remain largely unexplored.

Larval krill exploit ice-associated biota as a food source, but there may be other energetic and/or ecological explanations for their presence in the sea ice habitat that account for the larvae's small-scale distributional patterns (Frazer et al. in

press). Moreover, Hamner et al. (1989) suggested that sea ice facilitates the aggregation and social development of larval krill thus providing a mechanism for school formation. It is unlikely, however, that the earliest stage furcilia larvae (F1's and F2's, see Fraser 1936) exploit the ice and its associated resources, and thus remain in the water column even if sea ice is present. Additional studies on the ecology and behavior of larval krill are needed to shed new light on the mechanisms associated with the formation and/or maintenance of larval krill aggregations.

Quantitative sampling of krill in austral winter is problematic, and the relative partitioning of larvae between the ice habitat and the water column below the ice is not known (cf. Smetacek et al. 1991). Direct counts of larval krill by divers indicate that krill can be numerous under the ice, particularly in late winter (Frazer et al. in press, Quetin et al. in press). However, larval krill are also captured with nets from the water column in this same habitat type (Guzman 1983, Daly and Macaulay 1991). No direct comparisons of these abundance estimates and/or the two methods have been made, although Quetin et al. (1992) and Frazer et al. (in press) suggested that nets may undersample larvae in ice-covered seas.

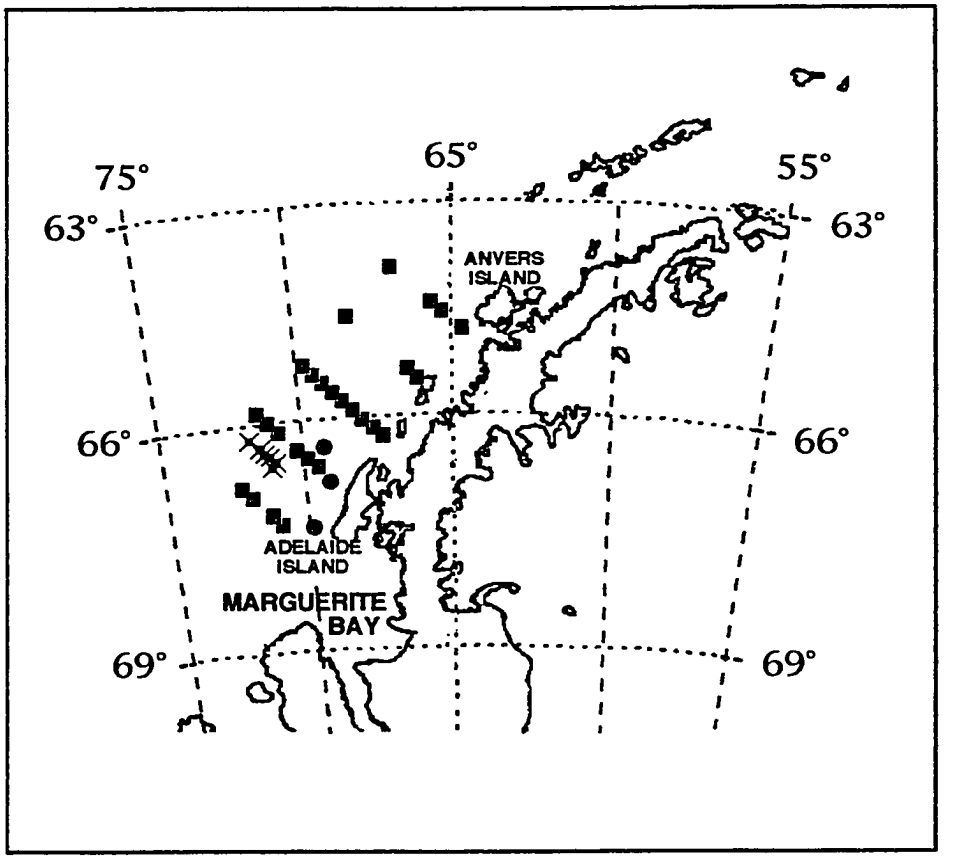
As part of this study, a comparison of net catch information and quantitative data collected by divers is made. Comparisons of size and stage frequency distributions for net-collected and diver-collected samples are used to test the prediction that early stage furcilia are absent from the ice habitat. Additional data on the small-scale patch characteristics of larval krill in the ice habitat is synthesized to expand on a recent report (Frazer et al. in press) on the abundance and distribution of larval krill associated with sea ice west of the Antarctic Peninsula. Unresolved questions relating to the ecology of larval krill in winter are addressed in the discussion.

METHODS

Study Area

Samples were collected from ice-covered seas west of the Antarctic Penisula during September 1991, June 1993 and September 1993 (Figure 1). Diver-collected and net-collected samples at any particular station were generally within 5 km of each other. During September 1991 and June 1993, sampling was restricted to a relatively small area offshore of

Figure 1. Sampling stations occupied during September 1991 (X), June 1993 (•) and September 1993 (▪). All sampling was conducted west of the Antarctic Peninsula in a region between Anvers Island and Marguerite Bay.



Adelaide Island over the continental shelf. During September 1993, a mesoscale survey was conducted between Anvers Island and Marguerite Bay; this area is in the center of the Peninsula grid (Waters and Smith 1992) of the Palmer Long-Term Ecological Research (LTER) site. Palmer LTER sampling stations are primarily over the continental shelf, although a few stations are over the slope.

Diver-collected samples

Quantitative Visual Census. Larval krill associated with annual sea ice were censused visually with SCUBA. At each sampling station, larvae were censused along 2-m wide by 30-m long transects to a depth of 3 m below the nearest ice surface. Usually three replicate transects at each sampling station were completed during a single 30 to 60 minute dive. Additional details and discussion of the method are reported by Frazer et al. (in press). Larval abundances were calculated as the number of individuals per m^2 and are reported as the mean of replicate transect counts at each sampling station.

Size and Stage Frequency Distributions. Larval krill collected for analyses of size distribution and stage frequency were

collected at sampling stations corresponding with those for diver censuses (Figure 1). Collections were made either on the census dive or on subsequent dives at the same station. Larval krill were captured by divers with hand-held aquarium nets (mesh size ca. 680 μm), and collections were taken from discrete patches of larvae (generally > 100 individuals). All of the larval krill, or a random subsample (minumum number = 88) from each collection, were staged according to Fraser (1936), and measured under a dissecting microscope for total length (TL: tip of rostrum to end of the uropod) to the nearest 0.1 mm. Measurements were recorded from fresh specimens, as well as animals preserved in 10% buffered formalin.

Characterization of Patch Structure. Locations of discrete aggregations of larval krill (> 2 larvae) associated with sea ice were recorded to the nearest 1 m along each 30-m transect, and the numbers of individuals within each aggregation estimated as part of the in situ census method described above. The spatial orientation of the ice surface(s) occupied by larval krill within each aggregation was recorded. In some instances, larval krill within an aggregation did not exhibit an obvious orientation to any ice surface although individuals within the group were generally less than 0.3 m from one or more ice surfaces. Isolated individuals (89 total observations; not

included in subsequent analyses), and small clusters of larval krill (< 10) in close proximity to, but without obvious orientation to one another, were sometimes observed swimming in the water column less than 1 m from overlying sea ice. No larger aggregations (> 10) of larval krill were observed swimming in a directed manner below sea ice, i.e., no polarized schools were seen (cf. Hamner et al. 1989). Discrete patches of larvae were categorized as very small (< 10 larvae), small (tens of larvae), medium (hundreds of larvae) or large (thousands of larvae). A very large aggregation of larval krill (> 10,000 individuals) was observed on only one occassion (September 1991). This observation was placed into the "large aggregation" category for subsequent analyses.

Net Sampling

Collection of Larval Krill. Oblique tows, with nets attached to rectangular frames, were generally made to 300 m (Table 1). All tows in September 1993 were with a 1-m^2 (mouth area) net (333 µm mesh). In September 1991, a 1 m x 2 m frame with paired nets (1-m diameter, 505-µm mesh) was towed obliquely at two stations, and a bongo frame with paired-nets (0.6-m diameter, 505-µm mesh) was towed vertically at four of

six stations. During June 1993, a 1-m diameter ring net (505-µm mesh) was fished vertically to collect larval krill. In all cases, nets were rigged with a General Oceanics flow meter, and fished both up and down without an opening and closing mechanism.

Differences in net sampling methods among the three cruises were a consequence of sampling regimes established for other objectives or to accommodate variable ice conditions as during September 1991. Nets with mesh sizes of 333 µm and 505 µm will both retain larval krill > 3 mm in length (R. Ross, personal communication), and there is no reported data to suggest that any of the above methods are more or less selective for larval krill in the Southern Ocean. Vertical tows made in heavy sea ice will filter less water than oblique tows to comparable depths, but differences in escape responses of larval krill are not anticipated.

Quantitative Catch Information. For vertical net tows, the number of larvae captured was expressed per m^2 over the depth range sampled, i.e., generally 0-300 m (see Table 1). For each oblique net haul the entire catch of larval krill was enumerated and the volume of water filtered determined so as to derive an estimate of the number of larval krill per m^3. The

Table 1. Summary of cruises, dates and locations where quantitative sampling was conducted. Estimates of larval abundance as determined by divers and from net catches are arranged as paired comparisons for each sampling station.

	DIVER-COLLECTED DATA				NET-COLLECTED DATA				
CRUISE	DATE	LATITUDE (°S)	LONGITUDE (°W)	LARVAE PER m^2	DATE	LATITUDE (°S)	LONGITUDE (°W)	LARVAE PER m^2	NOTES
WCIV	22 Sept 91	66.517	070.917	20.4	21 Sept 91	66.529	070.925	15.92	4
WCIV	22 Sept 91	66.667	071.083	2.99	24 Sept 91	66.599	071.086	38.92	4
WCIV	19 Sept 91	66.450	071.1	14.4	20 Sept 91	66.468	071.112	12.38	4
WCIV	26 Sept 91	66.400	071.283	95.62	26 Sept 91	66.396	071.280	3.54	4
WCIV	18 Sept 91	66.317	071.383	6.19	18 Sept 91	66.317	071.390	162.50	5
WCIV	17 Sept 91	66.183	071.7	18.21	17 Sept 91	66.182	071.680	260.50	5
WCV	08 June 93	67.45	069.867	1.57	08 June 93	66.442	069.287	0.00	2
WCV	13 June 93	66.85	069.183	0.02	12 June 93	66.778	069.286	32.50	2
WCV	17 June 93	66.43	069.283	4.52	15 June 93	67.421	069.892	15.00	3
LTER93C	21 Sept 93	67.380	070.907	46.02	NO NET COLLECTION			N/A	
LTER93C	21 Sept 93	67.248	071.221	19.08	NO NET COLLECTION			N/A	
LTER93C	22 Sept 93	66.982	071.838	12.80	NO NET COLLECTION			N/A	
LTER93C	22 Sept 93	66.848	072.142	42.17	NO NET COLLECTION			N/A	
LTER93C	17 Sept 93	66.634	069.555	5.76	17 Sept 93	66.641	069.570	2.46	1
LTER93C	16 Sept 93	66.505	069.867	0.74	16 Sept 93	66.516	069.889	24.82	1
LTER93C	16 Sept 93	66.375	070.175	14.40	16 Sept 93	66.390	070.200	12.17	1

Table 1. continued

LTER93C	15 Sept 93	66.245	070.481	1.17	NO NET COLLECTION			N/A	
LTER93C	15 Sept 93	66.114	070.783	6.49	15 Sept 93	66.104	070.769	12.17	1
LTER93C	14 Sept 93	66.982	071.082	1.81	15 Sept 93	65.968	071.067	1.91	1
LTER93C	14 Sept 93	66.850	071.378	1.25	14 Sept 93	65.836	071.358	8.36	1
LTER93C	09 Sept 93	66.254	067.337	5.42	NO NET COLLECTION			N/A	
LTER93C	09 Sept 93	66.129	067.655	67.44	NO NET COLLECTION			N/A	
LTER93C	10 Sept 93	66.004	067.971	8.89	10 Sept 93	66.008	067.940	21.03	1
LTER93C	11 Sept 93	65.878	068.283	14.22	11 Sept 93	66.876	068.279	106.98	1
LTER93C	12 Sept 93	65.751	068.592	14.50	11 Sept 93	65.763	068.582	28.18	1
LTER93C	13 Sept 93	65.496	069.202	7.93	12 Sept 93	65.493	069.199	3.84	1
LTER93C	13 Sept 93	65.367	069.502	11.64	13 Sept 93	65.365	069.492	0.00	1
LTER93C	13 Sept 93	65.238	069.800	12.71	13 Sept 93	65.228	069.770	127.32	1
LTER93C	08 Sept 93	65.479	066.149	3.72	06 Sept 93	65.484	065.477	7.09	1
LTER93C	07 Sept 93	65.357	066.464	11.04	07 Sept 93	65.348	066.452	50.05	1
LTER93C	04 Sept 93	64.610	068.293	13.12	04 Sept 93	64.613	068.308	2.53	1
LTER93C	03 Sept 93	64.815	064.717	21.78	03 Sept 93	64.807	064.715	144.99	1
LTER93C	01 Sept 93	64.575	065.341	45.69	01 Sept 93	64.581	065.330	21.44	1
LTER93C	01 Sept 93	64.455	065.650	37.1	01 Sept 93	64.461	065.626	22.25	1
LTER93C	30 Aug 93	63.966	066.856	7.99	30 Aug 93	63.955	066.834	29.04	1

1. Oblique not tow (0-300m), fished both up and down, 333-μm mesh, 1-m^2 rectangular net
2. Vertical net tow (0-300m), fished both up and down, 505-μm mesh, 1-m diameter ring net
3. Vertical net tow (0-150m), fished both up and down, 505-μm mesh, 1-m diameter ring net
4. Vertical net tow (0-300m), fished both up and down, 505-μm mesh, 0.6-m diameter bongo net
5. Oblique not tow (0-300m), fished both up and down, 333-μm mesh, 2-m^2 rectangular net

number of larvae per m^3 for each catch was then standardized so as to reflect the number of individuals per m^2 over the integrated depth range of the tow (generally 0-300 m). Larval abundances were expressed as the number per m^2 of sea surface, assuming that the maximum depth of occurrence or the full depth of the water column had been sampled. Larval abundance estimates for the entire water column would then be compared directly to estimates made by a diver of larval krill occupying the shallow layer associated with the ice habitat in the same general location.

Sizes and Stage Frequency Distributions. Larval krill used for analyses of size distribution and stage frequency were from the same net collection used for the abundance estimates above (see Figure 1). All of the larval krill, or a random sample (minimum number = 35) from each collection, were staged and measured for total length as described above for diver-collected samples. Measurements were of animals preserved in 10% buffered formalin.

Comparisons and Analyses

Larval Krill Abundance (Diver Census vs. Net Catch). A sign test (Sokal and Rohlf 1981) was used to assess the probability that nets undersample larval krill in the ice habitat. Paired estimates of larval krill abundance, from direct diver observation and net sampling, were used to test a null hypothesis that positive and negative differences occurred in equal proportions, i.e., empirical data were compared to an expected binomial distribution. A similar non-parametric procedure, Wilcoxon's Signed-Ranks Test for two groups, arranged as paired comparisons (Sokal and Rohlf 1981), was used to determine if mean abundance estimates differed for the two sampling procedures. Statistical significance was accepted at the 5% level.

Larval Size Distribution (Diver Census vs. Net Catch). Mean sizes of larvae collected by divers and larvae sampled with nets were compared with a T-test assuming equal variances. Samples were pooled into groups of either dive or net-collected larvae for within each of the three sampling periods for each comparison. Statistical significance was accepted at the 5% level.

Larval Stage Frequency (Diver Census vs. Net Catch). The larval stage frequency distribution for pooled diver-collected and pooled net-collected samples were compared with a Chi-square test (Sokal and Rohlf 1981) for each cruise. Statistical significance was accepted at the 5% level.

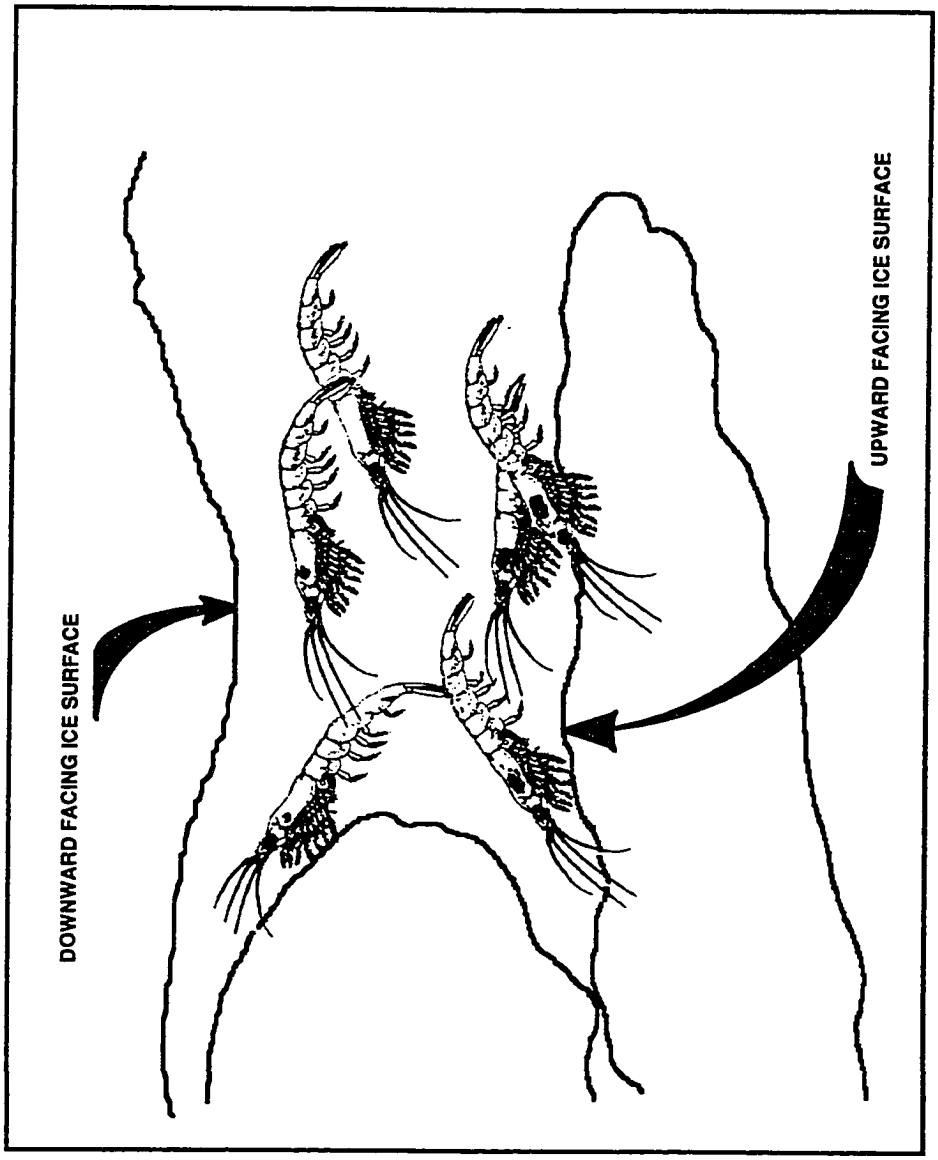
Patch Characterization of Larval Krill in the Ice Habitat. The mean number of larval aggregations, i.e., patches, per transect (2 m wide by 30 m long) for each sampling station was used to calculate the mean number of aggregations per m^2 (note, however, that the scale of sampling at each station is on the order of 100 m^2) for each of the three cruises. A Kruskal-Wallis non-parametric procedure with Chi-square approximation (Sokal and Rolf 1981) was used to compare the numbers of larval aggregations.

The association of larval aggregations with ice surfaces of different spatial orientation (relative to the plane of the air/water interface) was recorded secondary to the count data above. In some instances, no surface orientation data were recorded. In other instances, larvae were noted simply as occupying an eroded area or complex habitat; the implication being that larvae occupied an area where two or more surfaces of different spatial orientation were present. If krill were

reported to occupy an upward facing surface (see Figure 2) then, by implication, an adjacent downward facing ice surface must have been present. If, however, larvae were reported to occupy a downward facing surface, it could not be determined subsequently if an upward facing ice surface had been present below.

The null hypothesis that larval aggregations occupy areas of complex structure, i.e., areas with two or more ice surfaces, in equal proportion to smooth, downward facing ice surfaces was tested with a Chi-square goodness of fit procedure with data pooled from all three cruises. With regard to the analysis of these data, an assumption was made that krill occupied downward surfaces only in those areas where ice was not over-rafted and/or eroded. This assumption allowed for a conservative test of the hypothesis since smooth, unilayer floes with only downward facing surfaces were generally most common during the three cruises (personal observation).

Figure 2. Aggregations of larval krill (late stage furcilia are depicted here) tend to occupy upward facing ice surfaces and/or more structurally complex habitats (see text) that occur, for example, when pans of sea ice are over-rafted. Note that krill do not generally occupy downward facing ice surfaces, particularly if there is no additional structural character to the ice.



RESULTS

Krill Abundance (Diver Census vs. Net Catch)

Estimates of larval krill abundance as determined by net sampling techniques were usually greater than those obtained by direct observation at the same location (Figure 3). However, paired-comparisons made within cruises (September 1991 and 1993) were not statistically significant (sign-test, $P > 0.05$ for each year). Only three paired-comparisons were available for June 1993, an early winter sampling period, and in two instances, the greater estimate of larval krill abundance resulted from a net sample (see Table 1). Although the mean numbers of larval krill per m^2 as estimated from net catch data was consistently greater than estimates made by divers (Table 2), statistical comparisons were not significant ($P > 0.05$ for all three cruises).

Figure 3. A comparison of larval krill abundances as estimated by direct observation and from net catch information. Paired estimates resulting from both methods are expressed as the number of larval krill per m^2, although net data reflect the number of larval krill over an integrated depth range (generally 0 to 300 m, see Table 1), and diver estimates are only of those animals associated with the surfaces of sea ice. Points above the unity line occur when net estimates exceed counts made by divers at a given sampling station, and points below the line occur when counts made by divers exceed the corresponding net estimate.

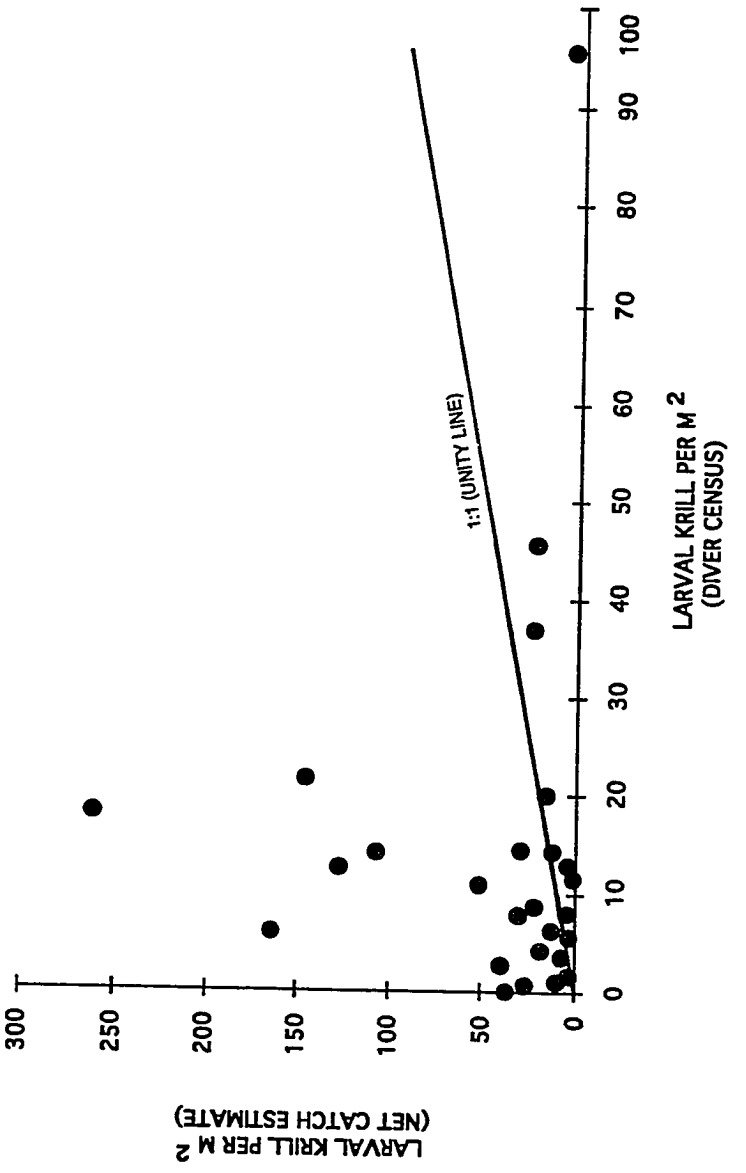


Table 2. Mean numbers (± SE) of larval krill for all samples collected during each of the three cruise periods. Note that statistical comparisons of abundances within sampling periods were made with Wilcoxon's Signed-Ranks Test using paired data (see text, and also Table 1), not the summary data below.

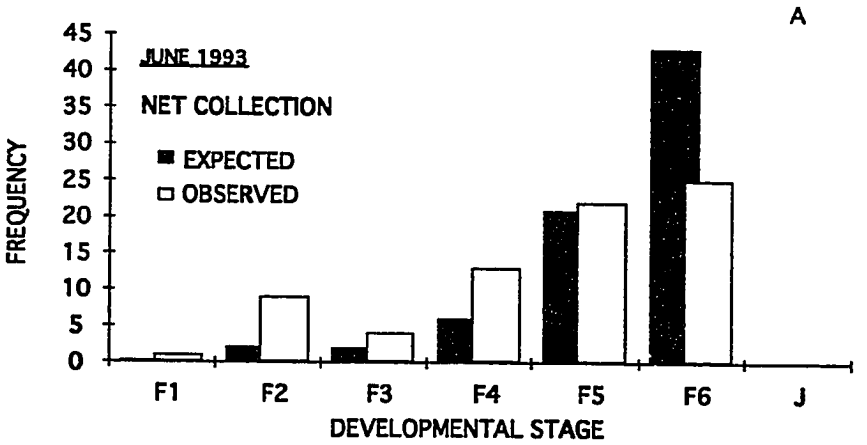
	JUNE 1993	SEPT 1993	SEPT 1991
NET ESTIMATE	15.83 (± 9.39)	32.98 (± 10.06)	82.29 (± 43.04)
DIVER ESTIMATE	2.04 (± 1.32)	16.72 (± 3.39)	24.60 (± 12.42)

Larval Sizes and Stage Frequency Distributions (Diver Census vs. Net Catch)

Within each of the three cruises, larval krill collected by divers were significantly larger (mean TL) than those sampled with nets (Figures 4, 5 and 6). In addition, a comparison of the mean lengths of larval krill collected by divers during June 1993 and September 1993 showed that larval krill captured during early winter (June 1993) were significantly larger than those collected during late winter of the same year (September 1993) (T-test, $P < 0.001$).

Within sampling periods, the stage composition of larval krill was dependent on the collection procedure. Net-collected samples contained a disproportionate number of early furcilia larvae during early winter, i.e., June 1993 (Figure 7), but during both late winter sampling periods, September 1991 and September 1993, nets sampled more juveniles than were expected (Figures 8 and 9); expected values for each cruise were generated from pooled dive-collected and net-collected data.

Figure 4. Size frequency distributions for larval krill collected with (A) nets and by (B) divers during June 1993. Descriptive statistics are as follows: N = total number of krill measured, X = the mean size (TL) of larvae, and SE = the standard error of the mean estimate. Note scale differences between (A) and (B).



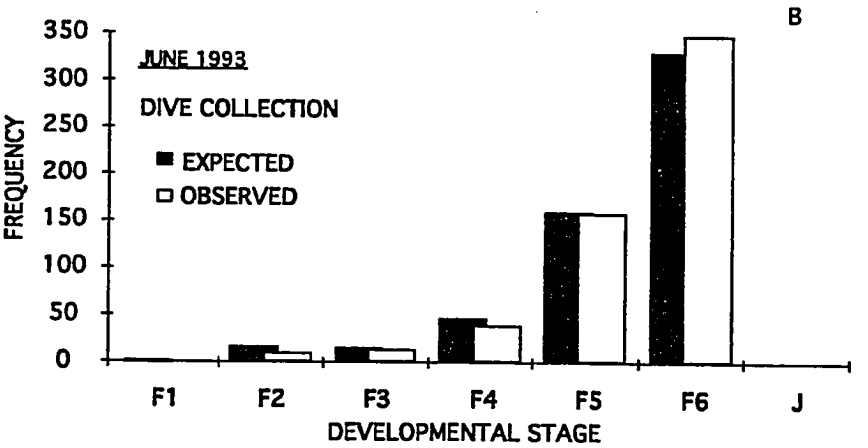
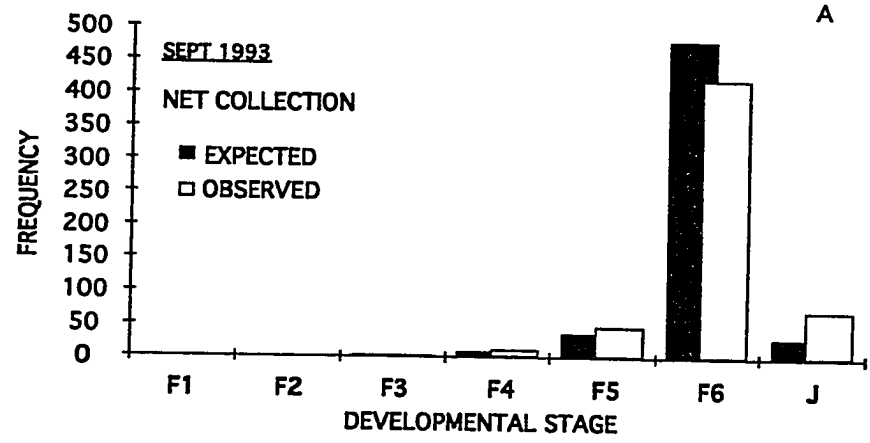


Figure 5. Size frequency distributions for larval krill collected with (A) nets and by (B) divers during September 1993. Descriptive statistics are as in Figure 4.



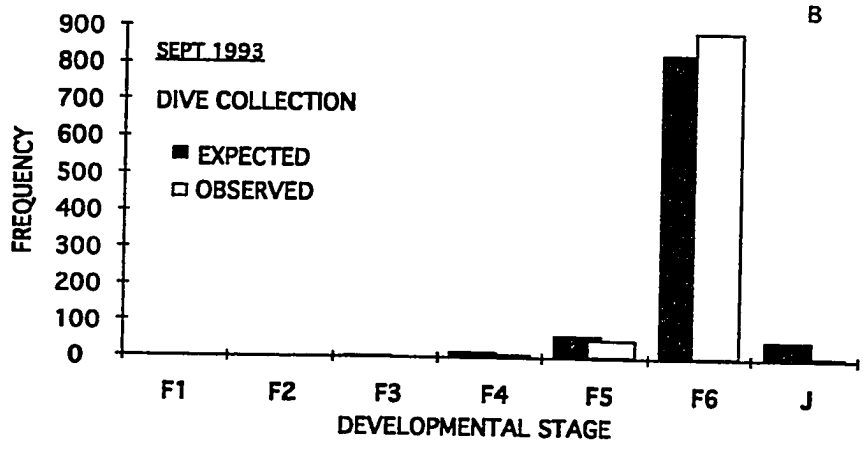
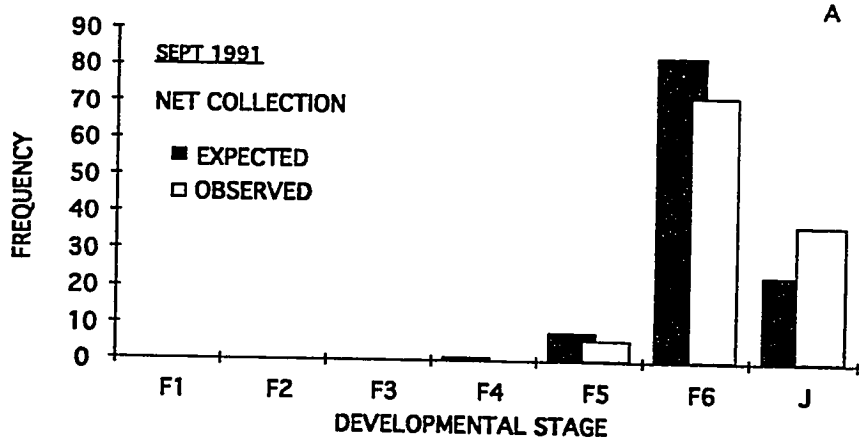


Figure 6. Size frequency distributions for larval krill collected with (A) nets and by (B) divers during September 1991. Descriptive statistics are as in Figure 4.



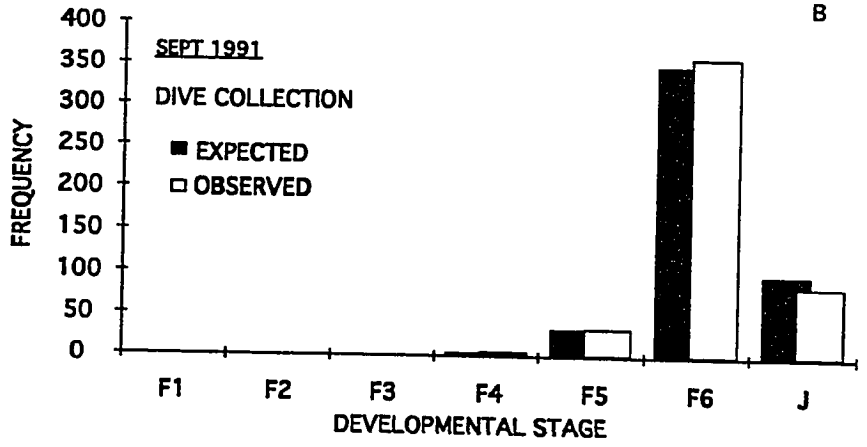
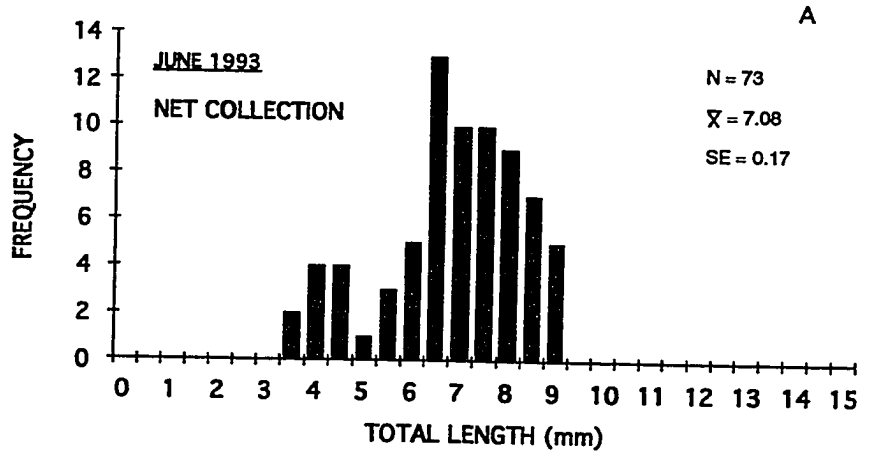


Figure 7. Expected and observed frequencies of different larval stages collected with (A) nets and by (B) divers during June 1993. Furcilia stages 1 through 6 are abbreviated as F1 through F6. The two observed frequency distributions are significantly different at the $P < 0.001$ level ($X^2 = 44.19$, df = 5).



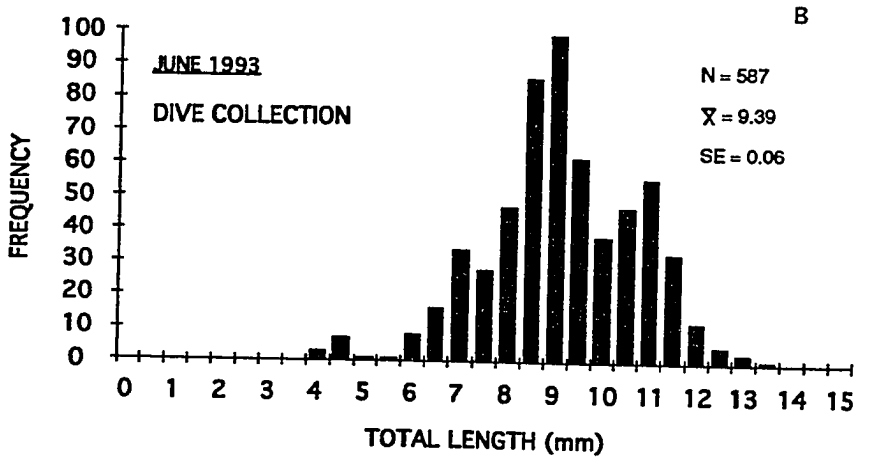
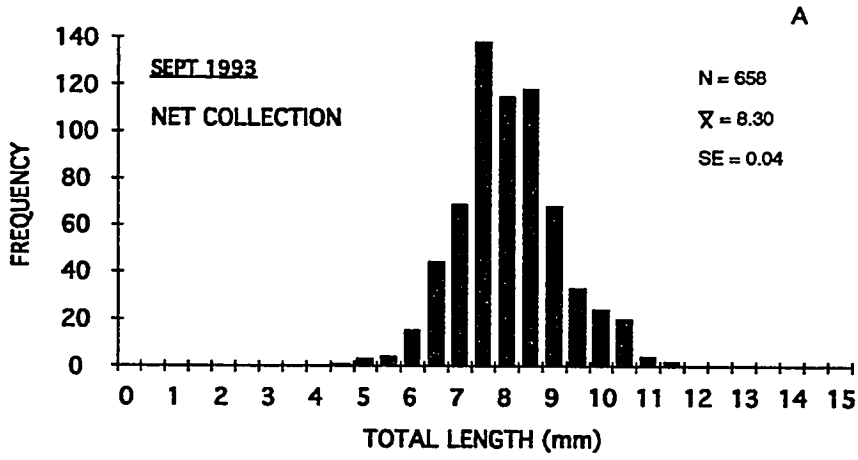


Figure 8. Expected and observed frequencies of different larval and post-larval stages collected with (A) nets and by (B) divers during September 1993. Furcilia stages 1 through 6 are abbreviated as F1 through F6, and young-of-the-year juvenile krill abbreviated with the letter J. The two observed frequency distributions are significantly different at the $P < 0.001$ level ($X^2 = 119.37$, df = 4).



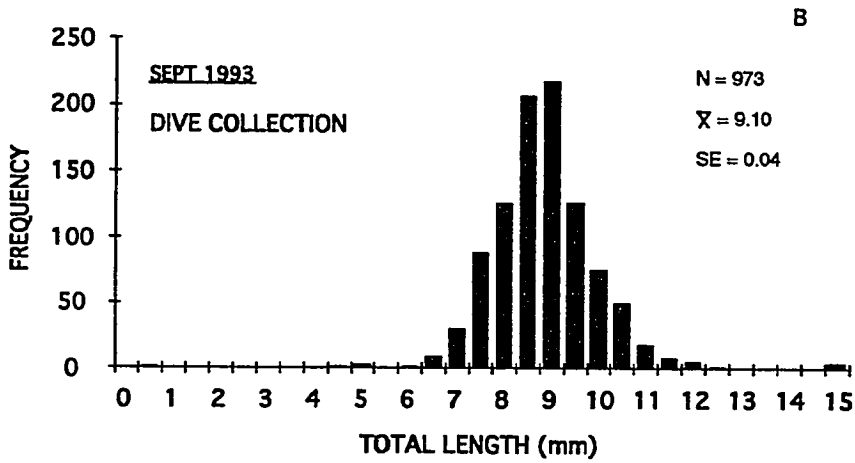
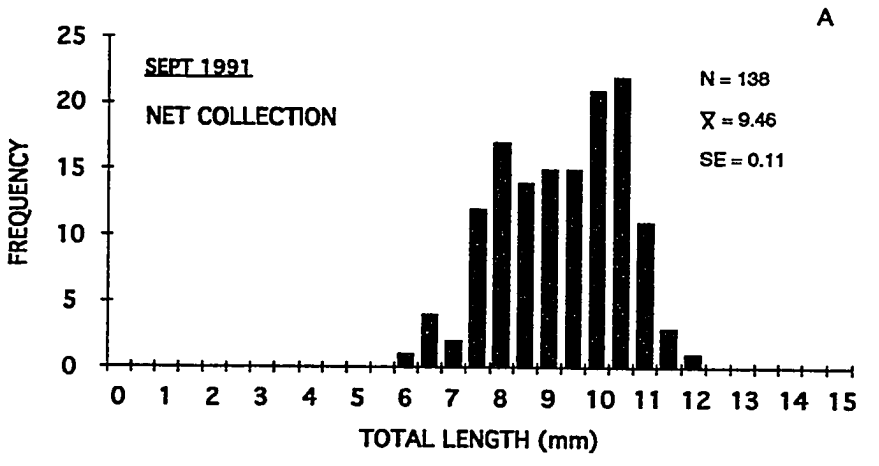
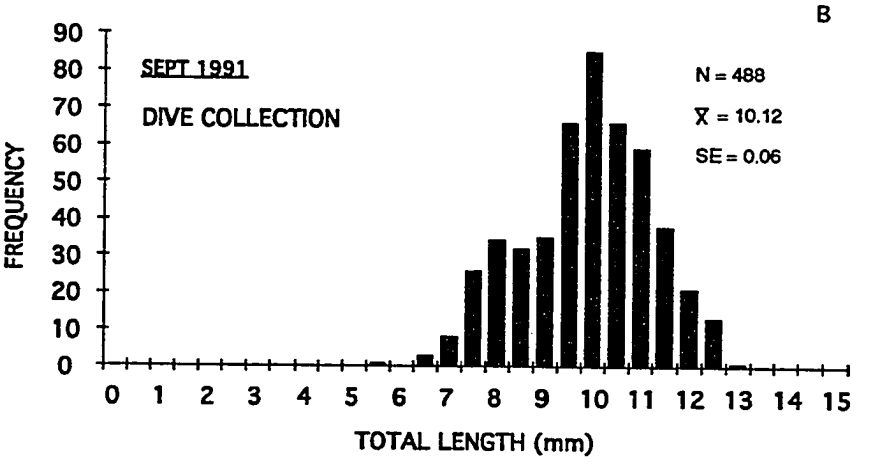


Figure 9. Expected and observed frequencies of different larval stages collected with (A) nets and by (B) divers during September 1991. Abbreviations are as in Figure 8. The two observed frequency distributions are significantly different at the $P < 0.01$ level ($X^2 = 44.19$, df = 3).





Patch Characteristics of Larval Krill in the Ice Habitat

The mean number of aggregations per m^2 for each sampling period depended, of course, on the size classification scheme that was employed (Figure 10). There were more total aggregations, and larger aggregations during late winter sampling periods (September 1991 and 1993) than in early winter (June 1993). In fact, no large aggregations were observed in early winter, and on only two occasions were medium sized aggregations censused by divers during the same period. The mean numbers of aggregations and the relative proportions of aggregations of varying sizes were remarkably similar among the two late winter sampling periods.

Aggregations of larval krill occupied upward facing ice surfaces and structurally complex microhabitats, i.e., areas with two or more adjacent ice surfaces, more often than they occupied smooth, downward facing ice surfaces (Figure 11, X^2_{pooled} = 64.10, df = 1, $P < 0.001$).

Figure 10. Mean number of larval aggregations per m^2 for all sampling stations during each of the three sampling periods: June 1993, September 1993, and September 1991. Four situations are presented: (1) total aggregations (all observations of two or more larval krill), (2) aggregations of 10 or more larvae, (3) aggregations of 100 or more larvae, and (4) aggregations of 1,000 or more larvae.

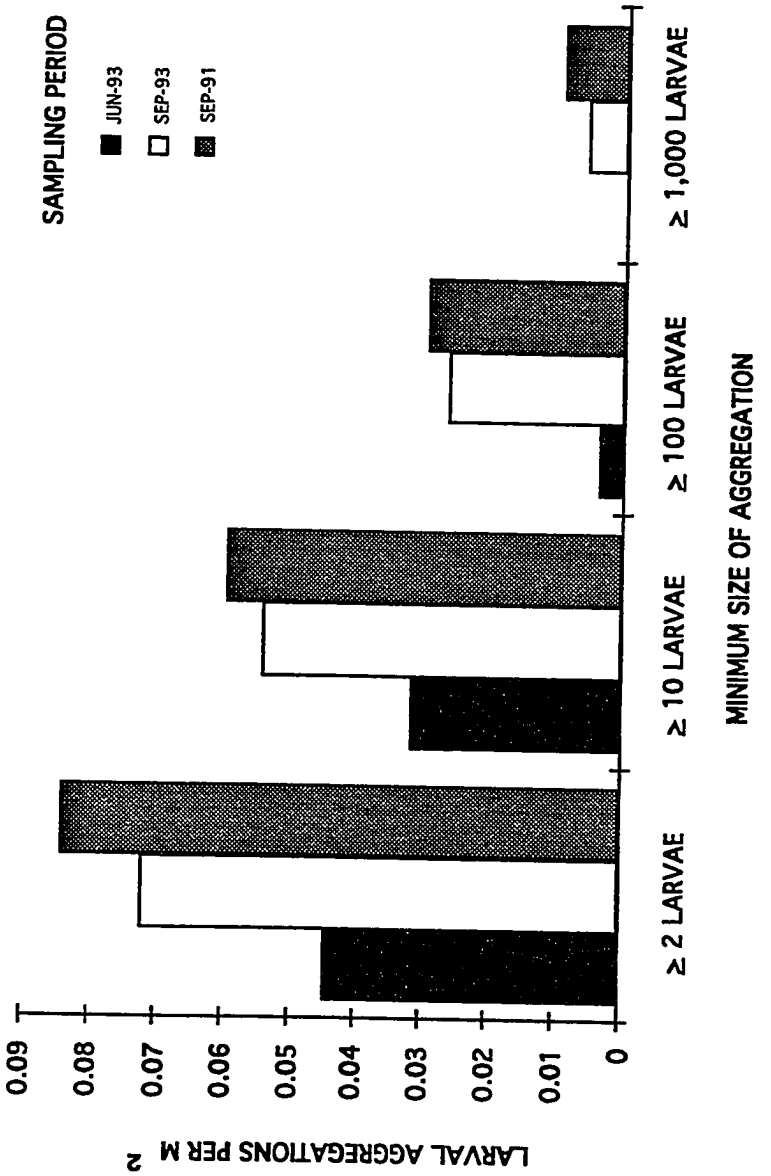
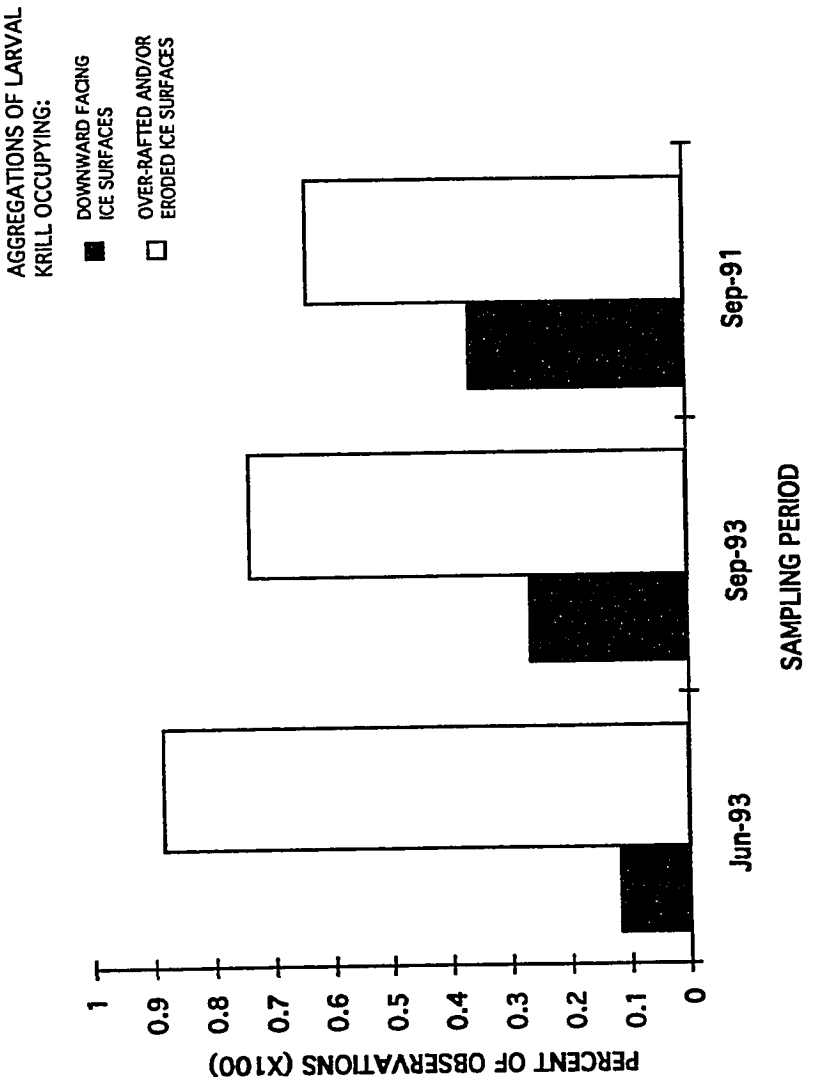


Figure 11. The relative proportion of larval aggregations observed to occupy smooth, downward-facing ice surfaces or more structurally complex areas. Ice-surface orientation was recorded for 17 larval aggregations in June 1993, 245 larval aggregations in September 1993, and 66 larval aggregations in September 1991.



DISCUSSION

Larval Krill Abundance (Diver Census vs. Net Catch)

As a research vessel is maneuvered through areas of heavy and/or consolidated ice it is probable that animals, larval krill in particular, are decoupled from the ice and often washed into the turbulent wake of the moving ship. These displaced larvae are likely captured in nets without opening and closing mechanisms, and net catches in this study may have consisted of larval krill from both the water column and ice habitat. Comparisons of larval krill abundance as estimated from net catches and by divers should be viewed in this light, and quantitative interpretations of the data reported here warrant caution. The fact that nets, in this study, often yielded more larval krill than divers estimated to be associated with sea ice suggests, however, that some portion of the larval population occupies the water column below sea ice during winter. Future investigators are advised to use nets with opening and closing mechanisms to more effectively sample larval krill from the water column in ice-covered seas.

Larval Size and Stage Frequency Distributions (Diver Census vs. Net Catch)

In both early and late winter, larval krill captured by divers were larger than those collected with nets. This finding further suggests that net-collected larvae are not just a random sample of animals recently decoupled from the ice. In view of the discussion above, it must be concluded that some portion of the total larval population under the ice occupies the water column, and is not closely associated with the ice.

Differences in the size distribution and stage composition of larval krill collected by divers and with nets in early winter (June 1993) were expected given the wide range of larval stages encountered, furcilia (F) 1-6. No early stage furcilia have been collected from the sea ice habitat prior to this study. Hamner et al. (1989) noted that larval aggregations associated with sea ice in April of 1986 did not contain larvae younger than developmental stage F4, though earlier furcilia stages, F1-3, were captured as isolates from the surface waters in adjacent areas. Daly and Macaulay (1991) reported collecting F4's and earlier developmental stages from the water column, but only juveniles (age class 0) were collected from ice surfaces in late March of the same year; F3's and later developmental

stages were collected from ice floes on a subsequent cruise (June/July 1988), but a wider range of developmental stages (calyptopis 3 through juvenile) was collected from the water column.

The results of the present study indicate that development stages as early as F2 can be found among larval aggregations in the ice. The frequency of occurrence, however, was less than expected given the total number of early furcilia in both net collected and diver collected samples. The earliest furcilia stage (F1) was not collected by divers in any of the above studies (i.e., Hamner et al. 1989, Daly and Macaulay 1991, data herein), but F1's were present in the water column in each case. The ability of early furcilia to exploit ice-associated food resources is not clear, but the evidence suggests that the earliest stage furcilia are not closely coupled with the ice, and may not be capable of efficient ice-scraping behavior. As a consequence, krill spawned late in the summer or fall are not likely to develop quickly enough to exploit the ice and its associated resources even if ice is available. An energetic comparison of filter-feeding and ice-scraping modes of feeding for larval krill, subjected to low temperatures (< -1.5°C) and different food regimes, might prove useful in further

understanding when and why krill enter and/or leave the ice habitat (see discussion below).

Larval/juvenile krill collected by divers during late winter (September 1991 and 1993) were significantly larger than those collected with nets, despite the higher proportion of juveniles (age class 0) in the latter collections. The disproportionate number of later developmental stages, i.e., juveniles, in the net samples would be expected to be reflected in a larger mean size. Thus it appears that size (TL) is not the determining factor in krill's (age class 0) departure from the ice habitat. It is more likely that a physical process and/or developmental change accompanies the krill's habitat shift from the ice to the water column. The nuances in form and function between larval and post-larval developmental stages of E. superba have received little attention, but are areas deserving of future research.

The comparison of mean sizes of larval krill between early and late winter of 1993 suggest that a reduction in body size may have occurred during winter for much of the larval population in the study region west of the Antarctic Peninsula. It is difficult, of course, to know with certainty that the same larval population was sampled during both cruises in 1993, and

the above interpretation of the data warrants caution. However, shrinkage of adult krill has been documented (e.g., Ikeda and Dixon 1982, Quetin and Ross 1991), and Frazer (unpublished data, Chapter 4) has reported a reduction in the mean wet weight of late stage furcilia in the laboratory under starved conditions. It is likely that late stage furcilia (F4-6), under natural conditions, exhibit shrinkage when food-limited in the Southern Ocean, though Ross et al. (1987) found that populations of larvae held in the laboratory during winter continued to advance in development even at low food concentrations. Early furcilia are less tolerant of starvation (Ross and Quetin 1991), however, and may die rather than molting to a smaller size.

Interannual variation in the timing of formation and extent of annual sea ice is expected to have consequences for size and condition of larval krill during the austral winter (see Ross and Quetin 1991). Annual sea ice formed relatively late over much of the area sampled in September 1993 (Stammerjohn and Smith in press), and little ice-algae seemed to be present during either June or September of that year (personal observation based on ice-coloration). Sea ice formed earlier along the Antarctic Peninsula in 1991 (Stammerjohn and Smith in press), and ice-algal abundance appeared to be

greater than in 1993 (personal observation based on ice-coloration). The above data and observations are suggestive of more favorable ice conditions for larval krill in 1991 than in 1993, and may explain the size differences reported here for larvae collected in late winter during the two different years. Data on the winter growth rates of larval krill are lacking (see Quetin et al. 1994 for review), but are much needed information.

Patch Characteristics of Larval Krill in the Ice Habitat

The term 'aggregation', as it is used here to describe discrete patches of larval krill, is synonymous with the term 'swarm' or 'group', and does not imply polarization or schooling of individuals. Schools of polarized larval krill have been observed by Hamner et al. (1989), but larvae censused in this study were generally oriented to the structure of sea ice rather than to one another. Hamner et al.'s observations of schooling furcilia in relation to ice may be a reflection of the predominant ice types present during April, i.e., small, isolated bits of ice and/or moving brash ice. Both of the above types of ice and the resources provided are extremely ephemeral in nature, and fundamentally different than the large floes and

consolidated pans of annual sea ice surveyed in this study during austral winter. Hamner et al.'s (1989) suggestion that the presence of ice provides cover and inhibits directed horizontal swimming of furcilia implies that schooling of larval krill may be a social behavior exhibited primarily in the absence of sea ice or during winter transitional periods when ice and its associated resources are more patchy in nature. However, schooling behavior likely occurs throughout the austral winter and may facilitate encounters of larval aggregations with patchy food resources in the annual sea ice habitat (see related discussion below).

Appreciable movement and coalescing of larval aggregations within the annual sea ice habitat is necessary to explain the increase in numbers and sizes of larval aggregations observed during late winter. Clearly, if we are to understand the mechanisms associated with the formation of large patches of larvae in the ice, then the small-scale movement patterns of larval krill must be characterized. Frazer et al. (in press) reported that aggregations of larval krill associated with sea ice were site attached at small spatial (< 1 m) and temporal (< 1 h) scales during daylight hours. One conclusion to be drawn from the above observations is that larger-scale movements of larval krill in the ice, and within the

study region, are restricted to periods of darkness or extreme low light. The above hypothesis is consistent with the idea that sea ice functions both as a refuge from predation and as a food source for larval krill.

Larval krill associated with sea ice are too small to be an important food source for large vertebrate predators (see e.g., Croxall et al. 1985, Lowry et al. 1988). In this regard, larvae may have a refuge in their size (Hamner et al. 1989, Quetin and Ross 1991). Sea ice, however, may afford larval krill a refuge from smaller invertebrate predators, such as ctenophores and amphipods (see Hamner et al. 1989), that are common in the water column below sea ice during the austral winter (personal observation). This study clearly illustrates the affinity of larval krill for areas of upward facing sea ice and more structurally complex microhabitats, and the associated refuge, though other reasons may also explain, to varying degrees, krill's tendency to aggregate in these areas; energetic and ecological considerations have been discussed elsewhere (see Frazer et al. in press).

The structural characteristics of sea ice and the patch characteristics of ice-associated flora on a scale relevant to the biology and ecology of larval krill have not been described.

Such information is needed, however, to more fully understand the behavior of krill in the sea ice habitat, and to further evaluate the role of ice-associated algae in the ecology of this important species. Diving has proven to be a requisite research tool to investigate the krill/ice interaction, and future advances in our understanding of this significant ecological linkage will rely on our ability to further exploit this method.

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CHAPTER III

Stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$) of larval krill, Euphausia superba, and two of its potential food sources in winter

ABSTRACT

Natural abundances of ^{13}C ($\delta^{13}C$) and ^{15}N ($\delta^{15}N$) were measured in larval krill (Euphausia superba), suspended particulate organic matter (POM) and ice-associated POM during early and late winter along the west coast of the Antarctic Peninsula. Larval krill were enriched in ^{13}C ($\delta^{13}C \geq$ -27 ‰) relative to both larvae and adults sampled during summer months ($\delta^{13}C$ generally $\leq$ -27 ‰). Elevated $\delta^{13}C$ were recorded also in suspended POM ($\delta^{13}C \geq$ -21 ‰) during early winter. These data imply: (1) seasonal shifts in the isotopic composition of larval krill need not result from changes in diet, and (2) mechanisms other than CO_2 limitation in the ice can account for ^{13}C enrichments in ice-associated POM. Stable carbon isotopes could not be used, in this study, to discern between suspended POM and ice-associated POM as alternative food sources for larval krill. During one early winter sampling period, larval krill were markedly depleted in ^{15}N ($\delta^{15}N <$ 1 ‰) suggesting that they are primarily herbivorous prior to exploiting ice-associated food resources. Mechanisms are proposed to explain variation in the isotopic composition of POM and larval krill, and will be of particular interest to those investigating food web dynamics and biogeochemical processes in the region.

INTRODUCTION

Seasonal variation in ice coverage is thought to affect the winter-over survival and subsequent recruitment of larval and post-larval krill to the adult population (Ross and Quetin 1991). Larval krill feed on ice-associated biota, algae in particular, and may be obligate consumers of this food resource during austral winter when phytoplankton levels in the water column are extremely low (Stretch et al. 1988; Daly 1990; Smetacek et al. 1990; Quetin and Ross 1991). Quantifying the role of ice biota in the early life history of Euphausia superba is necessary to understand more fully the the effects of large-scale differences in ice cover on the population dynamics of krill.

Carbon and nitrogen stable isotope ratios might be useful to characterize the role of potential alternative food resources in the winter diet of E. superba. These isotope ratios in consumers largely reflect those assimilated, and can be used to trace and/or discriminate among dietary sources with distinct isotopic composition (Peterson and Fry 1987). Reported measures of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ in suspended particulate organic matter (POM) from the Southern Ocean and POM associated with sea ice, two alternate food sources, often differ (Fischer 1991; Rau et al. 1991b). This difference suggests that

concurrent isotopic analyses of suspended POM, ice-associated POM and krill larvae collected at appropriate time and space scales might be used to resolve the relative importance of ice-associated food resources in the diet of larval krill.

During the austral summer, December through March, suspended POM in the Southern Ocean is characteristically depleted in ^{13}C (Sackett et al. 1965, Rau et al. 1982, 1989, 1991c, Wada et al. 1987, Fischer 1991, Fontugne et al. 1991, Wada and Hattori 1991) and is isotopically lighter than POM in or associated with sea ice. Explanatory mechanisms center on inorganic carbon and its availability as a substrate for photosynthesizing microalgae. Elevated levels of CO_2 (aq) in waters south of the polar front are thought to underlie low $\delta^{13}C$ in phytoplankton (see Rau et al. 1989, 1991c), whereas algae growing in or associated with sea ice are often assumed to be CO_2-limited, and, as a result, relatively enriched in ^{13}C (see Wada et al. 1987; Fischer 1989, 1991).

Less is known about stable nitrogen isotopes. Wada et al. (1987) confirmed a general pattern of ^{15}N enrichment with increased trophic level, but noted variable values in phytoplankton-dominated particulate matter. Rau et al. (1991b) also reported large $\delta^{15}N$ variations in POM. However,

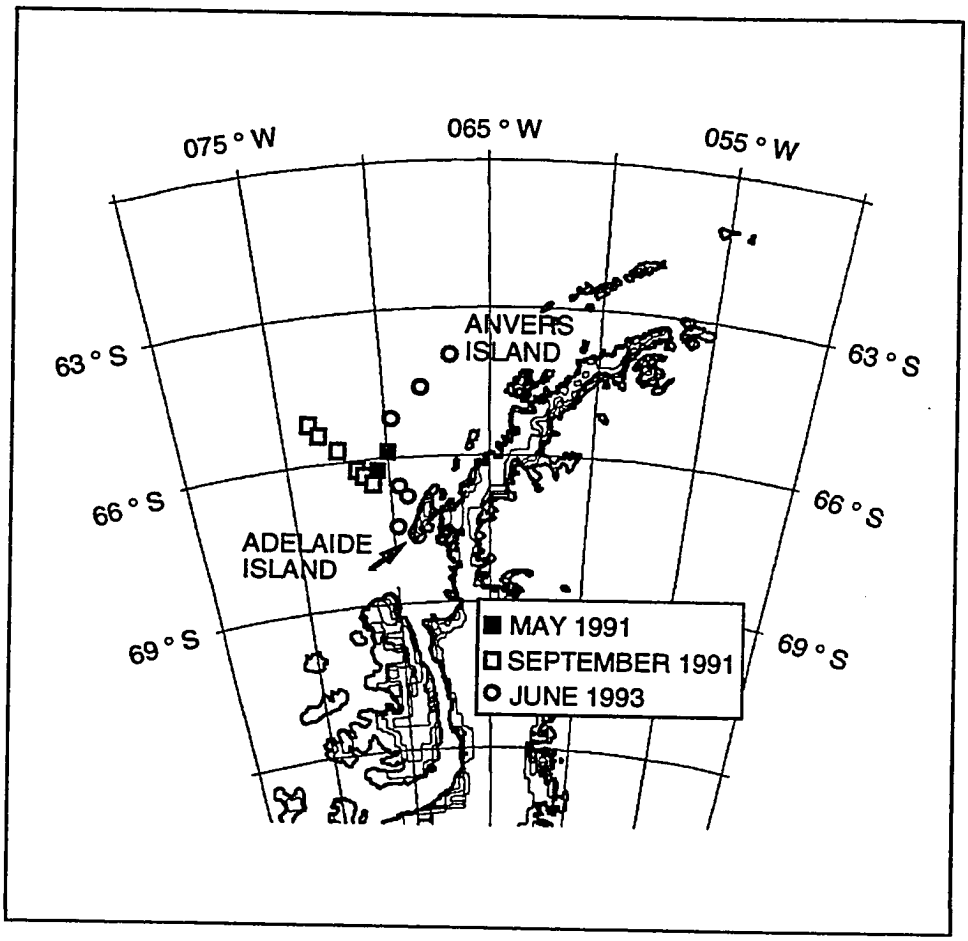
elevated values were found exclusively in POM in or associated with sea ice. The authors suggested that spatial and temporal changes in the concentrations and isotopic abundances of ammonium may underlie their observations. The reported isotopic variability within the food base provides an avenue for further investigating the coupling between larval krill and their ice-associated food resources.

Presented here are isotopic data and its interpretation from three winter cruises to a region west of the Antarctic Peninsula. I evaluate the use of stable isotopes of carbon and nitrogen as indicators of ice-associated food resources in the diet of larval krill and, in light of the findings, discuss alternative mechanisms to explain temporal and spatial variation in the isotopic ratios of POM source material and krill.

METHODS

Study Area and Sampling Overview. Larval krill and two likely food sources were sampled during three separate cruises to an offshore region northwest of Adelaide Island along the west coast of the Antarctic Peninsula (Figure 1). From May 26-29, 1991 (early winter), larval krill and ice-associated POM were collected opportunistically by divers in areas of rapidly forming sea ice. Suspended POM was collected from ice-free surface water approximately 80 km north of a recognizable ice edge zone. During September 13-29, 1991 (late winter, early spring), samples of krill and POM were collected at selected stations along a transect originating in ice free waters and penetrating into an area of heavy pack ice. Water samples were also collected at these stations for determination of inorganic nutrient concentrations and isotopic measurements of dissolved inorganic carbon. Samples of krill and POM in both open and ice-covered waters were also collected during June 9-23, 1993 (winter).

Figure 1. Sampling stations occupied during each of the three winter cruises.



Collection and Preparation of Samples

Krill. Animals in open waters and areas of "light" pack ice were collected with either a 1.6 m x 0.8 m trawl fitted with paired 505-μm mesh nets or a 1-m diameter ring net with the same mesh size. Nets were towed obliquely between 300 m and the surface. In "heavy" pack ice, either a 60-cm diameter bongo net or a 1-m ring net (505-μm mesh) was towed vertically from the same depth. Krill closely associated with the undersurfaces of sea ice were collected by divers with hand-held aquarium nets. Larval krill were held in filtered sea water for 12 to 24 hours after collection to allow the majority of food materials in their digestive systems to clear. Individuals were then staged (Fraser 1936) and measured for total length (tip of rostrum to the end of uropods) under a dissecting microscope. Larvae of like stage were combined (five or more per sample), placed directly in glass vials and dried at 60°C in preparation for stable isotope analyses, i.e., $\delta^{13}C$ and $\delta^{15}N$.

Suspended Particles. In May 1991, suspended POM was collected with a 10-cm diameter phytoplankton net (20-μm mesh) towed vertically between 0 and 100 m until clogged.

Concentrated particulate matter was transfered to 20-ml glass scintillation vials via pipette, stored frozen (< -20°C) and subsequently dried at 60°C.

In September 1991, seawater was collected at 60 m with either 5 or 10-l Niskin bottles. Suspended particles were concentrated via filtration (vacuum $\leq$ 7 psi) on precombusted (500°C, 1 h) glass fiber filters (GF/F, 2.5 cm, 0.7 µm pore size), stored frozen (< -20°C) and subsequently dried at 60°C.

In June 1993, suspended POM was collected with a phytoplankton net (15-cm diameter, 20-µm mesh) towed vertically to 100 m and also with 10-l Niskin bottles. In the latter case, water from four discrete depths (20, 40, 60 and 80 m) was taken in equal amounts and combined to obtain enough material for analysis. In all instances, particulate matter was concentrated on precombusted glass fiber filters via filtration (see above) and dried at 60°C.

Ice-associated Particles. Particulate matter was collected from sea ice surfaces where krill were observed feeding. Methods of collection differed in May, June and September due to the physical nature of sea ice. In May, unconsolidated ice crystals often formed a loose substrate, i.e., frazil ice, in which

concentrations of entrained microalgae were readily visible (see Garrison et al. 1983). The unconsolidated ice layer, while irregular in thickness (0 to tens of centimeters), was easily sampled with the same aquarium nets used for krill collection. Sampled material was stored in the dark at approximately 1°C and allowed to thaw (< 24 h). Settled particles were concentrated, stored frozen and subsequently dried at 60°C prior to analysis.

During September, ice was generally hard to the touch and particulate matter was suctioned from ice surfaces and the immediate (estimated $\leq$ 5 cm) underlying substrate by divers with a pneumatically driven pump. Ice surfaces were agitated with a rigid section of PVC pipe and loose material evacuated through connected tubing. Samples (a mixture of loose ice crystals, associated particles and adjacent seawater; the proportion of each was not quantified) were housed in opaque plastic bags and immediately prepared for analyses aboard ship. Particles were concentrated on precombusted glass fiber filters and prepared for isotopic analysis in the same manner as described above for suspended materials.

In June 1993, ice was again hard to the touch and particulate matter was suctioned from ice surfaces where krill

were feeding. Ice surfaces were agitated with a rigid section of pipe and loose material evacuated into a polyethylene flask through connected tubing. Suction was created by releasing vacuum pressure in the 500-ml flask. Particles from the flask were concentrated on glass fiber filters and prepared for subsequent analyses as decribed above.

Water Samples. In September 1991, water from 60 m was collected with either 5 or 10-l Niskin bottles. Interstitial water was drawn from the underside of annual sea ice by divers with hand held syringes (50 cc, $n \geq 10$ at each station). Water from each selected station was passed through a precombusted (500°C, 1 hr) glass fiber filter (GF/F, 4.25 cm, 0.7 μm pore size), subdivided, and then prepared for subsequent determination of inorganic nutrient concentrations and $\delta^{13}C$ of total dissolved inorganic carbon. Subsamples for inorganic nutrients were stored frozen ($\leq$ -20°C) in 20-ml plastic scintillation vials for up to four months. Water to be used for the measure of dissolved inorganic carbon parameters (250 ml) was transfered to glass jars, poisoned with 1 ml $HgCl_2$, sealed with electrical tape and refrigerated in the dark prior to analyses.

Isotopic Analyses. Measurements of carbon and nitrogen stable isotope ratios were made at the Stable Isotope Laboratory of the Marine Biological Laboratory (MBL) in Woods Hole, MA. Results are reported in standard δ notation and calculated as follows:

$$\delta^{13}C \text{ or } \delta^{15}N\ (‰) = [(R_{sample}/R_{standard}) - 1)] \times 1000$$

where $R = (^{13}C/^{12}C)$ or $(^{15}N/^{14}N)$, respectively. PeeDeeBelemnite and atmospheric dinitrogen served as reference standards. The analytical precision of the reported measures is ≤ 0.3 ‰.

Krill collected during all three cruises and POM collected during the May cruise were dried directly in glass vials and homogenized prior to analysis. Suspended POM and ice-associated POM samples concentrated onto precombusted glass-fiber filters in September 1991, and June 1993, were combusted in their entirety and subsequently blank corrected. Samples were not acidified as part of the preparation procedure. All organic samples were analyzed with an automated system for coupled $\delta^{13}C$ and $\delta^{15}N$ measurements (Fry et al. 1992). Procedures for the isotopic analysis of dissolved inorganic carbon are described by Kroopnick (1974).

Nutrient Analyses. Concentrations (μM) of inorganic nitrate, nitrite and silicate were measured with a flow injection system (Johnson et al. 1985) at the Marine Science Institute's analytical facility at the University of California, Santa Barbara. Concentrations of total dissolved inorganic carbon (μmoles C/kg) from poisoned water samples were determined at MBL simultaneously with measures of isotopic composition ($\delta^{13}C$).

RESULTS

Larval krill sampled during winter were generally enriched in ^{13}C relative to krill sampled during summer months (Figure 2). During one early winter sampling period (June 1993), larvae were also markedly depleted in ^{15}N (Figure 2). Comparative summer data is from Frazer et al. (unpublished data) and Rau et al. (1991a).

$\delta^{13}C$ of larvae sampled during the three winter cruises (Table 1) did not differ among cruises (ANOVA, $F = 0.96$, $df = 2$, $p > 0.05$). $\delta^{15}N$ of larvae sampled during June 1993, on the other hand, were significantly lower than those collected during May or September 1991 (Table 1, ANOVA, $F = 55.99$, $df = 2$, $p << 0.01$). The isotopic composition of larvae

Figure 2. $\delta^{13}C$ vs. $\delta^{15}N$ for krill irrespective of size: June 1993 (▪), September 1991 (□) and May 1991 (+). Summer measurements are from Frazer et al. (unpubl. data) and Rau et al. (1991a) for larvae (○) and adults (●), respectively. The latter data are provided as a comparative reference.

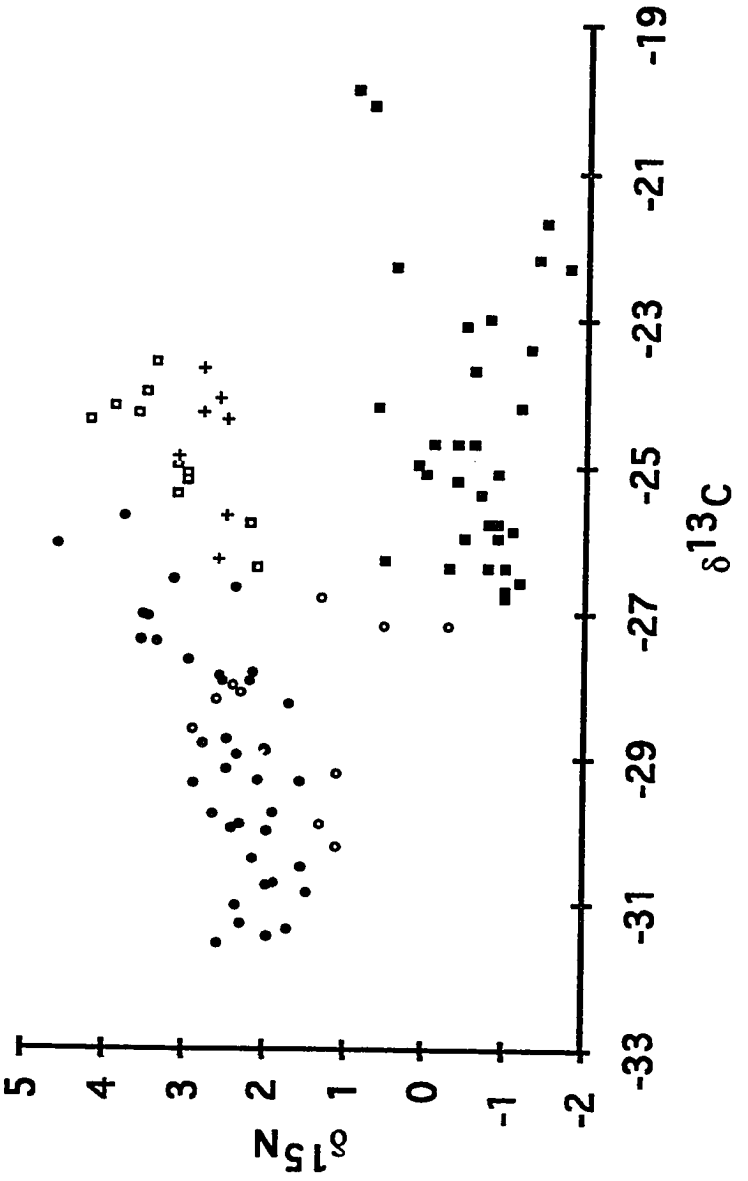


Table 1. Sample sizes and mean (± SD) $\delta^{13}C$ and $\delta^{15}N$ for suspended POM, ice-associated POM and larval krill collected during each of the three winter cruises. In those instances where n ≤ 4, data for individual samples are reported immediately below the summary statistics.

	SUSPENDED POM			ICE-ASSOCIATED POM			LARVAL KRILL		
	n	$\delta^{13}C$	$\delta^{15}N$	n	$\delta^{13}C$	$\delta^{15}N$	n	$\delta^{13}C$	$\delta^{15}N$
MAY 1991	3	-20.9 (0.0)	-1.0 (0.1)	4	-22.1 (0.1)	1.9 (0.2)	8	-24.9 (0.9)	2.7 (0.2)
		-20.9	-0.9		-21.9	2.1			
		-20.9	-1.0		-22.2	1.9			
		-20.9	-0.9		-22.0	1.8			
					-22.4	1.7			
SEPT 1991	2	-32.0 (1.4)	6.35 (0.1)	3	-24.7 (0.3)	5.2 (1.1)	10	-24.7 (0.9)	3.3 (0.7)
		-31.0	6.4		-24.6	4.0			
		-33.0	6.3		-24.5	5.6			
					-25.0	6.1			
JUNE 1993 *	8	-27.3 (1.0)	4.4 (2.4)	2	-25.2 (1.8)	14.0 (5.6)	32	-24.5 (1.9)	-0.6 (0.7)
					-26.5	17.9			
					-23.9	10.0			

* Additional information provided in Table 2

collected in the water column showed essentially the same distribution as those collected from ice surfaces in September 1991 and June 1993. Thus, no effort was made to separate data on the basis of habitat type.

There was no statistical relation between krill total length and $\delta^{13}C$ for animals collected in winter (Figure 3, Pearson r = 0.07). There was, however, a weak positive correlation between total length of larvae (mm) and $\delta^{15}N$ for animals collected in winter (Figure 4, Pearson r = 0.58). Within sampling periods, the isotopic composition of krill larvae was independent of size or life history stage (Figure 5).

Considerable variation in the isotopic composition of POM samples was evident and there were few similarities with krill larvae collected during like sampling periods (Table 1). Suspended POM sampled during May 1991 was slightly enhanced in ^{13}C relative to ice-associated POM and more so relative to larval krill collected on the same cruise. Suspended POM in September 1991 was significantly depleted in ^{13}C relative to ice-associated POM and larval krill and tended also to be enriched in ^{15}N relative to the latter two. During June 1993, POM samples varied widely with respect to $\delta^{15}N$ and measures were dependent on

Figure 3. $\delta^{13}C$ of krill as a function of total length (mm). Symbols are defined in Figure 2. Larval sizes during summer are approximations based on life history size distributions at the time of collection (Frazer et al. unpubl. data). Eggs were collected during January and February 1993, and size approximated according to Hofmann et al. (1992). Data for animals > 20 mm are from Rau et al. (1991a).

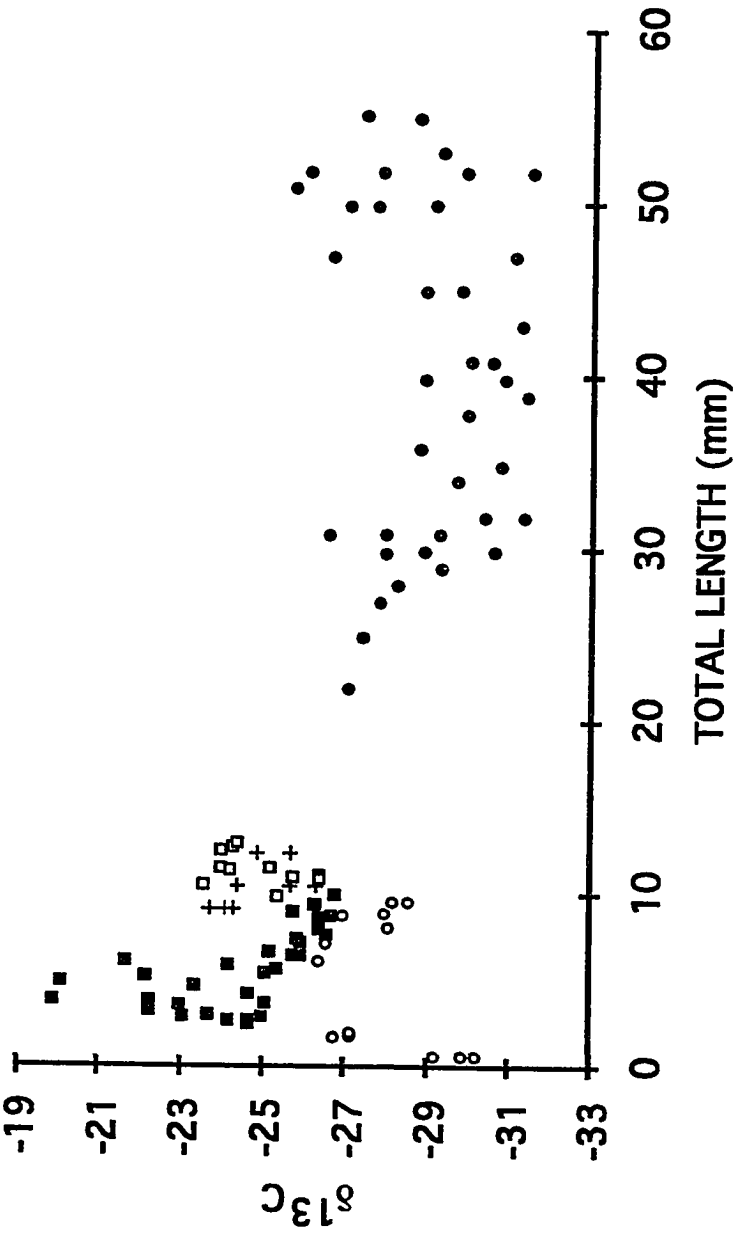


Figure 4. $\delta^{15}N$ of krill as a function of total length (mm). Symbols are defined in Figure 2. Larval sizes during summer are approximations based on life history size distributions at the time of collection (Frazer et al. unpubl. data). Eggs were collected during January and February 1993, and size approximated according to Hofmann et al. (1992). Data for animals > 20 mm are from Rau et al. (1991a).

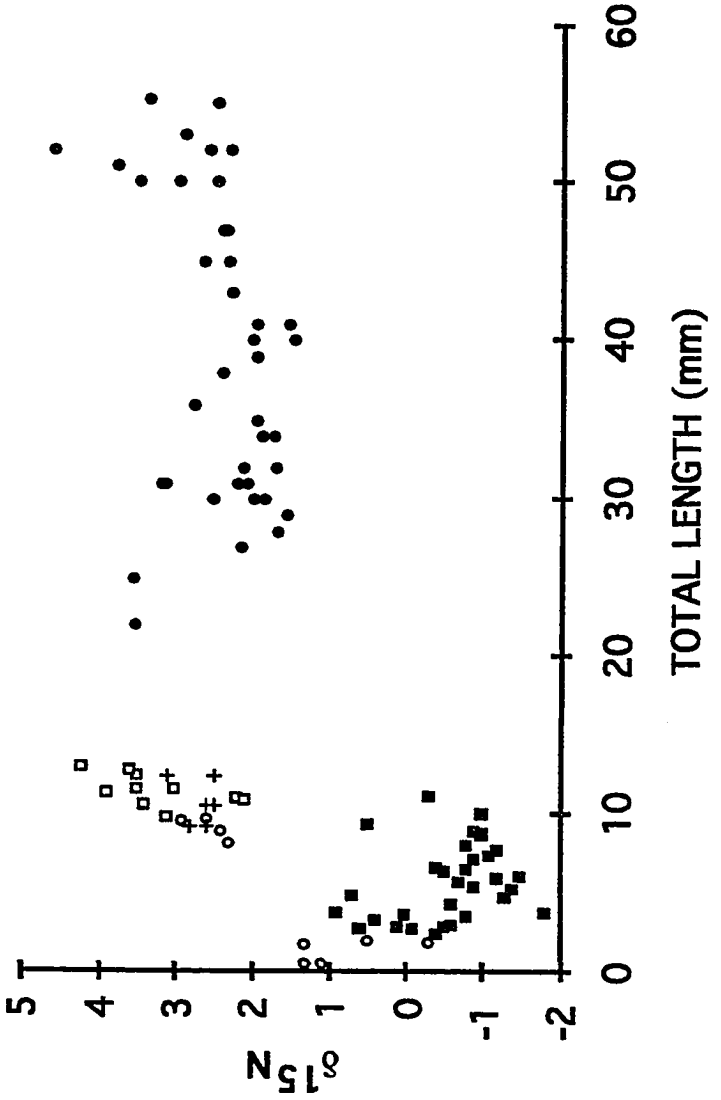
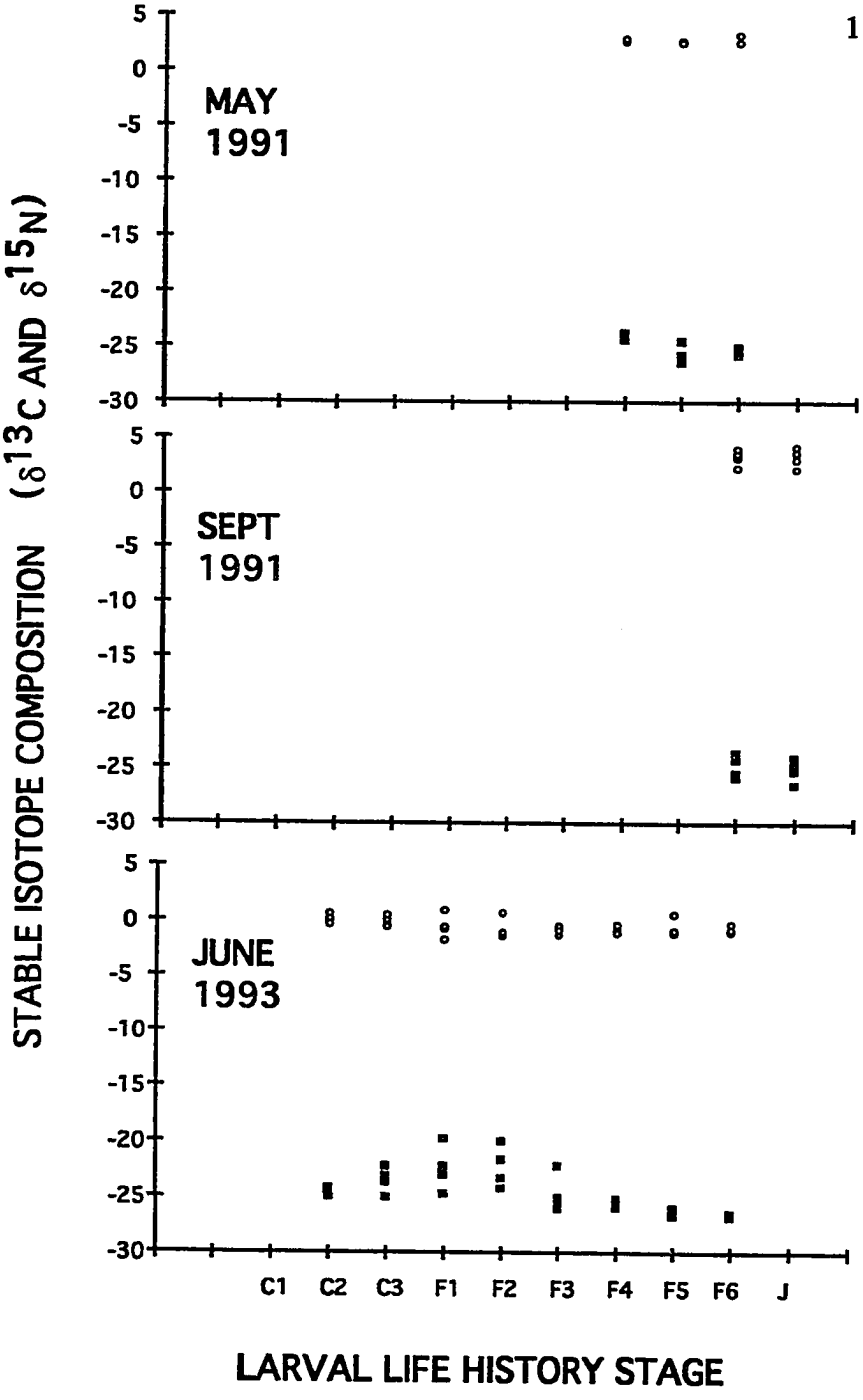


Figure 5. $\delta^{13}C$ (■) and $\delta^{15}N$ (○) for calyptopis (C) and furcilia (F) stage larvae collected during three winter cruises.



habitat type as well as collection method. POM sampled with phytoplankton nets was isotopically lighter, with respect to nitrogen, than POM sampled with Niskin Bottles (Table 2). Comparisons among winter sampling periods are suggestive of a similar pattern (see Table 1).

Concentrations of total dissolved inorganic carbon in the interstices of sea ice were less than the those in the water column during September 1991 (Table 3, Mann-Whitney rank sum test, $P = 0.008$). $\delta^{13}C$ of total dissolved inorganic carbon in water samples from ice and open water habitats did not differ (Table 3, Mann-Whitney rank sum test, $P = 0.258$) Water column measures of nitrate, nitrite and silicate were similar to those from the interstices of sea ice (Table 4). Nitrate and silicate concentrations, though variable, were generally greater than 10 and 30 μM, respectively. Nitrite concentrations were at or below the limit of detection, i.e., 0.1 μM.

TABLE 2. Mean (± SD) $\delta^{13}C$ and $\delta^{15}N$ for POM sampled with Niskin bottles and phytoplankton nets (20-μm mesh) at four stations during June 1993. Data for individual samples are reported immediately below the summary statistics.

	NISKIN BOTTLE		PHYTOPLANKTON NET	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
	-26.9 (1.3)	6.6 (0.5)	-27.7 (0.5)	2.2(0.2)
STA 1	-25.2	7.0	-27.5	2.0
STA 2	-28.2	5.9	-27.8	2.5
STA 3	-26.7	6.7	-28.4	2.1
STA 4	-27.3	6.9	-27.2	2.3

TABLE 3. Concentrations (μmoles/kg) and isotopic composition ($\delta^{13}C$) of total dissolved inorganic carbon in seawater and sea ice during September 1991.

	[TOTAL CO_2]	$\delta^{13}C$
SEAWATER		
median value	2196	1.3
sample #1	2188	1.6
sample #2	2198	1.4
sample #3	2202	1.3
sample #4	2196	1.4
sample #5	2219	1.3
sample #6	2187	1.3
sample #7	2183	1.3
SEA ICE		
median value	2162	1.7
sample #1	2176	1.2
sample #2	2140	1.7
sample #3	2162	1.8

TABLE 4. Concentrations (μM) of nitrate, nitrite and silicate in seawater and sea ice during September 1991.

	NITRATE	NITRITE	SILICATE
SEAWATER			
median value	13.4	- *	32.5
sample #1	13.1	- *	31.1
sample #2	11.2	- *	34.3
sample #3	18.9	- *	38.4
sample #4	17.3	- *	32.5
sample #5	24.8	- *	55.1
sample #6	10.7	- *	24.9
sample #7	13.4	0.1	28.9
SEA ICE			
median value	11.8	- *	30.0
sample #1	11.0	- *	30.0
sample #2	23.1	0.1	45.2
sample #3	11.8	- *	26.0

* Below the limit of detection

DISCUSSION

Elevated $\delta^{13}C$ in suspended POM and larval krill during early winter imply that (1) seasonal shifts in the isotopic composition of larval krill need not result from changes in diet, and (2) mechanisms other than CO_2 limitation in the ice can account for ^{13}C enrichments in ice-associated POM, at least initially. Because $\delta^{13}C$ of larval krill collected during May 1991 and June 1993 (early winter sampling periods) were not statistically different from $\delta^{13}C$ of larvae collected during September 1991 (late winter), stable carbon isotopes, in this study, failed to distinguish suspended POM from ice-associated POM as alternative food sources for larval krill.

Stable nitrogen isotope ratios shed little light on the relative roles of suspended POM and ice-associated POM in the winter diet of larval krill. $\delta^{15}N$ of larval krill differed significantly between the two early winter sampling periods, and, moreover, the isotopic signatures of larvae sampled in early winter and later winter of the same year did not differ.

Data reported here do support the contention that larval krill, prior to encountering sea ice, are primarily herbivorous. Larvae collected during summer months in the region of this

study (Frazer et al., unpublished data) were isotopically comparable to adult krill sampled during summer in other regions of the Southern Ocean (Wada et al. 1987, Rau et al. 1991a). By early winter, however, larval krill were relatively enriched in ^{13}C and often markedly depleted in ^{15}N. This latter observation is inconsistent with a shift to a carnivorous mode of feeding as increases in trophic level are generally associated with a stepwise enrichment in ^{15}N (DeNiro and Epstein 1981, Wada et al. 1987). Since detrital material too, is enriched in the heavy nitrogen isotope relative to phytoplankton dominated POM (B. Fry, personal communication), it is unlikely that detritivory by larval krill accounts for observed the pattern. These data lead to the suggestion that isotopic variation within the phytoplankton component of POM is the primary determinant of isotopic composition in larval krill.

Although there was some evidence for a positive relationship between $\delta^{15}N$ and total length of larval krill sampled in winter, there was a clear clustering of data based on time of collection. This is further support for pronounced seasonal variation in the isotopic composition of algal food resources exploited by krill. However, the extent to which geographic differences in the isotopic composition of food

and/or consumer might be represented in these data is not known.

The relative depletion of ^{15}N in krill larvae sampled during early winter (June 1993, see above) suggests that the animals had been feeding on an isotopically light component of the particulate pool for an extended period of time. Rates of isotopic turnover and accumulation in tissues of larval krill have not been measured, but are essential information for a complete interpretation of field collected data. Fry and Arnold (1982) demonstrated, in the laboratory, that brown shrimp, Panaeus aztecus, reflected the $^{13}C/^{12}C$ ratios of a new diet after a fourfold increase in wet tissue weight. Growth related parameters for krill larvae during winter are not well established, but reported values often equate to $\leq$ 5% of wet weight per day (see Quetin et al. 1994) and suggest that isotopic shifts for furcilia stage krill larvae occur on a time scale of months.

Rau and coworkers (1989) argued that abundant CO_2 (aq) underlies characteristic ^{13}C depletion in suspended particles from the Southern Ocean. Elevated $\delta^{13}C$ in suspended POM during early winter (this study) suggests that other factors may, at times, control the stable carbon isotope compostion of

suspended POM. Elevated $\delta^{13}C$ could result from changes in the carbon fixation pathways of the dominant microalgae. High rates of ß-carboxylation will increase $\delta^{13}C$ of algae (Goericke et al. 1994) and low light levels during the austral fall and/or early winter probably induce such activity. Fontugne et al. (1991) reported significant ß-carboxylase activity (PEPC + PEPCK) in POM samples from the Weddell sector of the Southern Ocean, i.e., the pathway exists, and Mortain-Bertrand (1988) found that an Antarctic diatom, Nitzschia tugiduloides, exhibited increased rates of ß-carboxylation under low light regimes providing support for the proposed mechanism above. Moreover, recent work by Thompson and Calvert (1994) showed that carbon-isotope discrimination by a marine diatom, Thalassiosira pseudonana, varied with daylength and irradiance; discrimination (against ^{13}C) was minimal at low irradiances (25-50 µmol photons m^{-2} s^{-1}). Thompson and Calvert (1994) suggest that their results "demonstrate a substantial role for irradiance rather than $[CO_2]$ (aq) in the physiology of ^{13}C incorporation of a marine diatom".

Suspended POM, collected as part of this study, was not acidified prior to isotopic analysis and a potential for inflated ^{13}C in the samples is recognized. However, a qualitative microscopic examination of the particles provided little

evidence that $CaCO_3$ containing organisms, e.g., foraminifera, contributed substantially to the biomass of the samples, regardless of collection period. In fact, all POM samples appeared to consist primarily of diatoms and prymnesiophytes with a smaller detrital component (Frazer, unpublished data). On one occasion (September 1991), this qualitative assessment of the algal make-up was corroborated with a subsequent charcterization of the pigment composition using HPLC analysis (Frazer, unpublished data).

With respect to nitrogen isotope ratios, Wada and Hattori (1991) noted that $\delta^{15}N$ of diatoms cultured under low-light intensities were less than those grown under high light intensities. Light is the most significant environmental factor affecting the growth and physiology of planktonic algae in the Southern Ocean and light-limited growth of phytoplankton during the austral fall may result in large isotope fractionations associated with the uptake of nitrate that are not observed during the primary growth season (see Wada et al. 1987, Goericke et al. 1994, and also Altabet and Francois 1994). An alternative explanation for low $\delta^{15}N$ in phytoplankton during late fall and/or winter is a greater reliance on ammonium (see Rau et al. 1991b), a more reduced nitrogenous nutrient, for growth. Even though nitrate concentrations generally exceed

those of ammonium by more than an order of magnitude in the region of this study, the energy cost associated with nitrate metabolism in extreme low-light environments may restrict its use by phytoplankton. Additional information on the isotopic compositon of nitrate and ammonium in Antarctic surface waters is necessary to further investigate this possibility.

Broad scale shifts in the isotopic signature of phytoplankton may also result from changes in species composition. Phytoplankton assemblages in the Southern Ocean are extremely diverse with regard to both taxonomy and size (Smith and Sakshaug 1990) and both variables can influence the isotopic signature of a sample (Montoya 1990, Rau et al. 1990). It is not unreasonable to expect that the $\delta^{15}N$ of a fall bloom consisting primarily of diatoms and/or other large species would differ appreciably when compared to a community dominated by small flagellates. A more quantitative characterization of the species assemblages associated with the POM samples collected as part of this study (see discussion above) would have been needed to address this possibility.

It is difficult to reconcile the differences reported here in $\delta^{15}N$ from POM sampled with Niskin bottles and phytoplankton nets. Rau et al. (1990) found that large particles were isotopically heavier than small ones and it does not seem likely that nets would discriminate against large particles nor that Niskin bottles would discriminate against small particles. Either of these two scenarios would have to occur to explain the relative abundance of $\delta^{15}N$ in net collected particulate matter. Isotopic differences in suspended particles collected with different methods is of obvious concern, and data reported here are interpreted with caution. Future investigators are advised to recognize the potential for isotopic variation in suspended POM as a result of sampling methodology.

Low concentrations of total CO_2 in sea ice during September 1991 (Table 3) are consistent with a hypothesis that algae growing in or associated with sea ice may at times become CO_2-limited and, as a consequence, exhibit elevated $\delta^{13}C$ (see Wada et al. 1987; Fisher 1989, 1991). However, suspended POM can clearly be enriched in ^{13}C prior to its incorporation into annual sea ice (May 1991), and other mechanism(s) can explain elevated $\delta^{13}C$ in ice-associated POM (see discussion above). The above observation calls into question the generality of aforementioned hypothesis. High

nitrate concentrations both in the ice habitat and water column do not support the idea of nitrogen limitation. However, ammonium concentrations were not measured and it is possible that relative differences in the concentrations and uptake rates of the two nitrogen species accounts for temporal and spatial variation in $\delta^{15}N$ of POM (see Rau et al. 1991b). Other explanations, e.g., increased heterotrophy and/or changes in the microbial assemblage, remain to be explored.

CONCLUDING REMARKS

Stable carbon isotopes in larval krill were found to be a poor indicator of the significance of ice-associated food resources in its diet. The measure of stable nitrogen isotopes, however, did provide evidence for herbivory over alternative modes of feeding (carnivory and/or detritivory) prior to winter. Spatial and temporal variations in the isotope ratios reported here and mechanisms posited to explain them are compelling from both a biological and geochemical perspective and deserving of further investigation.

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CHAPTER IV

Turnover of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) during growth of larval krill, Euphausia superba

ABSTRACT

Using natural abundances of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) as tracers, carbon and nitrogen turnover rates were determined for larval krill, Euphausia superba, maintained in the laboratory. Experimental populations of larvae were reared at +1.5°C and -1.5°C on foods of known isotopic composition and subsampled weekly (8-10 weeks) for a determination of wet weight and isotopic composition. Metabolic turnover of carbon ($\delta^{13}C$) was tied closely to temperature. There was a strong tendency for animals to conserve nitrogen ($\delta^{15}N$) in all treatment groups, irrespective of temperature or growth rate. These are the first coupled measures of carbon and nitrogen turnover, using a stable isotope approach, for any marine animal, and are essential information for the interpretation of field collected data. In addition to the feeding experiments, animals were starved for two months at +1.5°C and -1.5°C. Starved krill exhibited little isotopic change. This finding suggests that starvation cannot account for large temporal variations observed in the isotopic composition of larval krill collected from the field.

INTRODUCTION

Changes in the stable carbon and nitrogen isotope composition of an animal generally result from dietary shifts, though the timescales associated with such changes are not well documented. There is, of course, a lag time before the isotopic signature of new food sources can be detected in animal tissues, and often the stable isotope signature of an animal is intermediate between that of alternative food items. Intermediate isotopic values without ancillary information, e.g., growth history of the animal and associated rates of isotopic ($\delta^{13}C$ and $\delta^{15}N$) turnover in its tissues, are of limited value. In most instances the timing of dietary shifts cannot be identified nor can the contribution of specific food resources be quantified without such rate measurements.

As an aid to interpreting the analysis of stable carbon isotopes in mobile animals that switch diets, Fry and Arnold (1982) "fed brown shrimp isotopically distinct diets, and monitored the rate at which shrimp tissues shifted to isotopically match the new diets." The authors found that after a four-fold increase in weight, shrimp resembled the isotopic composition of their new diet. Subsequent investigations on the rapidity of change and turnover of stable isotopes in animal

tissues have been few (e.g., Gleason 1986, Montoya et al. 1991). As a consequence, quantitative interpretations of stable isotope measurements in food web investigations have been compromised.

As part of this study, rates of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) turnover were determined for larval krill, Euphausia superba, maintained in the laboratory at two different temperatures, +1.5°C and -1.5°C, on foods of known isotopic composition. Parallel experiments addressed the hypothesis that starvation might account for observed differences in the isotopic composition of larval krill collected from the field (see Frazer submitted). Coupled measures of stable carbon and nitrogen turnover have not been reported for any marine animal and, to our knowledge, controlled experiments using natural abundances of stable isotopes to directly measure nitrogen turnover rates have not been attempted for any zooplankton species. These results provide a valuable cross species comparison to the earlier work of Fry and Arnold (1982) on carbon turnover in brown shrimp, and shed new light on the potential effects of environmental temperatures on the expression of stable nitrogen isotopes in marine zooplankton.

METHODS

Collection and Laboratory Handling of Larval Krill

Larval krill were collected west of the Antarctic Peninsula offshore of Adelaide Island during June 1993 (Table 1). Larvae were sorted quickly to minimize handling stress and transferred with a large-bore pipette (ca. 7 mm inside diameter) to 2-l glass jars filled with filtered (0.2 μm) seawater. Jars with 25 - 50 late-stage furcilia larvae were held at ambient temperature (≤ -1.2 °C) in a running seawater table. Animals were held without the addition of food for up to 15 days prior to arriving at Palmer Station, Antarctica.

At Palmer Station, larvae collected at the same time and location on the cruise were placed in clean holding containers (see Elias 1990), and floated in 2-m diameter fiberglass tanks with flowing seawater for at least two days. Seawater was pumped through a sand filter to reduce particle concentration to near zero. Temperature of the water and light conditions varied with ambient conditions. Prior to experimentation, early furcilia (as identified by their small size) and animals that had obviously cannibalized another (as evidenced by red

Table 1. Dates and locations of collection for larval krill used in feeding and starvation experiments, with temperature, food type (if any) and start-up date for each experiment.

EXP #	Collection of Larval Krill			Experimental Information			NOTES
	DATE	LATITUDE (S)	LONGITUDE (W)	START-UP DATE	TEMP °C	FOOD TYPE[a]	
1	23 June 93	63°57'	66°53'	29 June 93	- 1.5	MIXED	b
2	12 June 93	66°46'	69°47'	01 July 93	+ 1.5	AP100	c
3	12 June 93	66°46'	69°47'	08 July 93	+ 1.5	MIXED	c
4	16 June 93	67°25'	69°17'	16 July 93	+ 1.5	NONE	c
5	12 June 93	66°46'	69°20'	23 July 93	- 1.5	NONE	c

[a] further details in text

[b] krill collected with nets (see Frazer, submitted)

[c] krill collected by SCUBA divers (see Frazer, submitted)

coloration of the digestive gland) were culled from sample populations. All sorting was done by eye with krill still in holding containers so as to minimize temperature fluctuations.

Larval krill used in each experiment were drawn from specific sample populations (Table 1). A total of five experiments were completed. Experiments were run at either + 1.5 °C (two with food, one with no food) or at - 1.5 °C (1 with food, 1 with no food) in a temperature controlled environment. Animals were held in the dark except during daily maintenance periods.

Turnover Experiments

Each of the feeding experiments (N=3) was carried out over a period of 8 to 10 weeks during July, August and September (Table 1). Initial larval numbers in each experiment were $\geq$ 330 larvae. Ten groups of larvae (30 - 33 individuals per group), drawn from the same sample population, were placed in 2-l glass jars with filtered (0.2 μm) seawater. An eleventh group of 30 larvae was sampled at the beginning of each experiment. The 2-l jars were placed on a roller-stirrer and rotated at 1 rpm to keep food in suspension. Jars were

inspected daily, molts counted and removed, and food added. Every two days, larvae were transferred with a large bore pipette to clean jars with filtered seawater at which time food was also added. At weekly sampling intervals, larval krill from one jar from each experiment were starved (ca. 24 h) to allow food in their digestive tracts to clear. Larvae were then staged, measured and weighed in preparation for isotopic analysis (described below).

Two commercial larval shrimp feeds of known isotopic composition were used for the experiments: (1) AP100 formulated by Zeigler Bros., Inc., and (2) A250 formulated by BioKyowa, Inc. Larvae from one experiment at each temperature (+1.5 °C and -1.5 °C) were fed a mixture (1:1 by weight) of the two foods. Larvae in another experiment (+1.5 °C) were fed only the AP100 variety (Table 1). All food was homogenized and sieved through a 100-μm mesh Nitex screen to yield particle sizes appropriate for larval krill. Food was weighed out daily, mixed with 50 ml of filtered sea water and poured directly into 2-l glass jars. Daily food ration was approximately 25% of the total body carbon of all larvae in a 2-l jar. Food was visible in the jars at all times, suggesting that daily rations of 25% were saturating for larval krill in these experiments.

Starvation Experiments

Larval krill were starved in two experiments, carried out in parallel, at +1.5 and -1.5 °C. Starvation experiments differed from those in which krill were fed: (1) only 100 animals were used for each experiment; (2) larvae were placed as individuals in separate 500-ml glass jars to prevent cannibalism; (3) glass jars were not rotated; (4) larvae were transferred to clean water less frequently (two week intervals) to decrease handling time and (5) the sampling interval was monthly. Jars were inspected daily for molts and molts were removed since captive larvae are known to consume them (personal observation). Sample processing was the same as for feeding experiments.

Growth Measures

Larval krill were staged according to Fraser (1936) under a dissecting microscope. Total length, from the tip of the rostrum to the end of the uropod, was measured to the nearest 0.1 mm under a dissecting microscope. Individual larvae were then rinsed in deionized water, carefully blotted dry and immediately placed on a piece of preweighed weighing paper.

Larval krill lose moisture quickly. To obtain accurate wet weights, individual larvae were placed on a Cahn microbalance and their weight was recorded in μg at 20, 40 and 60 s after being blotted dry. Initial wet weight (time zero) for an individual was taken as the y-intercept of the resulting linear regression of wet weight on time (see Quetin and Ross 1989). Growth of larval krill over the course of each experiment was estimated from regression analyses using the wet weights above. Predicted weights from these latter regressions were used in subsequent calculations of carbon and nitrogen turnover (see below) since composite samples were used for isotopic analyses.

Isotopic Analysis

After krill were measured and weighed, they were placed directly in 0.5 dram glass vials and dried at 60 °C. Ten larvae were combined in each sample vial and homogenized prior to analysis to ensure a representative sample of adequate size. All samples were analyzed with an automated system for coupled $\delta^{13}C$ and $\delta^{15}N$ measurements (Fry et al. 1992). Results are reported in standard δ notation where:

$$\delta^{13}C \text{ or } \delta^{15}N \text{ (‰)} = [(R_{sample}/R_{standard}) - 1)] \times 1000$$

and $R = (^{13}C/^{12}C)$ or $(^{15}N/^{14}N)$, respectively. Carbonate PeeDeeBelemnite and atmospheric nitrogen served as reference standards. The reproducibility of the reported measures in this study was to within ≤ 0.3 ‰.

Calculations for Turnover Rates of Carbon and Nitrogen

Turnover rates for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were determined as outlined by Fry and Arnold (1982) with some modifications. Calculations are reiterated here for clarity, and all assumptions made and/or deviations from the original approach are noted.

Step 1. A theoretical dilution equation (eq. 1) was calculated

$$\delta = \delta_f + (\delta_i - \delta_f)\ (w_i/w_t) \qquad \text{(eq. 1)}$$

where

δ_i = the initial $\delta^{13}C$ or $\delta^{15}N$ for larval krill,

w_i = the initial wet weight of larval krill in each experiment,

δ_f = the asymptotic value ($\delta^{13}C$ or $\delta^{15}N$) attained after extended growth,

w_t = the wet weight attained by larval krill at the time of sampling, and

δ = the isotopic value ($\delta^{13}C$ or $\delta^{15}N$) at the time of sampling.

The asymptotic value (δ_f) was assumed, since larval krill in the experiments described here did not reach isotopic equilibrium with their diets. Animals exhibit little isotopic fractionation of carbon ($\delta^{13}C$) when assimilating food items, and a value of +1‰ relative to the food source was used for δ_f (DeNiro and Epstein 1978). Since animals are generally enriched in ^{15}N relative to their diet by 3-4 ‰ (see Owens 1987), and Wada et. al. (1987)

reported a trophic level enhancement of 3.3 ‰ for consumers in the Southern Ocean, a value of 3.3 ‰ relative to the food source was used for δ_f when calculating nitrogen turnover. A lower value for δ_f was required when calculating nitrogen turnover for the feeding experiment at -1.5°C (see results for further explanation).

Step 2. A power function (eq. 2) was fitted to empirical data with nonlinear, iterative techniques and the aid of a computer software package (Microsoft EXCEL, version 4.0); the solver application was used to vary the value of the dimensionless exponent (c) to minimize the sum of the squared differences between observed and fitted data; the exponent was constrained such that its value was always $\leq$ -1.0; a value of -1.0 would imply that no turnover had occured. Regression coefficients (r^2) for each of the fitted equations were $\geq$ 0.95, suggesting that the assumed values were adequate.

$$\delta = \delta_f + (\delta_i - \delta_f)\ (w_t/w_i)^c \qquad \text{(eq. 2)}$$

Step 3. The dilution equation (eq. 1) was modified into a form (eq. 3) capable of fitting the empirical data.

$$\delta = \delta_f + (\delta_i - \delta_f)\ (T(w)(w_i/w_t)) \qquad \text{(eq. 3)}$$

Step 4. A turnover function was then calculated by equating eq. 2 and eq. 3 and solving for T(w);

$$T(w) = (w_i/w_t)^{c+1} \qquad \text{(eq. 4)}$$

T(w) is the fraction of initial carbon or nitrogen (δ_i) remaining after growth. The rationale for expressing carbon and nitrogen turnover in terms of weight, rather than in units of time, is given by Fry and Arnold (1982). Confidence intervals for turnover estimates might be calculated by subjecting eq. 2 to a linear transformation, and analyzing the transformed data with linear regression methods. However, the true confidence interval around each estimate is apt to be inflated with this procedure. Because of this possibility, and the extremely high r^2 values ($\geq$ 0.95) associated with a non-linear equation representing the data, confidence intervals are not reported here.

RESULTS

Growth and Mortality

Growth rates for larval krill in each of the three feeding experiments were affected by both temperature and food type (Figure 1). Larval krill reared at +1.5°C, and fed a mixed food source, approximately doubled their wet weight in 10 weeks (Figure 1A). Larval krill in the other two feeding experiments grew only half as fast (Figures 1B and 1C). The slopes of the regression lines for the three experiments were statistically different (ANCOVA, df = 2, $P < 0.05$). In each experiment, mortality was less than 5%. Larval krill in the three feeding experiments were primarily stage 6 furcilia (F6) throughout the study even though each experimental population passed through 3 molt cycles (Frazer, unpublished data). Direct development from stage 6 furcilia to juvenile did not occur in these feeding experiments.

Mean wet weight of larval krill (primarily F6) in the starvation experiments decreased with time (Figure 2). Larvae starved at +1.5°C were approximately 20% lighter after two months. Larval krill starved at -1.5°C were approximately 5% lighter over the same time interval. Starved animals continued

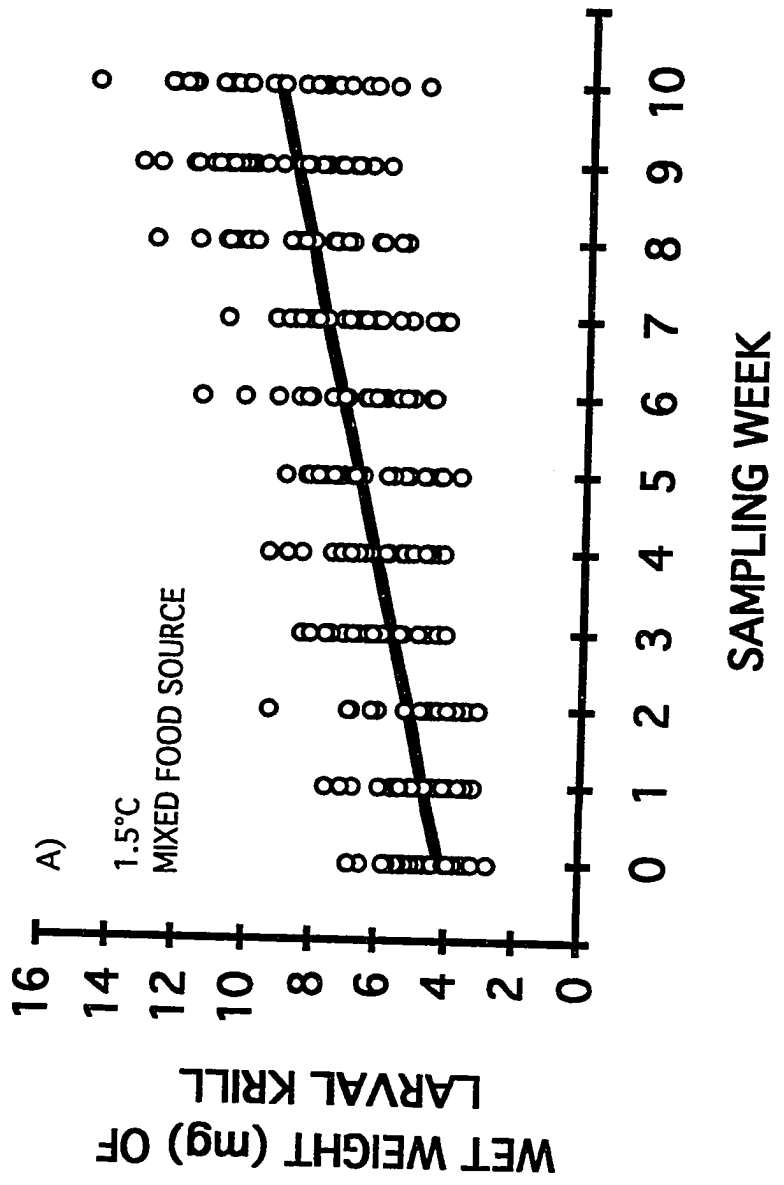
Figure 1. Wet weight (mg) as a function of time for larval krill in three separate feeding experiments: (A) animals reared at +1.5°C and fed a mixed food source, (B) animals reared at +1.5°C and fed a single food source, and (C) animals reared at -1.5°C and fed a mixed food source. Regression equations with r^2 for each of the above relationships are as follows:

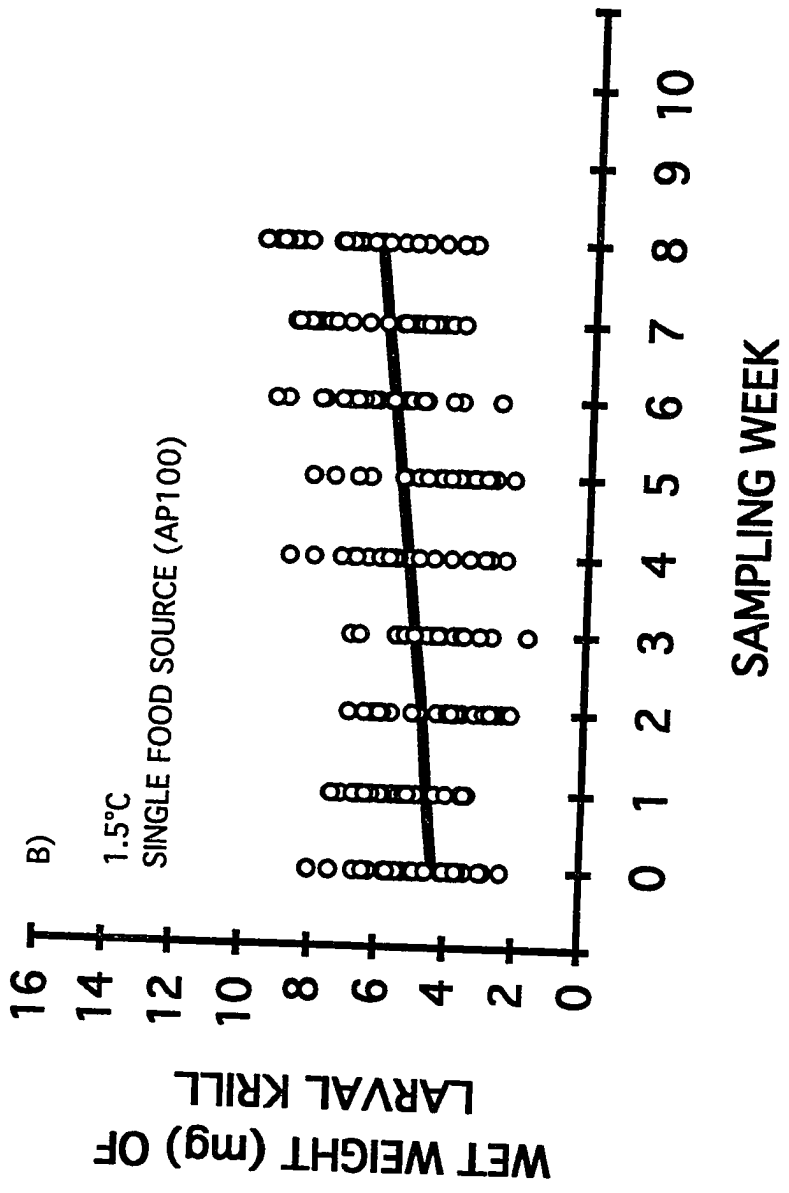
(A) $y = 4.137 + 0.521x$; $r^2 = 0.51$,

(B) $y = 4.321 + 0.253x$; $r^2 = 0.15$, and

(C) $y = 3.363 + 0.269x$; $r^2 = 0.38$.

Each equation is significant at a level of $P < 0.001$.





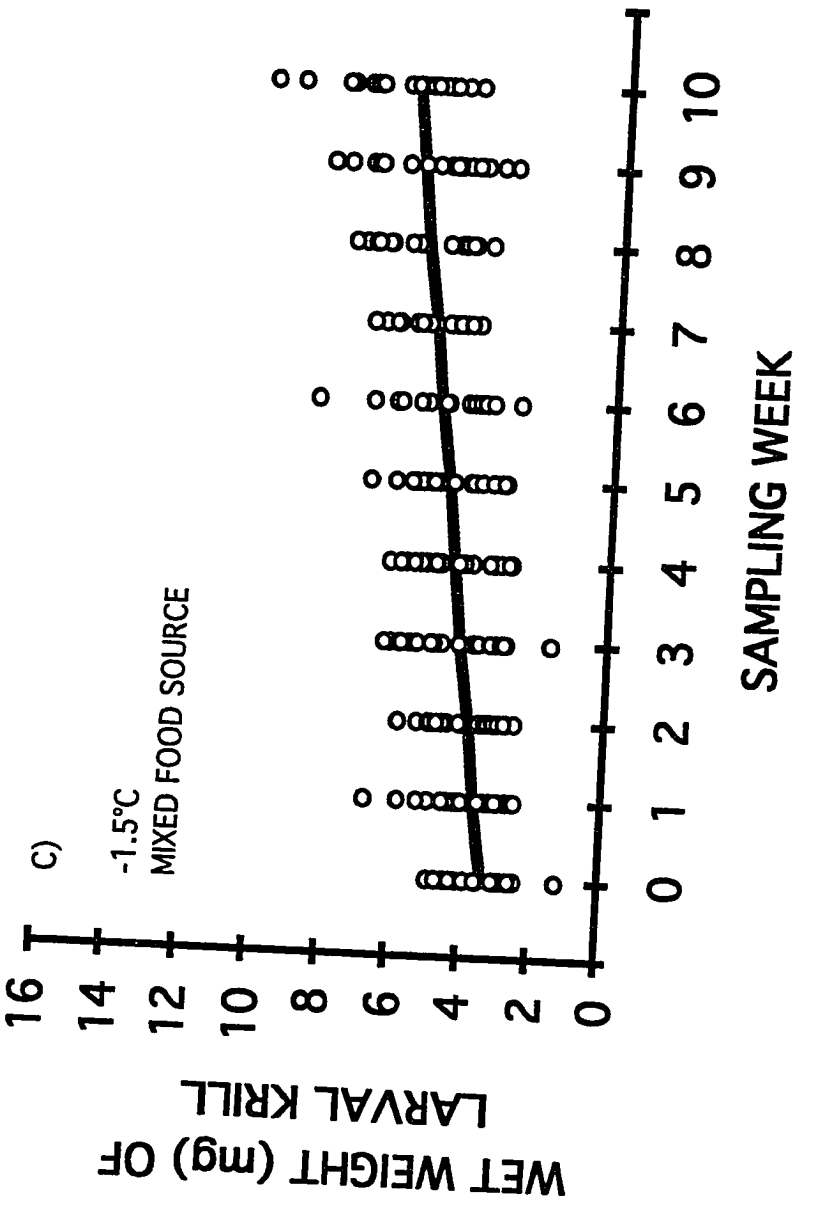
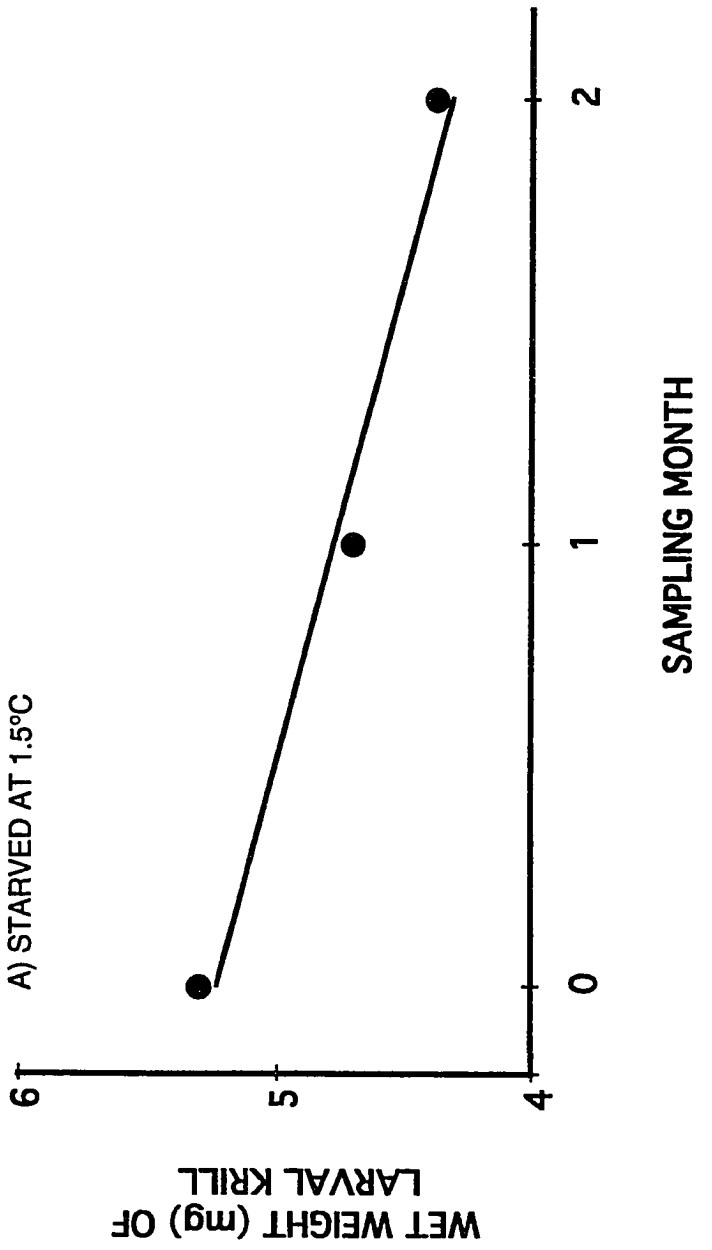
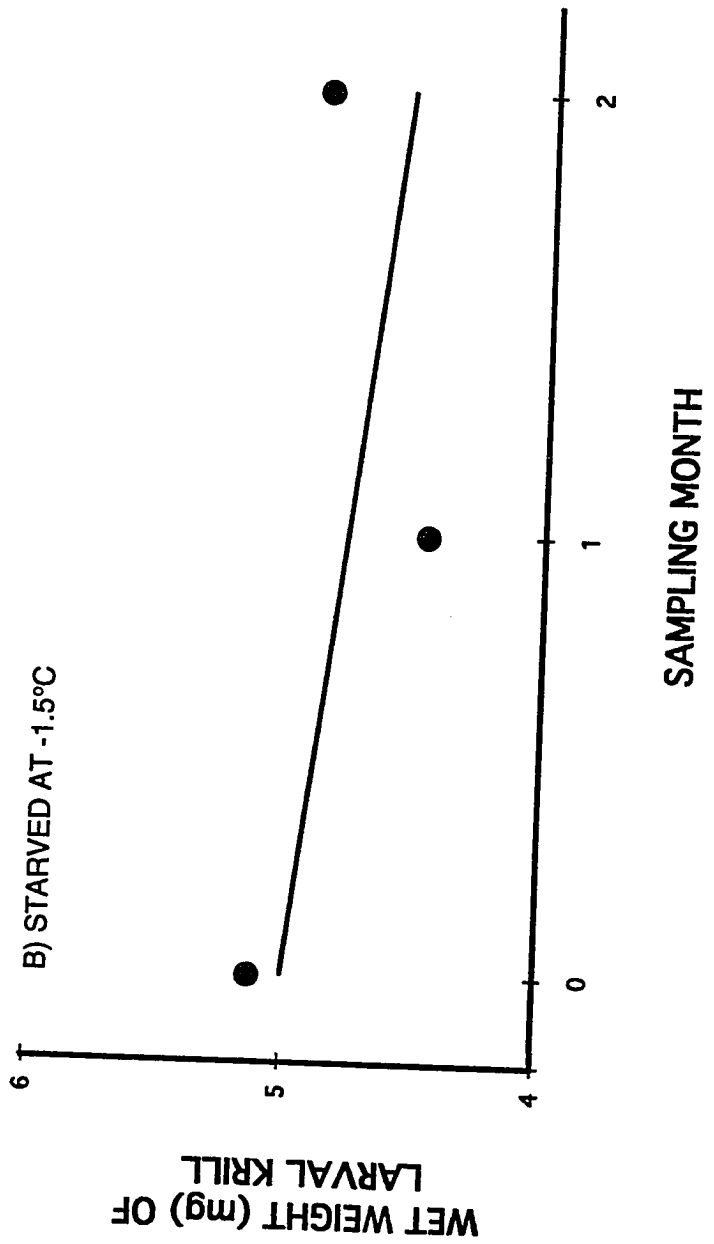


Figure 2. Wet weight (mg) as a function of time for larval krill in starvation experiments: (A) +1.5°C and (B) -1.5°C. Mean values for each sampling period are given. Equations for the regression lines are as follows:

(A) $y = 5.236 - 0.461x$; $r^2 = 0.19$, and
(B) $y = 4.998 - 0.215x$; $r^2 = 0.02$.

The equations are significant at a level of $P < 0.001$.



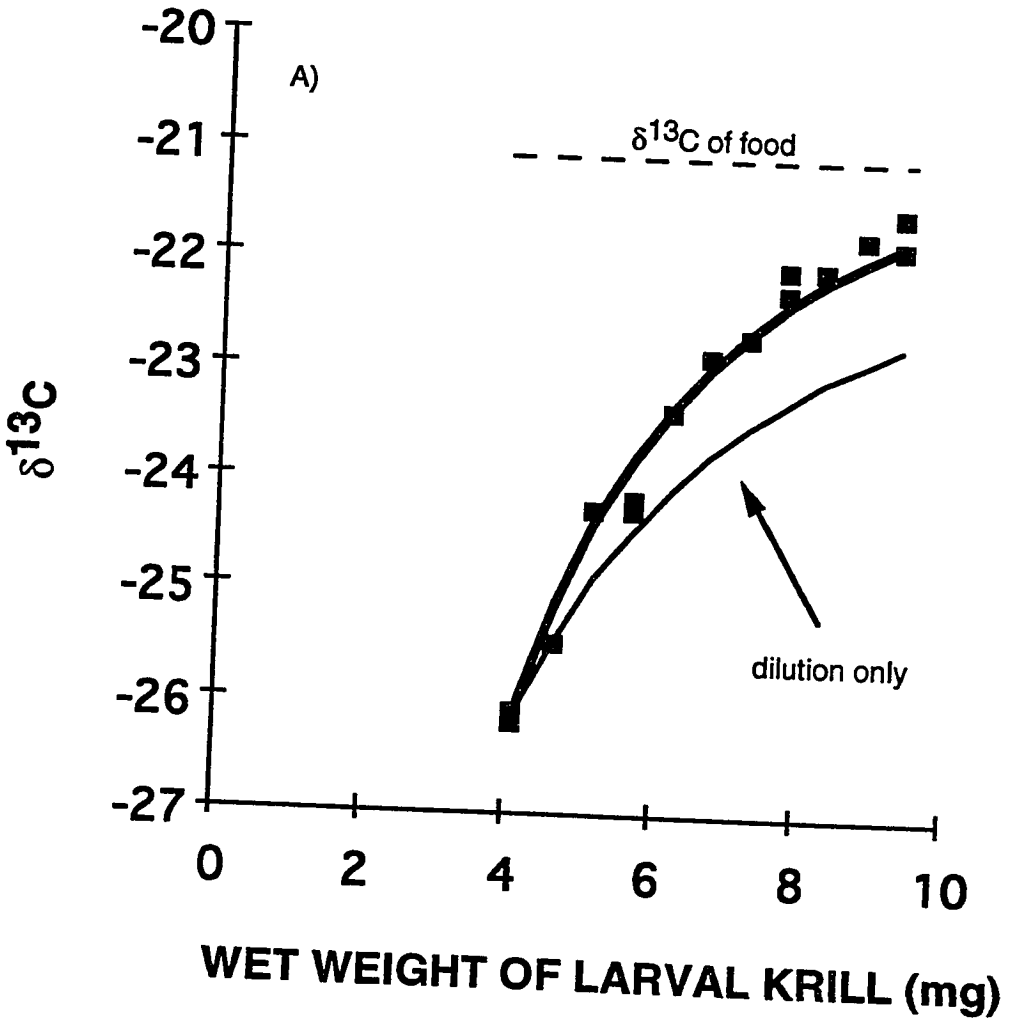


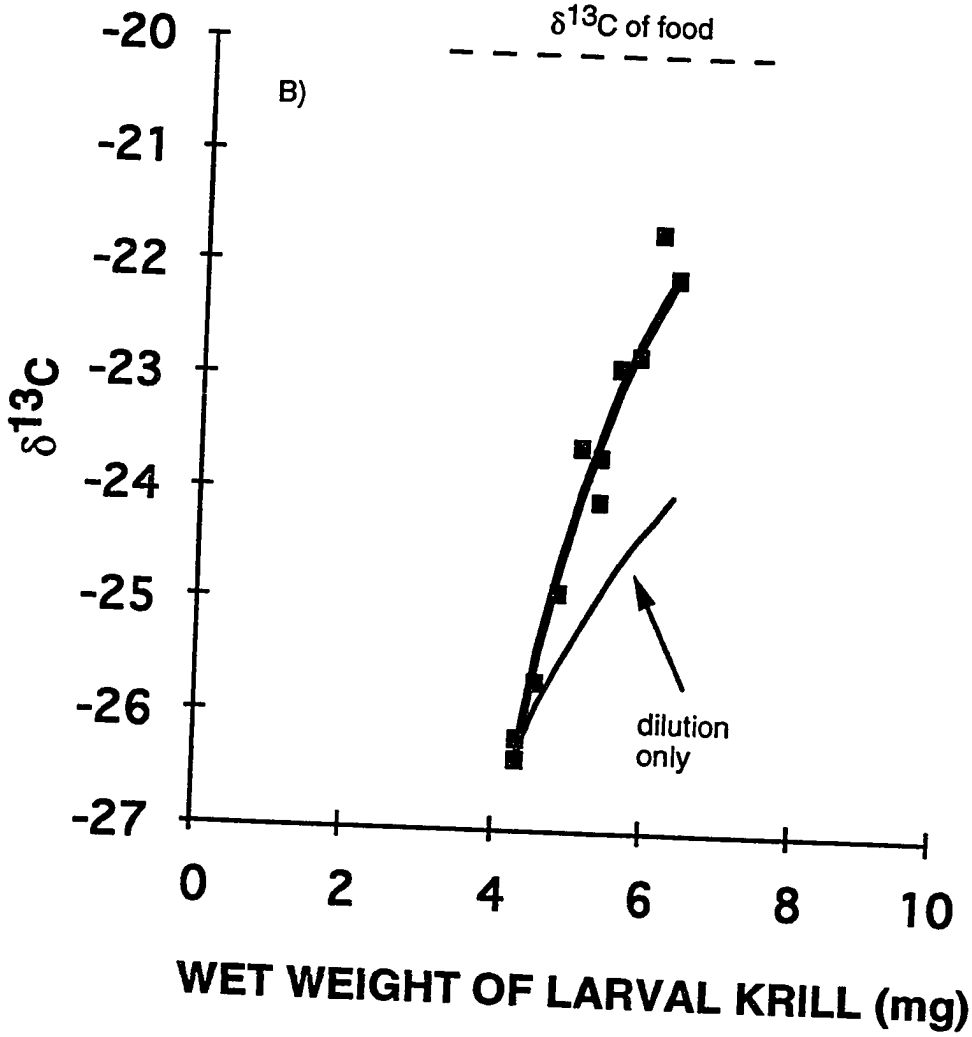
to molt, but less frequently than fed larvae (Frazer, unpublished data). The isotopic composition of starved krill remained relatively unchanged over the course of the experiment. Mortality was higher in starvation experiments than in feeding experiments; ca. 40% at -1.5°C and 20% at +1.5°C.

Carbon ($\delta^{13}C$) Turnover

The stable carbon isotope composition ($\delta^{13}C$) of larval krill approached that of their food. Only larvae reared at +1.5°C on a mixture of the two food types, however, came close to reaching an isotopic equilibrium with their diet (Figure 3). Metabolic turnover (T(w)) of carbon ($\delta^{13}C$) in larval krill was strongly affected by temperature (Table 2). Animals reared at +1.5°C had replaced approximately 35-40% of their original carbon after eight to ten weeks, whereas animals reared at -1.5°C had replaced less than 4% of their original body carbon at the conclusion of the experiment. Starved krill exhibited little change in stable carbon isotope ($\delta^{13}C$) composition with time (Figure 4).

Figure 3. Stable carbon isotope ($\delta^{13}C$) composition of larval krill as a function of wet weight over the experiment: (A) animals reared at +1.5°C and fed a mixed food source, (B) animals reared at +1.5°C and fed a single food source, and (C) animals reared at -1.5°C and fed a mixed food source. The thin solid line is a theoretical dilution curve corresponding to eq. 1 in the text. The dashed line is the stable carbon isotope ($\delta^{13}C$) composition of the food.





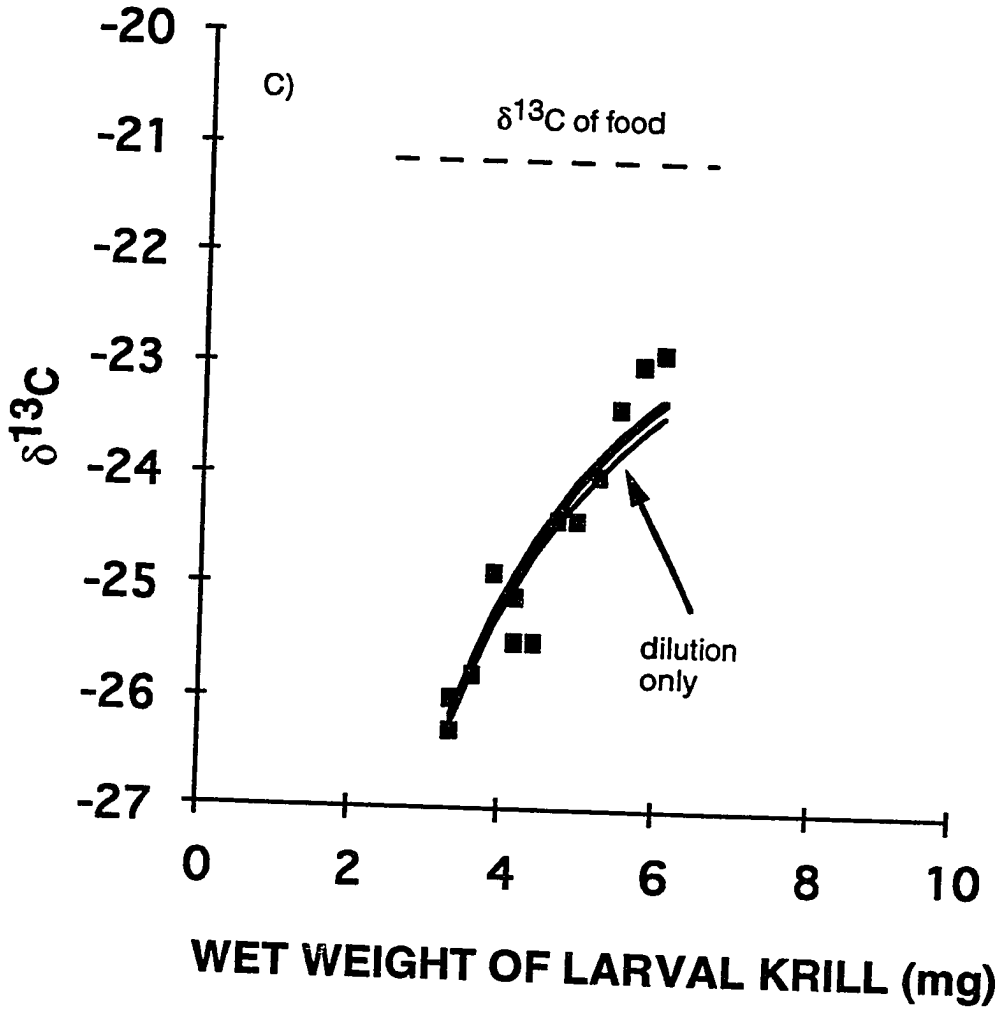
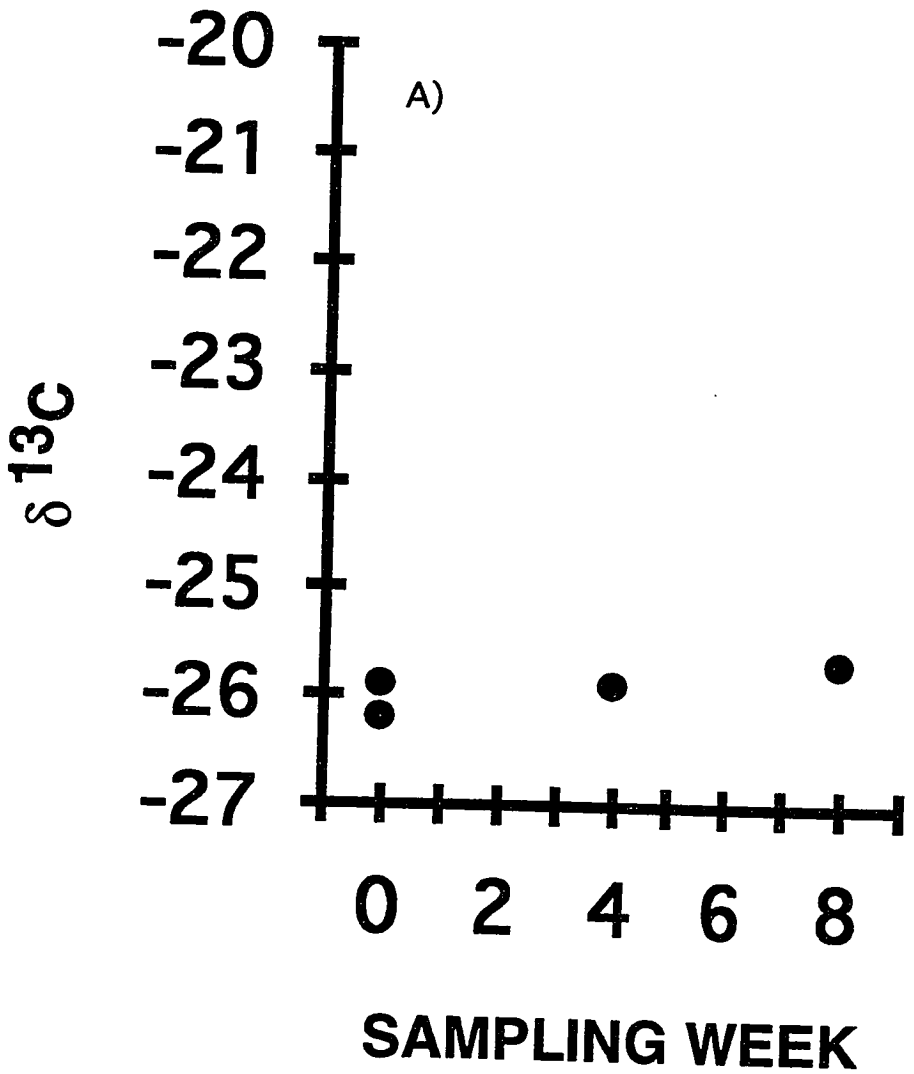
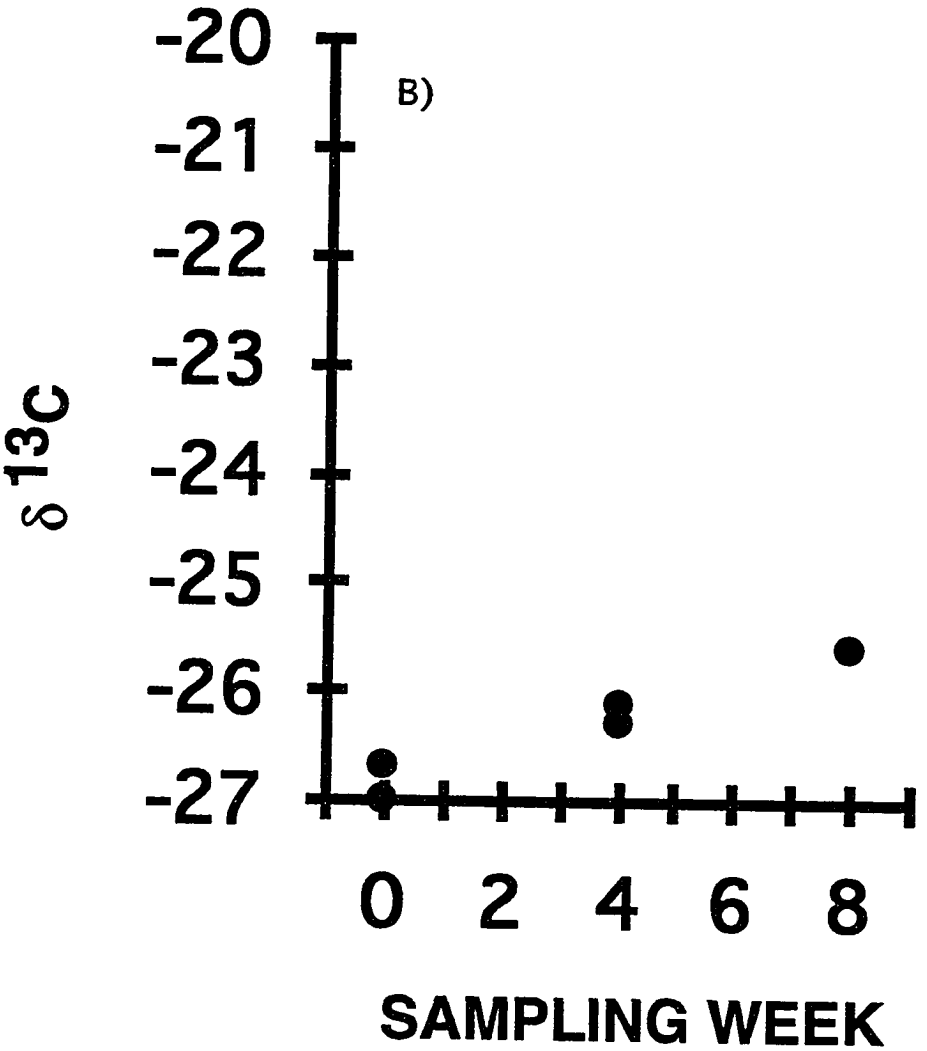


Table 2. $\delta^{13}C$ and $\delta^{15}N$ of larval krill and their food source for each of three feeding experiments. The isotopic composition of the food was measured only at the beginning (t_o) and end (tf) of each experiment. Replicate measures of krill at t_o and tf are given. T(w) is the estimated fraction of initial carbon and nitrogen remaining in krill tissue at the end (tf) of each experiment.

EXP #	TEMP °C	$\delta^{13}C$ FOOD (t_o)	$\delta^{13}C$ FOOD (tf)	$\delta^{13}C$ KRILL (t_o)	$\delta^{13}C$ KRILL (tf)	T(w) CARBON	$\delta^{15}N$ FOOD (t_o)	$\delta^{15}N$ FOOD (tf)	$\delta^{15}N$ KRILL (t_o)	$\delta^{15}N$ KRILL (tf)	T(w) NITROGEN
1	-1.5°C	-21.1	-21.0	-26.3, -26.0	-22.9, -22.9	0.96	7.2	7.2	-0.4, -0.5	3.7, 4.0	1.00
2	+1.5°C	-20.1	-20.1	-26.4, -26.2	22.1, 22.1	0.60	8.5	8.1	-0.3, 0.0	4.0, 3.7	0.94
3	+1.5°C	-21.1	-21.0	-26.1, -26.2	-21.9, -21.6	0.65	7.2	7.2	0.2, 0.2	6.4, 6.4	1.00

Figure 4. Stable carbon isotope ($\delta^{13}C$) composition of larval krill during the starvation experiments: (A) +1.5°C and (B) -1.5°C.

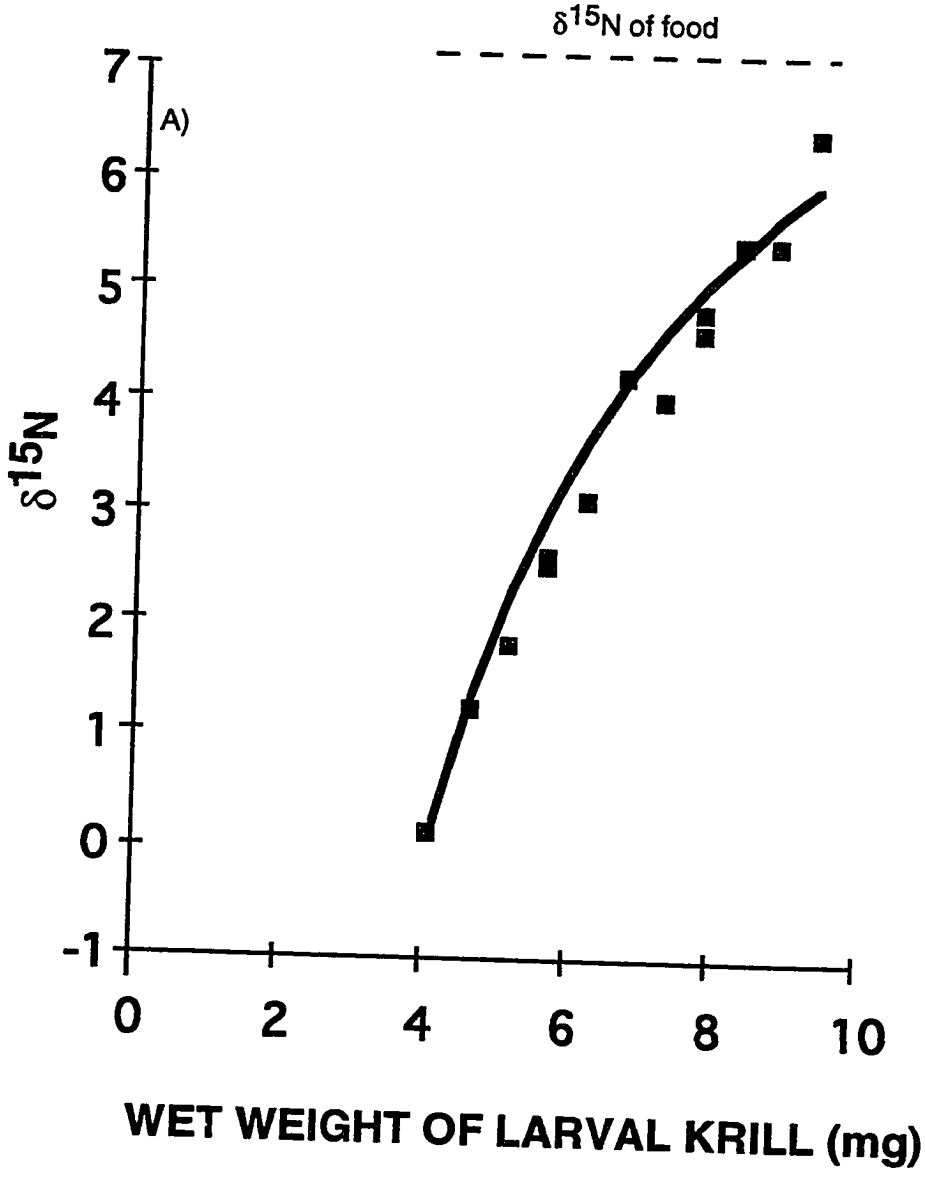


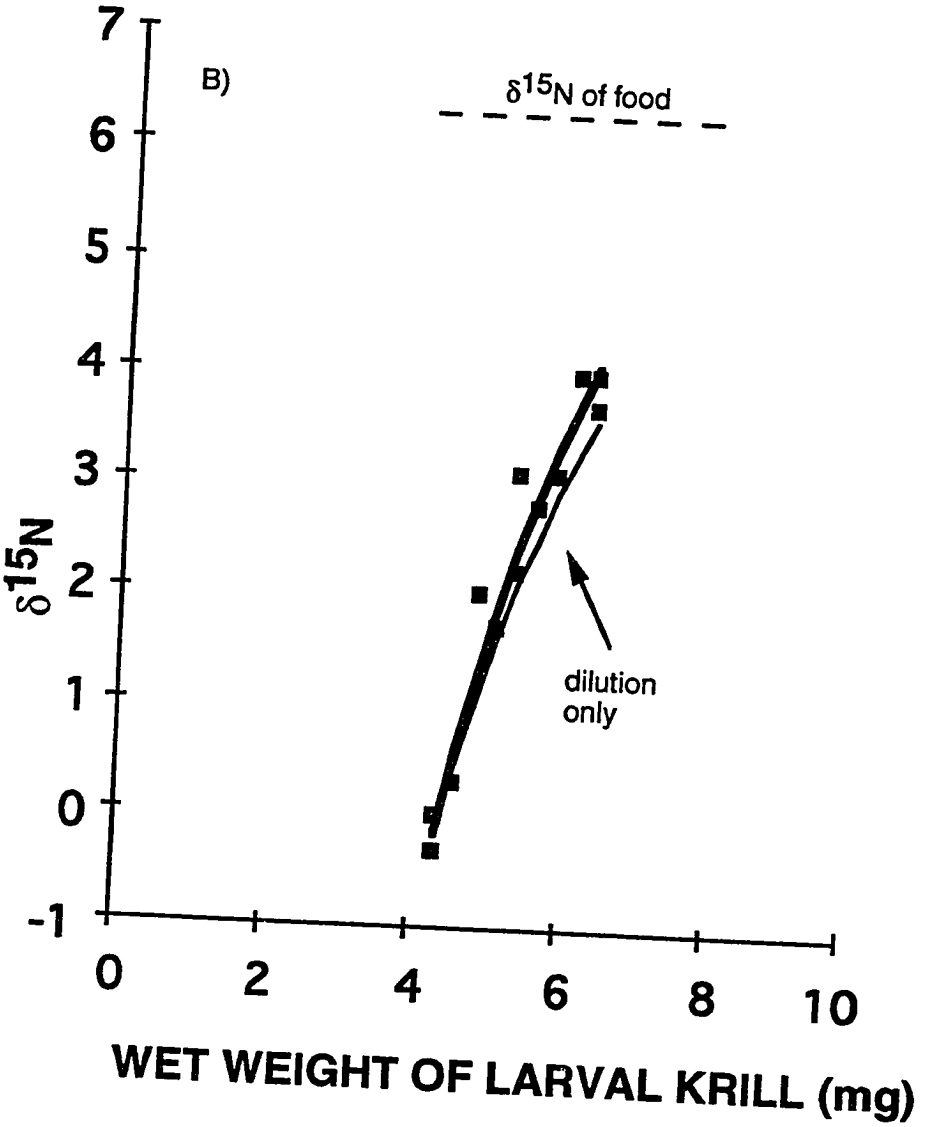


Nitrogen ($\delta^{15}N$) Turnover

The stable nitrogen isotope composition ($\delta^{15}N$) of larval krill in each of the three feeding experiments changed with growth (Figure 5). There was, however, little or no metabolic turnover of nitrogen in the krill (Table 2), irrespective of the experimental treatment. Thus, changes in $\delta^{15}N$ paralleled those of a theoretical dilution equation (eq. 1). In order to fit a power function to the empirical data when animals were fed a mixed food source and reared at -1.5°C (experiment #1), and satisfy the constraint on the exponent c (eq. 2), the fixed term δf was set at 8.2 ‰ in each of the equations (1-3). This value was substantially lower, relative to the food (+ 1 ‰, refer to Table 2), than that used in the other two feeding experiments (+ 3.3 ‰ relative to the food), and is suggestive of a temperature effect on the expression of $\delta^{15}N$ in the tissues of larval krill. Starved krill exhibited little change in stable nitrogen isotope ($\delta^{15}N$) composition with time (Figure 6).

Figure 5. Stable nitrogen isotope ($\delta^{15}N$) composition of larval krill as a function of wet weight: (A) animals reared at +1.5°C and fed a mixed food source, (B) animals reared at +1.5°C and fed a single food source, and (C) animals reared at -1.5°C and fed a mixed food source. The thin solid line is a theoretical dilution curve corresponding to eq. 1 in the text. When this line is not visible, it lies directly below the best fit line of the empirical data. The dashed line is the stable carbon isotope ($\delta^{15}N$) composition of the food.





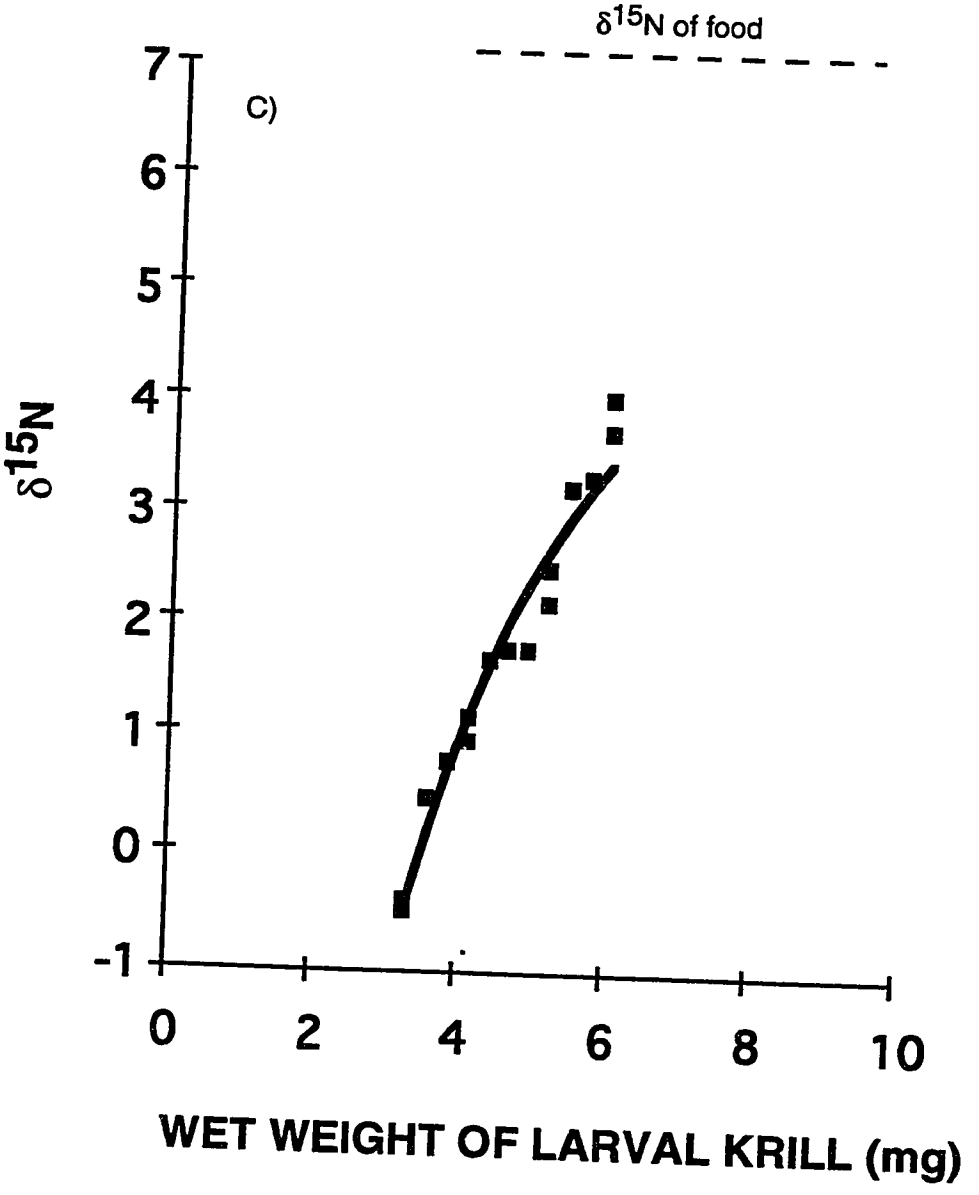
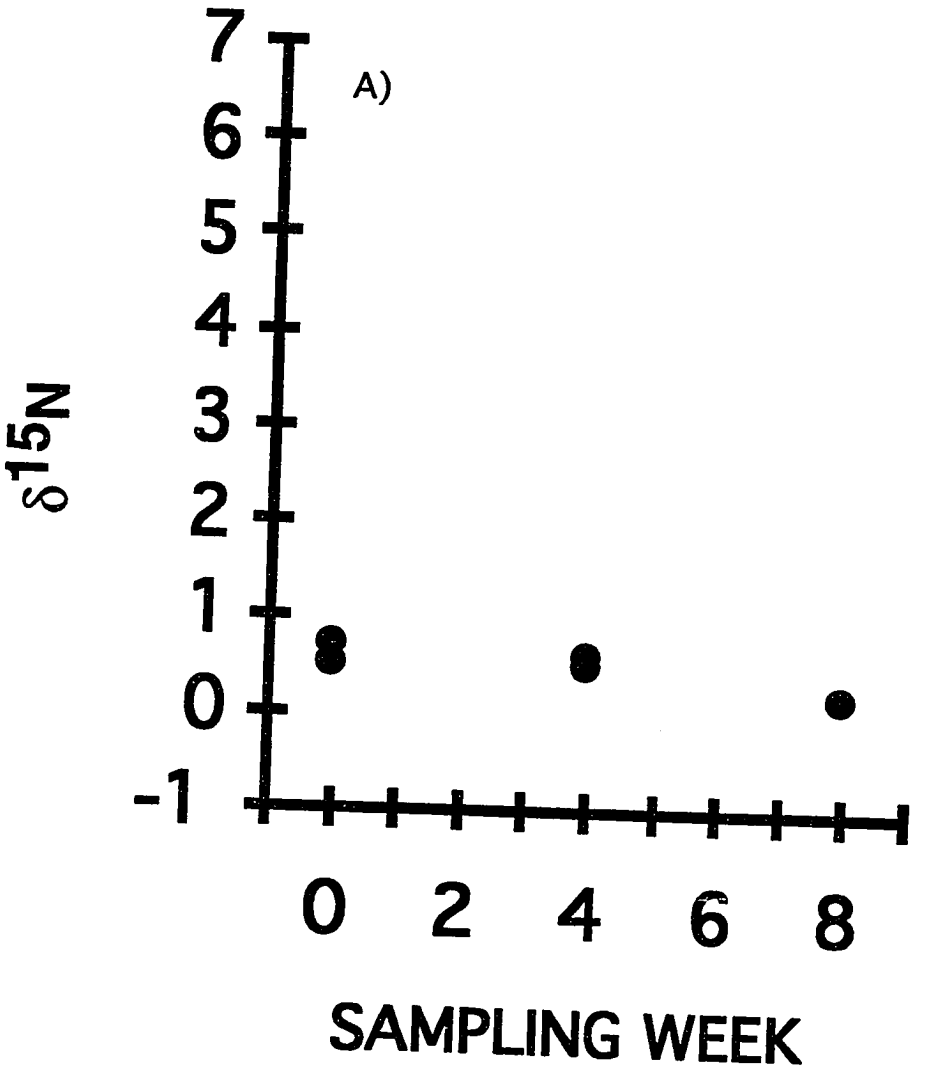
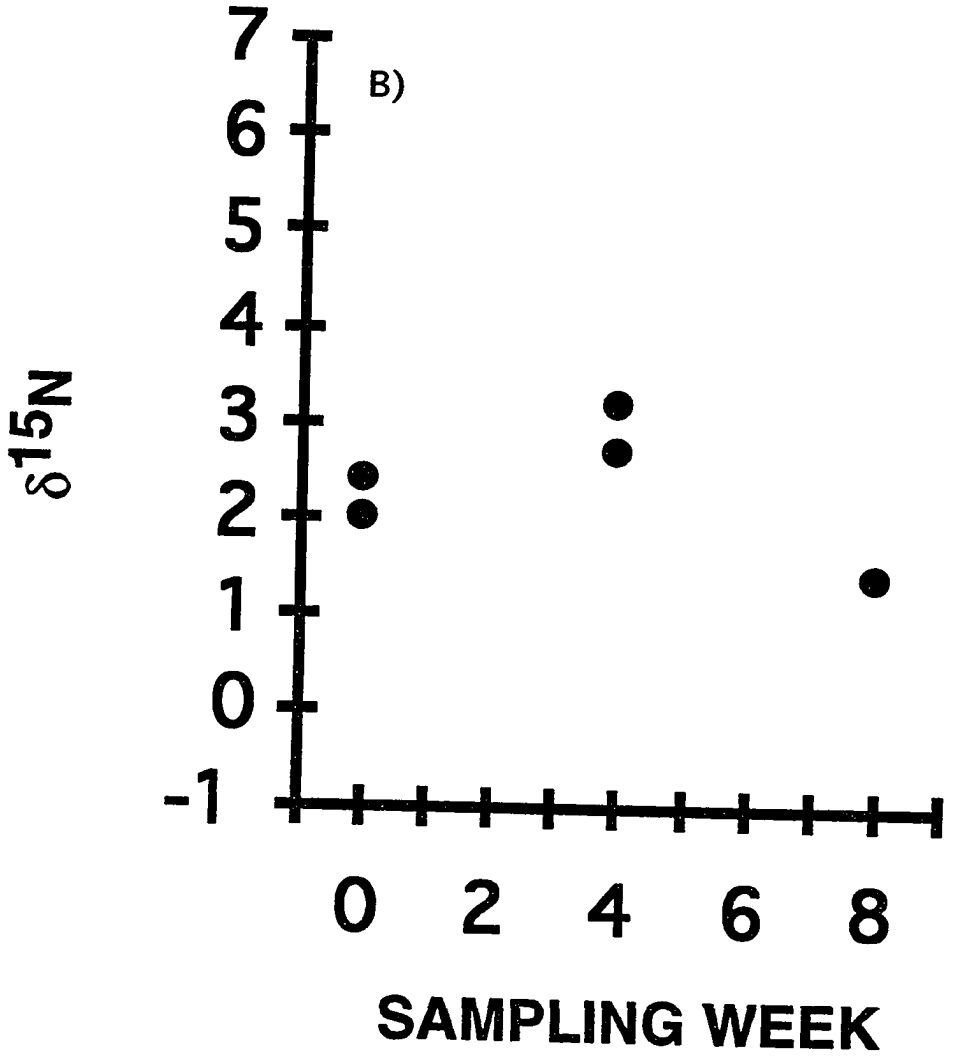


Figure 6. Stable nitrogen isotope ($\delta^{15}N$) composition of larval krill during the starvation experiments: (A) +1.5°C and (B) -1.5°C.





DISCUSSION

Effects of food and temperature on larval growth

The combined effects of food and temperature on the growth of larval krill have received little attention. Both variables influence growth (see Quetin et al. 1994), although Elias (1990) found that temperature had little effect on growth of larval krill at low food concentrations. In Elias' study, experiments were run at +1.1°C and at about -1.3°C (ambient temperature during much of the experiment), and growth rates were generally much less than 0.1 mg wet weight per day. In this sudy, feeding experiments were run at +1.5°C and -1.5°C, and food was always available to the larvae. Growth rates ranged from 0.036 to 0.075 mg wet weight per day and a significant temperature effect was observed; larvae reared at +1.5°C on a mixed food diet gained weight twice as fast as those reared at -1.5°C on the same food source. This finding suggests that for larval krill occupying the undersurfaces of winter sea ice, where water temperatures are generally $\leq$ -1.8°C, growth potential may be slowed significantly even if food is not limiting. Temperature may, in fact, be the primary determinant of larval growth during the austral winter (cf. Daly 1990). This suggestion, though subject to constraints in space

and time, is counter to a more generally accepted view that krill is food limited in the Southern Ocean (see Clarke 1983). The effects of extreme low temperatures on the physiology and behavior of larval krill will provide further insight into the role of sea ice in the winter ecology of this organism.

Carbon ($\delta^{13}C$) Turnover

Slow growth and low rates of metabolic turnover of carbon for larval krill at -1.5°C will limit the utility of stable carbon isotopes ($\delta^{13}C$) in dietary studies during the austral winter because long investigation periods will be necessary to detect isotopic changes. It should be emphasized, however, that this finding does not preclude the use of stable carbon isotopes in related food web investigations at other times of the year. In fact, the result of increased metabolic turnover of carbon concomitant with increased growth suggests that changes in diet might be quickly manifested in krill during non-winter months (cf. Fry and Arnold 1982).

More rapid, temperature dependent, turnover of somatic carbon by krill during spring/summer may confer several advantages on this organism at different stages of its life

history. Immature krill, for example, could use food sources with high C:N ratios to fuel the costs of basal metabolism and activity without sacrificing relatively less abundant building components, nitrogen in particular. The ability to quickly mobilize ingested carbon compounds will lessen the need for excessive storage products and may explain, in part, why krill generally have few lipid reserves (see Quetin et al. 1994). Similarly, reproductive females might ingest food sources with low nitrogen content, often considered of poor food quality, to fuel production of lipid-rich eggs with little consequence for growth. Turnover experiments with adult krill, comparable to those reported here for larvae, would help to evaluate these ideas.

In a broader context, rapid metabolic turnover of carbon implies a potential for high rates of respiration. This is corroborated by much experimental data with adult krill, especially at higher temperatures (see Quetin et al. 1994). The consequence, of course, is an increased rate of CO_2 return to the upper water column. Krill plays a central role in the trophic structure of the Antarctic food web and because of its abundance is likely to have a profound effect on the flow of carbon in the Southern Ocean.

Nitrogen ($\delta^{15}N$) Turnover

Roman (1983) suggested that marine invertebrates use non-nitrogenous substrates to meet their basal metabolic requirements, thus sparing assimilated proteins for growth and reproduction. The extent to which nitrogen is conserved for growth among marine zooplankton, however, is not generally known. The use of stable nitrogen isotopes in larval krill to demonstrate nitrogen conservation represents a novel application of this contemporary ecological tool. The results of this study suggest that assimilated nitrogen, once it has been allocated for somatic growth in larval krill, is not mobilized for subsequent metabolic processes, at least if food is readily available. The implications of nitrogen conservation are significant, and deserve further discussion.

No metabolic turnover of nitrogen ($\delta^{15}N$) suggests that changes in the isotopic composition during growth of larval krill, may at times, be characterized by a simple dilution equation (see Fry and Arnold 1982 and eq. 1 herein). In such situations, natural abundance measurements of stable nitrogen isotopes might be used to measure growth, i.e., secondary production. Direct measures of secondary production are few (Parsons and Takahashi 1973) and particularly difficult to

obtain for most mobile zooplankters, particularly crustaceans and gelatinous species. Natural abundance measurements of stable nitrogen isotopes thus provide a promising avenue for further investigations of material transfer in pelagic marine environments (see Montoya et al. 1991). Rates of nitrogen ($\delta^{15}N$) turnover have not been measured directly for any other marine zooplankton species and additional studies with other organisms, across broad taxa, are necessary to evaluate the generality of these results.

It is widely accepted that animals are enriched in ^{15}N relative to their food, but the factors underlying isotopic fractionation of nitrogen in animal tissues are not completely understood. Excretion of isotopically light NH_4^+ is thought to account for the observed pattern in zooplankton (e.g. Checkley and Miller 1989, Altabet and Small 1990), but complete nitrogen isotope budgets have not been directly formulated for any marine species. Consistent trophic level enhancements of approximately 3.5 ‰ (e.g., DeNiro and Epstein 1981, Minagawa and Wada 1984, Wada et al. 1987, Hobson and Welch 1992), coupled with our observation of nitrogen conservation in larval krill, imply that the $\delta^{15}N$ of excretory products and feces might vary in response to rates of food intake and its subsequent allocation for growth. In essence, as gross growth efficiencies

(K_1; expressed in terms of nitrogen) decline for larval krill, so too will the difference between the $\delta^{15}N$ of excreted ammonium and food; maximal differences will result from highest values of K_1. Only under certain conditions would larval krill contribute significantly to a pool of ^{15}N-depleted ammonium in surface waters of the Southern Ocean (cf. Checkley and Miller 1990).

Complete mass and isotope budgets for different zooplankton species under controlled laboratory conditions are needed to understand the mechanisms of nitrogen isotope fractionation in zooplankton. Because nitrogen ($\delta^{15}N$) is conserved in the tissues of larval krill, we hypothesize that $\delta^{15}N$ of excretory and fecal products will vary predictably as a result of different assimilation and growth efficiencies. This dynamic is necessary to account for the partitioning of nitrogen as a result of metabolic activity and to maintain a mass and isotope balance between ingested material and that allocated to growth, excretion and fecal production (cf. Checkley and Entzeroth 1985; Altabet and Small 1990).

Potential Effects of Temperature on the Expression of Stable Nitrogen Isotope Ratios in Larval Krill

Data reported here suggest that temperature influences directly the relationship between the nitrogen isotope composition ($\delta^{15}N$) of larval krill and its food source(s). This leads me to question whether the feeding behaviors of animals (which are often accompanied by shifts in microhabitat) might affect their $\delta^{15}N$ signatures? Consider, for example, a zooplankter that exhibits a diurnal vertical migration. If the zooplankter acquires its ration above a thermocline, and subsequently processes the food below it (in relatively cold water), then one might expect little or no isotopic fractionation associated with assimilation of the food source, i.e., the $\delta^{15}N$ signature of the animal and its food would be similar. Now consider a non-migratory zooplankter feeding in the same area on the same food source. This zooplankter would acquire its ration and process it in the relatively warm water above the thermocline. In this case, one might expect to see a more characteristic trophic level enhancement in the $\delta^{15}N$ signature of the non-migratory zooplankter. The effect in a larger, ecosystem context, would be to impose isotopic variation among taxa of the same trophic level. This, in turn, could obscure the view of the food web as delineated by stable

isotopes. Little work has been done on the effects of environmental variables on the expression of stable isotope ratios in animals at the organismal level, but there is clearly a justification to do so.

Effects of Starvation on $\delta^{15}N$ and Survival of Larval Krill

If larval krill catabolize their body nitrogen during starvation then a concomitant change in body tissue $\delta^{15}N$ might be expected as a result of excreting isotopically light ammonium (see discussion above). Starved krill, however, exhibited little evidence of isotopic change with time. The hypothesis that starvation might account for observed isotopic variations in larval krill is not supported by the findings reported here. These findings do support our assertion that consistent ^{15}N enhancements in zooplankton result primarily from isotopic fractionation during the production of new tissue, i.e., isotopically light ammonium appears a consequence only of anabolic processes. Such subtleties may serve to further our understanding of stable nitrogen isotope dynamics in marine systems.

With regard to survival, it is clear from these data and those of Elias (1990) that late stage larval krill (F6's), maintained in the laboratory, are able to tolerate periods of low food availability of two months or more. Winter-over survival of larval krill during years of little or no sea ice is likely then a consequence of size and stage distribution prior to winter. An early spawn during the previous summer would appear to facilitate larval growth, winter-over survivorship, and recruitment into the post-larval population.

Interpreting Isotopic Variation in Larval Krill

A fairly broad range of stable isotope values has been reported for krill from various sectors of the Southern Ocean (e.g., Wada et al. 1987, Rau et al. 1991), though variation, thus far, is most pronounced in larvae collected west of the Antarctic Peninsula (Frazer submitted). Temporal variation among larvae collected during summer and early winter is particularly difficult to interpret as larvae collected during winter are, in general, enhanced in the heavy carbon isotope (^{13}C), and sometimes depleted with respect to ^{15}N when compared to krill collected during summer months. To explain these observations, Frazer (submitted) put forth several hypotheses, but favored the

hypothesis that low-light induced changes in carbon fixation pathways and nitrogen uptake and metabolism of phytoplankton were largely responsible. The results of this study suggest, however, that changes in the stable nitrogen isotope composition of krill need not result entirely from isotopic shifts in the food source; seasonal changes in temperature may affect the expression of stable nitrogen isotope ratios in this animal.

Stable carbon isotope values for larval krill range from less than -30 ‰ to greater than -20 ‰ (Frazer submitted) with lightest values observed in summer and heaviest values in winter. To account for an extreme seasonal shift in the $\delta^{13}C$ of krill (> 10 ‰), growth rates during the austral fall must be substantial; larvae would have to more than double their weight in an eight to twelve week period assuming the $\delta^{13}C$ of their suspended food source during that time was constant at approximately -20 ‰. Such values have been reported for suspended POM in the Southern Ocean (Frazer submitted, Kopczynska et al. 1995) and suggest that the more characteristic summer values for suspended POM in the Southern Ocean (< -27 ‰; Sackett et al. 1965, Rau et al. 1982, 1989; Wada et al. 1987, Wada and Hattori 1991, Fischer 1991,

Fontugne et al. 1991) may not persist into the fall and/or winter.

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