Lipid biochemistry of Antarctic euphausiids – energetic adaptations and a critical appraisal of trophic biomarkers

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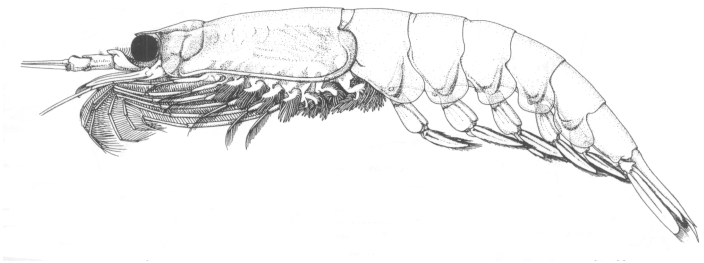

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We always can tell a likely spot to look for whales by the colour of the water. Whales feed upon insects which swarm in good whale water in myriads, making the water look quite thick and dark brown. When we see the water have this appearance we keep a good lookout for fish. If there is no food in the water you may be sure you won't see no fish, unless they happen to be passing on their way up or down the country, or are on the search for good feeding ground. It's my opinion a whale can go a long time without meat, but when they do feed they swallow a tremendous quantity. We often take bucketsful of whales' food out of their throats and mouths when cutting out the whalebone.

Whales' food consists of small red insects or animalcules, or whatever you may choose to call them, of a regular, uniform reddish colour, and spindle–shaped, tapering away to the tail. They are found principally in the Arctic and Antarctic Seas, where they exist in enormous numbers. They don't exceed an inch in length, yet they are the principal food of these great fish.

From the remarks of John Gravill, captain of the Arctic whaleship 'Diana' of Hull in 1866–67, cited in Marr (1962).

Contents

Publications

Abbreviations

ABW	Antarctic Bottom Water
ACC	Antarctic Circumpolar Current
ANOVA	Analysis of variance
ASW	Antarctic Surface Water
BHT	Butylhydroxytoluol
C	Carbon
CDW	Circumpolar Deep Water
Chl *a*	Chlorophyll *a*
CHOL	Cholsterol
CI-III	Calyptopis larvae I-III
DAG	Diacylglycerol
DCM	Dichloromethane
DM	Dry mass
EDTA	Ethylene diamine tetra-acetic acid
FAME	Fatty acid methyl ester
FFA	Free fatty acids
FI-VI	Furcilia larvae I-VI
FID	Flame ionisation detection
HCl	Hydrogen chloride
(HP) TLC	(High performance) Thin layer chromatography
IKMT	Isaac Kidd Midwater Trawl
KCl	Potassium chloride
LPC	Lysophosphatidylcholine
MeOH	Methanol
MUFA	Monounsaturated fatty acids
N	Nitrogen
n	Number of individuals/samples
NaCl	Sodium chloride
O	Oxygen
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PF	Polar Front

PI	Phosphatidylinositol
PL	Polar lipids
POM	Particulate organic material
PS	Phosphatidylserine
PUFA	Polyunsaturated fatty acids
RMT	Rectangular Midwater Trawl
SE	Sterol esters
SFA	Saturated fatty acids
ST	Sterols
TAG	Triacylglycerols
TL	Total lipid
V	Volume
WE	Wax esters

Preface

This cumulative dissertation is composed of six publications as listed below. It further includes a general introduction, a section with more detailed information on the applied methods, and a synoptic discussion. It is part of a joint project investigating the seasonal feeding strategies of the Antarctic krill, *Euphausia superba*, funded by the German Ministry of Education and Research (BMBF, Project No. 03PL025B/4).

Chapter 1

Stübing D, W Hagen, and K Schmidt

On the use of lipid biomarkers in marine food web analyses: An experimental case study on the Antarctic krill, *Euphausia superba*

The concept of this study was developed together with the second author. I created the experimental set–up and carried out the experiments, partially in collaboration with Dr. Katrin Schmidt, who used half of the 1999 experimental krill for the determination of stable isotopes (see Chapter 6). I performed the analyses, and both evaluated the results and wrote the manuscript with scientific and editorial help of Prof. Hagen. This article was published in Limnology and Oceanography 48: 1685–1700.

Chapter 2

Stübing D and W Hagen

Fatty acid biomarker ratios – suitable trophic indicators in Antarctic euphausiids?

The idea of this study was devised by myself. I carried out the analyses and evaluated the results. The manuscript was written with scientific and editorial advice by Prof. Hagen. It was published in Polar Biology 26: 774–782.

Chapter 3

Meyer B, A Atkinson, D Stübing, B Oettl, W Hagen, and UV Bathmann

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

I shared the field work with the first authors and supplied crucial data on the energetic condition of the larvae. The lipid analyses were carried out by myself, as well as the evaluation and interpretation of their results. I was also involved in the writing of the manuscript. This article was published in Limnology and Oceanography 47: 943–952.

Chapter 4

Atkinson A, B Meyer, D Stübing, W Hagen, K Schmidt, and UV Bathmann

Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter–II. Juveniles and adults

The samples were collected together with the other authors. I provided the data on the lipid content and composition and was involved in the interpretation of the respective results. This article was published in Limnology and Oceanography 47: 953–966.

Chapter 5

Stübing D, T Kreibich, and W Hagen

Energy storage via phosphatidylcholine (lecithine) in Antarctic euphausiids – preliminary biochemical and histochemical evidence

The original idea for this study and the appropriate methodology for addressing the central questions were developed by myself. The second author provided data on the lipid composition of isolated sub–cellular fractions and contributed to the evaluation and discussion of the results. I wrote the manuscript with editorial help of Prof. Hagen. It was prepared for submission to Marine Ecology Progress Series.

Chapter 6

Schmidt K, A Atkinson, D Stübing, JW McClelland, JP Montoya, and M Voss

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

Sampling and feeding experiments were carried out together with the first author. We have evaluated and discussed the results in close collaboration by comparison with the respective lipid data (see Chapter 1). I provided editorial help during the writing of the manuscript. This article was published in Limnology and Oceanography 48: 277–289.

Abstract

The energetic and physiological condition of various ontogenetic stages of Antarctic euphausiids was examined at the onset of winter, applying lipid analytics as the main investigative tool. *Euphausia superba* was the key species of the studies. Despite the comparatively large amount of literature on the biology and ecology of *E. superba*, major uncertainties persist concerning the overwintering strategies of this primarily herbivorous species. Combined field and experimental data were used to evaluate the relative importance of the alternative strategies for the different developmental stages. Furcilia III larvae were characterised by low lipid levels, predominantly phospholipids (PL), high metabolic and feeding activities, with the main diet being diatoms. Hence, the larvae exhibited a "business as usual" strategy at the onset of winter. A clearly deviating behaviour was observed in juvenile and adult *E. superba*, with high lipid depots, mainly composed of triacylglycerols (TAG) and PL, and low metabolic and feeding rates as compared to summer values. The trend of reduced and more carnivorous feeding with progressing ontogeny was still discernible in the postlarvae. These data indicated a compromise overwintering strategy between switch feeding and energy conservation for juvenile and adult *E. superba*.

The application of both fatty acid and stable isotope trophic biomarkers in the past as well as during this study led to contradictory results. Long–term feeding experiments ($\leq$ 44 days) were carried out to examine the effect of various diets on the lipids and stable isotopes of larval, juvenile, and adult *E. superba*. Total lipid content, lipid class, fatty acid and stable isotope compositions showed very little variation with the different feeding regimes in postlarval krill. The weak signal of the trophic marker fatty acids was attributed to the large lipid reserves buffering short–term variations in dietary lipid supply on the one hand, and inefficient utilisation of the offered food on the other hand. Furciliae were influenced more strongly by the fatty acid and stable isotope signatures of their food. Changes in fatty acid composition, combined with significant lipid build–up, could be induced experimentally and were also detected in field samples from different locations. Trophic marker fatty acids were indicative of either diatom or flagellate feeding. The faster growth, as well as the active food utilisation and assimilation of furciliae in conjunction with their lower lipid levels favour the incorporation and detection of trophic biomarkers as compared to postlarval *E. superba*.

The fatty acids 16:1(n-7) and 18:4(n-3) were found best suited as trophic markers in *E. superba*, since they were rapidly accumulated and depleted according to their abundance in the diet. Most fatty acid ratios proved to be only of limited use as

trophic indices, since they were strongly dependent on total lipid contents, which may vary severely with factors unrelated to specific feeding preferences.

In many polar euphausiids, phosphatidylcholine (PC) is known to linearly accumulate with increasing lipid content similar to the main neutral storage lipids. The present study added further evidence to the hypothesis that PC, besides its important role in biomembranes, also serves as supplementary depot lipid. Histochemical analyses revealed extensive lipid storage, both as neutral and phospholipids, in tissue surrounding the hepatopancreas. As demonstrated by the biochemical analyses, the major part of the phospholipids occurred in the free form, i.e. not incorporated into membranes. Whether its role lies in providing additional energy or rather in presenting a depot for structural requirements could not be fully resolved yet.

In contrast to the wealth of information available on *E. superba*, only fragmentary knowledge exists of the biology and ecology of the sympatric *Thysanoessa macrura* and especially the two more northerly distributed species *E. frigida* and *E. triacantha*. Autumn lipid levels of *T. macrura* exceeded those of *E. superba* and wax esters were the dominant neutral lipid class. The higher energy reserves of *T. macrura* were attributed to its spawning in late winter uncoupled from phytoplankton input. As compared to the high–Antarctic species, *E. frigida* and *E. triacantha* had moderate lipid levels reflecting the less pronounced seasonality of food availability with decreasing latitude. Seasonal variations of total lipid content and composition with higher neutral lipid levels in autumn illustrated the importance of lipids in the life histories of these two species. The lipid composition of *E. frigida* was only weakly influenced by dietary marker fatty acids, whereas *E. triacantha* lipids clearly reflected omnivory with a strong dependence on calanid copepods particularly in autumn.

The present study significantly contributes to the understanding of lipid dynamics and their ecological implications in Antarctic euphausiids.

Zusammenfassung

Im Zentrum dieser Arbeit steht die Untersuchung des energetischen und physiologischen Zustands verschiedener Entwicklungsstadien antarktischer Euphausiaceen zu Beginn des Winters. Diese Fragestellung wurde vorrangig mithilfe lipidbiochemischer Methoden bearbeitet, wobei das Hauptaugenmerk auf *Euphausia superba*, der weltweit häufigsten Euphausiaceenart, lag. Trotz einer verhältnismäßig umfangreichen Literatur zur Biologie und Ökologie von *E. superba*, bestehen noch immer Unsicherheiten bezüglich der Überwinterungsstrategien dieser überwiegend herbivoren Art. Anhand eines integrativen Ansatzes aus Feld- und experimentellen Daten wurde die relative Bedeutung der alternativ diskutierten Überwinterungsstrategien vergleichend für die verschiedenen ontogenetischen Stadien untersucht. Furcilia III–Larven zeichneten sich durch geringe Lipidgehalte und dementsprechend hohe Phospholipidanteile aus, sowie durch eine hohe Stoffwechsel- und Fraßaktivität, wobei Diatomeen den Hauptbestandteil ihrer Nahrung bildeten. Zu Beginn des Winters verfolgten die Furcilien demnach eine sogenannte "Business as usual"–Strategie, d.h. dass sie ihr Fraß- und Aktivitätsverhalten im Verhältnis zum Sommer nicht verändert hatten. Klar davon abweichend verhielten sich hingegen juvenile und adulte *E. superba*, die große Lipidreserven, überwiegend in Form von Triacylglycerinen, angelegt hatten und deutlich reduzierte Stoffwechsel- und Fraßraten zeigten im Vergleich zu entsprechenden Sommerdaten. Diese Reduktion war bei den Adultstadien noch ausgeprägter als bei den Juvenilen, zusammen mit einer Tendenz zunehmender Karnivorie. Diese Daten deuten auf eine "Kompromiss"–Überwinterungsstrategie postlarvaler *E. superba* hin, die die Nutzung alternativer Nahrungsquellen mit Energiesparmechanismen kombiniert.

Die Anwendung von Fettsäuren und stabilen Isotopen als trophische Biomarker hat sowohl in früheren Studien als auch in dieser Arbeit zu widersprüchlichen Ergebnissen geführt. Mithilfe von Langzeitexperimenten ($\leq$ 44 Tage) wurde der Einfluss verschiedener Nahrungsorganismen auf die Lipid- und Isotopenzusammensetzung larvaler, juveniler und adulter *E. superba* untersucht. In unterschiedlichen Fütterungsansätzen konnten bei postlarvalen Tieren nur sehr geringe Änderungen des Gesamtlipidgehalts, der Lipidklassen-, Fettsäure- und Isotopenzusammensetzung induziert werden. Das schwache Signal trophischer Markerfettsäuren in den Krilllipiden wurde einerseits auf die hohen Lipidreserven der Tiere zurückgeführt, die kurzzeitige Schwankungen von Nahrungslipiden abpuffern, und andererseits auf die ineffiziente Nutzung der in den Experimenten angebotenen Nahrung. *E. superba* Furcilien hingegen wurden stärker durch die Fettsäure- und Isotopensignatur ihrer Nahrung beeinflußt. Veränderungen ihrer Fettsäurezusammensetzung in Verbindung mit signifikanter Lipidakkumula-

tion konnten sowohl experimentell induziert, als auch in Feldproben unterschiedlicher Stationen nachgewiesen werden. Anhand der trophischen Markerfettsäuren wurden entweder Diatomeen oder Flagellaten als Hauptnahrung abgeleitet. Das schnellere Wachstum der Furcilien und ihre effektive Nutzung und Assimilation der verfügbaren Nahrung in Verbindung mit ihrem geringen Lipidgehalt begünstigen den Einbau und letztlich auch die Anwendbarkeit trophischer Biomarker im Vergleich zu den postlarvalen *E. superba*. Als besonders geeignete trophische Marker in den Lipiden von *E. superba* erwiesen sich die Fettsäuren 16:1(n-7) und 18:4(n-3), da sie schnell akkumuliert und wieder abgebaut wurden, entsprechend ihrer Konzentration in der Nahrung. Die meisten Indizes, die aus den Verhältnissen zweier Markerfettsäuren gebildet und in der Literatur zur Klassifizierung marinen Zooplanktons in Ernährungstypen herangezogen werden, waren nur bedingt anwendbar für *E. superba*, da sie stark von ihrem Gesamtlipidgehalt abhingen. Der wiederum ist deutlichen Schwankungen unterlegen, die nicht zwangsläufig mit der Ernährung zusammenhängen.

Von vielen polaren Euphausiaceenarten ist bekannt, dass Phosphatidylcholin (PC) mit zunehmendem Gesamtlipidgehalt linear ansteigt. Die Ergebnisse der vorliegenden Arbeit untermauern die Hypothese, dass PC, neben seiner essentiellen Rolle als Grundbaustein in Biomembranen, als zusätzliches Speicherlipid dient. Histochemische Analysen ließen ausgedehnte Lipidspeicher, sowohl Neutral- als auch Phospholipide, im Gewebe um die Mitteldarmdrüse erkennen. Mithilfe von biochemischen Methoden wurde nachgewiesen, dass der Großteil der Phospholipide in freier Form, d.h. nicht an Membranen gebunden, vorlag. Worin die spezifischen ökophysiologischen Vorteile der PC–Speicherung liegen und ob PC vorrangig als zusätzliche Energiereserve oder als Speicher für strukturelle Bedürfnisse dient, konnte noch nicht abschließend geklärt werden.

Im Gegensatz zu der Fülle an Literatur über *E. superba* ist das Wissen über die Biologie und Ökologie der sympatrischen Art *Thysanoessa macrura* und besonders der beiden nördlicheren Arten *E. frigida* und *E. triacantha* nur fragmentarisch. *T. macrura* erreicht im Herbst höhere maximale Lipidwerte als *E. superba*, wobei Wachsester das Hauptneutrallipid waren. Die höheren Energiereserven von *T. macrura* sind auf ihre Reproduktionsweise zurückzuführen. Diese Art laicht im Spätwinter ab, entkoppelt also von Nahrungszufuhr aus dem Phytoplankton. Verglichen mit den beiden hochantarktischen Arten *E. superba* und *T. macrura* haben *E. frigida* und *E. triacantha* nur mäßige Lipidgehalte, was die geringere Saisonalität der Nahrungsverfügbarkeit mit abnehmender geographischer Breite widerspiegelt. Dennoch spielen Lipide auch im Lebenszyklus dieser beiden Arten eine wichtige Rolle, was durch die saisonalen Schwankungen von Lipidgehalt und -zusammensetzung mit höheren Neutrallipidanteilen im Herbst verdeutlicht wird. Die Lipidzusammensetzung von *E. frigida* zeigt nur eine geringe Abhängigkeit von trophischen Markerfettsäuren, während das Fettsäuremuster von *E. triacantha* auf eine omnivore Ernährungsweise mit einem zunehmenden Einfluss von calaniden Copepoden im Herbst schließen lässt.

Die vorliegende Arbeit liefert einen wesentlichen Beitrag zum Verständnis der Lipiddynamik und ihrer ökologischen Bedeutung in antarktischen Euphausiaceen.

Acknowledgements

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1 Introduction

1.1 The Antarctic Ocean

Hydrography

Polar regions are characterised by low temperatures and a strong seasonality of solar irradiation alternating between perpetual darkness in winter and continuous daylight during the summer. In contrast to the Arctic Ocean the Southern Ocean is, strictly speaking, not truly polar: even under the shelf ice it does not extend further than 82° S and the south pole is covered by land. On average the continent extends to approximately 70° S and it is only in the Ross and Weddell Seas, where real high–Antarctic conditions prevail. While the Southern Ocean is bounded by the Antarctic continent southward, there are no fixed borders to the north. Therefore, the Polar Front (PF, frequently also termed Antarctic Convergence) is defined as the northern limit, which is located between 48-60° S and generally close to about 55° S. The PF is part of the Antarctic Circumpolar Current (ACC). The ACC is the largest current system of the world's oceans with a mean volume transport of $135 \times 10^6\,\mathrm{m^3\,s^{-1}}$ and acts as a hydrographical barrier, isolating the Antarctic Ocean from the adjacent parts of the three main oceans. The PF represents a significant faunal boundary in the Southern Ocean at least as far as near–surface organisms are concerned (Deacon 1982, Errhif et al. 1997, Pakhomov et al. 2000). The ACC is wind–driven and therefore synonymously called West Wind Drift.

Opposed to the ACC there is a counter–current flowing westward along the continent: the Antarctic Coastal Current or East Wind Drift. The transition between these two currents induces a divergence with continuous upwelling. Apart from these major current systems there are three subpolar gyres: the Weddell, Ross, and Amery Gyres.

The surface temperatures in the circum–Antarctic water belt generally decrease meridionally from about +5 °C at the PF to −1.8 °C in ice covered waters (Olbers et al. 1993).

Another characteristic feature of the Southern Ocean are the deep shelf regions. Antarctica is covered by a massive ice sheet pressing the continent down into the earth's crust. Therefore with a depth of 400–500 m the shelf is significantly deeper than those of the other continents.

Three main water masses are identifiable between the surface and the sea floor, namely the Antarctic Surface Water (ASW), the Circumpolar Deep Water (CDW) and the Antarctic Bottom Water (ABW) (Knox 1994, Fahrbach 1995). The melting of sea ice in summer and oceanic cooling in winter determine the properties of the

ASW. It typically extends down to 200 m depth, has a temperature ranging down to freezing point and a salinity of less than 34.5. Below the ASW and extending to the bottom is the CDW. Its temperature ranges from between 0 and 0.5 °C at the bottom to 2 °C at 300 to 600 m. CDW is transported around the Antarctic continent by the eastward flowing ACC. The majority of ABW is formed in the Weddell and Ross Seas. ABW is a mixture of water masses from the continental shelves of these two seas and the CDW. Due to the high density of the shelf waters caused by the accumulation of salt during ice formation, ABW flows down the continental slope and into the South Atlantic. There is also some eastward flow into the southern Indian and Pacific Oceans.

Sea ice

Large areas of the Southern Ocean are covered by sea ice in winter. There is a pronounced seasonality in the extent of sea ice cover: while a maximum area of $20 \times 10^6\,km^2$ is ice covered in late winter (September), only $4 \times 10^6\,km^2$ remain ice covered all year–round (Spindler 1990). Thus, only a small fraction of the Antarctic sea ice is multi–year ice.

Sea ice affects the primary productivity of the Southern Ocean in various ways. On the one hand, a closed ice cover drastically reduces the light available for photosynthesis in the under ice water. On the other hand, there is considerable primary production in the sea ice itself. Furthermore, the release of freshwater during ice melt favours stratification of the water column with a shallow stable surface layer and hence the potential development of phytoplankton blooms. This positive effect is enhanced by the simultaneous release of ice algae which may aid the initiation of a bloom ("seeding effect") (e.g. Sakshaug and Holm-Hansen 1984, Lizotte 2001).

Sea ice also represents an important factor in the life cycle of the Antarctic krill. *Euphausia superba* is known to feed on ice algae during austral winter and early spring, when phytoplankton is scarce (Marschall 1988, Stretch et al. 1988, Daly 1990). Moreover, sea ice crevices may provide shelter and rest (pseudo–benthic life style) for *E. superba* (Marschall 1988, Hagen et al. 2001).

Primary production

Due to the continuous upwelling caused by the two opposing current systems, the Southern Ocean is very rich in nutrient salts. The concentrations of nitrogen, phosphate and silicate are the highest found anywhere in surface waters (Sakshaug and Holm-Hansen 1984). And yet, the Antarctic Ocean is characterised by a low overall phytoplankton biomass. This phenomenon is known as the "Antarctic paradoxon". Although there is a decrease in nutrient concentrations during phytoplankton growth in summer, the nutrients are never depleted as they typically are in other regions. The depth of the mixed surface layer, which is often too high to allow the development of phytoplankton blooms, is assumed to be the most important limiting factor of primary production in Antarctic waters (Lochte and Smetacek 1995). However, this question is still not ultimately resolved; possibly also trace elements such as iron limit phytoplankton growth in the Antarctic Ocean (e.g. de Baar et al. 1995).

In the Arctic, spring phytoplankton blooms regularly develop along the ice edge (e.g. Sakshaug and Holm-Hansen 1984). In the Southern Ocean, however, such ice–edge blooms do not always develop. Dense phytoplankton blooms occur annually in protected shallow inshore bays, while low algal concentrations persist particularly in oceanic regions. The importance of water column stability has been emphasised by Lancelot et al. (1993): the development of marginal ice zone blooms is primarily controlled by the depth of the mixed layer, their intensity however is controlled by grazing pressure.

One region where favourable conditions for algal growth regularly develop is the Weddell–Scotia–Confluence (Bianchi et al. 1992): the Weddell Gyre transports sea ice northward, where it meets the warm Scotia water and melts. The continuous supply with freshwater from melting sea ice leads to enhanced water column stability early in spring, long before such conditions exist in other areas. Other regions with high chlorophyll concentrations can be found along the Antarctic Peninsula, in the Ross Sea, as well as in the northern parts of the Ross Gyre (Lochte and Smetacek 1995).

Phytoplankton blooms are usually dominated by large diatoms or *Phaeocystis* colonies, whereas in non–bloom conditions nanoplankton ($< 20\,\mathrm{nm}$) may constitute more than 50% of the biomass. One third of the latter can be heterotrophic flagellates (Sakshaug and Holm-Hansen 1984), which underlines the importance of the microbial loop in the Antarctic food web.

1.2 Antarctic euphausiids

Euphausiids, commonly called krill, play a key role in the Southern Ocean ecosystem. Although copepods can also be quite abundant numerically, krill clearly dominate the Antarctic zooplankton community in terms of biomass. They form an important part of the diet of many animals including fishes, seals, whales, penguins and other sea birds and thus represent a major link between primary producers and higher trophic levels.

The order Euphausiacea comprises 86 species (Baker et al. 1990), six of which live in Antarctic waters: *Euphausia frigida*, *Euphausia triacantha*, and *Thysanoessa vicina* have the northernmost distribution, *Euphausia superba* and *Thysanoessa macrura* occur mainly within the seasonal pack ice zone and *Euphausia crystallorophias* usually inhabits neritic waters with permanent sea ice cover (Mauchline and Fisher 1969, Kirkwood 1982).

Four species were investigated in the present work. Their distribution, biology, and ecology are briefly summarised in the following sections.

Euphausia superba

Euphausia superba is the most abundant of the world's euphausiids. Estimating abundance and biomass of *E. superba* is inherently problematic due to its patchy distribution and the formation of dense swarms. However, the overall summer standing stock is believed to amount to the order of hundreds of millions of tonnes (Miller and Hamp-

ton 1989, Nicol and Endo 1999). Due to its enormous abundance *E. superba* is not only of central importance for the Antarctic ecosystem but also of interest for human exploitation. As a result, *E. superba* is the most extensively studied krill species and its distribution and life–cycle are already well understood, although many questions still remain.

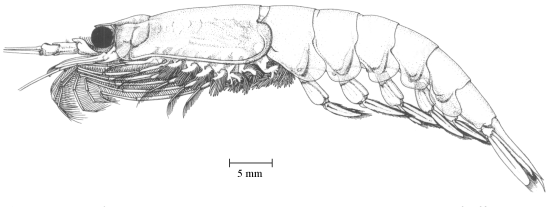

Figure 1.1: *Euphausia superba*

Like all Antarctic species, *E. superba* has a circumpolar distribution, mainly inhabiting the waters between the coast and the seasonal pack ice edge (Mauchline and Fisher 1969, Knox 1994). There are several areas of higher concentrations, often associated with water circulatory patterns favourable for the retention of zooplankton (Miller and Hampton 1989, Knox 1994, Siegel 2000a) and in part also for the development of phytoplankton blooms (Bianchi et al. 1992, Nicol et al. 2000). These areas often coincide with areas of high chlorophyll concentrations (see section Primary Production). They are in the East Wind Drift, the Scotia Sea, the Weddell Drift, around South Georgia and north of the Ross Sea (see Knox 1994, for review). *E. superba* is an epipelagic species; highest abundances are found in the upper 100 m (e.g. Daly and Macaulay 1988, Siegel et al. 1990, Nordhausen 1994b).

An average maximum life span of five to six years is now acknowledged (e.g. Siegel 2000b). Along with its extraordinary age *E. superba* attains also the largest size among the epipelagic euphausiids. With a maximum length of up to 65 mm (Baker et al. 1990, Nicol and Endo 1999) this species is considerably larger than its Antarctic congeners. Females mature in their third summer season, males even one year later (see Siegel 2000b, for review). In *E. superba*, spawning is restricted to the summer season. Unlike the other Antarctic euphausiids, *E. superba* does not rely on internal reserves for fuelling reproduction but is dependent on external resources and makes use of the summer phytoplankton bloom (Quetin et al. 1994, Siegel 2000b, Hagen et al. 2001). Eggs are specifically heavier than sea water and sink to depths of 1000–2000 m depending on temperature and hence embryonic developmental rate (Ross and Quetin 1989, 1991). At depth the eggs hatch and the nauplius larvae commence their reascent to the surface while developing via the metanauplius stage to the calyptopis larvae. This "developmental ascent" was first proposed by Marr (1962) and further studied by Marschall and Hirche (1984) and Hempel and Hempel (1986). Embryogenesis and development of the first three larval stages is fuelled by yolk reserves of the eggs. Calyptopis I, the stage which reaches the euphotic zone, is the first stage capable of feeding (Marschall 1985). Ontogenesis proceeds via two more calyptopes, followed

by six furcilia stages, before *E. superba* moults to the juvenile in its second year of life (Siegel 1987). Recruitment success depends on sea ice extent: good recruitment is positively correlated with early seasonal spawning, which is in turn favoured by extensive sea ice in the preceding winter (Smetacek et al. 1990, Loeb et al. 1997). Larval survival is again higher during cold winters with prolonged sea ice coverage, since sea ice algae may provide an important feeding ground for *E. superba* (Marschall 1988, Stretch et al. 1988), particularly for the furciliae (Daly 1990, 1998, Ross and Quetin 1991, Quetin et al. 1994).

E. superba is still considered to feed primarily on phytoplankton during the productive season (e.g. Hopkins et al. 1993, Mayzaud et al. 1998), although there are many recent studies that illustrate its opportunistic feeding behaviour, making use of any kind of food available (Atkinson and Snÿder 1997, Perissinotto et al. 1997, Ligowski 2000). This flexibility is probably one of the reasons for the success of *E. superba* in the highly seasonal environment of the Antarctic Ocean.

Euphausia frigida

In contrast to the wealth of information available on *Euphausia superba*, there are only few studies dealing with *E. frigida*. This species is mainly distributed to the north of the pack ice edge (Mauchline and Fisher 1969, Knox 1994). Its vertical distribution lies between 250–500 m during the day, however at night *E. frigida* ascends to the upper 50–100 m of the water column (Mauchline 1980). It is the smallest euphausiid south of the Antarctic Convergence with a maximum length of 27 mm.

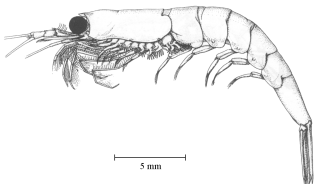

Figure 1.2: *Euphausia frigida*

With a two–year life cycle *E. frigida* has also the shortest life span of the Antarctic species. It reaches maturity in its second year of life (Siegel 1987) and thus experiences only a single breeding season. Although not as well known as in *E. superba*, larval development of *E. frigida* apparently follows similar patterns including a developmental ascent (Makarov 1977, cited in Makarov 1979), as for most of the other oceanic euphausiids (Siegel 2000b). Spawning starts in October and larval development is completed by April/May, before the onset of winter. Only little information exists on the feeding ecology of *E. frigida*. According to Hopkins et al. (1993) it is an omnivorous species, mainly feeding on diatoms but occasionally preying on protozoans and copepods.

Euphausia triacantha

Among the four species considered in this thesis, *E. triacantha* has the northernmost distribution. While *E. frigida* is confined to Antarctic waters, *E. triacantha* can also be found north of the Antarctic Convergence (Mauchline and Fisher 1969, Knox 1994). At night–time, *E. triacantha* is predominantly found between 0 and 250 m depth, migrating down to 500 m during the day (Mauchline 1980).

Siegel (1987) investigated the life history of this species. He reports a three–year life cycle and a maximum length of 42 mm. Consistent with its more northerly distribution, *E. triacantha* is growing continuously throughout the year, in contrast to *E. superba* for which growth is largely confined to the summer season. Starting in September, *E. triacantha* is among the first Antarctic euphausiid species to spawn.

E. triacantha is assumed to be an omnivorous species ingesting algae as well as foraminiferans, molluscs, and crustaceans (it was found to prey also on *E. superba* furciliae) (Pavlov 1976, cited in Mauchline 1980).

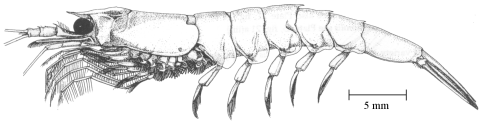

Figure 1.3: *Euphausia triacantha*

Thysanoessa macrura

The distributional range of *T. macrura* is very similar to that of *E. superba*, extending from the Antarctic coast to the pack ice edge. It is more ubiquitous than *E. superba*, albeit not occurring in such large numbers. Its distribution is normally more dispersed (Nordhausen 1994b), but it may also occur in swarms (Daly and Macaulay 1988). *T. macrura* mainly inhabits the upper 200 m of the water column. It has a life–span of four years and attains a maximum length of 36 mm (Siegel 1987). Females mature after 13 months (Nordhausen 1994a). *T. macrura* shows a prolonged spawning period starting as early as the more northerly species *E. triacantha* in late winter/early spring. The recruitment follows the receding ice edge as suggested by Makarov (1979) and supported by observations of Daly and Macaulay (1988). They found gravid and spent females under the ice and consequently also spawning must take place under the pack ice. Ontogenetic vertical migrations have been described also for this species (Makarov 1979), although the eggs do not seem to sink as deep as those of *E. superba* (Siegel 2000b). In consequence of the early spawning as compared to *E. superba*, food competition between the larvae of these two sympatric species is reduced (Makarov 1979). Larval development is completed by March and *T. macrura* spends its first winter already as juveniles (Nordhausen 1994b).

The anatomy of the thoracopods with the conspicuously elongated second pair is distinct from the feeding basket of the genus *Euphausia* and suggests a different feeding behaviour of the *Thysanoessa* species. Accordingly, metazoans, particularly

crustaceans, were reported to make up an essential part of the diet of *T. macrura*, although phytoplankton has also been found in their intestines (Hopkins 1985, 1987, Hopkins and Torres 1989). This species has therefore been classified as omnivorous with a strong carnivorous tendency (see also Mayzaud et al. 1985, Rau et al. 1991).

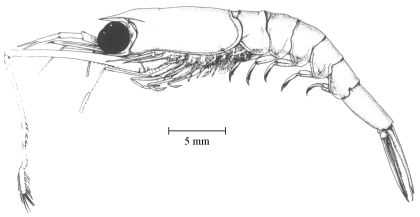

Figure 1.4: *Thysanoessa macrura*

1.3 Lipids

Lipids serve a variety of vital functions in organisms. Due to their diversity and physico–chemical properties they are important components in biomembranes. Lipids are very efficient energy stores, they release twice the energy content as compared to proteins and carbohydrates (Stryer 1999). Moreover, due to their hydrophobic nature neutral lipids can be stored free of water. Efficient lipid storage is particularly important for organisms living at high latitudes, where food availability is controlled by a strong seasonality in environmental parameters. Due to their low density, lipids may also provide buoyancy for pelagic organisms (see Falk-Petersen et al. 2000). Ontogenetic and seasonal trends of lipid content and composition can elucidate life cycle characteristics, e.g. whether organisms overwinter actively or in diapause, or the timing of reproduction (e.g. Clarke 1980, Sargent et al. 1981).

1.3.1 Lipid classes

Lipids comprise a variety of different classes from relatively simple molecules such as hydrocarbons and fatty acids over compound lipids like wax esters and the derivatives of glycerol, triacylglycerols and phosphoglycerides, to complex structures such as sphingolipids, glycolipids, and sterols.

Fatty acids are the essential building blocks of many of the complex lipids (see below). Most fatty acids in plants and animals contain between 12 and 32 carbon atoms per molecule (e.g. Sargent and Whittle 1981). They are either saturated (containing no double bonds) or unsaturated (containing one to six double bonds). Polyunsaturated fatty acids are generally thought to be only synthesised *de novo* by photosynthetic organisms (Sargent and Whittle 1981) and are therefore considered essential to heterotrophic organisms. Only a small portion of an organism's fatty acids occurs in the free form; the majority is bound to various other molecules. Elevated levels of

free fatty acids suggest enhanced lipid metabolic activity (anabolism or catabolism), or may also indicate poor sample treatment enabling autolytic processes.

Wax esters (WE) are simple esters of a fatty acid and a fatty alcohol. The reduction of fatty acids to their corresponding fatty alcohol is a fast mechanism to reduce the amount of free fatty acids and avoid the negative feed–back reaction on fatty acid biosynthesis. Wax ester storage is therefore a sophisticated way of efficiently exploiting pulses of intense food availability (e.g. Sargent 1978). Accordingly, many herbivorous polar copepod and euphausiid species contain high amounts of WE (up to 45% of their dry mass, e.g. Hagen 1988). It has been proposed that WE are more slowly catabolised than triacylglycerols (Lee et al. 1971, 1974, Sargent et al. 1977, Sargent 1978). WE are therefore considered as long–term energy reserves primarily utilised by species that spend the winter months with phytoplankton paucity in a dormant state and cease feeding (Conover 1988, Båmstedt et al. 1990).

Triacylglycerols (TAG) consist of three fatty acids esterified to a glycerol molecule. They are the major energy reserve of all terrestrial animals and play an important role also in many marine organisms. Digestion of TAG occurs via hydrolysis into diacylglycerols (DAG), monoacylglycerols, glycerol, and fatty acids which are absorbed through the intestinal wall (Chang and O'Connor 1983). These products are then either incorporated in the organisms' own lipids or metabolised for energy. TAG is generally accumulated during feeding and depleted during starvation. It is therefore a useful indicator of an organism's nutritional and physiological condition (Fraser 1989).

The biosynthesis of phospholipids (PL) follows the same pathway as that of TAG until the intermediate product DAG. In place of the third fatty acid in TAG, PL contain a phosphate group, which in turn is bound to other molecules (e.g. choline or ethanolamine). This group is hydrophilic and hence PL are amphipathic in nature, having the tendency for self assembly in bilayers in aqueous media. The hydrophobic fatty acid chains are oriented to the inside of the bilayers and free ends spontaneously fuse, leading to the rapid formation of vesicles or cell compartments (Stryer 1999). Due to these properties PL are the essential building blocks of biomembranes. There are different PL classes, each fulfilling specific functions in biomembranes. For example, membrane fluidity can be regulated via differential incorporation of phosphatidylcholine or phosphatidylethanolamine (Shinitzky 1984).

Being an important component of membrane lipids, cholesterol is the most common sterol in marine zooplankton (e.g. Phleger et al. 1998, Mühlebach et al. 1999, Nelson et al. 2001). It is abundant in plasma membranes of eucaryotic cells, whereas in the membranes of the cell organelles it is only of minor importance (Stryer 1999). The fluidity of plasma membranes can be regulated via the incorporation of cholesterol (Shinitzky 1984). Plants contain a wide range of sterols typically with a characteristic taxonomic composition, which makes them suitable as trophic biomarkers (e.g. Sargent and Whittle 1981, Skerratt et al. 1997, Mühlebach et al. 1999, Hamm et al. 2001).

1.3.2 Trophic marker fatty acids

Various fatty acids that are characteristic for specific taxonomic groups and that are transferred unaltered through the food chain can be classified as biomarkers. The trophic biomarker concept has been established upon observations that such fatty acids are incorporated largely unmodified in the consumers' lipids, which thus retain a signature of their dietary origin (Lee et al. 1971, Sargent and Whittle 1981, Sargent et al. 1987, Graeve et al. 1994b, Dalsgaard et al. 2003).

Diatoms are particularly rich in 16:1(n-7) and 20:5(n-3) (e.g. Nichols et al. 1993, Dunstan et al. 1994, Skerratt et al. 1995, Mock and Kroon 2002) and these fatty acids are considered as trophic markers for this algal group (Sargent et al. 1987, Graeve et al. 1994a,b). 18:1(n-7) can result from chain elongation of the diatom marker 16:1(n-7) and they are therefore often used in combination as diatom markers. In addition, diatoms are also characterised by elevated amounts of C16 polyunsaturated fatty acids (PUFAs) (e.g. Sargent et al. 1987).

The lipids of dinoflagellates usually contain high amounts of 18:4(n-3) and 22:6(n-3) (Sargent et al. 1987, Graeve et al. 1994b). Prymnesiophytes, of which *Phaeocystis* spp. can occur in high abundances in the Antarctic Ocean, and cryptomonads can be rich in these fatty acids as well (Sargent et al. 1987, Volkman et al. 1989, Virtue et al. 1993a). 18:5(n-3) has also been described as a potential marker fatty acid for dinoflagellates (Mayzaud et al. 1976) or *Phaeocystis* (Virtue et al. 1993a).

The C20 and C22 monoenes are typical components both as fatty acids and fatty alcohols of the wax esters and triacylglycerols of herbivorous calanid copepods (Kattner et al. 1994, Kattner and Hagen 1995, Albers et al. 1996). These trophic markers can be found predominantly in the neutral lipids of zooplanktivorous species such as *Meganyctiphanes norvegica* (e.g. Ackman et al. 1970, Virtue et al. 2000) or North Atlantic herring and North Sea sprat (see Sargent and Henderson 1995, for review).

18:1(n-9) is a common fatty acid in metazoans (e.g. Sargent and Falk-Petersen 1981, Falk-Petersen et al. 1990) and assumed to become particularly enriched in secondary consumers. The ratio 18:1(n-9)/18:1(n-7) is thus frequently used to estimate the degree of herbivory versus carnivory (Graeve et al. 1997, Cripps et al. 1999, Mayzaud et al. 1999, Falk-Petersen et al. 2000, Nelson et al. 2001). Another index, the ratio of polyunsaturated to saturated fatty acids has been proposed to be a measure of carnivory in *Euphausia superba* (Cripps and Atkinson 2000).

The fatty acid biomarker approach can provide information, where the classical gut content analysis fails (e.g. soft–bodied organisms, advanced digestion). Instead of a snap–shot impression, biomarkers integrate the trophic information over a longer time scale of several weeks. However, lipid signatures usually do not have the precision to identify species–specific interactions. Thus, they provide trophic information rather on the level of larger taxonomic groups.

1.4 Objectives

The present study was conducted to gain insight into several aspects of krill life history using lipid analytics as the main investigative tool. It was part of a joint project on the seasonal feeding strategies of *Euphausia superba*. One major objective of this project was to characterise the physiological condition and feeding behaviour of the Antarctic krill prior to the critical overwintering period.

Overwintering success is a key factor determining population size. As a predominantly herbivorous species with a high energy throughput (Kils 1981, Quetin et al. 1994), *E. superba* has to cope with an extended period of phytoplankton scarcity during the dark winter months. Two categories of overwintering mechanisms were suggested: non–feeding strategies and switching to alternative food sources. Possible non–feeding strategies include reduction of the metabolic rate (Kawaguchi et al. 1986, Quetin and Ross 1991, Torres et al. 1994a), utilisation of lipid reserves (Torres et al. 1994b, Hagen et al. 1996, 2001) and body protein resulting in shrinkage (Ikeda and Dixon 1982, Quetin and Ross 1991, Nicol et al. 1992). Feeding strategies on the other hand comprise switching to sea ice biota (Marschall 1988, Stretch et al. 1988, Daly 1990), zooplankton (Price et al. 1988, Huntley et al. 1994, Atkinson and Snÿder 1997, Perissinotto et al. 1997), or sea floor detritus (Kawaguchi et al. 1986, Ligowski 2000). The simultaneous examination of elemental and biochemical composition, feeding behaviour, respiration and excretion rates, both in the field and in controlled feeding and starvation experiments, aimed at assessing the relative importance of the various behavioural adaptations for the energy budget of overwintering *E. superba*. Different developmental stages were studied to reveal potential changes during ontogeny.

In contrast to *E. superba*, the biology and ecology of the two more northerly distributed *Euphausia* species, *E. frigida* and *E. triacantha*, is still poorly known. Only few studies dealt rather circumstantially with their lipid biochemistry (Clarke 1984b, Phleger et al. 1998, 2002). Total lipids, lipid class and fatty acid compositions were examined in summer and autumn in order to evaluate, whether seasonal lipid dynamics reveal specific life cycle strategies.

Trophic biomarkers are frequently used to characterise feeding behaviour integrated over different time scales. However, interpretations can be misleading and inference from marker fatty acids or stable isotope signatures should ideally be accompanied by experimental calibration. Therefore, another major objective of this study was to test the applicability of these two trophic biomarker approaches for *E. superba* in comparison to other Antarctic euphausiid and mesozooplankton species. The following questions were addressed by means of combined field and experimental data: Are there species– and/or stage–specific differences in the degree, to which dietary components become incorporated? Which factors control the assimilation of specific biomarkers and hence potentially favour or limit the use of these biochemical approaches? Which fatty acids are best suited as dietary markers, what digestive modifications may occur, and how useful are fatty acid ratios to identify feeding preferences?

Unique to euphausiids is the interesting phenomenon of phosphatidylcholine (PC) accumulation with increasing lipid content (e.g. Hagen et al. 1996, Mayzaud 1997). From its similarity to wax ester or triacylglycerol accumulation in many polar species, the hypothesis was inferred that PC, in addition to its important role in biomembranes, also serves as supplementary depot lipid. However, this conclusion has been questioned (Sargent and Henderson 1995) and the present study has attempted to clarify some points essential to understanding the ecological significance of these PC reservoirs. Major sites of PC accumulation were identified, since the proximity to specific organs may provide information on the function of PC. Further questions included the nature of the accumulated PC, i.e. whether it is entirely membrane–bound, or whether it also occurs as free lipid aggregations (e.g. vesicles) similar to neutral lipids. Is PC storage a phenomenon common to all Antarctic euphausiids or are there exceptions? And finally, what are the specific advantages over pure neutral lipid stores?

2 Material and Methods

In the following, the detailed description of methodologies will be restricted to those techniques that were applied by myself. A variety of additional approaches was used by my colleagues to obtain the results presented in Chapters 3, 4, and 6. They are amply explained in the Methods–paragraphs of the respective chapters.

2.1 Sampling

Euphausiids were sampled during two autumn cruises with RV *Polarstern*. Between March 26 and May 4 1999 the investigation areas covered the Polar Frontal Zone (∼49° S, 20° E), the Weddel Gyre (∼60° S, 10° E) and the shelf break in the south-west Lazarev Sea (∼69° S, 5° W). In 2001, euphausiids were collected west of the Antarctic Peninsula and in the Bellingshausen Sea (18 April – 1 May 2001). Summer samples were obtained during a cruise with RV *Yuzhmorgeologiya* off the South Shetland Islands from 18 February – 12 March 2000 (see Fig. 1 in Chapter 2 for a map of the investigation areas and depiction of the sampling stations). Detailed information about the programmes and the station data has been published by Bathmann et al. (2000), Lipsky (2001), and Bathmann (2002). In 1999, slow vertical bongo net tows (335 μm mesh, 5 litre closed cod end, Fig. 2.1a) were made in the top 150 m at night. In 2000, double oblique tows with an Isaac Kidd midwater trawl (IKMT, mesh size 505 μm, Fig. 2.1b) were performed in the top 170 m. In 2001, zooplankton was sampled by double oblique rectangular midwater trawl (RMT) 1+8 hauls (mesh size 325 and 4500 μm, respectively, 20 litre closed cod end, Fig. 2.1c), as well as vertical bongo net tows, and *E. superba* larvae were also collected by a hand–hauled Apstein net (mesh size 50 μm).

Zooplankton was thus obtained in excellent condition and immediately transferred to the cool lab, where it was sorted. After a ±24 h defecation period in 1 μm filtered seawater *E. superba* specimens were either frozen at −80° C or transferred to the experimental containers. Prior to freezing, developmental/maturity stage (after Kirkwood 1982), sex, and length (tip of rostrum to end of telson) were recorded and the animals were briefly rinsed with deionised water and blotted dry. Samples of juveniles and adults represent individual specimens, whereas 5–95 furciliae were pooled for each sample, depending on size and availability.

In 1999, both juvenile and adult *E. superba* were caught, as well as larvae (predominantly Furcilia III). Sampled *E. frigida* and *E. triacantha* consisted mainly of adults, while of *T. macrura* predominantly furcilia stages were caught. During the summer cruise in 2000, IKMT catches yielded almost exclusively mature male and

(a) Bongo net (b) IKMT (c) RMT 1+8

Figure 2.1: Sampling gear

female *E. superba*, very few calyptopis larvae, as well as some adult *E. triacantha* and *T. macrura*. In 2001, *E. superba* furciliae were present in all stages (FI–FVI) and clearly dominated the catches. Only very few postlarval *E. superba* could be collected. *E. triacantha* were locally quite abundant (Bathmann 2002) and mainly postlarvae, as well as adult *T. macrura* were used for lipid analysis.

2.2 Feeding experiments

The design of the feeding experiments had to be a compromise between the quantitative determination of feeding and the logistical feasibility, which depended on the availability of animals as well as of the large–volume containers. Therefore, emphasis was lain on the induceability of changes in lipid composition, and food–uptake was monitored rather qualitatively to verify feeding activity of *Euphausia superba*.

Adult and juvenile *E. superba*, caught during the 1999 expedition, were maintained in aerated 170 litre tanks and furcilia larvae in 18 litre containers. The experiments were carried out in a cold room ($0 - 2^{\circ}$ C) in dim light. Three batches of about 50 mixed juvenile and adult krill were incubated with freshly caught copepods or sea ice biota or were starved in 1 μm filtered seawater (Fig. 2.2a). The treatments for furciliae (about 200 per batch) were starvation or a diet of ice biota and mixed phytoplankton, respectively (Fig. 2.2b). In 2001, a monoalgal culture of the common Antarctic ice diatom *Fragilariopsis cylindrus* was fed to furcilia IV–V larvae. Ice biota were obtained by slowly thawing brown pieces of sea ice in at least the threefold volume of filtered seawater (according to Garrison and Buck 1986) and subsequent screening through a 55 μm sieve.

Copepods were picked out daily from bongo net tows excluding damaged animals and large carnivorous species such as *Pareuchaeta* spp.. Thus, with the northward

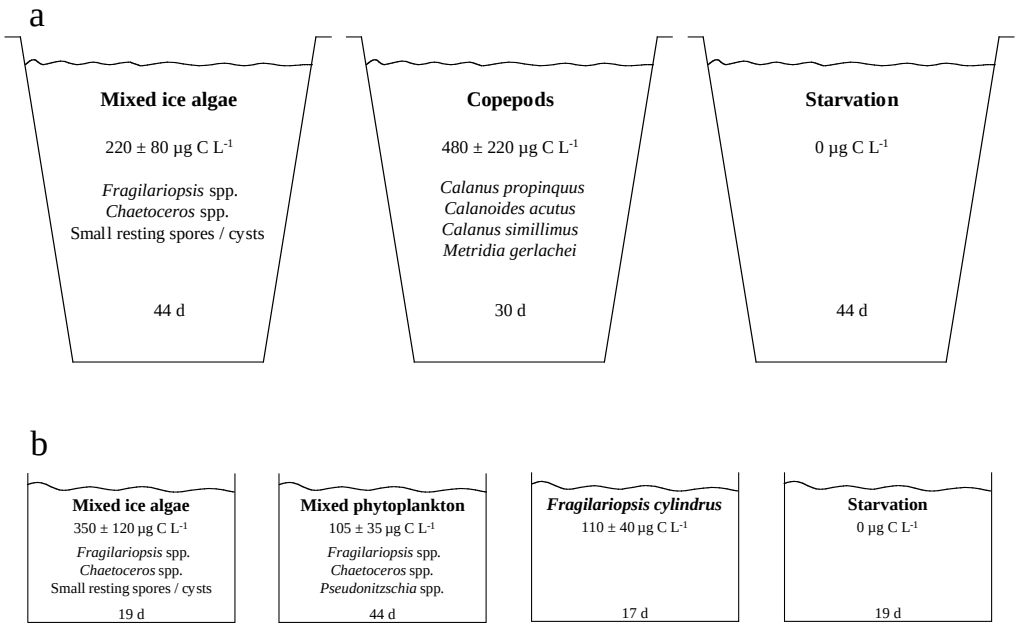

Figure 2.2: *Euphausia superba*: Experimental set–up with food types, food concentrations, dominant species, and duration (days) for (a) juveniles and adults and (b) furcilia III–V larvae

moving vessel the copepod diet consisted of varying species compositions dominated at first by the more southern species *Calanus propinquus* and *Calanoides acutus* (mainly CIV and CV) and later, while operating in the Polar Frontal Zone, by *Calanus simillimus* and *Metridia gerlachei* (CIII–CV).

Control experiments without krill were conducted with the phytoplankton and ice algae mixtures as well as with the *F. cylindrus* culture. At the end of each feeding period (after 48 h) the incubation water was thoroughly mixed to stir up potentially settled cells and subsamples of the control and the experiment were taken. One litre was filtered on 25 mm GF/F filters (Whatman), which were sonicated on ice for 30 s in 10 ml of 90% aqueous acetone and centrifuged at 700 g for 3 min. The supernatant was used for the determination of chlorophyll *a* (Chl *a*) with a Turner 700D fluorometer. Carbon content was calculated from chlorophyll *a* values assuming a C:Chl *a* ratio of 50. This ratio is in the middle of the range determined for similar phytoplankton and ice algae assemblages (Chapter 3). Additionally, another subsample of 250 ml was preserved in 1% Lugol's solution for cell counting. Two replicates of 25 ml and 50 ml for the ice algae and the phytoplankton treatment, respectively, were settled and analysed by inverted microscopy (after Utermöhl 1958). Comparison of the Chl *a* concentrations and the cell numbers in the control and experimental containers provided the amount of food ingested. Additionally, subsamples of the three algal cultures were taken every two days, centrifuged at 3,600 g for 10 min and the pelleted cells were frozen in dichloromethane/methanol under nitrogen atmosphere at −80° C.

The copepods were enumerated and a subsample was frozen for identification before they were fed to *E. superba*. In regular intervals another subsample was taken for lipid analysis. At the end of each feeding period, the remaining copepods were frozen for later enumeration and identification.

Every 48 h, *E. superba* specimens were transferred to a new batch of food or filtered seawater. Animals in poor condition as well as faecal strings were removed and frozen in dichloromethane/methanol under nitrogen atmosphere at $-80°$ C. Maximum duration of the experiments was 44 days depending on the availability of food (the copepod experiment was terminated after 30 days), or on the condition of the experimental krill (the furcilia–ice algae and –starvation experiments were terminated after 19 days, as well as the female–starvation experiment). Subsamples of three juveniles and adults, respectively were taken after 19 and 30 days, and of $3 \times 8 - 9$ furciliae after 19 days (phytoplankton treatment). Only healthy animals were considered in the results presented here.

Krill from these experiments were used for lipid analyses (Chapter 1) or for stable isotope measurements (Chapter 6).

2.3 Lipid analyses

After lyophilisation for 48 h, the samples were weighed and total lipid was extracted with dichloromethane/methanol (DCM/MeOH) (2:1 by volume + 0.01% butylhydroxytoluene (BHT) as antioxidant) and determined gravimetrically (Hagen 2000). In order to obtain a correction value for the BHT, ten blank aliquots of extraction solvent were treated in the same way as the samples and the mean blank mass (=0.33 mg) was subtracted from the total lipid mass of each sample. Due to the interference of BHT with sterol esters (see below), it was not added as antioxidant to the 2001 samples. Since those samples did not show any traits of oxidative degradation (e.g. elevated levels of free fatty acids, break–down of long–chain polyunsaturated fatty acids), the omittance of BHT did apparently not have any harmful consequence on the lipid composition.

Neutral lipid classes were analysed in duplicate by thin–layer chromatography (TLC)–flame ionisation detection (FID) on an Iatroscan Mk V according to Fraser et al. (1985). Since different lipid classes give different FID–responses (Volkman et al. 1986, Parrish 1987), two mixtures of commercial standards (Sigma), which approximated the lipid class compositions of the analysed samples, were prepared for calibration: Phosphatidylcholine:triolein:cholesterol:cholesteryl oleate:oleic acid = 49:35:8:5:3 (V:V) for juveniles and adults and = 64:25:6:4:1 (V:V) for furciliae. As BHT co–runs with sterol esters, a dilution series of the BHT blanks was analysed by Iatroscan and the sterol ester amounts of the samples concerned were corrected by the appropriate values.

As the separation of polar lipids remained unsatisfactory by TLC–FID, the phospholipid composition was determined by high performance (HP) TLC–scanning densitometry (modified after Olsen and Henderson 1989). Five μl of the total lipid

extracts were applied by means of a CAMAG Linomat IV in duplicate or triplicate on pre–developed HPTLC–plates (silica gel 60, Merck). The plates were developed in a horizontal chamber with isopropanol:methylacetate:chloroform:methanol:0.25% KCl (25:25:25:10:9, V:V) for 17 min and dried for 30 min in an evacuated desiccator. The plates were then immersed for 5 s in a post–chromatographic derivatisation reagent with a CAMAG Chromatogram Immersion Device III and charred at 200° C for 20 min. The derivatisation reagent was prepared by dissolving 1.2 mg manganese(II)–chloride in 180 ml of deionised water and adding 180 ml methanol and 12 ml concentrated sulphuric acid. The lipid bands were quantified with a CAMAG TLC–Scanner 3 at 550 nm wavelength and calibrated using commercial standards for each detected lipid class.

For the fatty acid analysis of single lipid classes, aliquots of the total lipid extracts were spotted on self–coated glass plates (silica gel Merck H60, film thickness 750 μm) by a CAMAG Linomat IV. In order to keep the run time as short as possible, neutral and polar lipids were developed separately. The developing solvent was evaporated with nitrogen and the lipid bands visualised by iodine vapour. The bands were scraped off with a teflon spatula and extracted according to the total lipid extraction. The purity of the isolated lipid classes was verified by TLC. Lipids were hydrolysed and the fatty acids converted to their methyl ester derivatives (FAME) in methanol containing 3% concentrated sulphuric acid at 80° C for 4 h (Kattner and Fricke 1986). After cooling, 4 ml of deionised water were added and FAMEs and free fatty alcohols were extracted with hexane (3 × 1.7 ml), analysed in a gas chromatograph (HP 6890A) equipped with a DB–FFAP column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness) using temperature programming and helium as carrier gas. FAMEs and fatty alcohols were detected by flame ionisation and identified by comparing retention time data with those obtained from standard mixtures.

2.4 Sub–cellular fractionation

Deep–frozen euphausiid specimens were homogenised in approx. the three–fold volume of ice–cold buffer (50 mM Tris-HCl, 5 mM EDTA, 250 mM D(+)-sucrose, pH 7.4) by three brief strokes of an ultra–turrax. The samples were then subjected to a discontinuous density gradient centrifugation: 1.1 M, 0.9 M, and 0.7 M sucrose (3 ml each), prepared in Tris–buffer, were layered in a 12 ml centrifuge tube (Nalgene PA). The top layer consisted of 3 ml sample homogenate. The gradients were centrifuged at 100,000 g (35,000 rpm) and 4° C for 16 h (Optima LE-80 K Ultracentrifuge, Beckman Coulter) using a 6 × 12 ml swing–out rotor (SW 40, Beckman). The separated materials were collected at each interface. The first fraction was transferred to a 10 ml centrifuge tube (Beckman) and diluted 1:1 (by volume) with ice–cold sucrose–free buffer containing 150 mM NaCl and 10 mM Tris-HCl (pH 7.4). This suspension was centrifuged at 100,000 g (45,000 rpm) and 4° C for 2 h (Optima MAX-E Ultracentrifuge, Beckman) using an angle rotor (MLA-80, Beckman). The resulting supernatant was

separated from the pellet. Thus, five fractions were obtained in total (see Fig. 2.3 for the fractionation protocol).

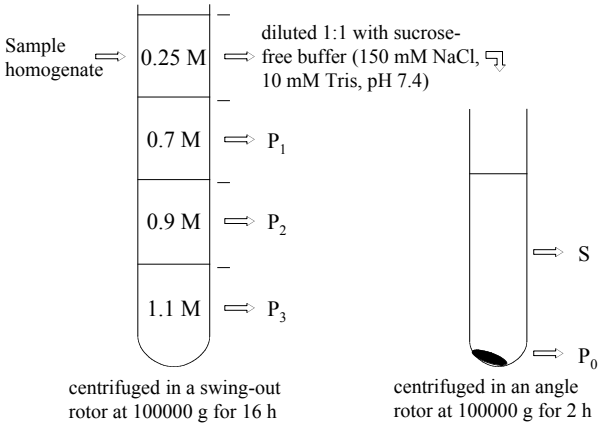

Figure 2.3: Protocol for the sub–cellular fractionation

For *Euphausia superba* an aliquot of the homogenate (ca. 3 ml) was used for analysis and the lipid yield of the different fractions was multiplied by the appropriate factor to account for the rest of the homogenate. For the other two species, the entire homogenates (ca. 3 ml for *Euphausia triacantha* and 2.5 ml for *Thysanoessa macrura*) were used for analysis.

2.5 Histochemistry

To prepare sections of a tissue, it is necessary to harden the specimen and to halt enzymatic activity including autolysis. In conventional histology, this is achieved by embedding the sample in paraffin wax or some similar matrix. This involves the use of hydrophobic solvents, leading to the loss of lipids. The alternative way of hardening tissue, so it can be sectioned even without embedding, is by cooling it to a suitably low temperature. This is a critical step, since slow freezing leads to the formation of ice crystals and disruption of the cells. Chayen and Bitensky (1991) recommend a procedure that ensures rapid freezing via supercooling of the protoplasm. Hexane is chilled in a bath of alcohol saturated with solid carbon dioxide to a maximum temperature of -65° C and the sample is dropped into it. It is then transferred to a pre–cooled vial and stored at -70° C or below.

This sophisticated method was logistically not applicable on board the research vessels. Instead, the samples were either directly frozen at -80° C (on RV *Yuzhmorgeologiya*) or first immersed in liquid nitrogen before being stored at -80° C (on RV *Polarstern*). Therefore, some degree of tissue damage cannot be completely ruled out.

During freeze–sectioning, care must be taken to avoid thawing of the samples. Cryosections were performed with a Leica CM3050 S cryomicrotome. The cutting–

chamber containing all instruments used was cooled to $-25°$ C. The cephalothorax was dissected from the deep–frozen specimens, trimmed to remove the cuticle, and frozen to the cutting–block. The knife was orientated towards the sample in an angle of $20 - 25°$. The sample blocks were trimmed for a few mm and then a series of cross–sections of $8 - 9\,\mu$m thickness was made. When holding a warm slide (room temperature) close to the section, it jumps the gap and attaches to the slide, leaving an imprint of melted water on the knife. The section is thus freeze–dried and safe against enzymatic degradation processes. The slides were immersed in coplin jars containing the different dyes. Neutral lipids were stained with "oil red O" and mounted in glycine, phospholipids were stained with acid haematein and mounted in Euparal (according to Chayen and Bitensky 1991).

2.6 Statistics

Analysis of variance (ANOVA) was performed to detect significant differences between the means of the different experimental treatments. Using the SPSS software package for Macintosh, one–way ANOVA was calculated and the Games–Howell post–hoc test was applied for multiple comparisons. Multivariate statistical analyses were applied to the percentage fatty acid composition of the total lipids of *Euphausia superba*. They were carried out with the software "Primer" (Clarke and Warwick 1994). The Bray–Curtis–Index was used to calculate a similarity matrix, which was the basis for the cluster analysis (hierarchical agglomerative, group–average linkage).

3 Synoptic discussion and conclusions

The present thesis has examined energetic and nutritional condition of various species and ontogenetic stages of Antarctic krill. Emphasis was placed on *Euphausia superba* (target species of the joint project). Additionally, comparative studies were carried out on the lipid dynamics of three other Antarctic euphausiids, *E. frigida*, *E. triacantha*, and *Thysanoessa macrura*. This discussion is organised in two sub–chapters. First, energetic aspects of krill life history and physiological condition of various ontogenetic stages of *E. superba* at the onset of winter are discussed. Second, comprehensive information on the feeding behaviour of krill is summarised and the applicability of two trophic biomarker approaches is critically evaluated.

3.1 Energetics and physiological condition

3.1.1 ***Euphausia superba*** (Chapters 1, 3, 4, and 5)

Chapters 3 and 4 focus on the overwintering strategies of larval and postlarval *Euphausia superba*, respectively. In these studies, a wide range of techniques was applied to evaluate the relative importance of the various behavioural adaptations of the Antarctic krill to winter conditions.

In the Lazarev Sea in autumn, total lipid contents were on average 35–40% of dry mass (DM) in adults and between 34 and 37% DM in juveniles. West of the Antarctic Peninsula, even higher levels (45% DM) were reached in adult *E. superba*. These values are at the top end of the seasonal range reported for the various stages (Hagen et al. 1996, 2001). It has often been disputed that lipids play a pivotal role in the life cycle of *E. superba* and serve as an important energy source during the winter (Ikeda and Dixon 1982, Clarke 1984a, Ikeda and Bruce 1986, Virtue et al. 1993b, 1997). However, these conclusions are largely based on summer data or starvation experiments. Our results reconfirm the important role of lipids for overwintering of *E. superba* as emphasised by Hagen et al. (2001).

Year–round feeding has been deduced from the absence of wax esters (Clarke 1980, Sargent et al. 1981). However, this might as well reflect the different reproductive strategy as compared to the other Antarctic species, which do accumulate wax esters (see also Hagen and Kattner 1998, Kattner and Hagen 1998, Hagen et al. 2001). The timing of reproduction in *E. superba* is unique. In contrast to the other euphausiids, *E. superba* reproduces comparatively late in the season (Siegel 1987), and gonad maturation and egg production are fuelled by early spring primary production and summer phytoplankton (Cuzin-Roudy and Labat 1992). Hence, *E. superba* utilises its energy reserves only for overwintering and not for reproduction.

Lipid content of FIII larvae averaged 14–16% DM in autumn 1999, comparable to data by Hagen et al. (2001). High ratios of oxygen to nitrogen in the larvae imply rapid lipid turnover, indicative of feeding on lipid–rich organisms (senescent diatoms have elevated lipid contents (e.g. Mock and Kroon 2002)). The high carbon–loss and low nitrogen–loss of freshly caught furciliae indicates that the lipids of ingested food are metabolised for energy production, whereas the protein is incorporated into body tissue. This also explains the comparatively low amounts of 16:1(n-7) in the furciliae, which were primarily feeding on diatoms. Furthermore, it is consistent also with the results from furciliae growth (as inferred from dry mass) and lipid accumulation, which revealed preferential investment of dietary energy in somatic growth and intensified lipid build–up only after a certain body mass (approx. 1.2 mg) has been attained (Fig. 2 in Chapter 1 and unpubl. data). This is typical of the early developmental stages of pelagic crustaceans (Falk-Petersen 1981, Kattner and Krause 1987, Hagen 1988).

Lipid reserves of furciliae are considerably lower than those of postlarval individuals (Chapters 1 and 3). At the same time, mass specific metabolic rates of the larvae are significantly higher (Chapters 3 and 4). Based on metabolic carbon losses, a maximum survival time of 24 days without food uptake was calculated (Chapter 3), which basically agrees with estimations by Quetin et al. (1994) and Hagen et al. (2001). Winter feeding is therefore essential for the early life stages, but its importance decreases with ontogeny (Daly 1990, Hofmann and Lascara 2000).

In 2001, a high spatial variability of total lipid contents was observed in furciliae (between 12 and 23% DM for furcilia III-IV, Chapter 1) as well as in adults (21±8% DM versus 45±3% DM at two different stations in the Bellingshausen Sea (unpubl. data)). This illustrates the pronounced individual variability in energetic condition with which the animals face the critical winter period. Favourable feeding conditions in autumn are essential for both ontogenetic stages to enhance winter survival. They enable the furciliae to deposit some energy for overwintering after most of the ingested food has been invested in somatic growth and development (see above). Adults have to replenish their reserves after the energy demanding reproductive activity during the summer (Nicol et al. 1995, Virtue et al. 1996).

Torres et al. (1994b) classified overwintering strategies of Antarctic zooplankton and micronekton into three categories: “business as usual” involves continued feeding and growth, whereas at the other extreme was dormancy with cessation of feeding and energy conservation. A third “compromise” strategy involves a mixture of reduced, opportunistic feeding and reduced metabolism. The present study confirmed, that furciliae perform “business as usual” during the winter (as inferred from April data, when phytoplankton concentrations were very low, i.e. comparable to winter levels), staying metabolically active and exploiting mainly ice algae as alternative food source (Daly 1990, Quetin et al. 1994, Frazer 1996). It also clearly elaborated the ontogenetic shift in behavioural adaptations to winter conditions. Corroborating previous categorisations (Torres et al. 1994b, Hagen et al. 2001), adult and juvenile *E. superba* were ascribed to follow a “compromise” strategy. The potential problems inherent

in such a strategy, concerning the relative gains and losses of a moderately reduced activity, are highlighted in Chapter 4.

Due to the linear accumulation of phospholipids (PL), namely phosphatidylcholine (PC) with increasing lipid content, it has been hypothesised that PL/PC serve as additional energy depot in Antarctic euphausiids (Saether et al. 1986, Hagen 1988, Hagen et al. 1996, Mayzaud 1997). This conclusion has been questioned by Sargent and Henderson (1995). They considered the voluminous, PL–rich hepatopancreas responsible for the high PL levels in *E. superba*. The present study further consolidated the original hypothesis. It was shown that vast parts of the cephalothorax of lipid–rich adult *E. superba* likewise contain neutral lipids and PL, demonstrating that the hepatopancreas is not the largest source of PL in the lipid–rich anterior body part of *E. superba*. Further evidence was provided by the large portions of non–membrane bound PC (Chapter 5), implying that these do not serve structural functions but are available as depot. The specific advantages of this phenomenon are still a matter of speculation. Saether et al. (1986) proposed that PL might be easier mobilised. This suggestion was further specified by Olsen et al. (1991). According to these authors, the smaller PL molecules are more easily transferred across the plasma membranes than the larger triacylglycerols, which have to be hydrolysed first. The phenomenon of PC storage is plausibly linked to the fascinating life history of krill. Assuming that shrinkage is a common mechanism also in the field (e.g. Nicol et al. 1992), PL requirements will be elevated during regrowth. During times of food plenty, these costly compounds can be synthesised and deposited and upon demand, they are quickly available as essential structural components. However, further comparative studies including species from Arctic and subpolar regions combined with experimental evidence are required to consolidate such conclusions and to improve our understanding of the ecophysiological role of PC storage in the complex patterns of energetic and life history adaptations in polar euphausiids.

Conclusions

- Substantial lipid reserves are accumulated before the critical overwintering period in juvenile and adult *E. superba*.
- Furciliae have only low to medium lipid stores associated with high (lipid) metabolic activity.

⇒ Juvenile and adult *E. superba* follow a “compromise” overwintering strategy versus “business as usual” of furciliae.

- Non–membrane bound phosphatidylcholine is accumulated as energy reserve additional to triacylglycerols

3.1.2 Other species (Chapters 2 and 5)

Maximum autumn lipid levels of the other euphausiid species investigated can be inferred from Figure 2 in Chapter 2. *Thysanoessa macrura* has the highest lipid contents with up to 57% DM, followed by *Euphausia triacantha* with up to 50% DM and *Euphausia frigida* with maximum lipid levels of 32% DM. *T. macrura* has recently received growing attention and its ontogenetic and seasonal lipid dynamics are already well documented (Kattner et al. 1996, Hagen and Kattner 1998, Falk-Petersen et al. 1999, 2000). Along with its high lipid content, *T. macrura* accumulates large amounts of storage lipids, mainly as wax esters (WE) but also as phosphatidylcholine (PC) (Chapter 5, Hagen et al. 1996). However, these high lipid reservoirs present in autumn are apparently only contingently utilised for energy production during the winter as inferred from a moderate decline of total lipids during the dark months (Torres et al. 1994b). Energy saving mechanisms, i.e. reduced metabolic activity (Torres et al. 1994a), combined with omnivorous feeding behaviour (Hopkins 1985, 1987, Hopkins and Torres 1989, Rau et al. 1991) are sufficient to ensure winter survival of *T. macrura* largely without mobilising lipids. Thus, the internal energy reserves are saved for fuelling reproduction processes such as gonad maturation, egg production, and mating in late winter prior to the vernal phytoplankton bloom. This is documented by a strong decline in total lipid levels from September to November (Hagen and Kattner 1998).

The lipid biochemistry of *E. frigida* and *E. triacantha* has been studied less intensively. Sporadic summer data on total lipids are available, but mostly given in percent of wet mass (Clarke 1984b, Phleger et al. 1998, 2002) and hence not directly comparable to our data. In contrast to the results presented here, Phleger et al. (1998) found higher lipid levels in *E. frigida* as compared to *E. triacantha*. However, neither length nor stage were indicated, so comparison is difficult. *E. triacantha* stores primarily triacylglycerols (TAG, up to 50% of total lipids (TL), Fig. 2 in Chapter 5) and to a lesser extent WE (max. 30% TL in adult specimens, unpubl. data). These values are only slightly higher than summer data reported by Phleger et al. (2002), but considerably higher than those of an earlier summer study (Phleger et al. 1998). PC does not seem to be as extensively accumulated in *E. triacantha* as in *E. superba* or *T. macrura*, as indicated by PC levels of $< 10\%$ DM (Fig. 2b in Chapter 5), as compared to about 15 and $> 30\%$ DM in *E. superba* and *T. macrura*, respectively (Fig. 2a and c in Chapter 5, see also Hagen et al. 1996). Preliminary results from the sub–cellular fractionation indicate that these trends in total PC levels are mirrored in the relative proportions of free, non–membrane bound PL, which are as well maximum in *T. macrura* (89% of total PL), followed by *E. superba* with 83%, and lowest in *E. triacantha* (65%, Fig. 4 in Chapter 5).

E. frigida attains the lowest maximum lipid contents among the Antarctic euphausiids studied here. Accordingly, also depot lipids, mainly TAG, did not amount to such high levels (on average around 50% of total lipids in autumn). Phleger et al. (1998) found a similar lipid class pattern in summer 1996, while TAG values were consider-

ably lower during summer 1998 (Phleger et al. 2002). The role of PC as additional storage lipid is slightly more important in *E. frigida* than in *E. triacantha*, as indicated by the steeper slope when regressing PC against TL (unpubl. data). Maximum PC levels of *E. frigida* were around 10% DM and hence markedly lower than those of *E. superba* and particularly *T. macrura*. The significantly lower relative amounts of polyunsaturated fatty acids (PUFAs) in *E. frigida* show an interesting difference to the other species (Fig. 3 in Chapter 2). High levels of PUFAs can be considered as an adaptation to low temperatures, because the incorporation of a double bond into the hydrocarbon chain affects its melting point, a mechanism to regulate membrane fluidity (e.g. Shinitzky 1984, Sargent and Henderson 1995). It therefore seems contradictory that the truly Antarctic *E. frigida* has lower PUFA levels than *E. triacantha*, which also occurs in sub–Antarctic waters with considerably higher temperatures. This is possibly a matter of evolutionary time scale. Zane and Patarnello (2000) found a closer relationship of *E. frigida* to the sub–Antarctic *Euphausia vallentini* than to the other Antarctic *Euphausia* species, which clearly clustered in one group. According to the molecular clock, the separation of these groups occurred before the formation of the Antarctic Circumpolar Current and *E. frigida* presumably migrated southward over the Antarctic Convergence into its present habitat. Hence, the lower lipid content and dependence on PUFAs might reflect original adaptations to a more subpolar climate.

Conclusions

- Total lipid content is highest in *T. macrura*, intermediate in *E. triacantha*, and lowest in *E. frigida* mirroring the latitudinal differences of their distribution.
- Wax esters are the main storage lipids in *T. macrura*, wax esters and triacylglycerols in *E. triacantha*, and triacylglycerols in *E. frigida*.
- The importance of phosphatidylcholine as additional storage lipid decreases from *T. macrura* over *E. triacantha* to a minimum in *E. frigida*.

3.2 Feeding ecology

Quantitative information on feeding in Antarctic krill is difficult to obtain, since each method has its specific drawbacks and may yield biased results. Gut content analyses give a direct impression of the recently ingested food. However, results may be biased by feeding occurring in the net, identification of food items can be difficult, soft bodied or rapidly digested components are underrepresented and inference is possible only on ingestion, not on assimilation (e.g. Boyd et al. 1984). Furthermore, this method provides only snap–shot information on recently ingested food (Båmstedt et al. 2000). Incubations provide insight into feeding rates and selectivity but could suffer from container artefacts. In captivity, zooplankton and their prey can behave unnaturally (Boyd et al. 1984) and it is not always simple to simulate natural food assemblages in experiments. Biomarkers are an alternative tool to study food webs. This approach makes use of the fact that specific biochemical components, e.g. fatty acids or stable isotopes, are characteristic of specific groups of phytoplankton or zooplankton. These biomarkers are incorporated largely unaltered into the consumers, which thus retain a signature of their dietary origin (Sargent and Whittle 1981, Sargent et al. 1987, Graeve et al. 1994b). However, these biochemical methods also suffer from specific problems. Fatty acid compositions are not precisely species–specific, hence they provide trophic information rather on the level of larger taxonomic groups (e.g. Dalsgaard et al. 2003). Some fatty acids, that are used as biomarkers can also be synthesised by the consumers (e.g. 16:1(n-7)) and finally, some degree of modification of the ingested lipids cannot be ruled out. Problems in stable isotope studies may emerge due to varying isotope fractionation according to species or food source (e.g. DeNiro and Epstein 1981, Gorokhova and Hansson 1999).

In Chapters 3 and 4 three of these methods, namely gut content analyses, feeding experiments, and fatty acid biomarkers, were applied in order to obtain a realistic picture of the feeding behaviour of *E. superba*. In Chapters 1, 2, and 6 the applicability of fatty acid and stable isotope trophic biomarkers for elucidating the feeding habits of *E. superba* is critically evaluated.

3.2.1 Application of trophic marker fatty acids (Chapters 1, 3 and 4)

Juvenile and adult *Euphausia superba* did not markedly differ with respect to their fatty acid compositions. Individual variability exceeded ontogenetic differences. No clear trend in the relative abundance of trophic marker fatty acids was discernible. Levels of 18:4(n-3) were generally low, consistent with low flagellate abundance in the phytoplankton at that time. 16:1(n-7), a marker for diatoms (e.g. Sargent et al. 1987), exhibited pronounced variations, but tended to be higher in adult *E. superba*. This did not agree with the findings from parallel gut content analyses, which revealed a higher degree of herbivory in juveniles and more crustacean fragments in the stomachs of adults. These differences were again not mirrored by the fatty acids typical of calanid copepods (the C20 and C22 monoenes, e.g. Kattner et al. 1994, Kattner and Hagen

1995, Albers et al. 1996), which mostly occurred in traces only. This is consistent with previous studies on the fatty acid composition of *E. superba* (Phleger et al. 1998, Hagen et al. 2001). Apparently, *E. superba* is not adapted to make full use of the energy–rich copepod lipids (see below, Section 3.2.2).

Trophic indices calculated from biomarker ratios did not provide any further insight into feeding preferences. In comparison with the larvae, they yielded rather confounding results, since one of the indices for carnivory (the ratio of polyunsaturated to saturated fatty acids (PUFA/SFA, Cripps and Atkinson 2000) was higher in the herbivorous furciliae than in the postlarvae. While this might be partly due to feeding on diatoms, which also contain high concentrations of PUFAs (e.g. Nichols et al. 1993, Dunstan et al. 1994), this ratio is inherently problematic (see Section 3.2.2).

As discussed above (Section 3.1.1), despite diatoms constituting the major fraction in the stomachs, 16:1(n-7) comprised only small portions ($< 5\%$) of the furciliae fatty acids. These values are in line with those published by Hagen et al. (2001). Since even postlarval krill did not contain significant amounts of copepod fatty acids, an unexpected result was the occurrence of the 22:1 isomers in some of the furcilia samples (Table 4 in Chapter 3). Although these moieties presumably originated from the stomach contents (because these samples were not maintained to evacuate their guts) rather than from assimilated body lipids, it demonstrates the principle capability of raptorial feeding already of the early *E. superba* stages.

In contrast to *E. superba*, adult *E. triacantha* exhibited seasonal variations in dietary fatty acid markers (unpubl. data). In summer, when total lipid levels were significantly lower than in autumn (maximum values of 12% DM and 32% DM, respectively), typical membrane fatty acids (20:5(n-3), 16:0, and 22:6(n-3)) prevailed. In autumn, there was a significant increase of neutral lipid moieties, particularly of 18:1(n-9) and the 22:1 isomers (fatty acids as well as fatty alcohols), but also of 16:1(n-7). Indicating omnivory (also proposed by Pavlov 1976, cited in Mauchline 1980), these findings also illustrate the importance of heterotrophic prey including calanid copepods in the food spectrum of *E. triacantha* at that time of the year. Apart from the near absence of copepod markers in furciliae and most juveniles, there were no clear indications of ontogenetic differences in feeding behaviour. The diatom marker 16:1(n-7) attained highest portions in lipid–rich juveniles and adults and overall the abundance of marker fatty acids was rather a function of total lipid content than of developmental stage. These results are in accordance with the fatty acid data reported by Phleger et al. (1998), although they reported even higher percentages of the C20 monoenes, particularly of 20:1(n-9), which is an abundant fatty acid in *Calanoides acutus* (Kattner et al. 1994, Albers et al. 1996, Falk-Petersen et al. 1999).

In *E. frigida*, despite neutral lipid accumulation in autumn as compared to the summer, the fatty acid composition was essentially similar in both seasons (unpubl. data). Hence, no seasonal shift in feeding behaviour could be detected via the fatty acid biomarker approach. The absence of copepod marker fatty acids and the slightly higher value of 16:1(n-7) as compared to *E. triacantha* indicate a more herbivorous feeding mode of *E. frigida*, although its fatty acid pattern showed only little trophic

influence (see also Phleger et al. 2002). δ^{15}N values of *E. frigida* furciliae implied a more omnivorous feeding behaviour than *E. superba* furciliae (Table 2 in Chapter 6).

Conclusions

- Comparison of fatty acid biomarkers and stomach contents produced inconclusive results in *E. superba*.
- Adult *E. superba* showed the lowest feeding activity and highest degree of carnivory in autumn, compared to intermediate feeding activity and omnivory in juveniles, and highest feeding activity and degree of herbivory in furciliae.
- *E. triacantha* is omnivorous with a strong tendency towards carnivory (calanid copepods) in autumn
- There is only a weak influence of dietary fatty acids on the lipid pattern of adult *E. frigida*, albeit some indications of omnivory.

3.2.2 Evaluation of trophic biomarkers (Chapters 1, 2, and 6)

As described in the previous section (3.2.1), fatty acid compositions of postlarval krill were quite homogeneous and did not reflect recent feeding as detected through stomach content analyses. Similar results were obtained via stable isotope measurements (Chapter 6). Below, the limitations of these biomarker approaches are outlined and potential reasons are discussed with respect to ontogenetic differences. In Chapters 1 and 6 an experimental approach was chosen to study the suitability of the two biomarker methods. Chapter 2 has an empirical background and is based on statistical evaluations of total lipid and fatty acid data.

The fatty acid compositions of adult and juvenile krill did not respond to different food sources even after prolonged exposure. Low assimilation, despite observed feeding, could be inferred from decreasing dry and lipid masses especially in the first phase of the experiments (Fig. 2a–d in Chapter 1). While this might have been one reason for the poor response, the influence of diet on the fatty acid composition of postlarval *E. superba* appears to be generally low. Various field studies (Cripps and Hill 1998, Cripps et al. 1999, Hagen et al. 2001) found only little variation of fatty acid composition attributable to feeding. The same applied for stable isotope signatures (Chapter 6). While the majority of the mesozooplankton tended to respond to the marked δ^{15}N variations of POM, postlarval krill integrated them. In the feeding incubations, *E. superba* juveniles and adults showed a very long lag time before any changes in stable isotope patterns could be observed. After 30 days, there was no response of adult specimens, and juveniles had only started to shift towards higher δ^{13}C and δ^{15}N values.

Difficulties of these biomarker approaches arise with the size and the life style of the animals studied. Low C and N turnover rates of the large, slow growing adult *E. superba* result in slow equilibration with their diet. The large lipid depots of juvenile and adult *E. superba* in autumn buffer short–term variations in dietary lipid composition. Moreover, further accumulation of lipids was probably of minor importance, since at the time of sampling phytoplankton biomass was very low and feeding rates of postlarval krill significantly reduced (Chapter 4). The fatty acid composition of individuals with lower total lipid levels better reflect feeding habits as indicated by diet–induced changes in furciliae (Table 2 in Chapter 1) or in starved postlarvae (Virtue et al. 1993a). Advection or migration from regions characterised by different trophic biomarker patterns can have further confounding effects. These characteristics of postlarval euphausiids, namely their slower turnover/growth rates and their ability to migrate, might confuse trophic effects with those of a temporally/spatially changing baseline.

Irrespective of the problems inherent in these trophic biomarker approaches, fatty acid biomarkers can be classified into those that are principally well suited to reflect dietary influences and those that are likely to produce ambiguous results. Among the marker fatty acids for diatoms, 16:1(n-7) and 20:5(n-3) (e.g. Nichols et al. 1993, Dunstan et al. 1994), the former is better suited since its abundance in *E. superba* lipids is subjected to large variations and it can apparently be depleted when krill feed on other food sources (Chapter 1, see also Hagen et al. 2001). 20:5(n-3) on the other hand is usually one of the dominant fatty acids in the total lipids of *E. superba*. Like the second dominant essential long–chain fatty PUFA 22:6(n-3) it is tightly conserved, i.e. losses via catabolism are low (Sargent and Whittle 1981, Sargent and Henderson 1995). The same holds true for the two marker fatty acids for flagellates, 18:4(n-3) and 22:6(n-3) (Sargent et al. 1987, Graeve et al. 1994b). Since (n-3)–PUFAs can only be synthesised *de novo* by plants (Sargent and Whittle 1981, Sargent and Henderson 1995), 18:4(n-3) is an even more unequivocal trophic marker than 16:1(n-7), which can also be synthesised by krill itself.

The C20 and C22 fatty acids and fatty alcohols are not applicable for detecting predation on calanid copepods by *E. superba*, since they are not assimilated but excreted with the faeces (see also Section 3.2.1). However, this may be different in the more carnivorous species that also contain wax esters, like *Thysanoessa macrura* and *E. triacantha*. Field samples of both species did contain these moieties, particularly the fatty alcohols (unpubl. data, see also Kattner et al. 1996, Hagen and Kattner 1998, for fatty acid and alcohol composition of *T. macrura*), however conclusions on potential differences in enzymatic configuration as compared to *E. superba* need to be consolidated by appropriate experimental evidence.

Several fatty acid ratios have been proposed as distinct measures for the classification of marine invertebrates into feeding types. Such ratios include 16:0/16:1(n-7), which has been applied to discriminate between diatom versus flagellate feeding (St. John and Lund 1996, Cripps et al. 1999, Mayzaud et al. 1999, Nelson et al. 2001, Phleger et al. 2001, Auel et al. 2002). Correspondingly, high ratios of 20:5(n-3)/22:6(n-

3) are indicative of diatom food and low ratios of a flagellate–based nutrition (Nelson et al. 2001, Phleger et al. 2001, 2002). The ratio 18:1(n-9)/18:1(n-7) is frequently used to estimate the degree of carnivory versus herbivory (Graeve et al. 1997, Cripps et al. 1999, Mayzaud et al. 1999, Falk-Petersen et al. 2000, Nelson et al. 2001). Another index, the ratio of polyunsaturated to saturated fatty acids (PUFA/SFA), has been proposed to be a measure of carnivory in *E. superba* with possible extension to other euphausiid species (Cripps and Atkinson 2000). While some of these indices have proven very useful particularly when applied for herbivorous copepod species, others produced rather inconclusive results (Chapters 3 and 4, see also Auel et al. 2002). In Chapter 2, the applicability of these fatty acid biomarker ratios has been evaluated by empirical studies on four Antarctic euphausiids. In conclusion, there is a risk of misinterpretation inherent in the majority of these indices particularly when comparing individuals with markedly varying lipid levels, since the fatty acid composition of the investigated euphausiids was strongly related to their total lipid contents. Therefore, especially those fatty acids that constitute major membrane components, such as the long-chain PUFAs 20:5(n-3) and 22:6(n-3), but also 16:0 and 18:1(n-7) (Chapter 1, see also Mayzaud 1997, Mayzaud et al. 2000), may vary greatly with factors (e.g. reproductive processes) unrelated to feeding.

Possible refinements of the biomarker approaches might include the selection of organs or body parts with higher turnover rates as compared to those integrated over the whole animals. *E. superba* moults and faeces equilibrated faster with the stable isotope composition of the food (Chapter 6). While moults are generally unsuitable for fatty acid studies, because they are almost devoid of lipids, faecal strings might reflect predation on copepods, since their typical marker fatty acids are not assimilated (Fig. 8a in Chapter 1). The diatom marker fatty acids, however, are preferentially extracted from the food. The fatty acid composition of ingested diatoms is significantly altered during gut passage (Fig. 8b-d in Chapter 1) and hence their faeces badly reflect feeding on diatoms. In decapod crustaceans, the hepatopancreas is the organ directly involved in the uptake and processing of food–derived nutrients (e.g. Chandumpai et al. 1991). Thus, it would appear to be a particularly suitable site to detect potential dietary influence. However, the isolated hepatopancreas of adult *E. superba* did not reflect different feeding regimes better than the whole animal, neither with regard to stable isotopes (Chapter 6) nor to fatty acids (unpubl. data). Detailed studies on the dietary influence on the fatty acid composition of isolated organs are rare. Inconsistent with our results, Alonzo et al. (2003) report changes in the fatty acid content and the lipid class composition of the digestive gland associated with different diets. This is also mirrored by the fatty acid composition, although it is only specified for total *E. superba* samples and not for the hepatopancreas. Virtue et al. (1993b) found a decline of PUFAs in the digestive gland during short–term starvation. However, since the entire bodies were not studied for comparison, it cannot be decided whether the isolated organ provides information that might stay concealed when analysing the whole animal only.

A further potential refinement to the fatty acid biomarker approach is the analysis of isolated lipid classes instead of the total lipid extract. Dietary influences are considered to being primarily mirrored in the storage lipids (Sargent and Henderson 1986). Inversely, food–derived fatty acids can be masked by the presence of structural lipids, which are supposed to have a relatively stable fatty acid profile (Sargent et al. 1987). However, such analyses are extremely time–consuming and only feasible for limited sample sets. Moreover, such detailed information does not always yield clearer results. In feeding experiments with juvenile and adult *E. superba*, no statistical changes could be induced in the fatty acid composition of the storage lipid classes (Fig. 4 in Chapter 1). Although not correlated with diet, phosphatidylethanolamine, which serves exclusively membrane functions, exhibited the highest variability, which parallels previous results by Mayzaud et al. (2000). In field–caught *E. superba* larvae, trophic effects were better reflected in the triacylglycerols (TAG) only when TAG–typical fatty acids (16:1(n-7), 18:4(n-3)) were concerned, while for phospholipid (PL)–typical fatty acids (20:5(n-3), 22:6(n-3)) dietary effects were more evident in total lipid extracts (Fig. 6 and Table 2 in Chapter 1). In *E. superba* and in many other polar euphausiids, the differentiation between neutral storage lipids and structural polar lipids cannot be drawn as strictly as in other marine invertebrates, because of the elevated PC contents in the former (Section 3.1.1). Nevertheless, it is plausible that PL are also influenced by diet, since the long–chain PUFAs are essential to most marine zooplankton species (Sargent and Whittle 1981) and hence necessarily originate from the food. Evidence of dietary influences on the fatty acid composition of PL was also found in *Meganyctiphanes norvegica* (Virtue et al. 2000), in Antarctic amphipods (Graeve et al. 2001), and in the bivalve *Pecten maximus* (Soudant et al. 1996, 1997). Hence, in some cases such detailed fatty acid analyses may provide additional trophic information, but it appears that most tendencies will also be discernible from the total lipid extracts.

Conclusions

- Larval lipid and stable isotope compositions may reflect dietary signatures due to higher turnover rates and lower lipid levels as compared to postlarval *E. superba*.
- Neither fatty acids nor stable isotopes are reliable indicators for the recent feeding history of juvenile and adult *E. superba*.
- Biomarker ratios (e.g. 18:1(n-9)/18:1(n-7), PUFA/SFA) are only of limited suitability as trophic indices.
- Fatty acids unrelated to total lipid content (e.g. 16:1(n-7) and 18:4(n-3)) are best–suited as trophic markers.

4 Perspectives

During this study, several questions emerged, which could not be fully resolved within the framework of this thesis. They present particularly promising subjects for future research. One interesting topic is the predation on mesozooplankton, which was described as alternative feeding strategy for *Euphausia superba* during periods of phytoplankton shortage (see Chapter 1 and review of Ph.D., section 3.2.2). The results of the present study indicate, that *E. superba* is apparently not adapted to make full use of ingested copepods, which are very rich in lipid moieties not present in *E. superba* (wax esters, long–chain monounsaturated fatty acids). However, this conclusion could only be drawn from the absence of these fatty acids in the krill lipids and it remained unclear, whether they could not be assimilated or were rapidly catabolised for energy. A suitable approach could be the incubation of krill tissue with radioactively labelled wax esters as substrate and testing its enzymatic hydrolysis.

Calculations of energy gains based on total carbon content of the food can be misleading and the above described approach may yield valuable information on the energy budget of krill feeding on calanid copepods. Comparison with other species, that are supposed to be more carnivorous and that also contain wax esters (e.g. *Thysanoessa macrura* and *Euphausia triacantha*) or that apparently do assimilate the long–chain monounsaturated fatty acids (*Meganyctiphanes norvegica*) may help to understand species–specific lipid metabolic adaptations.

Chapter 5 has shed new light on the role of phosphatidylcholine (PC) in three Antarctic krill species. However, this is a preliminary study and some aspects require more detailed investigation. The sub–cellular fractionation technique might be optimised to yield pure membrane fractions of different cell compartments, which can be verified by measuring marker enzyme activities. The fatty acid composition of PC isolated from these membrane fractions can then be compared to that of the supernatant in order to reveal potential differences between structural and depot PC and thereby obtain information on the specific ecological advantages of PC–storage. The ultrastructure of the accumulated PC could be further studied by transmission electron microscopy. Thus, the nature of PC depots could be specified, i.e. whether it is membraneous or of some other form, and whether this is uniform throughout all PC–rich tissues and organs (hepatopancreas, fat body, gonads, eggs) or variable according to their specific demands. In combination with well designed feeding and starvation experiments such analyses may provide valuable insight in the metabolic pathways of PC accumulation or depletion.

Comparative analyses of the lipid class and fatty acid compositions of the eggs of different euphausiid species could further our understanding of the respective advan-

tages of different storage lipid classes with respect to the sinking behaviour off the eggs and to the energetic and structural requirements of specific lipids during embryonic and early larval development.

Such detailed studies could fill the gaps in our knowledge on the ecophysiological advantages connected with the utilisation of different lipids as energy reserves and provide essential information on the biochemical patterns behind the observed life histories of polar euphausiids.

References

Ackman RG, Eaton CA, Sipos JC, Hooper SN, Castell JD (1970) Lipids and fatty acids of two species of North Atlantic krill (*Meganyctiphanes norvegica* and *Thysanoessa inermis*) and their role in the aquatic food web. J Fish Res Bd Can 27: 513–533

Albers CS, Kattner G, Hagen W (1996) The compositions of wax esters, triacylglycerols and phospholipids in Arctic and Antarctic copepods: Evidence of energetic adaptations. Mar Chem 55: 347–358

Alonzo F, Nicol S, Virtue P, Nichols PD (2003) Lipids as trophic markers in Antarctic krill, I. Validation under controlled laboratory conditions. In: Huiskes AHL, Gieskes WWC, Rozema J, Schorno RML, van der Vies SM, Wolff WJ (eds) Antarctic Biology in a Global Context, Backhuys Publishers, Leiden, 121–128

Atkinson A, Snÿder R (1997) Krill-copepod interactions at South Georgia, Antarctica, I. Omnivory by *Euphausia superba*. Mar Ecol Prog Ser 160: 63–76

Auel H, Harjes M, da Rocha R, Stübing D, Hagen W (2001) Lipid biomarkers indicate different ecological niches and trophic relationships of the Arctic hyperiid amphipods *Themisto abyssorum* and *T. libellula*. Polar Biol 25: 374–383

Baker AdC, Boden BP, Brinton E (1990) A practical guide to the euphausiids of the world. British Museum (Natural History), London, 96 pp

Båmstedt U, Gifford DJ, Irigoien X, Atkinson A, Roman M (2000) Feeding. In: R Harris, P Wiebe, J Lenz, H Skjoldal, M Huntley, eds., ICES Zooplankton Methodology Manual, Academic Press, San Diego, 297–399

Båmstedt U, Håkannson JL, Brenner-Larsen J, Björnsen PK, Geertz-Hansen O, Tiselius P (1990) Copepod nutritional condition and pelagic production during autumn in Kosterfjorden, western Sweden. Mar Biol 104: 197–208

Bathmann U (2002) The expedition ANTARKTIS XVIII/5b of the research vessel "Polarstern" in 2001. Ber Polarforsch 407: 1–98

Bathmann U, Smetacek V, Reinke M (2000) The expeditions ANTARKTIS XVI/3-4 of the research vessel "Polarstern" in 1999. Ber Polarforsch 362: 1–139

Bianchi F, Boldrin A, Cioce F, Dieckmann G, Kuosa H, Larsson AM, Nöthig EM, Sehlstedt PI, Socal G, Syvertsen EE (1992) Phytoplankton distribution in relation to sea ice, hydrography and nutrients in the northwestern Weddell Sea in early spring 1988 during EPOS. Polar Biol 12: 225–235

Boyd CM, Heyraud M, Boyd CN (1984) Feeding of the Antarctic krill *Euphausia superba*. J Crust Biol 4: 123–141

Chandumpai A, Dall W, Smith DM (1991) Lipid-class composition of organs and tissues of the tiger prawn *Penaeus esculentus* during the moulting cycle and during starvation. Mar Biol 108: 235–245

Chang ES, O'Connor JD (1983) Metabolism and transport of carbohydrates and lipids. In: LH Mantel, ed., The Biology of Crustacea, Academic Press, New York, vol 5, 263–287

Chayen J, Bitensky L (1991) Practical Histochemistry. Wiley, Chichester, 321 pp

Clarke A (1980) The biochemical composition of krill, *Euphausia superba* Dana, from South Georgia. J Exp Mar Biol Ecol 43: 221–236

Clarke A (1984a) Lipid content and composition of Antarctic krill, *Euphausia superba* Dana. J Crust Biol 4: 285–294

Clarke A (1984b) The lipid content and composition of some Antarctic macrozooplankton. Br Antarct Surv Bull 63: 57–70

Clarke KR, Warwick RM (1994) Change in marine communities: An approach to statistical analysis and interpretation. Natural Environment Research Council, Bournemouth, 144 pp

Conover RJ (1988) Comparative life histories in the genera *Calanus* and *Neocalanus* in high latitudes of the northern hemisphere. Hydrobiologia 167: 127–142

Cripps GC, Atkinson A (2000) Fatty acid composition as an indicator of carnivory in Antarctic krill, *Euphausia superba*. Can J Fish Aquat Sci 57: 31–37

Cripps GC, Hill HJ (1998) Changes in lipid composition of copepods and *Euphausia superba* associated with diet and environmental conditions in the marginal ice zone, Bellingshausen Sea, Antarctica. Deep-Sea Res 45: 1357–1381

Cripps GC, Watkins JL, Hill HJ, Atkinson A (1999) Fatty acid content of Antarctic krill, *Euphausia superba*, at South Georgia related to regional populations and variations in diet. Mar Ecol Prog Ser 181: 177–188

Cuzin-Roudy J, Labat JP (1992) Early summer distribution of Antarctic krill sexual development in the Scotia-Weddell region: a multivariate approach. Polar Biol 12: 65–74

Dalsgaard J, St. John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Adv Mar Biol 46: 225–340

Daly KL (1990) Overwintering development, growth, and feeding of larval *Euphausia superba* in the Antarctic marginal ice zone. Limnol Oceanogr 35: 1564–1576

Daly KL (1998) Physioecology of juvenile Antarctic krill (*Euphausia superba*) during spring in ice-covered seas. Antarct Res Ser 73: 183–198

Daly KL, Macaulay MC (1988) Abundance and distribution of krill in the ice edge zone of the Weddell Sea, austral spring 1983. Deep-Sea Res 35: 21–41

Deacon GER (1982) Physical and biological zonation in the Southern Ocean. Deep-Sea Res 129: 1–15

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45: 341–351

de Baar HJW, Bakker DCE, Veth C, Bathmann U, Smetacek V, Jong JTMd, Löscher BM (1995) Importance of iron for plankton blooms and carbon dioxide drawdown in the Southern Ocean. Nature 373: 412–415

Dunstan GA, Volkman JK, Barrett SM, Leroi JM, Jeffrey SW (1994) Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). Phytochemistry 35: 155–161

Errhif A, Razouls C, Mayzaud P (1997) Composition and community structure of pelagic copepods in the Indian sector of the Antarctic Ocean during the end of the austral summer. Polar Biol 17: 418–430

Fahrbach E (1995) Die Polarmeere - ein Überblick. In: I Hempel, G Hempel, eds., Biologie der Polarmeere, Fischer, Jena, 24–44

Falk-Petersen S (1981) Ecological investigations on the zooplankton community of Balsfjorden, northern Norway: seasonal changes in body weight and the main biochemical composition of *Thysanoessa inermis* (Krøyer), *T. raschii* (M. Sars), and *Meganyctiphanes norvegica* (M. Sars) in relation to environmental factors. J Exp Mar Biol Ecol 49: 103–120

Falk-Petersen S, Hopkins CCE, Sargent JR (1990) Trophic relationships in the pelagic arctic food web. In: M Barnes, R Gibson, eds., Proceedings of the 24th European Marine Biology Symposium, Oban, Argyll, Scotland, University Press, Aberdeen, 315–333

Falk-Petersen S, Sargent JR, Lønne OJ, Timofeev S (1999) Functional biodiversity of lipids in Antarctic zooplankton; *Calanoides acutus*, *Calanus propinquus*, *Thysanoessa macrura* and *Euphausia crystallorophias*. Polar Biol 21: 37–47

Falk-Petersen S, Hagen W, Kattner G, Clarke A, Sargent J (2000) Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. Can J Fish Aquat Sci 57: 178–191

Fraser AJ (1989) Triacylglycerol content as a condition index for fish, bivalve and crustacean larvae. Can J Fish Aquat Sci 46: 1868–1873

Fraser AJ, Tocher DR, Sargent JR (1985) Thin-layer chromatography-flame ionization detection and the quantitation of marine neutral lipids and phospholipids. J Exp Mar Biol Ecol 88: 91–99

Fraser FG (1936) On the development and distribution of the young stages of krill (*Euphausia superba*). Discovery Rep 14: 1–192

Frazer T (1996) Stable isotope composition (δ^{13}C and δ^{15}N) of larval krill, *Euphausia superba*, and two of its potential food sources in winter. J Plankton Res 18: 1413–1426

Garrison DL, Buck KR (1986) Organism losses during ice melting: a serious bias in sea ice community studies. Polar Biol 6: 237–239

Gorokhova E, Hansson S (1999) An experimental study on variation in stable carbon and nitrogen isotope fractionation during growth of *Mysis mixta* and *Neomysis integer*. Can J Fish Aquat Sci 56: 2203–2210

Graeve M, Hagen W, Kattner G (1994a) Herbivorous or omnivorous? On the significance of lipid compositions as trophic markers in Antarctic copepods. Deep-Sea Res 41: 915–924

Graeve M, Kattner G, Hagen W (1994b) Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: Experimental evidence of trophic markers. J Exp Mar Biol Ecol 182: 97–110

Graeve M, Kattner G, Piepenburg D (1997) Lipids in Arctic benthos: does the fatty acid and alcohol composition reflect feeding and trophic interactions? Polar Biol 18: 53–61

Graeve M, Dauby P, Scailteur Y (2001) Combined lipid, fatty acid and digestive tract content analyses: a penetrating approach to estimate feeding modes of Antarctic amphipods. Polar Biol 24: 853–862

Hagen W (1988) Zur Bedeutung der Lipide im antarktischen Zooplankton. Ber Polarforsch 49: 1–129

Hagen W (2000) Lipids. In: R Harris, P Wiebe, J Lenz, H Skjoldal, M Huntley, eds., ICES Zooplankton Methodology Manual, Academic Press, San Diego, 113–119

Hagen W, Kattner G (1998) Lipid metabolism of the Antarctic euphausiid *Thysanoessa macrura* and its ecological implications. Limnol Oceanogr 43: 1894–1901

Hagen W, Van Vleet ES, Kattner G (1996) Seasonal lipid storage as overwintering strategy of Antarctic krill. Mar Ecol Prog Ser 134: 85–89

Hagen W, Kattner G, Terbrüggen A, Van Vleet ES (2001) Lipid metabolism of the Antarctic krill *Euphausia superba* and its ecological implications. Mar Biol 139: 95–104

Hamm C, Reigstad M, Wexels Riser C, Mühlebach A, Wassmann P (2001) On the trophic fate of *Phaeocystis pouchetii*. VII. Sterols and fatty acids reveal sedimentation of *P. pouchetii*-derived organic matter via krill fecal strings. Mar Ecol Prog Ser 209: 55–69

Hempel I, Hempel G (1986) Field observations on the developmental ascent of larval *Euphausia superba* (Crustacea). Polar Biol 6: 121–126

Hofmann EE, Lascara CM (2000) Modeling the growth dynamics of Antarctic krill *Euphausia superba*. Mar Ecol Prog Ser 194: 219–231

Hopkins TL (1985) Food web of an Antarctic midwater ecosystem. Mar Biol 89: 197–212

Hopkins TL (1987) Midwater food web in McMurdo Sound, Ross Sea, Antarctica. Mar Biol 96: 93–106

Hopkins TL, Torres JJ (1989) Midwater food web in the vicinity of a marginal ice zone in the western Weddell Sea. Deep-Sea Res 36: 543–560

Hopkins TL, Ainley DG, Torres JJ, Lancraft TM (1993) Trophic structure in open waters of the marginal ice zone in the Weddell-Scotia confluence region during spring (1983). Polar Biol 13: 389–397

Huntley ME, Nordhausen W, Lopez MDG (1994) Elemental composition, metabolic activity and growth of Antarctic krill *Euphausia superba* during winter. Mar Ecol Prog Ser 107: 23–40

Ikeda T, Bruce B (1986) Metabolic activity and elemental composition of krill and other zooplankton from Prydz Bay, Antarctica, during early summer (November-December). Mar Biol 92: 545–555

Ikeda T, Dixon P (1982) Body shrinkage as a possible over-wintering mechanism of the Antarctic krill, *Euphausia superba* Dana. J Exp Mar Biol Ecol 62: 143–151

Kattner G, Fricke HSG (1986) Simple gas-liquid chromatography method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. J Chromatogr 361: 263–268

Kattner G, Hagen W (1995) Polar herbivorous copepods – different pathways in lipid biochemistry. ICES J Mar Sci 52: 329–335

Kattner G, Hagen W (1998) Lipid metabolism of the Antarctic euphausiid *Euphausia crystallorophias* and its ecological implications. Mar Ecol Prog Ser 170: 203–213

Kattner G, Krause M (1987) Changes in lipids during the development of *Calanus finmarchicus* s. l. from copepodid I to adult. Mar Biol 96: 511–518

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

Kattner G, Hagen W, Falk-Petersen S, Sargent JR, Hendersen RJ (1996) Antarctic krill *Thysanoessa macrura* fills a major gap in marine lipogenic pathways. Mar Ecol Prog Ser 134: 295–198

Kawaguchi K, Ishikawa S, Matsuda O (1986) The overwintering strategy of Antarctic krill (*Euphausia superba* Dana) under the coastal fast ice off the Ongul Islands in Lützow-Holm Bay, Antarctica. Mem Nat Inst Polar Res Spec Iss 44: 67–86

Kils U (1981) Swimming behaviour, swimming performance and energy balance of the Antarctic krill *Euphausia superba*. BIOMASS Sci Ser 3: 1–122

Kirkwood JM (1982) A guide to the Euphausiacea of the Southern Ocean. ANARE Research Notes 1: 1–45

Knox GA (1994) The biology of the Southern Ocean. University Press, Cambridge, 444 pp

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

Lee RF, Nevenzel JG, Paffenhöfer GA (1971) Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Mar Biol 9: 99–108

Lee RF, Nevenzel JG, Lewis AG (1974) Lipid changes during life cycle of marine copepod, *Euchaeta japonica* Marukawa. Lipids 9: 891–898

Ligowski R (2000) Benthic feeding by krill, *Euphausia superba* Dana, in coastal waters off West Antarctica and in Admiralty Bay, South Shetland Islands. Polar Biol 23: 619–625

Lipsky J (2001) AMLR 1999/2000 field season report. NOAA-TM-NMFS-SWFSC 302: 1–139

Lizotte MP (2001) The contributions of sea ice algae to Antarctic marine primary production. Am Zool 41: 57–73

Lochte K, Smetacek V (1995) Was steuert die Produktivität des Planktons im Südpolarmeer? In: I Hempel, G Hempel, eds., Biologie der Polarmeere, Gustav Fischer Verlag, Jena, 104–115

Loeb V, Siegel V, Holm-Hansen O, Hewitt R, Fraser W, Trivelpiece W, Trivelpiece S (1997) Effects of sea-ice extent and krill or salp dominance on the Antarctic food web. Nature 387: 897–900

Makarov RR (1979) Larval distribution and reproductive ecology of *Thysanoessa macrura* (Crustacea: Euphausiacea) in the Scotia Sea. Mar Biol 52: 377–386

Marr JWS (1962) The natural history and geography of the Antarctic krill (*Euphausia superba* Dana). Discovery Rep 32: 33–464

Marschall HP (1985) Structural and functional analyses on the feeding appendages of krill larvae. In: WR Siegfried, PR Condy, RM Laws, eds., Antarctic nutrient cycles and food webs, Springer, Berlin, 346–354

Marschall HP (1988) The overwintering strategy of Antarctic krill under the pack-ice of the Weddell Sea. Polar Biol 9: 129–135

Marschall HP, Hirche HJ (1984) Development of eggs and nauplii of *Euphausia superba*. Polar Biol 2: 245–250

Mauchline J (1980) The biology of mysids and euphausiids. Adv Mar Biol 18: 1–677

Mauchline J, Fisher LR (1969) The biology of euphausiids. Adv Mar Biol 7: 1–454

Mayzaud P (1997) Spatial and life-cycle changes in lipid and fatty acid structure of the Antarctic euphausiid *Euphausia superba*. In: B Battaglia, J Valencia, DWH Walton, eds., Antarctic Communities: Species, Structure and Survival, Cambridge University Press, Cambridge, 284–294

Mayzaud P, Eaton CA, Ackman RG (1976) The occurrence and distribution of octadecapentaenoic acid in a natural plankton population. A possible food chain index. Lipids 11: 858–862

Mayzaud P, Farber-Lorda J, Corre MC (1985) Aspects of nutritional metabolism of two species of Antarctic euphausiids: *Euphausia superba* and *Thysanoessa macrura*. In: WR Siegfried, PR Condy, RM Laws, eds., Antarctic nutrient cycles and food webs, Springer Verlag, Berlin, 330–338

Mayzaud P, Albessard E, Cuzin-Roudy J (1998) Changes in lipid composition of the Antarctic krill *Euphausia superba* in the Indian sector of the Antarctic Ocean: influence of geographical location, sexual maturity stage and distribution among organs. Mar Ecol Prog Ser 173: 149–162

Mayzaud P, Virtue P, Albessard E (1999) Seasonal variations in the lipid and fatty acid composition of the euphausiid *Meganyctiphanes norvegica* from the Ligurian Sea. Mar Ecol Prog Ser 186: 199–210

Mayzaud P, Albessard E, Virtue P (2000) Environmental constraints on the lipid composition and metabolism of euphausiids: the case of *Euphausia superba* and *Meganyctiphanes norvegica*. Can J Fish Aquat Sci 57: 91–103

Miller DGM, Hampton I (1989) Biology and ecology of the Antarctic krill (*Euphausia superba* Dana): a review. BIOMASS Sci Ser 9: 1–166

Mock T, Kroon BMA (2002) Photosynthetic energy conversion under extreme conditions – I: important role of lipids as structural modulators and energy sink under N-limited growth in Antarctic sea ice diatoms. Phytochemistry 61: 41–51

Mühlebach A, Albers C, Kattner G (1999) Differences in the sterol composition of dominant Antarctic zooplankton. Lipids 34: 45–51

Nelson MM, Mooney BD, Nichols PD, Phleger CF (2001) Lipids of Antarctic Ocean amphipods: food chain interactions and the occurrence of novel biomarkers. Mar Chem 73: 53–64

Nichols DS, Nichols PD, Sullivan CW (1993) Fatty acid, sterol and hydrocarbon composition of Antarctic sea ice diatom communities during the spring bloom in McMurdo Sound. Antarct Sci 5: 271–278

Nicol S, Endo Y (1999) Krill fisheries: Development, management and ecosystem implications. Aquat Living Resour 12: 105–120

Nicol S, Stolp M, Cochran T, Geijsel P, Marshall J (1992) Growth and shrinkage of Antarctic krill *Euphausia superba* from the Indian Ocean sector of the Southern Ocean during summer. Mar Ecol Prog Ser 89: 175–181

Nicol S, De la Mare W, Stolp M (1995) The energetic cost of egg production in Antarctic krill (*Euphausia superba* Dana). Antarct Sci 7: 25–30

Nicol S, Pauly T, Bindoff NL, Wright S, Thiele D, Hosie GW, Strutton PG, Woehler E (2000) Ocean circulation off east Antarctica affects ecosystem structure and sea-ice extent. Nature 406: 504–507

Nordhausen W (1994a) Distribution and diel vertical migration of the euphausiid *Thysanoessa macrura* in Gerlache Strait, Antarctica. Polar Biol 14: 219–229

Nordhausen W (1994b) Winter abundance and distribution of *Euphausia superba*, *E. crystallorophias*, and *Thysanoessa macrura* in Gerlache Strait and Crystal Sound, Antarctica. Mar Ecol Prog Ser 109: 131–142

Olbers D, Gouretski V, Seiß G, Schröter J (1993) Hydrographic Atlas of the Southern Ocean. Alfred Wegener Institute, Bremerhaven, 82 pp

Olsen RE, Henderson RJ (1989) The rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densitometry. J Exp Mar Biol Ecol 129: 189–197

Olsen RE, Henderson RJ, Pedersen T (1991) The influence of dietary lipid classes on the fatty acid composition of small cod *Gadus morhua* L. juveniles reared in an enclosure in northern Norway. J Exp Mar Biol Ecol 148: 59–76

Pakhomov EA, Perissinotto R, McQuaid CR, Froneman PW (2000) Zooplankton structure and grazing in the Atlantic sector of the Southern Ocean in late austral summer 1993. Part l. Ecological zonation. Deep-Sea Res 147: 1663–1686

Parrish CC (1987) Separation of aquatic lipid classes by chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. Can J Fish Aquat Sci 44: 722–730

Perissinotto R, Pakhomov EA, McQuaid CD, Froneman PW (1997) *In situ* grazing rates and daily ration of Antarctic krill *Euphausia superba* feeding on phytoplankton at the Antarctic Polar Front and the Marginal Ice Zone. Mar Ecol Prog Ser 160: 77–91

Phleger CF, Nichols PD, Virtue P (1998) Lipids and trophodynamics of Antarctic zooplankton. Comp Biochem Physiol 120B: 311–323

Phleger CF, Nelson MM, Mooney BD, Nichols PD (2001) Interannual variations in the lipids of the Antarctic pteropods *Clione limacina* and *Clio pyramidata*. Comp Biochem Physiol 128B: 553–564

Phleger CF, Nelson MM, Mooney BD, Nichols PD (2002) Interannual and between species comparison of the lipids, fatty acids and sterols of Antarctic krill from the US AMLR Elephant Island survey area. Comp Biochem Physiol 131B: 733–747

Price HJ, Boyd KR, Boyd CM (1988) Omnivorous feeding behavior of the Antarctic krill *Euphausia superba*. Mar Biol 97: 67–77

Quetin LB, Ross RM (1991) Behavioral and physiological characteristics of the Antarctic krill, *Euphausia superba*. Am Zool 31: 49–63

Quetin LB, Ross RM, Clarke A (1994) Krill energetics: seasonal and environmental aspects of the physiology of *Euphausia superba*. In: S El-Sayed, ed., Southern Ocean ecology: the BIOMASS perspective, Cambridge University Press, Cambridge, 165–184

Rau GH, Hopkins TL, Torres JJ (1991) $^{15}N/^{14}N$ and $^{13}C/^{12}C$ in Weddell Sea invertebrates: Implications for feeding diversity. Mar Ecol Prog Ser 77: 1–6

Ross RM, Quetin LB (1989) Energetic cost to develop to the first feeding stage of *Euphausia superba* Dana and the effect of delays in food availability. J Exp Mar Biol Ecol 133: 103–127

Ross RM, Quetin LB (1991) Ecological physiology of larval euphausiids, *Euphausia superba* (Euphausiacea). Mem Queensl Mus 31: 321–333

Saether O, Ellingsen TE, Mohr V (1986) Lipids of North Atlantic krill. J Lipid Res 27: 274–285

Sakshaug E, Holm-Hansen O (1984) Factors governing pelagic production in polar oceans. In: O Holm-Hansen, L Bolis, R Gilles, eds., Marine phytoplankton and productivity, Springer, Berlin, vol 8, 1–18

Sargent JR (1978) Marine wax esters. Sci Prog 65: 437–458

Sargent JR, Falk-Petersen S (1981) Ecological investigations on the zooplankton community in Balsfjorden, northern Norway: Lipids and fatty acids in *Meganyctiphanes norvegica*, *Thysanoessa raschi* and *T. inermis* during mid-winter. Mar Biol 62: 131–137

Sargent JR, Henderson RJ (1986) Lipids. In: EDS Corner, SCM O'Hara, eds., Biological chemistry of marine copepods, Oxford University Press, Oxford, 59–108

Sargent JR, Henderson RJ (1995) Marine (n-3) polyunsaturated fatty acids. In: RJ Hamilton, ed., Developments in oils and fats, Blackie Academic and Professional, London, 32–65

Sargent JR, Whittle KJ (1981) Lipids and hydrocarbons in the marine food web. In: AR Longhurst, ed., Analysis of marine ecosystems, Academic Press, London, 491–533

Sargent JR, Gatten RR, Corner EDS, Kilvington CC (1977) On the nutrition and metabolism of zooplankton. XI. Lipids in *Calanus helgolandicus* grazing *Biddulphia sinensus*. J mar biol Ass UK 57: 525–533

Sargent JR, Gatten RR, Henderson RJ (1981) Lipid biochemistry of zooplankton from high latitudes. Océanis 7: 623–632

Sargent JR, Parkes RJ, Mueller-Harvey I, Henderson RJ (1987) Lipid biomarkers in marine ecology. In: MA Sleigh, ed., Microbes in the sea, Ellis Horwood, Chichester, 119–138

Shinitzky M (1984) Physiology of membrane fluidity, vol I, II. CRC Inc, Boca Raton, 331 pp

Siegel V (1987) Age and growth of Antarctic Euphausiacea (Crustacea) under natural conditions. Mar Biol 96: 483–495

Siegel V (2000a) Krill (Euphausiacea) demography and variability in abundance and distribution. Can J Fish Aquat Sci 57: 151–167

Siegel V (2000b) Krill (Euphausiacea) life history and aspects of population dynamics. Can J Fish Aquat Sci 57: 130–150

Siegel V, Bergström B, Strömberg JO, Schalk PH (1990) Distribution, size frequencies and maturity stages of krill, *Euphausia superba*, in relation to sea-ice in the northern Weddell Sea. Polar Biol 10: 549–557

Skerratt JH, Nichols PD, McMeekin TA, Burton H (1995) Seasonal and inter-annual changes in planktonic biomass and community structure in eastern Antarctica using signature lipids. Mar Chem 51: 93–113

Skerratt JH, Nichols PD, McMeekin TA, Burton HR (1997) Identification of dominant taxa in coastal Antarctic water and ice core samples using lipid signatures. In: B Battaglia, J Valencia, DWH Walton, eds., Antarctic Communities. Species, Structure and Survival, Cambridge University Press, Cambridge, 79–85

Smetacek V, Scharek R, N thig EM (1990) Seasonal and regional variation in the pelagial and its relationship to the life history cycle of krill. In: KR Kerry, G Hempel, eds., Antarctic ecosystems. Ecological change and conservation, Springer, Berlin, 103–114

Spindler M (1990) A comparison of Arctic and Antarctic sea ice and the effects of different properties on sea ice biota. In: U Bleil, J Thiede, eds., Geological History of the Polar Oceans: Arctic Versus Antarctic, Kluwers Academic Publishers, Dordrecht, 173–186

Soudant P, Moal J, Marty Y, Samain JF (1996) Impact of the quality of dietary fatty acids on metabolism and the composition of polar lipid classes in female gonads of *Pecten maximus* (L.). J Exp Mar Biol Ecol 205: 149–163

Soudant P, Moal J, Marty Y, Samain JF (1997) Composition of polar lipid classes in male gonads of *Pecten maximus* (L.). Effect of nutrition. J Exp Mar Biol Ecol 215: 103–114

St. John MA, Lund T (1996) Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. Mar Ecol Prog Ser 131: 75–85

Stretch JJ, Hamner PP, Hamner WM, Michel WC, Cook J, Sullivan CW (1988) Foraging behavior of Antarctic krill *Euphausia superba* on sea ice microalgae. Mar Ecol Prog Ser 44: 131–139

Stryer L (1999) Biochemie. Spektrum, Heidelberg, 1125 pp

Torres JJ, Aarset AV, Donnelly J, Hopkins TL, Lancraft TM, Ainley DG (1994a) Metabolism of Antarctic micronektonic Crustacea as a function of depth of occurrence and season. Mar Ecol Prog Ser 113: 207–219

Torres JJ, Donnelly J, Hopkins TL, Lancraft TM, Aarset AV, Ainley DG (1994b) Proximate composition and overwintering strategies of Antarctic micronektonic Crustacea. Mar Ecol Prog Ser 113: 221–232

Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Verh Int Limnol 9: 1–38

Virtue P, Nichols PD, Nicol S, McMinn A, Sikes EL (1993a) The lipid composition of *Euphausia superba* Dana in relation to the nutritional value of *Phaeocystis pouchetii* (Hariot) Lagerheim. Antarct Sci 5: 169–177

Virtue P, Nicol S, Nichols PD (1993b) Changes in the digestive gland of *Euphausia superba* during short-term starvation: lipid class, fatty acid and sterol content and composition. Mar Biol 117: 441–448

Virtue P, Nichols PD, Nicol S, Hosie G (1996) Reproductive trade-off in male Antarctic krill, *Euphausia superba*. Mar Biol 126: 521–527

Virtue P, Nichols PD, Nicol S (1997) Dietary-related mechanisms of survival of *Euphausia superba*: biochemical changes during long-term starvation and bacteria as a possible source of nutrition. In: B Battaglia, J Valencia, DWH Walton, eds., Antarctic Communities: Species, Structure and Survival, Cambridge University Press, Cambridge, 193–201

Virtue P, Mayzaud P, Albessard E, Nichols P (2000) Use of fatty acids as dietary indicators in northern krill, *Meganyctiphanes norvegica*, from northeastern Atlantic, Kattegat, and Mediterranean waters. Can J Fish Aquat Sci 57: 104–114

Volkman JK, Everitt DA, Allen DI (1986) Some analyses of lipid classes in marine organisms, sediments and seawater using thin-layer chromatography-flame ionisation detection. J Chromatogr 356: 147–162

Volkman JK, Jeffrey SW, Nichols PD, Rogers GI, Garland CD (1989) Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J Exp Mar Biol Ecol 128: 219–240

Zane L, Patarnello T (2000) Krill: a possible model for investigating the effects of ocean currents on the genetic structure of a pelagic invertebrate. Can J Fish Aquat Sci 57: 16–23

Chapter 1

On the use of lipid biomarkers in marine food web analyses: An experimental case study on the Antarctic krill, *Euphausia superba*

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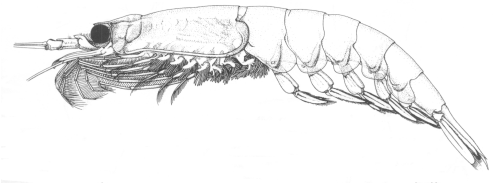

Euphausia superba adult male (from Baker et al. 1990)

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On the use of lipid biomarkers in marine food web analyses: An experimental case study on the Antarctic krill, *Euphausia superba*

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Abstract

The application of marker fatty acids to trace the feeding habits of *Euphausia superba* (krill) has produced contradictory results. We examined the effects of various diets on the fatty acid composition of larval, juvenile, and adult *E. superba* collected in April 1999 in the southwest Lazarev Sea and in April 2001 in the Bellingshausen Sea. Specimens were fed four different diets (mixed phytoplankton, mixed ice algae, the ice diatom *Fragilariopsis cylindrus,* and mixed copepod assemblages) or starved for up to 44 d. Total lipid content, lipid classes, and fatty acid composition showed very little variation in juvenile and adult krill with the different feeding regimes. Furcilia lipids were much more strongly influenced by the fatty acid signatures of their food. No stage-specific food preferences were detected in the larvae, and spatial patterns were mirrored by all furcilia stages. Comparison of the fatty acid profiles of the offered food with those of the subsequently excreted feces indicated preferential assimilation of polyunsaturated fatty acids by *E. superba.*

The analysis of lipid compositions has been applied successfully to reveal food web relationships in marine ecosystems. This trophic biomarker concept is based on observations that specific dietary lipid components, particularly fatty acids, are incorporated into the consumers' lipids largely unmodified (Sargent and Whittle 1981; Sargent et al. 1987; Graeve et al. 1994*b*). This approach can provide information where the classical gut content analysis fails (e.g., soft-bodied organisms, advanced digestion). Instead of a snap-shot impression, biomarkers integrate the trophic information over a longer time scale of several weeks. However, lipid signatures usually do not have the precision to identify species-specific interactions. Rather, they provide trophic information on the level of larger taxonomic groups.

Experimental studies have further consolidated the trophic biomarker approach. Clear changes in fatty acid compositions could be induced by different phytoplankton diets in Arctic copepods (Graeve et al. 1994*b*) and even traced up to secondary consumers, such as juvenile North Sea cod (St. John and Lund 1996). In recent years, an increasing number of studies have applied this method to identify trophic relationships in various marine ecosystems: among benthic species and communities (e.g., Graeve et al. 1997), in a variety of Arctic and Antarctic zooplankton groups (e.g., Scott et al. 1999; Falk-Petersen et al. 2002), and most extensively in polar copepods (e.g., Graeve et al. 1994*a*, Kattner and Hagen 1995; Scott et al. 1999) and euphausiids (Hagen and Kattner 1998; Kattner and Hagen 1998; Phleger et al. 1998; Virtue et al. 2000; *see* also Falk-Petersen et al. 2000 for review).

In Antarctic krill, *Euphausia superba,* investigations of feeding habits via fatty acid compositions produced contradictory results. Virtue et al. (1993*a*) found significant differences between adult krill fed on diatoms versus the flagellate *Phaeocystis*. Another feeding experiment with copepods yielded a strong increase in polyunsaturated fatty acids as compared to field krill (Cripps and Atkinson 2000). In other investigations, this approach did not provide such clear results. Cripps and Hill (1998) found that the fatty acid compositions of copepods clearly reflected dietary preferences and changing food availability, but there were no such relationships detectable for *E. superba.* Based on a multiseasonal data set, Hagen et al. (2001) concluded that the influence of dietary fatty acids on the lipid composition of *E. superba* is rather small. On the other hand, numerous studies traced feeding habits in *E. superba* with the biomarker approach (Virtue et al. 1997; Cripps et al. 1999; Cripps and Atkinson 2000; Falk-Petersen et al. 2000; Phleger et al. 2002).

Therefore, one major objective of this study was to clarify the applicability of fatty acids as trophic markers for *E. superba* and discuss the reasons for possible limitations in various ontogenetic stages. This study presents a comprehensive experimental approach to evaluate the influence of dietary fatty acids on the lipid composition of *E. superba.* Females, juveniles, and furciliae were caught in two autumn seasons

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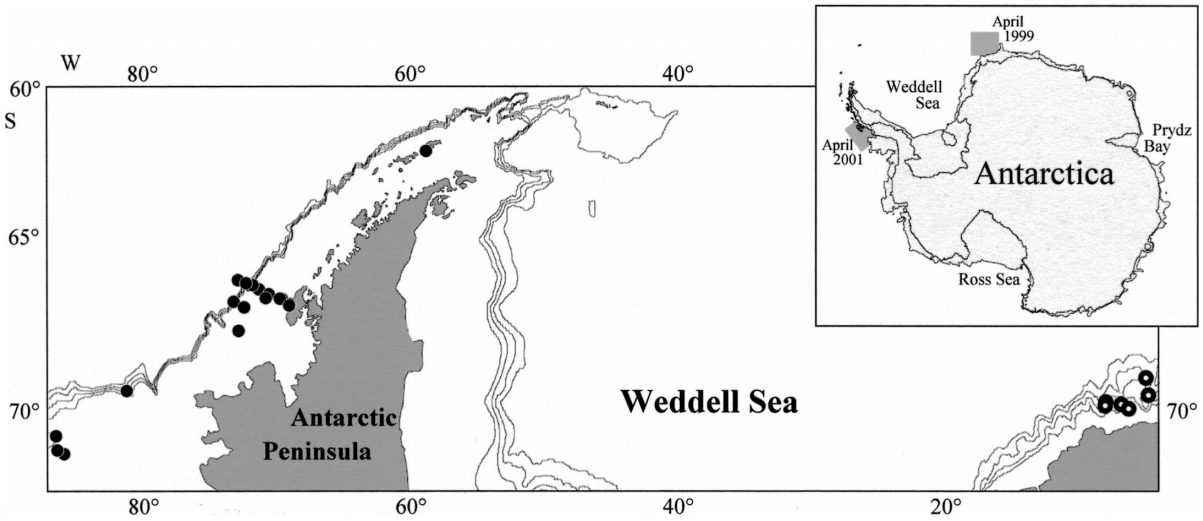

Fig. 1. Map of the investigation areas. Open circles mark stations sampled in the southwest Lazarev Sea in autumn 1999; filled circles indicate stations in the Bellingshausen Sea in autumn 2001.

and fed controlled diets (mixed phytoplankton, ice algae, or copepods) or starved for up to 44 d. Experimental data were compared with those of field samples, and the results are discussed with regard to lipid metabolism and ontogenetic differences.

Methods

Sampling—E. superba specimens were sampled during two autumn cruises with RV *Polarstern* (14–20 April 1999, open circles, and 18 April–1 May 2001, filled circles, Fig. 1) on transects across the shelf break in the southwestern Lazarev Sea and in the Bellingshausen Sea, respectively. In 1999, slow vertical bongo net tows (335-μm mesh, 5-liter closed cod end) were carried out in the top 150 m at night. In 2001, zooplankton was sampled by double oblique rectangular midwater trawl (RMT 1 + 8) hauls (mesh size 325 and 4,500 μm, 20-liter closed cod end), vertical bongo net tows, or a hand-hauled Apstein net. Zooplankton was thus obtained in excellent condition and immediately transferred to the cool lab for sorting. After a $\pm$24-h defecation period in 1-μm-filtered seawater *E. superba* specimens were either frozen at -80°C or transferred to the experimental containers. Prior to freezing, developmental/maturity stage, sex, and length (tip of rostrum to end of telson) were recorded, and the animals were briefly rinsed with deionized water and blotted dry. Samples of juveniles and adults represent individual specimens, whereas 5–95 furciliae were pooled for each sample, depending on size and availability.

Experiments—Adult and juvenile *E. superba* were maintained in aerated 170-liter tanks and furcilia larvae in 18-liter containers. The experiments were carried out in a cold room (0–2°C) in dim light. Three batches of about 50 mixed juvenile and adult krill were incubated with freshly caught copepods or sea ice biota or were starved in 1-μm-filtered seawater. The treatments for furciliae (about 200 per batch) were starvation or a diet of ice biota, a monoalgal culture of the common Antarctic ice diatom *Fragilariopsis cylindrus*, or mixed phytoplankton. Ice biota were obtained by slowly thawing brown pieces of sea ice in at least a threefold volume of filtered seawater and subsequently screening through a 55-μm sieve. Copepods were picked out daily from bongo net tows, excluding damaged animals and large carnivorous species such as *Pareuchaeta* spp. Thus, the copepod diet consisted of varying species compositions dominated at first by the more southern species *Calanus propinquus* and *Calanoides acutus* and later by *Calanus simillimus* and *Metridia gerlachei*. Both the mixed phytoplankton and the ice biota were dominated by diatoms: the first by *Fragilariopsis*, *Chaetoceros*, and *Pseudonitzschia* species; the latter also by *Fragilariopsis* spp. and *Chaetoceros* spp. as well as by small resting spores or cysts. The food concentrations approximated 480 $\pm$ 220 μg C L^{-1} for copepods, 105 $\pm$ 35 μg C L^{-1} for phytoplankton, 350 $\pm$ 120 (furciliae) or 220 $\pm$ 80 μg C L^{-1} (juveniles and adults) for ice algae, and 110 $\pm$ 40 μg C L^{-1} for *F. cylindrus*. Every 48 h, animals were transferred to a new batch of food or filtered seawater. Animals in poor condition, as well as fecal strings, were removed and frozen in dichloromethane/methanol under a nitrogen atmosphere at -80°C. Food uptake was monitored by chlorophyll measurements and counting of the phytoplankton cells and the copepods. Subsamples of the food were frozen in dichloromethane/methanol for fatty acid analyses.

Krill from these experiments were used either for lipid analyses (present study) or for stable isotope measurements (Schmidt et al. 2003).

Lipid analyses—After lyophilization for 48 h, the samples were weighed and total lipid was extracted with dichloro-

methane/methanol (DCM/MeOH) (2 : 1 [v/v] + 0.01% butylhydroxytoluene [BHT] as antioxidant) and determined gravimetrically (Hagen 2000). In order to obtain a correction value for the BHT, 10 blank aliquots of extraction solvent were treated the same way as the samples, and the mean blank mass (=0.33 mg) was subtracted from the total lipid mass of each sample. Because of the interference of BHT with sterol esters (*see below*), it was not added as antioxidant to the 2001 samples.

Neutral lipid classes were analyzed in duplicate by thin-layer chromatography (TLC)–flame ionization detection (FID) on an Iatroscan Mk V according to Fraser et al. (1985). Because different lipid classes give different FID responses, two mixtures of commercial standards (Sigma) that approximated the lipid class compositions of the analyzed samples were prepared for calibration: phosphatidylcholine : triolein : cholesterol : cholesteryl oleate : oleic acid at 49 : 35 : 8 : 5 : 3 (v/v) for juveniles and adults and 64 : 25 : 6 : 4 : 1 (v/v) for furciliae. Because BHT co-runs with sterol esters, a dilution series of the BHT blanks was analyzed by Iatroscan, and the sterol ester amounts of the 1999 samples were corrected by the appropriate values.

Because the separation of polar lipids remained unsatisfactory by TLC-FID, the phospholipid composition was determined by high-performance (HP) TLC-scanning densitometry (modified after Olsen and Henderson 1989). Five microliters of the total lipid extracts was applied by means of a CAMAG Linomat IV in duplicate or triplicate on predeveloped HPTLC plates (silica gel 60, Merck). The plates were developed in a horizontal chamber with isopropanol : methylacetate : chloroform : methanol : 0.25% KCl (25 : 25 : 25 : 10 : 9, v/v) for 17 min and dried for 30 min in an evacuated desiccator. The plates were then immersed for 5 s in a postchromatographic derivatization reagent with a CAMAG Chromatogram Immersion Device III and charred at 200°C for 20 min. The derivatization reagent was prepared by dissolving 1.2 mg manganese(II)-chloride in 180 ml of Aqua bidest and adding 180 ml methanol and 12 ml concentrated sulfuric acid. The lipid bands were quantified with a CAMAG TLC-Scanner 3 at 550 nm wavelength and calibrated using commercial standards for each detected lipid class.

For the fatty acid analysis of single lipid classes, aliquots of the total lipid extracts were spotted on self-coated glass plates (silica gel Merck H60, film thickness 750 μm) by a CAMAG Linomat IV. In order to keep the run time as short as possible, neutral and polar lipids were developed separately. The developing solvent was evaporated with nitrogen, and the lipid bands were visualized by iodine vapor. The bands were scraped off with a Teflon spatula and extracted according to the total lipid extraction. The purity of the isolated lipid classes was verified by TLC. Lipids were hydrolyzed, and the fatty acids were converted to their methyl ester derivatives (FAME) in methanol containing 3% concentrated sulfuric acid at 80°C for 4 h (Kattner and Fricke 1986). After cooling, 4 ml of Aqua bidest. were added, and FAMEs were extracted with hexane (3 × 1.7 ml), analyzed in a gas chromatograph (HP 6890A) equipped with a DB-FFAP column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness) using temperature programming and helium as the carrier gas. FAMEs were detected by flame ionization and identified by comparing retention time data with those obtained from standard mixtures.

Statistics—Analysis of variance was performed to detect significant differences between the means of the different experimental treatments. Using the SPSS software package for Macintosh, a one-way ANOVA was calculated and the Games–Howell post hoc test was applied for multiple comparisons.

Multivariate statistical analyses were applied to the percent fatty acid composition of the total lipids of *E. superba.* They were carried out with the software ''Primer.'' The Bray–Curtis index was used to calculate a similarity matrix, which was the basis for the cluster analysis (hierarchical agglomerative, group average linkage).

Results

Dry mass and total lipid content—Length, dry mass, and total lipid content of the *E. superba* used in the feeding/starvation experiments are given in Table 1. There was no optimal random distribution of the krill specimens to the various treatments; that is, the body length varied between treatments, with the largest animals in the field group representing time zero in the experiments. Accordingly, body mass and total lipid content also showed pronounced variations. In order to allow comparisons between the different treatments irrespective of length-dependent variations, values were calculated for standardized specimens of a certain length. For this purpose, regressions of dry mass and lipid mass versus total length were calculated separately for each time point of each experiment. Dry mass and lipid mass, respectively, of a medium-sized animal (females 44 mm, juveniles 31 mm) were then derived from these regressions. Figure 2 shows the development of dry mass and total lipid for such hypothetical animals in the different treatments. Dry mass and total lipid decreased during the experiments, indicating that the krill were not doing well in our experiments. Female dry mass decreased most under starvation conditions and least on the ice algae diet (Fig. 2a). Total lipid showed the reverse, with the strongest initial decline in individuals fed on ice algae (Fig. 2b). Although the dry mass continued to drop, lipid mass increased slightly toward the end of the ice algae experiment after 44 d.

Whereas the dry mass of females steadily decreased in the course of all experiments, this was only true for the starving juveniles. After an initial loss, juveniles feeding on copepods or ice algae increased again in body mass (Fig. 2c), almost entirely because of lipid accumulation (Fig. 2d).

Because of experimental constraints (a limited number of larvae per treatment, only 5–9 individuals could be frozen in DCM/MeOH), dry mass data were not available for the furcilia experiments from April 1999. Dry mass and total lipid mass strongly increased in furciliae feeding on the Antarctic ice diatom *F. cylindrus* (Fig. 2e,f). Although the slope of dry mass increase was steeper during the first 10 d of feeding, total lipid increased more rapidly between days 10 and 17, indicating that somatic growth is fueled first before substantial lipid accumulation occurs.

Table 1. *Euphausia superba.* Standard length (SL), dry mass (DM), and total lipid (TL), absolute and percent dry mass, of females, juveniles, and furciliae used in the different treatments. *n*, number of samples; n.d., not determined.

	Females					Juveniles					Furciliae				
	SL (mm)	DM (mg)	TL (mg)	TL (% DM)	*n*	SL (mm)	DM (mg)	TL (mg)	TL (% DM)	*n*	Stage	DM (mg)	TL (mg)	TL (% DM)	*n*
Field	50±2	208.9±27.4	74.0±18.5	35.1±5.8	11	33±3	60.5±15.5	22.8±7.3	34.7±5.4	19	FIII	0.47±0.07	0.07±0.02	13.8±2.6	8
Copepods	47±3	184.4±39.1	66.5±19.6	35.5±4.4	6	32±2	57.1±10.0	21.5±4.7	37.5±2.6	9	—	—	—	—	—
Ice algae	46±2	148.3±34.7	59.4±28.5	35.5±10.0	8	31±1	46.7±3.6	16.3±2.3	34.8±3.1	5	FIII	n.d.	0.06±0.02	n.d.	4
F. cylindrus	—	—	—	—	—	—	—	—	—	—	FIII–V	0.99±0.21	0.15±0.05	14.8±2.5	3
Phytoplankton	—	—	—	—	—	—	—	—	—	—	FIV–V	n.d.	0.17±0.04	n.d.	7
Starvation	45±3	144.7±14.3	53.1±2.7	37.0±3.0	3	31±2	46.6±15.1	16.4±7.3	34.2±5.1	10	FIII	n.d.	0.06±0.02	n.d.	3

After 19 d, the furciliae feeding on a diet of mixed ice biota and those under starvation did not show any significant changes in their lipid content, whereas the phytoplankton-fed animals showed a significant ($p \leq 0.001$) increase in lipid mass (Fig. 2f). Concomitantly, these furciliae continued to grow and molt, reaching stage IV to V at the end of the experiment after 44 d (Table 1).

Lipid class composition—Lipid class compositions were very uniform for females and juveniles and did not show any food-related differences. The variability of individuals from the same treatment was within the same range as for individuals from different treatments. Therefore, the means were averaged over all experiments (Fig. 3). Triacylglycerol (TAG) was the major lipid class with 20% of dry mass (DM) in juveniles and slightly less in females. In furcilia III–IV larvae, the proportion of TAG was <5% DM. Their lipids were dominated by phosphatidylcholine (PC) with >7% DM, which is equivalent to 47% of total lipids. The second prominent phospholipid, phosphatidylethanolamine (PE), was present in fairly equal levels of ~3% DM in all stages.

Fatty acid composition—Juveniles and adults: As for the lipid class compositions, the fatty acid compositions also did not show major differences between the experimental treatments (Table 2). This might be partly because of the similarity of the algal diets used in the feeding experiments, which all showed clear diatom fatty acid signals. However, the proportion of the essential polyunsaturated fatty acid (PUFA) 20:5(n-3) is lowest in the mixed phytoplankton and highest in the pure ice diatom culture (Fig. 8). Accordingly, one objective of our study was to find out whether it is possible to discriminate between feeding on phytoplankton or ice diatoms by applying fatty acid analyses (or stable isotope analyses, Schmidt et al. 2003).

The lipids of both juveniles and females were dominated by the fatty acids 16:0 and 20:5(n-3), followed by 18:1(n-9) and 14:0 with similar proportions of 12–16% of total fatty acids (TFA). Fatty acids 22:6(n-3) and 18:1(n-7) ranked next with 6–8% TFA. For females, only one significant difference could be detected: 22:1(n-9), one of the marker fatty acids for calanid copepods (e.g., Kattner and Hagen 1995), exhibited a significant increase in individuals feeding on copepods, as compared to the field samples. For juveniles, both 22:1 isomers reached significantly higher levels in the copepods treatment than in the field animals and the other treatments. Juveniles feeding on ice algae showed significantly higher proportions of 18:1(n-9) accompanied by lower levels of 20:5(n-3), as compared to field samples or animals feeding on mixed phytoplankton or starving ($p \leq 0.05$). This result is contrary to the expected changes because 20:5(n-3), with up to 33% TFA, is by far the dominant fatty acid of the ice algae, and 18:1(n-9) is only a minor constituent (Fig. 8).

Dietary influences on the fatty acid composition of total lipid extracts can be masked by the presence of structural lipids, which are supposed to have a relatively stable fatty acid profile (Sargent et al. 1987). Because there was no clear response observable for the total lipids from postlarval krill, particularly females, of the various feeding regimes (Table

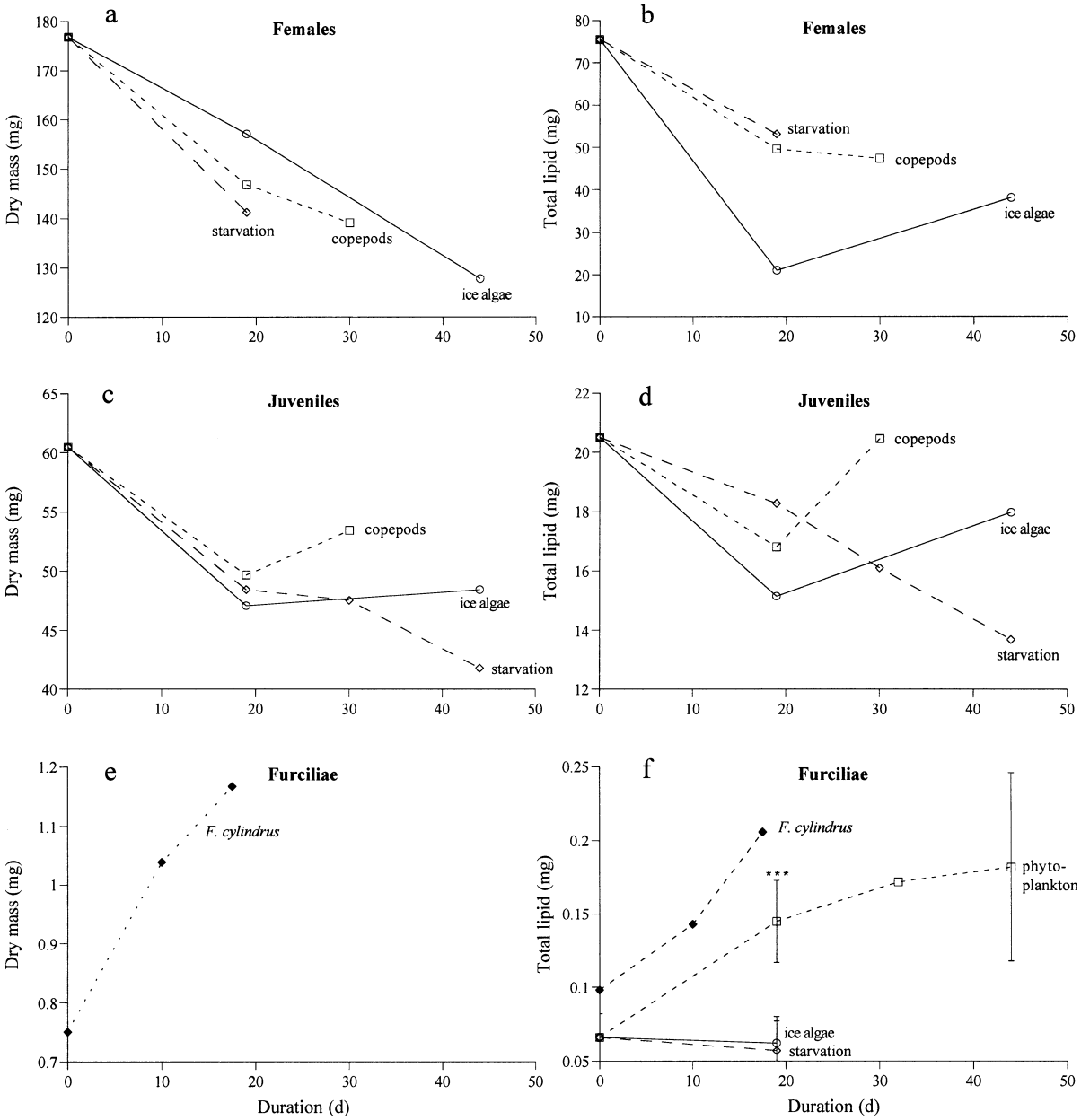

Fig. 2. *Euphausia superba*. (a,b) Development of dry mass and total lipid mass during the feeding/starvation experiments for a standardized female of 44 mm body length, (b,c) a standardized juvenile of 31 mm, and (e,f) for furciliae (not standardized). The significant increase in total lipid of furciliae feeding for 19 d on phytoplankton is *** $p \leq 0.001$.

2), the fatty acid compositions were analyzed separately for the dominant lipid classes. This is based on the assumption that the fatty acid composition is characteristic for each lipid class and that dietary influences are primarily mirrored in the storage lipids. Because TAG is the primary storage lipid of *E. superba* and phosphatidylcholine (PC) serves, besides its structural function in membranes, as an additional energy source (Hagen et al. 1996, 2001), the fatty acid composition of these two lipid classes should best reflect the animals' feeding habits. Figure 4 compares the dominant fatty acids of the main lipid classes TAG, PC, and phosphatidylethanolamine (PE). In contrast to the hypothesis, the fatty acid

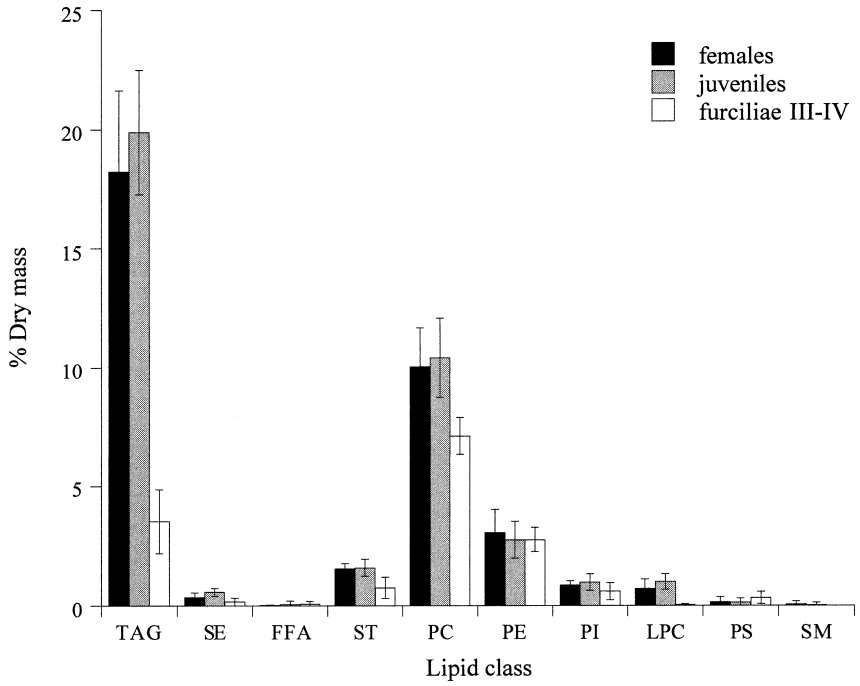

Fig. 3. *Euphausia superba*. Lipid class composition (means and standard deviations) of females, juveniles, and furciliae averaged over all treatments. SE, sterol ester; TAG, triacylglycerol; FFA, free fatty acids; ST, sterol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; LPC, lysophosphatidylcholine; SM, sphingomyelin.

compositions of TAG and PC were quite uniform and showed only very little variation with the different feeding regimes, both in juveniles and females. PE however, which is purely a membrane lipid and should thus be stable, was found to have the strongest variability, although not correlated with the typical dietary fatty acids.

Furciliae: Whereas no clear changes in the fatty acid profiles of juvenile and female krill could be induced by different feeding regimes, there was a higher variability exhibited by the furciliae. As Fig. 2f shows, from the 1999 experiments, the phytoplankton-fed furciliae should be most interesting because they were the only ones that obviously fed and grew during the experimental period. Compared to the field samples, their lipids increased significantly in the diatom marker 16 : 1(n-7) and in 18 : 1(n-7) (Table 2), the latter probably resulting from chain elongation of the former fatty acid. However, the portion of 20 : 5(n-3), the second diatom marker, decreased by almost 5%. This can be explained by the comparatively moderate levels of this long-chain PUFA in the lipids of the mixed phytoplankton culture (~18% TFA) compared to the ice algae (Fig. 8). However, the other two treatments also had observable changes in the fatty acid composition, although the total lipid content did not change significantly (Fig. 2f). The most pronounced changes concerned the two 18 : 1 isomers: whereas the (n-9) isomer decreased significantly, the (n-7) moiety showed a significant increase during 19 d of feeding on ice algae. Significant decreases compared to the field and the phytoplankton-fed individuals also were observed for the flagellate marker 18 : 4(n-3). This decrease was even stronger during starvation. Ice algae were the only diet which did not induce a decline in the proportion of 20 : 5(n-3), indicating that the furciliae had been feeding on a diet rich in this PUFA (i.e., ice algae) in the field.

In 2001, a large number of furciliae could be sampled at different locations, and varying fatty acid compositions could be related to different feeding histories. For the later furcilia stages (III–VI), multivariate statistics were applied on the percent compositions of all identified fatty acids of the total lipids to detect similarities. Three groups were identified by cluster analysis (Fig. 5a). Although most of the larvae showed fatty acid profiles typical of feeding on diatoms (Table 2; Fig. 5b), there were clear deviations toward a more flagellate-dominated diet at two stations. Group I comprises animals from station 301, the northernmost offshore station off Adelaide Island (Fig. 1). They were characterized by a relatively low lipid content of 12.0 ± 2.4% of dry mass (DM) and accordingly high levels of PUFAs (Table 2). The ratios 16 : 1(n-7)/18 : 4(n-3) and 20 : 5(n-3)/22 : 6(n-3) (=EPA/DHA) were intermediate and did not point to a diatom- or a flagellate-dominated diet. Group II represents furciliae IV–VI with a mean lipid content of about 20% DM and the lowest PUFA levels. They were caught on station

Table 2. *Euphausia superba*. Percent composition (means ± SD) of the major fatty acids (>0.5% of total fatty acids) of females, juveniles, and furciliae (FIII–FVI) from the different treatments. Significant differences between the means of the treatments are * $p \leq 0.05$, ** $p \leq 0.01$), or *** $p \leq 0.001$. Nonsignificant differences were left blank. Field/f, field krill; Ice/i, krill feeding on ice algae; Cops/c, krill feeding on copepods; Ppl/p, krill feeding on phytoplankton; Starv/s, starved krill. Numbers in parentheses denote sample size (*n*).

	Females 1999					Juveniles 1999									
	Field (3)	Ice (3)	Cops (6)	Starv (3)	ANOVA f:c	Field (3)	Ice (2)	Cops (6)	Starv (6)	ANOVA f:i	f:c	f:s	i:c	i:s	c:s
14:0	12.6±0.1	11.5±0.4	11.8±0.6	13.3±1.1		12.3±0.8	13.0±1.2	12.0±1.3	13.0±0.6						
16:0	21.6±0.3	21.4±1.3	19.4±2.1	18.9±3.4		21.0±0.8	23.2±1.5	19.9±1.6	19.6±2.8						
18:0	1.1±0.3	1.3±0.1	1.1±0.2	0.9±0.2		1.8±0.3	1.7±0.2	1.4±0.2	1.4±0.2						
16:1(n-7)	7.6±0.6	8.1±0.5	8.9±1.8	8.8±1.4		7.7±1.2	8.1±0.7	7.5±1.2	7.8±1.1						
18:1(n-9)	12.1±1.5	14.7±2.8	13.7±3.0	12.3±0.2		12.0±1.2	16.2±0.6	11.5±2.3	12.1±2.0	*			*	*	
18:1(n-7)	8.2±0.8	7.1±0.9	7.2±0.8	7.9±1.4		6.1±0.8	5.9±0.0	5.9±0.4	6.7±0.5						
20:1(n-9)	1.2±0.1	1.5±0.4	1.4±0.3	1.2±0.1		1.1±0.3	1.3±0.1	1.0±0.2	0.9±0.1						
22:1(n-11)	0.0±0.0	0.1±0.0	0.7±0.7	0.0±0.0		0.1±0.1	0.1±0.0	1.4±0.7	0.1±0.3		*		*		*
22:1(n-9)	0.7±0.1	1.0±0.2	1.2±0.3	0.8±0.2	*	0.6±0.2	0.8±0.0	1.2±0.3	0.5±0.2		*				**
16:2(n-4)	1.0±0.1	1.1±0.1	1.6±0.5	1.8±0.4		1.1±0.1	1.4±0.1	1.5±0.1	1.7±0.2						
18:2(n-6)	2.7±0.2	2.0±0.4	2.1±0.5	2.4±0.6		2.3±0.3	2.2±0.1	2.4±0.4	2.7±0.3						
16:4(n-1)	0.4±0.1	0.3±0.1	0.5±0.2	0.5±0.2		0.8±0.2	0.5±0.2	0.7±0.2	0.8±0.2						
18:4(n-3)	1.5±0.2	2.4±1.1	2.0±0.5	1.4±0.6		3.0±1.3	2.8±0.7	2.1±0.5	2.0±0.6						
20:4(n-6)	0.7±0.1	0.6±0.2	0.7±0.1	0.8±0.3		0.7±0.2	0.6±0.1	0.7±0.1	0.8±0.1						
20:5(n-3)	15.4±1.1	13.5±1.2	14.8±0.5	15.3±0.7		15.3±0.9	12.6±0.1	14.7±1.3	14.5±1.2	*			*	*	
22:6(n-3)	7.1±1.0	8.0±1.1	7.7±1.7	7.2±1.4		7.5±1.5	5.9±0.9	8.0±1.6	7.2±1.0						

	Furciliae 1999										Furciliae 2001					
	Field (4)	Ice (3)	Ppl (6)	Starv (3)	ANOVA f:i	f:p	f:s	i:p	i:s	p:s	Group I (5)	Group II (4)	Group III (8)	ANOVA I:II	I:III	II:III
14:0	3.7±0.9	2.3±0.5	3.4±1.7	1.7±0.4			*				5.5±1.0	7.2±0.7	6.6±1.2	*		
16:0	17.2±0.1	16.3±0.4	15.8±1.0	18.0±1.2							15.1±0.2	16.0±0.9	16.9±1.6		*	
18:0	0.8±0.1	1.0±0.0	1.0±0.2	1.1±0.1	*		*				1.0±0.1	1.1±0.1	1.2±0.1		*	
16:1(n-7)	3.3±0.6	3.6±0.8	6.3±2.0	2.1±0.6		*				*	4.7±0.8	3.5±0.8	7.9±0.9		***	***
18:1(n-9)	7.1±0.2	5.4±0.2	6.5±0.5	6.7±0.2	***			**	**		5.0±0.6	5.4±0.9	5.8±1.1			
18:1(n-7)	5.5±0.1	6.4±0.1	7.6±0.9	6.6±0.2	***	**	*				5.6±0.1	4.4±0.7	5.8±0.8			*
20:1(n-9)	0.8±0.1	0.5±0.1	0.7±0.2	0.4±0.0			*				1.2±0.1	1.1±0.2	0.6±0.1		***	**
16:2(n-4)	1.2±0.3	1.3±0.2	1.5±0.5	0.5±0.2			*		*	*	1.5±0.4	3.2±0.3	3.0±0.3	***	***	
18:2(n-6)	2.5±0.1	2.3±0.1	2.2±0.2	2.2±0.0			*				2.6±0.2	1.2±0.1	1.6±0.2	***	***	**
16:4(n-1)	0.5±0.2	0.5±0.3	0.6±0.4	0.1±0.1							1.5±0.6	1.5±0.3	3.5±0.8		**	***
18:4(n-3)	2.1±0.4	0.6±0.3	1.2±0.7	0.3±0.1	**		**				4.3±0.8	11.7±1.5	1.5±0.4	***	***	***
20:4(n-6)	1.2±0.1	1.9±0.1	1.5±0.3	1.8±0.2	***		*	*			0.9±0.2	0.3±0.1	0.8±0.1		**	***
20:5(n-3)	27.0±0.6	26.5±1.2	22.9±1.2	22.3±1.0		***	*	*	*		25.7±1.5	18.5±0.6	29.9±1.8	***	**	***
22:6(n-3)	19.9±1.5	20.0±1.7	18.3±3.3	22.4±0.2							17.0±2.7	15.6±2.1	8.4±2.0		***	**

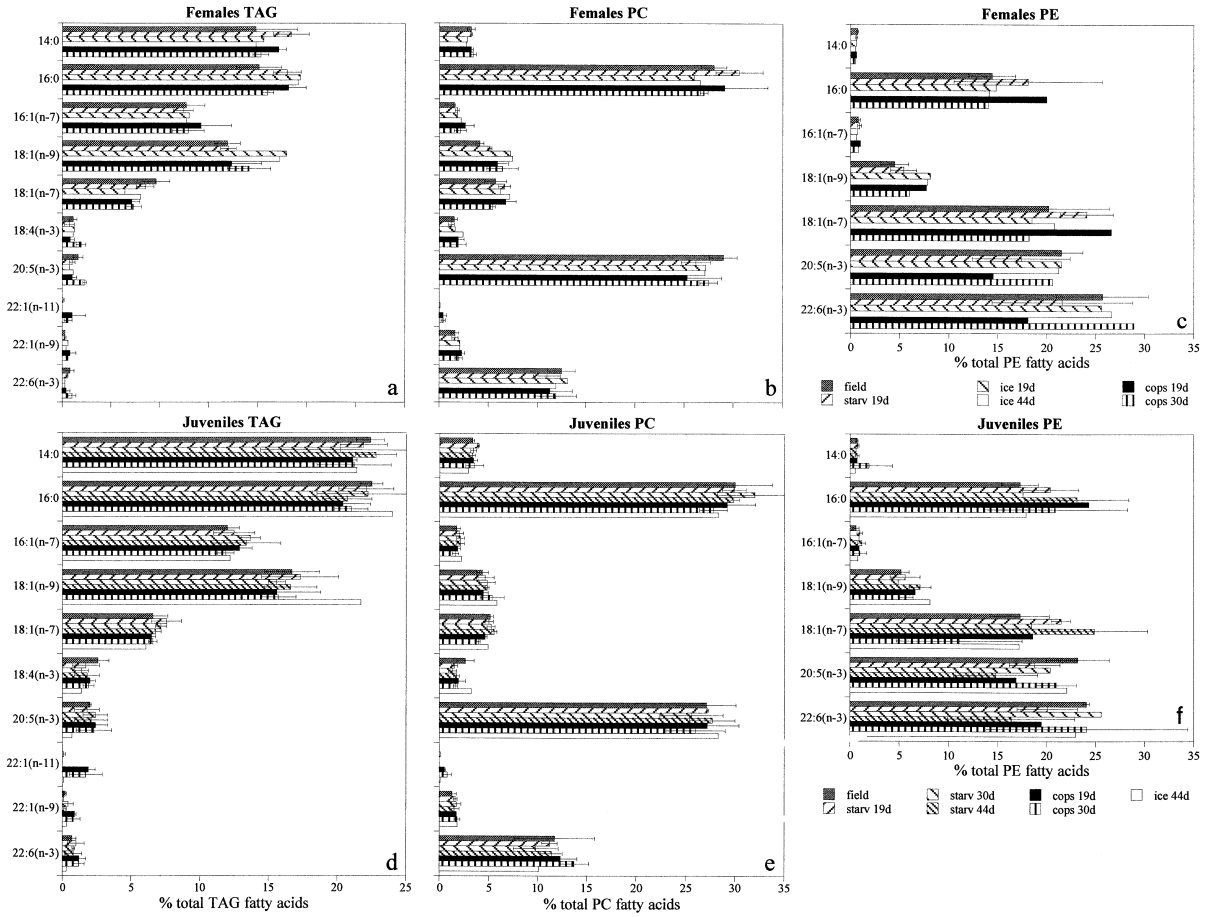

Fig. 4. *Euphausia superba*. Percentage fatty acid compositions of the three dominant lipid classes of (a–c) females and (d–f) juveniles compared for all experiments. TAG, triacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; starv, starvation; cops, feeding on copepods; ice, feeding on ice algae.

328, also on the northernmost transect, but further inshore and 2 weeks later than those from station 301. Their trophic indices were very low and clearly pointed to a flagellate-dominated diet. The last group is a mixture of larval stages III–V from different stations and from the feeding experiment with *F. cylindrus*. They share a high lipid content of 18.9 ± 4.6% DM and very high diatom/flagellate marker ratios.

In Fig. 5b, furciliae samples were plotted according to their ratios 16 : 1(n-7)/18 : 4(n-3) and EPA/DHA to discriminate between diatom (high values) versus flagellate feeding histories (low values). The same groups were identified by the multivariate analyses (Fig. 5a). The high variability in the diatom-feeding group is from differences in the total lipid content. The two samples with lower EPA/DHA ratios contained only 13.7 and 11.4% DM lipid compared to 21.0 ± 2.6% in the six samples with high ratios. The intermediate group was characterized by very low lipid content, and a 16 : 1(n-7)/18 : 4(n-3) ratio of about 1 points to a mixed diet consisting of diatoms and flagellates. In the third group, both ratios were very low, clearly indicating a flagellate-dominated diet.

In order to find out in which lipid class the flagellate marker fatty acids are accumulated, detailed analyses of the fatty acid compositions of the three main lipid classes (PC, PE, and TAG) were carried out for the four furcilia samples from station 328. Most of the flagellate marker 18 : 4(n-3) is accumulated in TAG (65%), with about one third in PC and only minor amounts in PE (Fig. 6). The second flagellate marker 22 : 6(n-3) is predominantly located in the phospholipids, especially in PC. Other typical TAG fatty acids are 14 : 0 and 16 : 2(n-4), which occur only in minor amounts in the phospholipids. Fatty acids 18 : 0 and 18 : 1(n-7) have the highest share in PE.

The feeding experiment with the Antarctic ice diatom *F. cylindrus* was carried out with furciliae caught at station 301 (group I, Table 2, Fig. 5). Their low lipid content and the weak diatom signal in their fatty acids represented an ideal

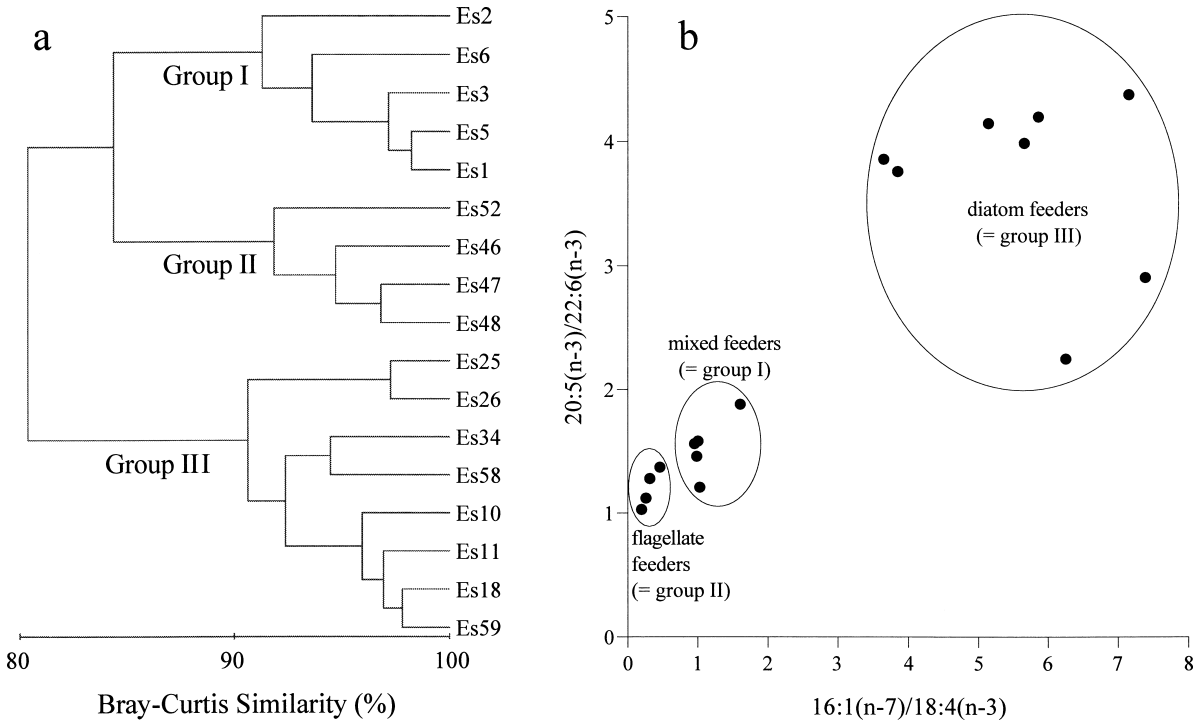

Fig. 5. *Euphausia superba.* Groupings of furciliae III–VI caught at different stations in 2001 (a) according to their percentage composition of the total fatty acids by cluster analysis and (b) according to their ratios of 20:5(n-3)/22:6(n-3) versus 16:1(n-7)/18:4(n-3).

background signature for this experiment. The development of the dominant fatty acids after 10 and 17 d of feeding is illustrated in Fig. 7. In the course of the experiment, the diatom markers 20:5(n-3) and 16:1(n-7), which are the major constituents of the lipids of *F. cylindrus* (Fig. 8b), increased markedly. The saturated short-chain fatty acids 14:0 and 16:0, as well as the 18:1 moieties, also showed a moderate increase in mass, but this did not cause a shift in the percent composition (Table 2). The absolute amount of 22:6(n-3) did not change throughout the experimental period, resulting in a relative decrease of over 50% from 15 to 7% TFA. Fatty acid 18:4(n-3) was largely depleted when feeding on *F. cylindrus*, which is poor in this fatty acid.

Biotransformation of fatty acids during gut passage— Comparisons of fatty acid compositions of food sources with those of the feces can help deduce whether specific dietary fatty acids are preferentially assimilated by *E. superba* (Fig. 8). Because the fatty acid composition of the prey copepods varied markedly with time, no means were calculated. Instead, the signature of the copepod assemblage, which had not been eaten by the end of the experiment, was compared with that of the fecal strings that were produced during the preceding 4 d. Whereas the short-chain saturated fatty acids 14:0 and 16:0 tended to be more abundant in the fecal strings of krill than in the prey copepods, the energy-rich long-chain polyunsaturated essential fatty acids 20:5(n-3) and 22:6(n-3) had been assimilated and occurred in low amounts in the feces. The two 22:1 isomers, typical marker fatty acids for calanid copepods, however, were apparently not selectively assimilated by the krill. They occurred in similar levels both in the prey copepods and in the feces (Fig. 8a). The lipids of both the mixed ice biota and the ice diatom *F. cylindrus* were clearly dominated by 20:5(n-3) followed by 16:1(n-7) (Fig. 8b,c). The fecal strings were depleted of both these marker fatty acids, as well as of the shorter chain polyunsaturated fatty acids 16:3(n-4) and 16:4(n-1). Again, the saturated moieties 16:0 and 18:0 were found in higher concentrations in the feces, and also 18:1(n-9) was not assimilated from the food. The lipids of the diatom-dominated phytoplankton mixture were also primarily composed of the typical diatom fatty acids, but with a more even distribution and a higher level of 16:0 (Fig. 8d). The essential fatty acids 20:5(n-3) and 22:6(n-3) had been extracted from the phytoplankton, resulting in a higher portion of short-chain saturated moieties in the fecal strings.

Discussion

The present study produced contradictory results on the potential of fatty acids as trophic biomarkers in krill. Whereas the fatty acid compositions of neither total lipids nor the storage lipid classes of female and juvenile *E. superba* were much influenced by different diets, those of larval krill were. The reasons for possible limitations of the biomarker approach and potential ontogenetic differences are outlined be-

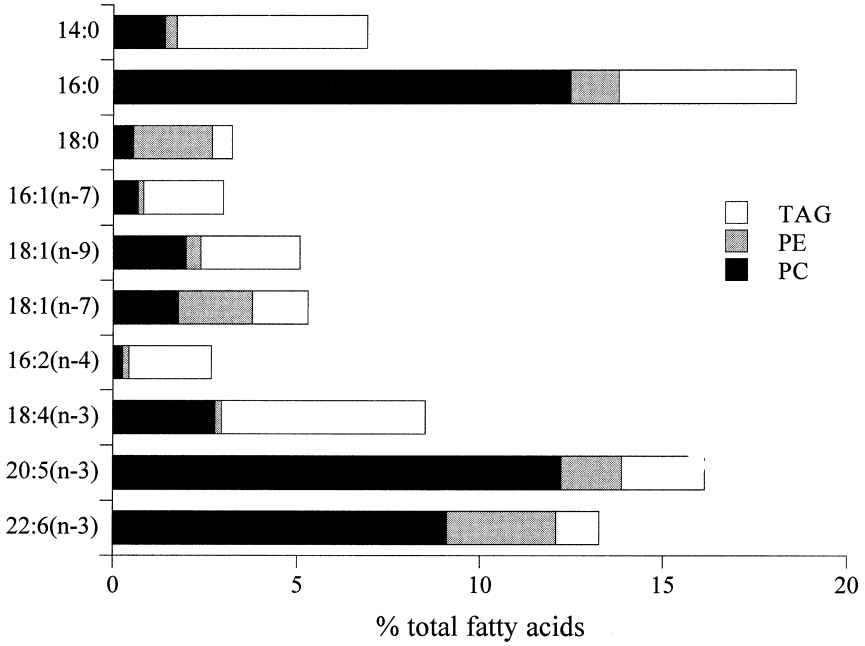

Fig. 6. *Euphausia superba*. Percentage contribution of the three main lipid classes to the total fatty acid composition of furciliae IV–VI from group II (*see Fig. 5*). TAG, triacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

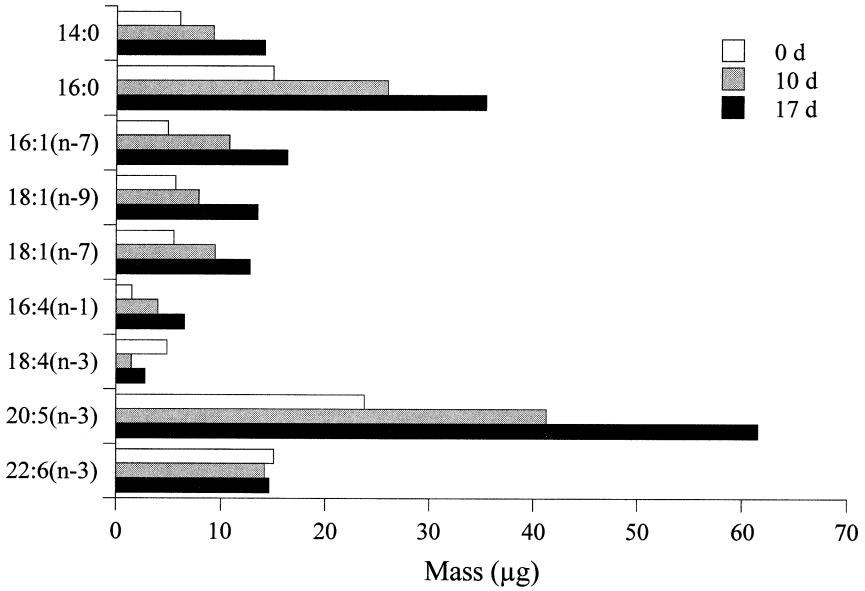

Fig. 7. *Euphausia superba*. Development of the main fatty acids of furciliae III–V feeding on *Fragilariopsis cylindrus* for up to 17 d.

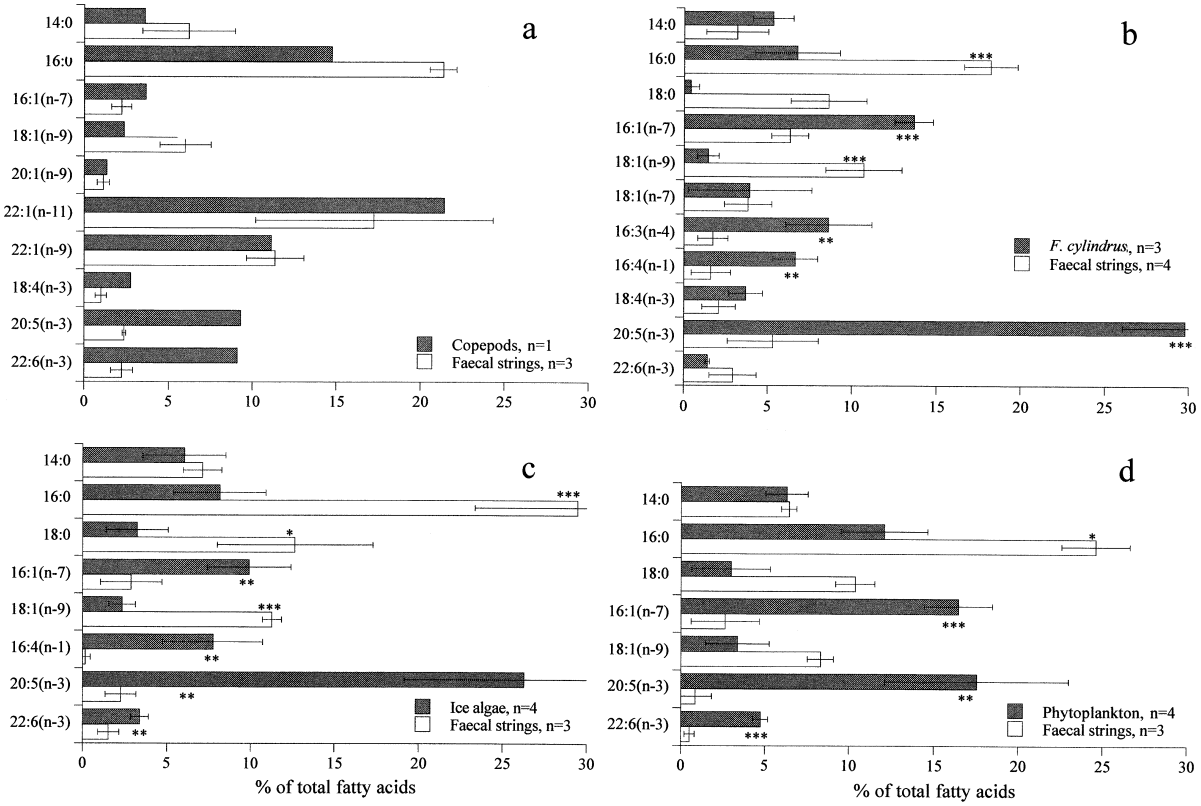

Fig. 8. *Euphausia superba.* Comparison of the main fatty acids from the different food sources with those of the feces. Significant differences between means are * $p \leq 0.05$, ** $p \leq 0.01$, or *** $p \leq 0.001$.

low and the specific marker fatty acids are discussed in terms of their respective usefulness for tracing feeding habits of the Antarctic krill.

Juvenile and adult krill—Our results are in line with the findings of a companion study on stable isotopes (Schmidt et al. 2003), which were measured in *E. superba* specimens from the same experiments as in the present study. Juvenile and adult *E. superba* did not significantly shift toward a heavier carbon (C) and nitrogen (N) signal when feeding on copepods or ice algae or when starving for 20 d. Only after 30 d had the juveniles from the two feeding regimes significantly increased in their δ^{15}N and δ^{13}C ratios. The poor response of postlarval krill, with regard to both lipid and stable isotope signals, to different diets can be attributed to various reasons. Although molting rates were higher than in starving krill (Schmidt et al. 2003) and fecal strings were produced, most of the experimental juveniles and adults from the 1999 treatments were apparently not feeding efficiently on the offered food, as indicated by their decreasing dry and lipid masses.

Parallel to our studies, Atkinson et al. (2002) have conducted feeding experiments with juvenile and adult *E. superba* sampled at the same location and time. They found very low feeding rates even after prolonged exposure to high food concentrations and a metabolic reduction of 60–80% compared to summer values. Thus, the juvenile and adult krill from our study were apparently already adjusting to winter conditions and did not efficiently use the food offered in the experiments. At the least, the diet was not utilized for further accumulation of storage lipid but rather for short-term energy requirements. This weakens the conclusions that can be drawn from our observed results of the feeding experiments.

However, there appears to be a generally low influence of dietary lipids on the fatty acid signatures of juvenile and adult krill, irrespective of feeding and metabolic activity. During a summer cruise to the South Shetland Islands (February 2000), adult krill were sampled at various stations, and POM fatty acids were compared to those of *E. superba.* Even from clearly deviating stations with strong diatom versus flagellate signatures, the krill lipids did not reflect these differences (data not shown). This is consistent with findings from Cripps and Hill (1998), who, along a transect from pack ice to the open ocean, investigated the effect of various food regimes on the fatty acid compositions of five Antarctic copepod species as well as *E. superba.* In contrast to the copepods, the fatty acid compositions of krill were essen-

tially the same at all stations. Hagen et al. (2001) drew similar conclusions from a multiseason data set, where the lipids of adult *E. superba* did not show pronounced dietary fatty acid signals. However, juveniles, to a certain extent, did reflect seasonal changes in algal composition, especially by variations of the marker fatty acid for flagellates, 18 : 4(n-3).

The comparatively large lipid reservoir of juvenile and adult krill seems to buffer short-term diet-induced variations. In addition, dietary lipids are probably modified to a larger degree; thus, a specific overall pattern is apparently maintained by the krill.

Larval krill—In contrast to the small effect of dietary lipids on the fatty acid compositions of juvenile and adult *E. superba*, furciliae lipids are markedly influenced by their food. Changes in fatty acid composition could be induced experimentally, by offering controlled diets, as well as detected in field samples from different locations. Trophic marker fatty acids were indicative of either diatom or flagellate feeding. Intermediate signatures combined with low lipid content demonstrated an opportunistic feeding behavior when overall phytoplankton biomass was presumably low.

The contrasting results between larval and postlarval krill are in line with findings by Hagen et al. (2001). Furciliae showed much stronger seasonal variations in the levels of phytoplankton marker fatty acids than adult krill. The same has been found for *Thysanoessa macrura*, another abundant Antarctic euphausiid (Hagen and Kattner 1998). The greater extent to which furciliae fatty acid compositions are influenced by dietary lipids could be due in part to their nonreduced feeding rates and metabolic activities in autumn (Meyer et al. 2002). It had already been shown that krill larvae do not enter dormancy, but continue to grow and feed during the winter (Daly 1990; Quetin and Ross 1991). The first winter is one of the most critical periods in the life cycle of *E. superba.* In contrast to adult and juvenile krill, the energy reserves of the furciliae are not sufficient to ensure survival during the long winter months without feeding.

However, irrespective of these activity differences, the low lipid levels facilitate the detection of dietary lipids. Future investigations must show whether these ontogenetic differences are due to different pathways of lipid metabolism (e.g., different degrees of modification of ingested fatty acids).

Trophic marker fatty acids—Marker fatty acids are a valuable tool for the identification of trophic relationships among polar plankton. It can provide information where the classical gut content analyses fail (e.g., soft-bodied organisms, advanced digestion) and integrates the trophic information over a longer time scale of several weeks. This approach has been applied in various studies on the trophic ecology of *E. superba* (Virtue et al. 1993*a*, 1997; Cripps and Hill 1998; Cripps et al. 1999; Cripps and Atkinson 2000; Phleger et al. 2002).

However, a lot of factors influence the fatty acid composition other than food (e.g., Sargent and Henderson 1995). Most dominant fatty acids were shown to clearly accumulate with increasing lipid levels, independent of the diet (Hagen et al. 2001). Therefore, fatty acids as trophic markers should be used with caution, and comparisons should only be made between specimens with a similar total lipid background.

In the following section we discuss the specific marker fatty acids for various plankton groups with regard to their applicability to trace feeding habits in *E. superba.* Measurements of preferential assimilation of specific fatty acids (i.e., comparison of the fatty acid compositions of the food with those of the feces) have helped to evaluate the actual usefulness of the taxon-characteristic fatty acids as trophic markers.

Diatoms: *E. superba* is still considered to feed primarily on phytoplankton (e.g., Mayzaud et al. 1998), although many recent studies illustrate the opportunistic feeding behavior of the Antarctic krill that use any kind of food available (e.g., Atkinson and Snÿder 1997; Perissinotto et al. 1997). Phytoplankton blooms in the Antarctic Ocean are principally dominated by diatoms (e.g., Clarke and Leakey 1996), although temporally, dinoflagellates and *Phaeocystis* can be abundant as well (Kang and Fryxell 1993; Clarke and Leakey 1996).

Diatoms are particularly rich in 16 : 1(n-7) and 20 : 5(n-3) (=EPA) (e.g., Nichols et al. 1993; Dunstan et al. 1994), and these fatty acids are considered trophic markers for this algal group (Sargent et al. 1987; Graeve et al. 1994*a*,*b*). Our comparative analyses of the diatom food with the subsequently excreted feces showed that both fatty acids were extracted from the food and thus confirmed their potential as trophic markers. However, autoxidation processes cannot be completely excluded. The highly unsaturated fatty acids are less stable than the saturates (SFAs) and monounsaturates (MUFAs). They are therefore more susceptible to oxidation, and their proportions would diminish in favor of the SFAs and MUFAs. However, the inconsistent behavior of similar fatty acids (e.g., the portions of 16 : 1[n-7] being always lower in the feces than in the food in contrast to 18 : 1[n-9]) suggests that chemical processes are an unlikely source of modification. To our knowledge, there is no quantitative study on the transformation of fatty acids during gut passage. Our conclusion is in accordance with findings by Olsen et al. (1991) that cod feces can contain significant amounts of polyunsaturated fatty acids (PUFAs).

EPA usually is one of the dominant fatty acids in the total lipids of *E. superba.* Like the second dominant essential long-chain PUFA 22 : 6(n-3), it is tightly conserved (i.e., losses via catabolism are low) (Sargent and Whittle 1981; Sargent and Henderson 1995). It is therefore only of limited use with regard to providing trophic information. In contrast, 16 : 1(n-7) can clearly be considered a trophic marker for diatoms because it is subjected to larger variations and can apparently be depleted when krill feed on other food sources. Although most marine animals also have the potential to synthesize 16 : 1(n-7) de novo, the strong uptake of 16 : 1(n-7) from the food (as demonstrated by its low abundance in the fecal strings) as well as its pronounced seasonal and regional variability in *E. superba* lipids (*see also* Hagen et al. 2001) confirm its suitability as a trophic marker in Antarctic krill.

In addition to 16 : 1(n-7), various C16 PUFAs also seemed to be preferentially assimilated from the food. However, their

proportions of *E. superba*'s total fatty acids remained rather small (<4% on average).

Despite clear differences in the fatty acid signatures of ice algae and phytoplankton (particularly of high levels of EPA in the former), the lipid biomarker approach is not sensitive enough to quantify the respective contributions of sympagic versus planktonic diatoms in the feeding history of *E. superba*.

Flagellates: The lipids of dinoflagellates usually contain high amounts of 18:4(n-3) and 22:6(n-3) (=DHA) (Sargent et al. 1987; Graeve et al. 1994*b*). Prymnesiophytes, of which *Phaeocystis* spp. can occur in high abundances in the Antarctic Ocean, and cryptomonads can be rich in these fatty acids as well (Sargent et al. 1987; Volkman et al. 1989; Virtue et al. 1993*a*). Fatty acid 18:5(n-3) has also been described as a potential marker for dinoflagellates (Mayzaud et al. 1976) or *Phaeocystis* (Virtue et al. 1993*a*). However, 18:5(n-3) was not detected in any of our krill samples. It is usually present in very low concentrations in zooplankton (e.g., Fraser et al. 1989; Virtue et al. 2000), and very few studies reported the occurrence of this fatty acid in krill (Mayzaud et al. 1976; Virtue et al. 2000; Phleger et al. 2002). A possible explanation for its low abundance was presented by Ghioni et al. (2001). They studied the fate of radio-labeled 18:5(n-3) in cultured fish cells and proposed its rapid conversion to 18:4(n-3), which can be further elongated and desaturated to 20:5(n-3). However, in the mixed phytoplankton culture that was fed to the krill furciliae, no 18:5(n-3) and only small amounts of 18:4(n-3) (<1% of total fatty acids) were detected. This is consistent with the low numerical abundance (<2%, data not shown) of flagellates as determined by the cell counts.

We did not carry out feeding experiments with flagellates. In an experimental study on a calanoid copepod feeding on a dinoflagellate species, assimilation efficiencies for different dietary lipid components were calculated (Harvey et al. 1987). The authors observed a very high assimilation efficiency for polyunsaturated fatty acids, with the flagellate markers 18:4(n-3) and 22:6(n-3) being almost completely removed from the food during the gut passage.

In *E. superba*, 18:4(n-3) is predominantly accumulated in the triacylglycerols (Hagen et al. 2001; this study). Because it seems to be rapidly metabolized when not refueled by exogenous sources, it is a valuable short-term trophic marker, in contrast to the efficiently retained long-chain polyunsaturated fatty acid DHA.

Copepods: It is now well established that *E. superba* can resort to carnivory during periods of phytoplankton shortage (Huntley et al. 1994; Atkinson and Snÿder 1997; Perissinotto et al. 2000; Atkinson et al. 2002). Particularly, copepods would represent an abundant high-energy food source because they can contain >50% of their dry mass as lipids (e.g., Hagen and Schnack-Schiel 1996). The C20 and C22 monoenes are typical components, both as fatty acids and fatty alcohols of the wax esters and triacylglycerols of herbivorous calanid copepods (e.g., Kattner and Hagen 1995). However, these components are not accumulated in *E. superba* lipids (Phleger et al. 1998; Hagen et al. 2001; this study). In contrast, other euphausiids and oil-rich fish preying on these copepods incorporate these moieties into their body lipids. They occur in significant proportions, especially in the triacylglycerols of *Meganyctiphanes norvegica* (e.g., Virtue et al. 2000), as well as in those of North Atlantic herring and North Sea sprat (*see* Sargent and Henderson 1995 for review). Hence, in contrast to the carnivorous *M. norvegica*, calanid copepods do not appear to constitute a regular food source for *E. superba*.

As the present study has demonstrated, fecal strings of krill feeding on copepods rich in the two 22:1 isomers contain high levels of these fatty acids, indicating that they are not assimilated. A quantitative determination of the amount of these fatty acids in the prey copepods before and after gut passage was not possible. Therefore, it remains unclear whether their low abundance in krill body lipids is due to their generally low absorption or whether the assimilated fatty acids are readily metabolized for energy production instead of deposition. Similar results have been published for small cod juveniles feeding on zooplankton rich in the C20 and C22 monoenes (Olsen et al. 1991). Until a certain age, their body lipids did not contain these fatty acids, and the authors suggested a limited ability to digest neutral lipids in the early life stages. High levels of wax esters, as well as 20:1(n-9) and 22:1(n-11) moieties in the fish feces confirmed their conclusion. The apparent weak assimilation of the long-chain monoenes makes these fatty acids unsuitable as trophic markers to detect feeding on calanid copepods by *E. superba*.

Cripps and Atkinson (2000) proposed that the ratio of polyunsaturated to saturated fatty acids (PUFA/SFA) is a useful indicator for carnivorous (high values) versus herbivorous feeding in krill. However, there is a clear relationship between the total lipid content and the levels of polyunsaturated fatty acids (PUFA) in Antarctic euphausiids (data not shown). Nevertheless, there is some individual variability, which could be attributable to differences in feeding histories. This underlines the importance of such indices only being applied to individuals with comparable lipid content. Sargent and Henderson (1995) reviewed literature on the origin and transfer of marine (n-3)-PUFAs and stated that diet is not the only factor responsible for variations in lipid composition. They emphasize the importance of total oil levels. They also point out that the lower the percentage of neutral lipid in total zooplankton lipids, the higher the content of (n-3) PUFA in total lipid.

The dietary imprint of copepods on *E. superba* lipids is rather small. But even if *E. superba* does switch to copepod prey during times of low phytoplankton availability, it remains doubtful whether they can efficiently utilize this lipid-rich food. It cannot be decided yet whether krill can enzymatically degrade and efficiently absorb copepod wax esters. The low portions of the monounsaturated C20 and C22 fatty acids in krill lipids and their high levels in the fecal material suggest a poor ability of *E. superba* to digest these high-energy lipids of copepods.

Starvation—Juvenile and adult krill show a high tolerance toward suboptimal feeding conditions and can survive long periods without food (Ikeda and Dixon 1982). The authors

observed a considerable reduction in metabolic activity of starved *E. superba* compared to wild specimens, but no change in chemical composition (C and N). This indicates that both body lipid and protein are used as energy sources in starving krill. Similar results have been found by Virtue et al. (1997). They reported a significant decrease of lipid mass per animal during 130 d of starvation, while the relative lipid portion in percentage of body dry mass remained constant, although it should be kept in mind that the initial lipid levels of the experimental specimens were already quite low. The percent composition of both lipid classes and fatty acids also showed no significant changes. In an earlier study by the same authors, the effect of short-term starvation on the lipid content and composition of the digestive gland of *E. superba* was examined (Virtue et al. 1993*b*). Again, absolute lipid levels significantly decreased, but on a lipid class basis, relative levels remained the same. However, there was a change concerning the fatty acid composition. The portions of the long-chain PUFAs 20 : 5(n-3) and 22 : 6(n-3) decreased in favor of 16 : 0. Our results agree with these literature data: Although absolute lipid levels decreased during starvation, the relative compositions remained largely unchanged in juvenile and adult krill. That there is no preferential utilization of either high-energy long-chain PUFAs or low-cost short-chain saturates further illustrates the balanced lipid metabolism of postlarval *E. superba.*

The concept of body shrinkage in *E. superba* during periods of food limitation (e.g., Ikeda and Dixon 1982; Nicol et al. 1992) agrees well with these findings that the relative biochemical compositions do not change. Shrinkage apparently occurs via a reduction in cell volume rather than the resorption of whole cells, as deduced by microscopic counts of cell nuclei (McGaffin et al. 2002). This implies an even reduction of the various cell constituents, including proteins as well as storage and membrane lipids, except for nucleic material.

We emphasize that these rather stable total lipid levels and relative lipid compositions under laboratory starvation conditions are not in contradiction to the pronounced seasonal lipid dynamics observed in the wild (Hagen et al. 1996, 2001; Falk-Petersen et al. 2000). This is a matter of different scales: laboratory investigations observe a reduction of absolute lipid mass by a factor of two or less (Virtue et al. 1997; this study), whereas those of field samples can vary by a factor of 10.

To our knowledge, the effects of starvation on the lipid biochemistry of *E. superba* furciliae have not been examined so far. Similar to the results of the feeding experiments, starvation also had a stronger effect on the furcilia larvae than on juvenile and adult krill. There was a significant reduction of the contribution of storage lipid (triacylglycerol and phosphatidylcholine) typical fatty acids, resulting in a relative increase of membrane lipid fatty acids. This suggests that larvae follow a different strategy than juvenile and adult krill. These furciliae utilize their lipid reserves and do not metabolize their body mass for energy production. Findings by Frazer et al. (1997) on stable isotopes of *E. superba* larvae (mainly Furcilia VI) corroborate this conclusion. During 8 weeks of starvation, there was no isotopic change toward a heavier δ^{15}N signal because of the excretion of isotopically light ammonium. This indicates that krill larvae do not catabolize their body nitrogen during starvation.

This study revealed only a weak influence of dietary fatty acids on the lipid compositions of juvenile and adult *E. superba.* This might be partly attributable to the late season and thus reduced feeding and metabolic activities, although weak trophic effects on the fatty acid compositions of postlarval *E. superba* have also been found for the summer season. The large lipid deposits seem to buffer variations in dietary lipid supply; hence, a specific fatty acid pattern is maintained.

The fatty acid compositions of larval krill, however, are much more clearly influenced by their food. An unaltered incorporation of dietary fatty acids in furciliae body lipids together with lower lipid levels allows the deduction of feeding habits through fatty acid analyses.

It is emphasized that the relative lipid compositions are strongly dependent on the total lipid contents. Trophic indices derived from marker fatty acids therefore should not be interpreted as absolute values but viewed in the physiological context of the animals.

References

ATKINSON, A., AND R. SNŸDER. 1997. Krill–copepod interactions at South Georgia, Antarctica, I. Omnivory by *Euphausia superba.* Mar. Ecol. Prog. Ser. **160:** 63–76.

———, B. MEYER, D. STÜBING, W. HAGEN, K. SCHMIDT, AND U. V. BATHMANN. 2002. Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter—II. Juveniles and adults. Limnol. Oceanogr. **47:** 953–966.

CLARKE, A., AND R. J. G. LEAKEY. 1996. The seasonal cycle of phytoplankton, macronutrients, and the microbial community in a nearshore Antarctic marine ecosystem. Limnol. Oceanogr. **41:** 1281–1294.

CRIPPS, G. C., AND A. ATKINSON. 2000. Fatty acid composition as an indicator of carnivory in Antarctic krill, *Euphausia superba.* Can. J. Fish. Aquat. Sci. **57:** 1–7.

———, AND H. J. HILL. 1998. Changes in lipid composition of copepods and *Euphausia superba* associated with diet and environmental conditions in the marginal ice zone, Bellingshausen Sea, Antarctica. Deep-Sea Res. I **45:** 1357–1381.

———, J. L. WATKINS, H. J. HILL, AND A. ATKINSON. 1999. Fatty acid content of Antarctic krill, *Euphausia superba,* at South Georgia related to regional populations and variations in diet. Mar. Ecol. Prog. Ser. **181:** 177–188.

DALY, K. L. 1990. Overwintering development, growth, and feeding of larval *Euphausia superba* in the Antarctic marginal ice zone. Limnol. Oceanogr. **35:** 1564–1576.

DUNSTAN, G. A., J. K. VOLKMAN, S. M. BARRETT, J.-M. LEROI, AND S. W. JEFFREY. 1994. Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). Phytochemistry **35:** 155–161.

FALK-PETERSEN, S., W. HAGEN, G. KATTNER, A. CLARKE, AND J. SARGENT. 2000. Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. Can. J. Fish. Aquat. Sci. **57:** 178–191.

———, AND OTHERS. 2002. Lipid biomarkers and trophic linkages between ctenophores and copepods in Svalbard waters. Mar. Ecol. Prog. Ser. **227:** 187–194.

FRASER, A. J., D. R. TOCHER, AND J. R. SARGENT. 1985. Thin-layer chromatography–flame ionization detection and the quantita-

tion of marine neutral lipids and phospholipids. J. Exp. Mar. Biol. Ecol. **88:** 91–99.

———, J. R. SARGENT, J. C. GAMBLE, AND D. D. SEATON. 1989. Formation and transfer of fatty acids in an enclosed marine food chain comprising phytoplankton, zooplankton and herring (*Clupea harengus* L.) larvae. Mar. Chem. **27:** 1–18.

FRAZER, T. K., R. M. ROSS, L. B. QUETIN, AND J. P. MONTOYA. 1997. Turnover of carbon and nitrogen during growth of larval krill, *Euphausia superba* Dana: A stable isotope approach. J. Exp. Mar. Biol. Ecol. **212:** 259–275.

GHIONI, C., A. E. A. PORTER, I. H. SADLER, D. R. TOCHER, AND J. R. SARGENT. 2001. Cultured fish cells metabolize octadecapentaenoic acid (all-*cis* delta 3,6,9,12,15–18 : 5) to octadecatetraenoic acid (all-*cis* delta 6,9,12,15–18 : 4) via its 2-*trans* intermediate (*trans* delta 2, all-*cis* delta 6,9,12,15–18 : 5). Lipids **36:** 145–152.

GRAEVE, M., W. HAGEN, AND G. KATTNER. 1994*a*. Herbivorous or omnivorous? On the significance of lipid compositions as trophic markers in Antarctic copepods. Deep-Sea Res. I **41:** 915–924.

———, G. KATTNER, AND W. HAGEN. 1994*b*. Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: Experimental evidence of trophic markers. J. Exp. Mar. Biol. Ecol. **182:** 97–110.

———, ———, AND D. PIEPENBURG. 1997. Lipids in Arctic benthos: Does the fatty acid and alcohol composition reflect feeding and trophic interactions? Polar Biol. **18:** 53–61.

HAGEN, W. 2000. Lipids, p. 113–119. *In* R. Harris, P. Wiebe, J. Lenz, H. Skjoldal, and M. Huntley [eds.], ICES Zooplankton Methodology Manual. Academic Press.

———, AND G. KATTNER. 1998. Lipid metabolism of the Antarctic euphausiid *Thysanoessa macrura* and its ecological implications. Limnol. Oceanogr. **43:** 1894–1901.

———, AND S. B. SCHNACK-SCHIEL. 1996. Seasonal lipid dynamics in dominant Antarctic copepods: Energy for overwintering or reproduction? Deep-Sea Res. I **43:** 139–158.

———, E. S. VAN VLEET, AND G. KATTNER. 1996. Seasonal lipid storage as overwintering strategy of Antarctic krill. Mar. Ecol. Prog. Ser. **134:** 85–89.

———, G. KATTNER, A. TERBRÜGGEN, AND E. S. VAN VLEET. 2001. Lipid metabolism of the Antarctic krill *Euphausia superba* and its ecological implications. Mar. Biol. **139:** 95–104.

HARVEY, H. R., G. EGLINTON, S. C. M. O'HARA, AND E. D. S. CORNER. 1987. Biotransformation and assimilation of dietary lipids by *Calanus* feeding on a dinoflagellate. Geochim. Cosmochim. Acta **51:** 3031–3040.

HUNTLEY, M. E., W. NORDHAUSEN, AND M. D. G. LOPEZ. 1994. Elemental composition, metabolic activity and growth of Antarctic krill *Euphausia superba* during winter. Mar. Ecol. Prog. Ser. **107:** 23–40.

IKEDA, T., AND P. DIXON. 1982. Body shrinkage as a possible overwintering mechanism of the Antarctic krill, *Euphausia superba* Dana. J. Exp. Mar. Biol. Ecol. **62:** 143–151.

KANG, S. H., AND G. A. FRYXELL. 1993. Phytoplankton in the Weddell Sea, Antarctica—composition, abundance and distribution in water-column assemblages of the marginal ice-edge zone during austral autumn. Mar. Biol. **116:** 335–348.

KATTNER, G., AND H. S. G. FRICKE. 1986. Simple gas–liquid chromatography method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. J. Chromatogr. **361:** 263–268.

———, AND W. HAGEN. 1995. Polar herbivorous copepods—different pathways in lipid biochemistry. ICES J. Mar. Sci. **52:** 329–335.

———, AND ———. 1998. Lipid metabolism of the Antarctic euphausiid *Euphausia crystallorophias* and its ecological implications. Mar. Ecol. Prog. Ser. **170:** 203–213.

MAYZAUD, P., C. A. EATON, AND R. G. ACKMAN. 1976. The occurrence and distribution of octadecapentaenoic acid in a natural plankton population. A possible food chain index. Lipids **11:** 858–862.

———, E. ALBESSARD, AND J. CUZIN-ROUDY. 1998. Changes in lipid composition of the Antarctic krill *Euphausia superba* in the Indian sector of the Antarctic Ocean: Influence of geographical location, sexual maturity stage and distribution among organs. Mar. Ecol. Prog. Ser. **173:** 149–162.

MCGAFFIN, A. F., S. NICOL, AND D. A. RITZ. 2002. Changes in muscle tissue of shrinking Antarctic krill. Polar Biol. **25:** 180–186.

MEYER, B., A. ATKINSON, D. STÜBING, B. OETTL, W. HAGEN, AND U. V. BATHMANN. 2002. Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter—I. Furcilia III larvae. Limnol. Oceanogr. **47:** 943–952.

NICHOLS, D. S., P. D. NICHOLS, AND C. W. SULLIVAN. 1993. Fatty acid, sterol and hydrocarbon composition of Antarctic sea ice diatom communities during the spring bloom in McMurdo Sound. Antarct. Sci. **5:** 271–278.

NICOL, S., M. STOLP, T. COCHRAN, P. GEIJSEL, AND J. MARSHALL. 1992. Growth and shrinkage of Antarctic krill *Euphausia superba* from the Indian Ocean sector of the Southern Ocean during summer. Mar. Ecol. Prog. Ser. **89:** 175–181.

OLSEN, R., AND R. HENDERSON. 1989. The rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densitometry. J. Exp. Mar. Biol. Ecol. **129:** 189–197.

———, ———, AND T. PEDERSEN. 1991. The influence of dietary lipid classes on the fatty acid composition of small cod *Gadus morhua* L. juveniles reared in an enclosure in northern Norway. J. Exp. Mar. Biol. Ecol. **148:** 59–76.

PERISSINOTTO, R., E. A. PAKHOMOV, C. D. MCQUAID, AND P. W. FRONEMAN. 1997. In situ grazing rates and daily ration of Antarctic krill *Euphausia superba* feeding on phytoplankton at the Antarctic Polar Front and the Marginal Ice Zone. Mar. Ecol. Prog. Ser. **160:** 77–91.

———, L. GURNEY, AND E. A. PAKHOMOV. 2000. Contribution of heterotrophic material to diet and energy budget of Antarctic krill, *Euphausia superba*. Mar. Biol. **136:** 129–135.

PHLEGER, C. F., P. D. NICHOLS, AND P. VIRTUE. 1998. Lipids and trophodynamics of Antarctic zooplankton. Comp. Biochem. Physiol. **120:** 311–323.

———, M. M. NELSON, B. D. MOONEY, AND P. D. NICHOLS. 2002. Interannual and between species comparison of the lipids, fatty acids and sterols of Antarctic krill from the US AMLR Elephant Island survey area. Comp. Biochem. Physiol. **131:** 733–747.

QUETIN, L. B., AND R. M. ROSS. 1991. Behavioral and physiological characteristics of the Antarctic krill, *Euphausia superba*. Am. Zool. **31:** 49–63.

SARGENT, J. R., AND R. J. HENDERSON. 1995. Marine (n-3) polyunsaturated fatty acids, p. 32–65. *In* R. J. Hamilton [ed.], Developments in oils and fats. Blackie Academic and Professional.

———, AND K. J. WHITTLE. 1981. Lipids and hydrocarbons in the marine food web, p. 491–533, *In* A. R. Longhurst [ed.], Analysis of marine ecosystems. Academic Press.

———, R. J. PARKES, I. MUELLER-HARVEY, AND R. J. HENDERSON. 1987. Lipid biomarkers in marine ecology, p. 119–138. *In* M. A. Sleigh [ed.], Microbes in the sea. Ellis Horwood.

SCHMIDT, K., A. ATKINSON, D. STÜBING, J. W. MCCLELLAND, J. P. MONTOYA, AND M. VOSS. 2003. Trophic relationships among Southern Ocean copepods and krill: Some uses and limitations of a stable isotope approach. Limnol. Oceanogr. **48:** 277–289.

SCOTT, C. L., S. FALK-PETERSEN, J. R. SARGENT, H. HOP, O. J. LØNNE, AND M. POLTERMANN. 1999. Lipids and trophic interactions of ice fauna and pelagic zooplankton in the marginal ice zone of the Barents Sea. Polar Biol. **21:** 65–70.

ST. JOHN, M. A., AND T. LUND. 1996. Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. Mar. Ecol. Prog. Ser. **131:** 75–85.

VIRTUE, P., P. D. NICHOLS, S. NICOL, A. MCMINN, AND E. L. SIKES. 1993*a*. The lipid composition of *Euphausia superba* Dana in relation to the nutritional value of *Phaeocystis pouchetii* (Hariot) Lagerheim. Antarct. Sci. **5:** 169–177.

———, S. NICOL, AND P. D. NICHOLS. 1993*b*. Changes in the digestive gland of *Euphausia superba* during short-term starvation: Lipid class, fatty acid and sterol content and composition. Mar. Biol. **117:** 441–448.

———, P. D. NICHOLS, AND S. NICOL. 1997. Dietary-related mechanisms of survival of *Euphausia superba*: Biochemical changes during long-term starvation and bacteria as a possible source of nutrition, p. 193–201. *In* B. Battaglia, J. Valencia, and D. W. H. Walton [eds.], Antarctic communities: Species, structure and survival. Cambridge Univ, Press.

———, P. MAYZAUD, E. ALBESSARD, AND P. NICHOLS. 2000. Use of fatty acids as dietary indicators in northern krill, *Meganyctiphanes norvegica*, from northeastern Atlantic, Kattegat, and Mediterranean waters. Can. J. Fish. Aquat. Sci. **57:** 104–114.

VOLKMAN, J. K., S. W. JEFFREY, P. D. NICHOLS, G. I. ROGERS, AND C. D. GARLAND. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J. Exp. Mar. Biol. Ecol. **128:** 219–240.

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Chapter 2

Fatty acid biomarker ratios – suitable trophic indicators in Antarctic euphausiids?

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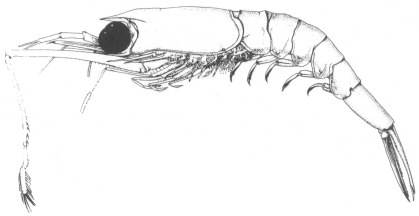

Thysanoessa macrura adult male (from Baker et al. 1990)

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ORIGINAL PAPER

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Fatty acid biomarker ratios—suitable trophic indicators in Antarctic euphausiids?

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Abstract Fatty-acid biomarkers are frequently used for the identification of trophic relationships among marine zooplankton. We have evaluated the suitability of five fatty-acid ratios [16:0/16:1(*n*-7), 16:1(*n*-7)/18:4(*n*-3), 18:1(*n*-9)/18:1(*n*-7), 20:5(*n*-3)/22:6(*n*-3), PUFA/SFA] that have been proposed as trophic indicators in the literature. Total lipid content and fatty-acid composition were determined in four Antarctic euphausiid species (*Euphausia superba*, *E. frigida*, *E. triacantha*, *Thysanoessa macrura*). There is a significant relationship between the lipid content and most of these ratios in the investigated euphausiids. Only the 16:1(*n*-7)/18:4(*n*-3) ratio exhibits no clear relationship to total lipids. Further exceptions occur in *E. triacantha*: the 18:1(*n*-9)/18:1(*n*-7) and the 20:5(*n*-3)/22:6(*n*-3) ratios are not correlated to the lipid content in this species. There is a weaker correlation between the fatty-acid ratios and the total lipid content in *E. superba* larvae than in the postlarvae, indicating a stronger dietary influence on the lipids of the younger stages. We conclude that those fatty-acid ratios that strongly depend on an animal's total lipid content (particularly PUFA/SFA), are only of limited use as trophic indices, since total lipid content may vary greatly with factors (such as reproductive processes) that are unrelated to specific feeding preferences.

Introduction

The analysis of lipid composition is a useful tool for deducing feeding relationships in marine ecosystems. This trophic biomarker approach makes use of the fact that specific lipid components, particularly fatty acids, are characteristic of specific groups of phytoplankton or zooplankton. These marker fatty acids are incorporated largely unaltered into the consumer's lipids, which thus retain a signature of their dietary origin (Sargent and Whittle 1981; Sargent et al. 1987; Graeve et al. 1994; Dalsgaard et al. 2003).

In order to have a distinct measure for classification into feeding types, a variety of fatty-acid biomarker ratios has been proposed. Tested via feeding experiments, but mostly based on field observations, such indices have been established to allow the assignment of different species (copepods, euphausiids, amphipods, and other zooplankton) into trophic categories. The ratio 16:0/16:1(*n*-7) has been applied to discriminate between diatom versus flagellate feeding (St. John and Lund 1996; Cripps et al. 1999; Mayzaud et al. 1999; Nelson et al. 2001; Phleger et al. 2001; Auel et al. 2002). This is based on the fact that diatoms are particularly rich in 16:1(*n*-7) (e.g. Nichols et al. 1993; Dunstan et al. 1994), while flagellates contain higher amounts of 16:0 (Sargent and Whittle 1981; Sargent et al. 1987; Virtue et al. 1993; Skerratt et al. 1997). Correspondingly, high ratios of 20:5(*n*-3)/22:6(*n*-3) (EPA/DHA) are indicative of diatom food and low ratios of a flagellate-based nutrition (Nelson et al. 2001; Phleger et al. 2001, 2002). Another ratio with the potential to differentiate between these two food sources may be the quotient of 16:1(*n*-7) to 18:4(*n*-3), since the latter fatty acid is found in higher percentages in flagellates (Sargent et al. 1987; Graeve et al. 1994). Although this ratio has as yet been used infrequently (Stübing et al. 2003), it may be applicable, since both fatty acids are particularly useful trophic biomarkers in Antarctic euphausiids (Kattner and Hagen 1998; Hagen et al. 2001).

The fatty acid 18:1(*n*-7) can result from chain elongation of the diatom marker 16:1(*n*-7), whereas the (*n*-9) isomer of 18:1 is a common fatty acid in metazoans (e.g. Falk-Petersen et al. 1990). The ratio 18:1(*n*-9)/18:1(*n*-7) is thus frequently used to estimate the degree of carnivory versus herbivory (Graeve et al. 1997; Cripps et al. 1999; Mayzaud et al. 1999; Falk-Petersen et al. 2000; Nelson et al. 2001). Another index, the ratio of

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polyunsaturated to saturated fatty acids (PUFA/SFA), has been proposed to be a measure of carnivory in *Euphausia superba*, with possible extension to other euphausiid species (Cripps and Atkinson 2000). While some of these indices have proven very useful, others produced rather inconclusive results (e.g. Atkinson et al. 2002; Auel et al. 2002; Meyer et al. 2002).

Combining the lipid data of different developmental stages of four Antarctic euphausiid species, we have investigated the relationship between the total lipid content and five biomarker fatty-acid ratios in order to evaluate their applicability as trophic markers for these euphausiids. The approach presented here is not based on experimental investigations but rather compiles empirical evidence for a general correlation between fatty-acid compositions and total lipid contents. The working hypothesis assumes that the concentrations of suitable trophic-marker fatty acids should be independent of the total lipid levels of the specimens analysed. Hence, if a tight relationship emerges between the respective fatty acids and the total lipid content, this would question their applicability as trophic markers.

Materials and methods

Sampling

Euphausiids were sampled during two autumn cruises with R.V. *Polarstern* (14–20 April 1999, and 18 April–1 May 2001, Fig. 1) in the Polar Frontal Zone and on transects across the shelf break in the southwest Lazarev Sea, and in the Bellingshausen Sea, respectively. Summer samples were obtained during a cruise with R.V. *Yuzhmorgeologiya* off the South Shetland Islands from 18 February–12 March 2000 (Fig. 1). Detailed information about the programmes and the station data have been published by Bathmann et al. (2000), Lipsky (2001), and Bathmann (2002). In 1999, slow vertical Bongo-net tows (335-µm mesh, 5 l closed cod end) were made in the top 150 m at night. In 2000, double oblique Isaacs-Kidd Midwater Trawl tows (mesh size 505 µm) were performed in the top 170 m. In 2001, zooplankton was sampled by double oblique Rectangular Midwater Trawl 1 + 8 hauls (mesh size 325 and 4,500 µm, respectively, 20 l closed cod end), vertical Bongo-net tows, or a hand-hauled Apstein net. Zooplankton was immediately transferred to the cool laboratory, where it was sorted. Stage or sex (after Kirkwood 1982), and length (tip of rostrum to end of telson) were recorded; the animals were briefly rinsed with deionised water, blotted dry and then frozen at −80°C until analysis.

Lipid analyses

After lyophilisation for 48 h, the samples were weighed and total lipid was extracted with dichloromethane/methanol (2:1 by volume) and determined gravimetrically (Hagen 2000). Lipids were hydrolysed and the fatty acids converted to their methyl ester derivatives (FAME) in methanol containing 3% concentrated sulphuric acid at 80°C for 4 h (Kattner and Fricke 1986). After cooling, 4 ml deionised water was added and FAME were extracted with hexane (3×1.7 ml), analysed in a gas chromatograph (HP 6890A) equipped with a DB-FFAP column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) using temperature programming and helium as carrier gas. FAME were detected by flame ionisation and identified by comparing retention time data with those obtained from standard mixtures.

Statistics

Five fatty-acid ratios were regressed against total lipid content (in % dry mass) using SPSS for Macintosh. Regression curves were best fitted by power (Figs. 2, 3) or quadratic functions (Fig. 5a) or by the Pearson's linear regression model which was applied for *E. superba* larvae and in Figs. 4, 5b–d, and 6.

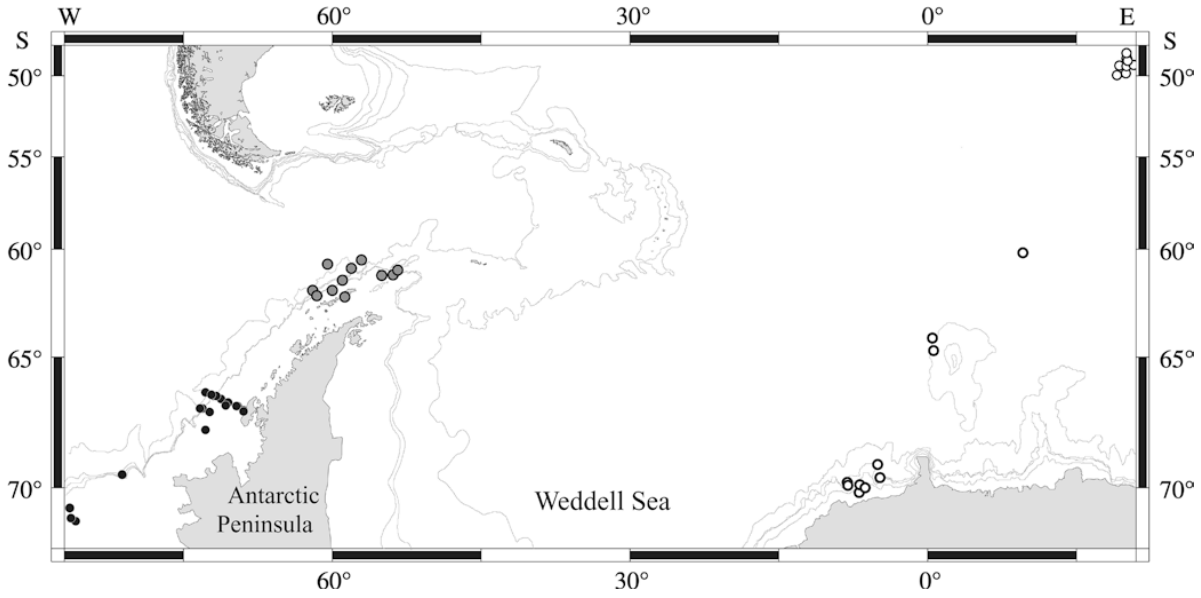

Fig. 1 Map of the investigation areas. *Unfilled circles* mark stations in the Polar Frontal Zone and in the southwest Lazarev Sea sampled in autumn 1999, *grey circles* stations off the South Shetland Islands in summer 2000, and *filled circles* stations in the Bellingshausen Sea in autumn 2001

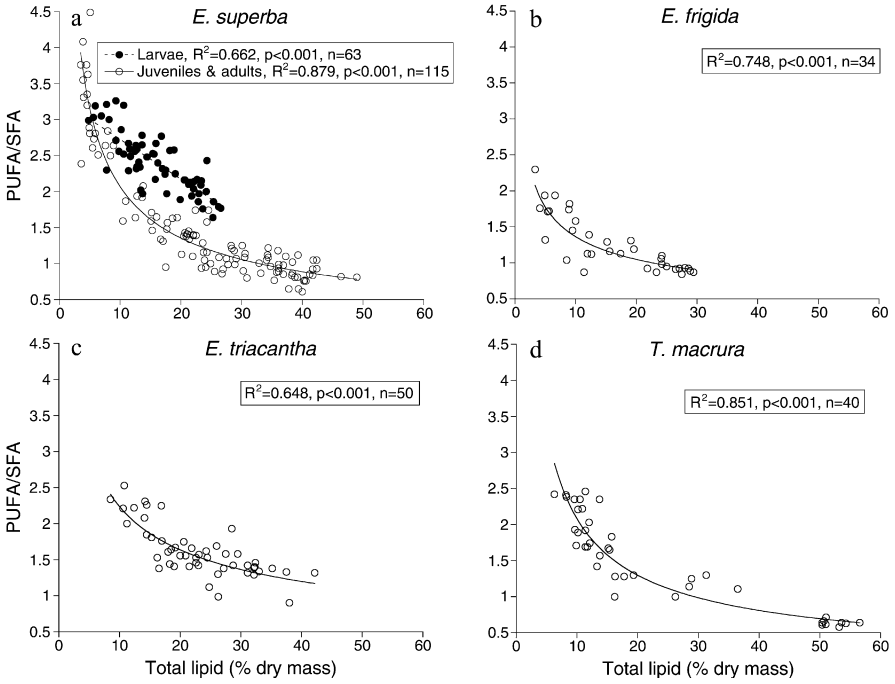

Fig. 2a–d Relationship between the ratio of polyunsaturated to saturated fatty acids (PUFA/SFA) and the total lipid content (% dry mass) of four Antarctic euphausiid species (*p* significance level, *n* number of samples)

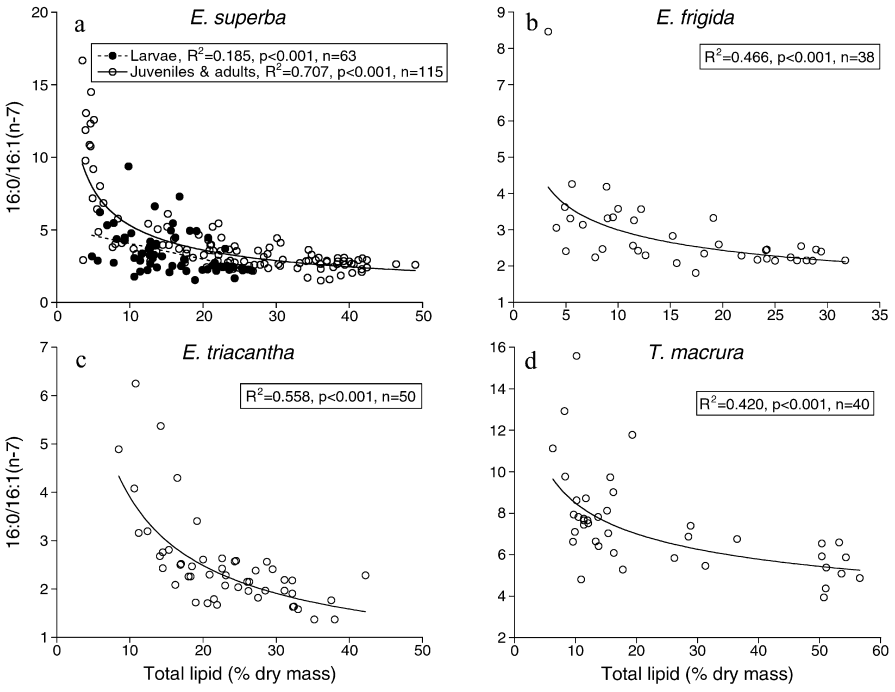

Fig. 3a–d Relationship between the 16:0/16:1(*n*-7) ratio and the total lipid content (% dry mass) of four Antarctic euphausiid species (*p* significance level, *n* number of samples). Note different axes

Results

Table 1 summarises dry mass (DM), total lipid, and fatty-acid data of the four euphausiid species sampled in various regions and seasons and grouped into broad developmental categories (larvae, juveniles and adults). *E. frigida*, the smallest species, is characterised by the lowest lipid contents, as opposed to *Thysanoessa macrura*, which may attain lipid contents nearly twice as high,

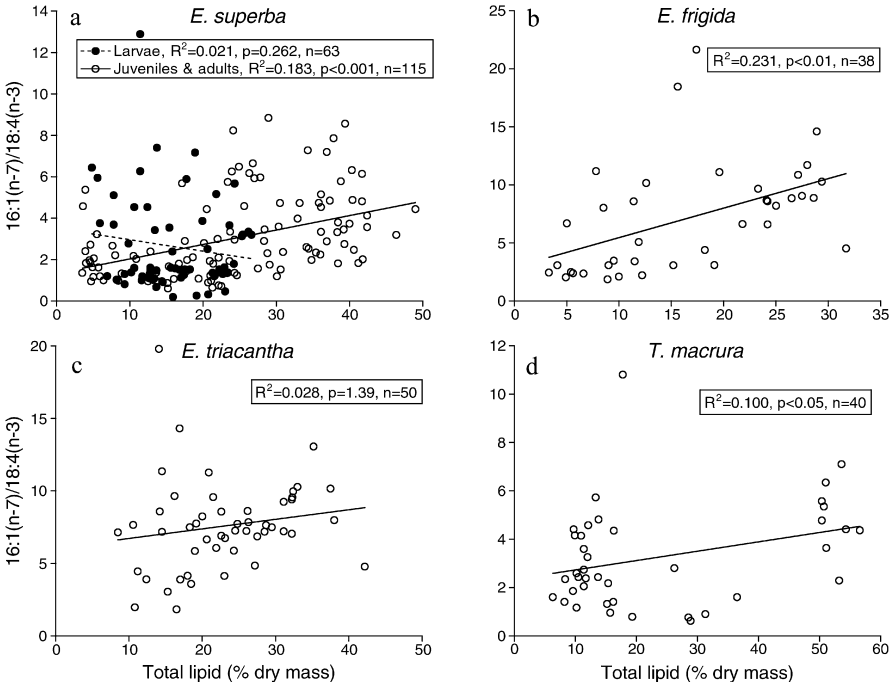

Fig. 4a–d Relationship between the 16:1(*n*-7)/18:4(*n*-3) ratio and the total lipid content (% dry mass) of four Antarctic euphausiid species (*p* significance level, *n* number of samples). Note different axes

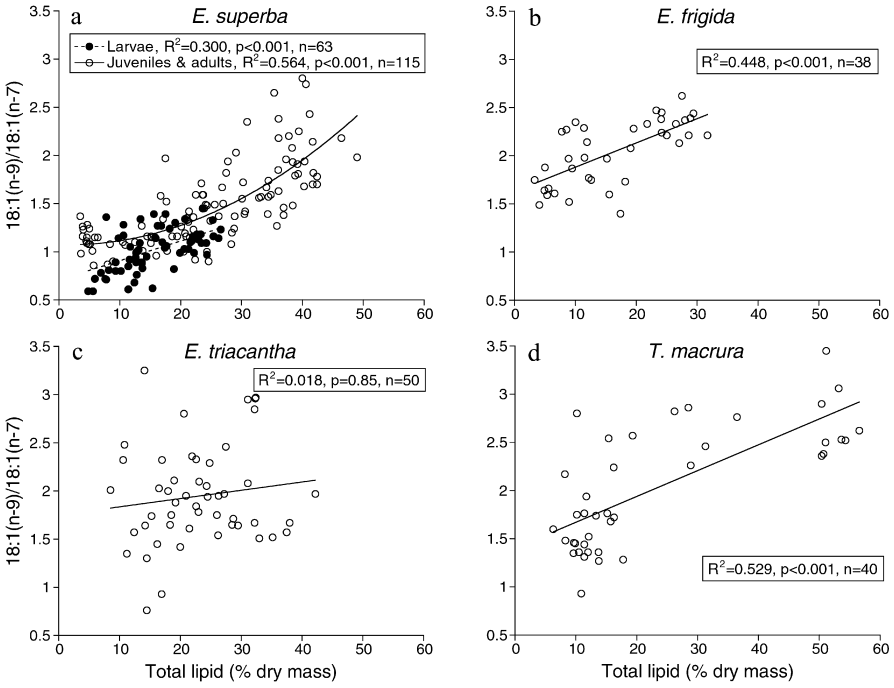

Fig. 5a–d Relationship between the 18:1(*n*-9)/18:1(*n*-7) ratio and the total lipid content (% dry mass) of four Antarctic euphausiid species (*p* significance level, *n* number of samples)

with maximum values of 57% DM. This does not only apply to adult specimens in autumn; the larvae of *T. macrura* also contain higher lipid contents than those of *E. superba*. In general, the larval stages are characterised by particularly high levels of the membrane-typical fatty acids 20:5(*n*-3) and 22:6(*n*-3), consistent with their overall lower lipid contents and higher fractions of membrane lipids as compared to juveniles and adults. Dominant fatty acids in *E. superba* juveniles and adults are the saturates (SFA) 16:0 and 14:0, the long-

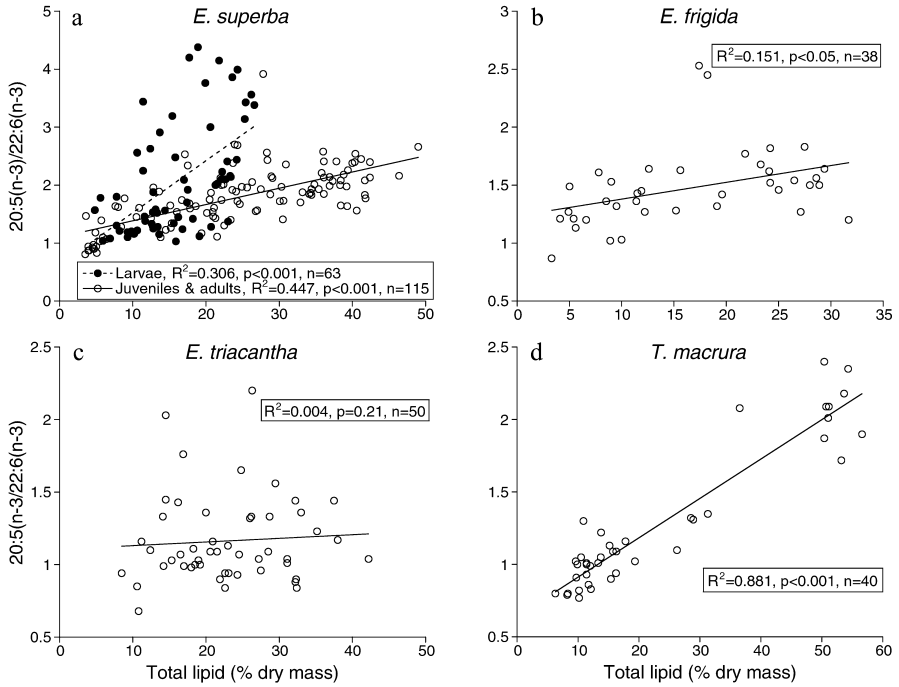

Fig. 6a–d Relationship between the 20:5(*n*-3)/22:6(*n*-3) ratio and the total lipid content (% dry mass) of four Antarctic euphausiid species (*p* significance level, *n* number of samples). Note different axes

Table 1 Dry mass (*DM*), total lipids (*TL*), and percent fatty-acid compositions (means, ranges in *parentheses*) of larvae (CI-FVI in *Euphausia superba*, FI-FVI in *Thysanoessa macrura*) and juveniles and adults of the four euphausiid species. Fatty acids < 1% and fatty alcohols (present in *E. triacantha* and *T. macrura*) not listed (*n* number of samples)

	E. superba		*E. frigida*	*E. triacantha*	*T. macrura*	
	Larvae (*n* = 63)	Juveniles + adults (*n* = 115)	Juveniles + adults (*n* = 38)	Juveniles + adults (*n* = 50)	Larvae (*n* = 31)	Juveniles + adults (*n* = 9)
Dry mass (mg)	0.683 (0.001–3.5)	184.1 (13.3–550.6)	12.8 (3.9–26.2)	36.7 (1.2–102.6)	0.9 (0.1–3.7)	34.0 (7.1–69.1)
TL (mg)	0.139 (0.0001–0.9)	46.4 (6.7–255.4)	2.6 (0.2–8.2)	10.8 (0.5–39.0)	0.2 (0.02–1.0)	17.9 (1.0–39.3)
TL (% DM)	16.0 (4.8–26.6)	24.3 (3.5–49.0)	16.4 (3.3–29.4)	23.1 (8.1–42.2)	14.5 (6.3–31.3)	51.3 (36.5–56.8)
14:0	5.1 (1.2–8.3)	10.2 (1.2–14.4)	8.6 (2.8–12.0)	5.8 (2.0–8.6)	8.6 (2.7–22.6)	21.8 (15.5–25.9)
16:0	16.0 (12.2–18.7)	19.7 (13.9–24.3)	20.0 (16.3–23.3)	16.3 (13.6–24.0)	20.7 (18.4–23.8)	21.7 (14.8–23.7)
18:0	1.0 (0.5–1.5)	1.1 (0.5–2.1)	1.0 (0.6–1.7)	1.0 (0.7–2.0)	1.0 (0.5–1.6)	0.6 (0.4–0.8)
16:1(*n*-7)	5.2 (1.5–9.1)	6.9 (1.0–11.3)	7.7 (2.2–11.7)	6.9 (2.4–11.8)	2.6 (1.5–4.1)	4.3 (3.1–5.7)
18:1(*n*-9)	6.1 (3.4–10.4)	11.3 (5.6–18.5)	11.5 (7.1–15.2)	14.8 (6.4–21.6)	7.0 (6.1–9.0)	9.6 (7.4–12.6)
18:1(*n*-7)	6.0 (3.7–8.6)	7.1 (4.8–9.2)	5.6 (4.4–7.4)	7.6 (4.3–10.0)	3.5 (2.7–4.7)	3.7 (2.7–5.9)
20:1(*n*-9)	1.0 (0.4–2.9)	1.1 (0.3–1.9)	0.9 (0.4–1.9)	2.1 (0.6–3.8)	1.0 (0.4–2.9)	1.1 (0.7–3.2)
16:2(*n*-4)	1.9 (0.4–3.6)	1.9 (0.2–4.1)	1.6 (0.7–3.1)	1.3 (0.6–3.8)	0.3 (0.1–0.5)	0.6 (0.3–0.8)
18:2(*n*-6)	2.1 (1.0–3.0)	2.2 (0.7–3.0)	2.2 (1.7–2.6)	1.6 (1.1–2.7)	2.1 (1.3–2.7)	1.4 (1.1–1.7)
16:4(*n*-1)	1.6 (0.1–4.5)	0.7 (0.2–3.8)	0.3 (0.0–0.8)	0.3 (0.1–0.7)	0.1 (0.0–0.2)	0.2 (0.1–0.4)
18:4(*n*-3)	3.0 (0.2–13.2)	2.0 (1.0–4.2)	1.4 (0.5–2.4)	1.1 (0.3–2.4)	1.9 (0.6–4.5)	1.0 (0.7–1.9)
20:5(*n*-3)	25.5 (16.2–35.2)	17.2 (12.1–26.2)	16.2 (12.5–22.4)	15.0 (9.9–21.8)	20.3 (15.7–24.1)	16.9 (14.1–23.6)
22:6(*n*-3)	15.6 (7.1–24.8)	10.3 (4.8–30.0)	11.6 (7.2–23.9)	13.9 (7.3–27.7)	20.4 (8.4–28.9)	8.2 (7.1–11.3)

chain polyunsaturates (PUFA) 20:5(*n*-3) and to a lesser extent 22:6(*n*-3), and the monounsaturate (MUFA) 18:1(*n*-9). Similar patterns also emerge in the other three species, although in *E. frigida* and *E. triacantha* the two long-chain PUFA tend to be more equally represented and 14:0 is less important. With respect to this short-chain saturate, the reverse is true for the lipid-rich juvenile and adult *T. macrura*, where it slightly exceeds the levels of 16:0.

The data vary within broad ranges, which is largely attributable to the different developmental or reproductive stages, whereas within stages and seasons the variability is less pronounced. Especially in autumn, the lipid compositions of juvenile and adult euphausiids can be remarkably uniform. In the larvae, there is a more-or-less steady increase in dry and lipid mass with advancing stage; however, their fatty-acid patterns are not entirely stage-specific. The fatty-acid data of *E. superba* calyp-

topes typically range close to the means listed in Table 1, while the variability tends to increase within the older furcilia stages. The broad range of lipid levels in postlarval *E. superba* is due to lipid-rich juveniles and adults caught in autumn at the upper end, and reproductive males (maturity stage m3B, according to Kirkwood 1982) at the other extreme. Having a dry mass of 210 ± 30 mg, these males range at the lowermost end with respect to lipid mass, resulting in extremely low lipid contents of 3.5–9.8% DM. Accordingly, their lipids are characterised by very high PUFA levels and are virtually devoid of fatty acids typical of neutral lipids [e.g. 14:0, 16:1(*n*-7)]. In *E. frigida* and *E. triacantha*, the lowest dry-mass and lipid values were recorded in small juveniles from autumn.

The broad range of lipid contents covered by each species allows the deduction of potential relationships between the fatty-acid composition and the total lipid content. All investigated Antarctic euphausiid species show a significant ($P < 0.001$) inverse correlation between the total lipid content and the ratio of polyunsaturated to saturated fatty acids (PUFA/SFA) (Fig. 2). High ratios are characteristic for lipid-poor individuals, and with increasing lipid content, the portion of PUFA continuously decreases in favour of SFA. In *E. superba*, there are pronounced differences between larval and postlarval stages: as in the other investigated species, the relationship is clearly non-linear in juveniles and adults, whereas in the larvae, the PUFA/SFA ratio decreases linearly with increasing lipid content. Furthermore, larvae contain higher amounts of PUFA than juvenile and adult *E. superba* at similar total lipid levels.

The strongest effects of the lipid content on the ratio occur in lipid-poor animals (< 10% dry mass), while the curve markedly flattens towards higher lipid levels, indicating a weaker relationship between the PUFA/SFA ratio and total lipid content in lipid-rich individuals. This is best detected in the postlarvae of *E. superba* and *T. macrura* (Fig. 2a, d) while in *E. frigida* and *E. triacantha*, the ratio range is much narrower (Fig. 2b, c). In the last species, this might be due to the lack of very lipid-poor specimens (< 8% DM), so the extension of the curve further to the left remains unclear. In *E. frigida*, however, even the extremely lipid-poor individuals do not show very elevated PUFA-levels. Within the covered range of lipid contents (3–29% DM), the PUFA/SFA ratios vary by a factor of 2.7 in *E. frigida*, as compared to 4.7 in *E. superba* for the same lipid range.

Besides demonstrating a general dependence of the PUFA/SFA ratio on the total lipid contents, Fig. 2 also provides information on the lipid metabolism of the investigated species. In spite of the steep negative slope of the curve at low lipid levels, further lipid accumulation at this stage occurs to a lower degree via SFA, but the valuable energy-rich PUFA are accumulated preferentially. With augmenting lipid levels, the curve gradually flattens. We can identify a threshold level above which PUFA and SFA are accumulated at almost equal rates, with the curve declining only slightly. It ranges at a lipid level of around 20% of dry mass in postlarval *E. superba*, *E. triacantha*, and *T. macrura*, whereas it is only 10% in *E. frigida*, which generally reaches lower maximum lipid levels than the other three species.

Figure 3 shows the relationship between the ratio of 16:0 to 16:1(*n*-7) and the total lipid content for the four species. Again, the values generally decrease significantly with increasing lipid levels, although the correlations are not as strong as for the PUFA/SFA ratio. There is a very high variability, particularly in *E. superba* larvae, but also in *E. frigida* and *T. macrura* individuals with lower lipid content.

In contrast, the 16:1(*n*-7)/18:4(*n*-3) ratio is not correlated with total lipid content (Fig. 4). The values scatter within a broad range, especially in *E. frigida* and *E. triacantha*, while in *E. superba* and *T. macrura*, the ratio variation is less pronounced. In *E. frigida*, there is a weak tendency towards higher ratios with increasing lipid contents.

The ratio 18:1(*n*-9)/18:1(*n*-7) exhibits a heterogeneous pattern among the four species (Fig. 5). In *E. superba*, *E. frigida* and *T. macrura*, there is a significant correlation between this ratio and total lipid content (although weaker in *E. superba* larvae), whereas there is no such relationship in *E. triacantha*.

The same applies to the ratio of the two long-chain PUFA 20:5(*n*-3)/22:6(*n*-3). It significantly increases with augmenting lipid levels in all species studied, except for *E. triacantha* (Fig. 6). While the increase is steep in *E. superba* larvae and in *T. macrura*, it is lower in juvenile and adult *E. superba* and in *E. frigida*. There is a particularly high degree of variance in *E. superba* larvae.

Discussion

In polar euphausiids and in many other marine zooplankton and fish species, the lipid composition is principally dependent on the total lipid content. This is particularly well documented for lipid classes (e.g. Clarke 1984; Saether et al. 1986; Hagen et al. 1996; Mayzaud 1997; Kattner and Hagen 1998), and it also applies to fatty-acid compositions (Sargent and Henderson 1995; Hagen and Kattner 1998; Kattner and Hagen 1998; Hagen et al. 2001). This must be taken into consideration for the selection of certain fatty acids as trophic biomarkers. The most suitable components will be those that are preferentially assimilated from the food, but depleted again when their dietary source is absent. Their abundance will thus be independent of total lipid levels. Fatty acids that serve certain functions in biomembranes and that are tightly conserved, such as the essential long-chain polyunsaturated fatty acids (Sargent and Henderson 1995), however, are only of limited use as trophic biomarkers. Their portions typically fluctuate characteristically with the total lipid content. In this study, we present a critical appraisal of several fatty-acid ratios that are commonly used as trophic indicators.

The ratio of polyunsaturated to saturated fatty acids (PUFA/SFA) has been proposed as an indicator of carnivory in *E. superba*, with possible extension to other euphausiids with similar diets such as *T. macrura* and *E. triacantha* (Cripps and Atkinson 2000). Following a field study, where large differences in the abundance of PUFA had been found among different groups of juvenile and adult *E. superba* (Cripps et al. 1999), the authors conducted experiments feeding *E. superba* with the Antarctic copepod species *Drepanopus forcipatus*. A significant increase in the proportion of PUFA could be induced and the PUFA/SFA ratio doubled within 16 days on a diet of copepods. However, in subsequent studies applying this index, the results were rather inconclusive. Atkinson et al. (2002) reported no differences between adult and juvenile *E. superba* (PUFA/SFA = 1.0), although stomach contents revealed a larger contribution of crustacean prey in adult specimens. In contrast, the clearly herbivorous furcilia larvae from the same study site had PUFA/SFA ratios that were 3 times higher than those of juveniles and adults (Meyer et al. 2002). This obvious contradiction to the above-described hypothesis of increasing PUFA/SFA ratios with increasing degree of carnivory (Cripps and Atkinson 2000) demonstrates the risk of misinterpretation when considering only the absolute values for classification into feeding types. First, it must be remembered that diatoms also contain very high levels of PUFA, especially 20:5(*n*-3) (e.g. Nichols et al. 1993; Dunstan et al. 1994) and flagellates may be a major source of 22:6(*n*-3) (e.g. Sargent et al. 1987; Graeve et al. 1994). But apart from this, the long-chain PUFA are in general not unambiguously suitable as trophic indicators, since these essential fatty acids are preferentially retained in the animals' lipids and their turnover is usually low (Sargent and Whittle 1981; Sargent and Henderson 1995). In the present study, we clearly demonstrate the tight correlation between the PUFA/SFA ratio and the total lipid content.

Cripps and Atkinson (2000) reported PUFA/SFA ratios of up to 5 in juvenile *E. superba* specimens from two stations off South Georgia. This is even higher than the maximum values detected in our study (4.5). The highest ratios (>3.3) were found in reproductive males (maturity stage m3B, according to Kirkwood 1982), which were also characterised by extremely low lipid contents. It has previously been reported that reproductive males have very low total lipid and triacylglycerol levels, as well as high mortality rates (Virtue et al. 1996; Phleger et al. 1998). Virtue et al. (1996) pointed out that these depleted energy reserves in the males did not result from starvation, but have probably been the consequence of processes associated with mating. Presumably, the lipid-biomarker approach is not applicable at all in these males, since their metabolism is so strongly affected by other processes that feeding has no influence on body lipids, but most of the ingested lipid is probably readily catabolised to satisfy immediate energy demands due to mating processes.

Nevertheless, there is some variability of the PUFA/SFA ratio among individuals with similar lipid contents, which may be attributable to differences in feeding histories. This applies in particular to the larval stages, as illustrated by the lower correlation coefficient in *E. superba* larvae. However, these differences are more clearly reflected by the other ratios (see below) than by the PUFA/SFA ratio, which seems to represent a more approximate estimate only.

The other indicator of carnivorous versus herbivorous feeding behaviour, the 18:1(*n*-9)/18:1(*n*-7) ratio, shows a linear increase with augmenting lipid content, except for *E. triacantha*. The (*n*-7) isomer is one of the major fatty acids in the membrane lipid phosphatidylethanolamine (PE) of *E. superba* (Mayzaud 1997; Mayzaud et al. 2000; Stübing et al. 2003). Hence, on the one hand, the rising slope is a reflection of the decreasing relative importance of PE with increasing lipid content. However, it also mirrors the increase of 18:1(*n*-9), typical of storage lipids. Furthermore, both isomers can also be derived from de novo synthesis: 18:1(*n*-9) via desaturation of 18:0 and 18:1(*n*-7) via chain elongation of the possibly also de novo-synthesised 16:1(*n*-7). Therefore, this ratio is only of limited suitability as a trophic index. However, the comparatively high variability might to a certain degree be due to differences in feeding, although such conclusions should be supported by other data.

The correlation curves for 16:0/16:1(*n*-7) are very similar to those of the PUFA/SFA ratio. Again, an important membrane fatty acid, 16:0 (Mayzaud 1997; Stübing et al. 2003), is part of this index, and hence a negative relationship to the total lipid content was to be expected. The same applies to the 20:5(*n*-3)/22:6(*n*-3) ratio, which is consequently also strongly correlated with total lipids in the investigated euphausiids, apart from *E. triacantha*. A better index for a diatom- versus a flagellate-based diet is the ratio of 16:1(*n*-7)/18:4(*n*-3), which exhibits large variations independent of total lipid levels. Both these fatty acids have already been shown to accumulate less strictly with total lipids than the other dominant fatty acids in *E. superba* (Hagen et al. 2001) and in *T. macrura* (Hagen and Kattner 1998).

The generally higher variability of the various ratios and hence weaker dependence on total lipid levels in larval *E. superba* reflect the stronger influence of diet on the lipid compositions of the younger stages. This is in accordance with findings from an experimental study, which showed that different diets had much stronger effects on the fatty-acid compositions of *E. superba* furciliae than on juveniles and adults (Stübing et al. 2003). In postlarval *E. superba*, the comparatively large lipid reservoirs obviously buffer relatively short-term variations in dietary lipid supply, and hence a certain fatty-acid pattern is maintained. In contrast, the lipid-poorer larval stages incorporate dietary fatty acids largely unmodified into their body lipids, especially later in the season, when energy reserves for the winter are extensively accumulated.

A potential refinement to this biomarker approach is the fatty-acid analysis of isolated lipid classes instead of the total lipid extract. Dietary influences are considered to be primarily mirrored in the storage lipids. Inversely, food-derived fatty acids can be masked by the presence of structural lipids, which are supposed to have a relatively stable fatty-acid profile (Sargent et al. 1987). However, such analyses are extremely time-consuming and only feasible for limited sample sets. Moreover, such detailed information does not always yield clearer results. In feeding experiments with juvenile and adult *E. superba*, no statistical changes could be induced in the fatty-acid composition of the storage lipid classes (Stübing et al. 2003). Although not correlated with diet, phosphatidylethanolamine, which serves membrane functions exclusively, exhibited the highest variability, which parallels previous results by Mayzaud et al. (2000). In field-caught *E. superba* larvae, trophic effects were better reflected in the triacylglycerols (TAG) only when TAG-typical fatty acids [16:1(*n*-7), 18:4(*n*-3)] were concerned, while for phospholipid (PL)-typical fatty acids [20:5(*n*-3), 22:6(*n*-3)], dietary effects were more evident in total lipid extracts (Stübing et al. 2003). It is plausible that PL are also influenced by diet, since the long-chain PUFA are essential to most marine zooplankton species (Sargent and Whittle 1981), and hence necessarily originate from the food. Evidence of dietary influences on the fatty-acid composition of PL was found by Virtue et al. (2000) and Graeve et al. (2001). Soudant et al. (1996, 1997) identified phosphatidylcholine as the polar lipid class with the strongest dietary dependence. Hence, in some cases such detailed fatty-acid analyses may provide additional trophic information, but it appears that most tendencies will also be discernible from the total lipid extracts. However, further experimental evidence, coupled with well-designed field studies, are needed to enable wider ecological interpretations.

We conclude that there is a strong dependence of the fatty-acid composition on the total lipid content in Antarctic euphausiids. There is a strong risk of misinterpretation when comparing ratio values of individuals with markedly different total lipid contents. The PUFA/SFA ratio particularly should not be interpreted as an absolute index for nutritional history but always be viewed in the context of total lipid levels. This does not mean that fatty-acid biomarker ratios are generally unsuitable as trophic indicators; however, with the results presented here, we have elucidated important interrelationships, which should be taken into account when applying this approach. This is especially important for those fatty acids that constitute major membrane components, such as the long-chain PUFA 20:5(*n*-3) and 22:6(*n*-3), but also 16:0 and 18:1(*n*-7). Therefore, fatty-acid ratios which include these moieties should always be used with caution when determining an animal's feeding habits or trophic position.

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References

Atkinson A, Meyer B, Stübing D, Hagen W, Schmidt K, Bathmann UV (2002) Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter. II. Juveniles and adults. Limnol Oceanogr 47:953–966

Auel H, Harjes M, da Rocha R, Stübing D, Hagen W (2002) Lipid biomarkers indicate different ecological niches and trophic relationships of the Arctic hyperiid amphipods *Themisto abyssorum* and *T. libellula*. Polar Biol 25:374–383

Bathmann U (2002) The expedition ANTARKTIS XVIII/5b of the research vessel "Polarstern" in 2001. Ber Polarforsch 407: 1–98

Bathmann U, Smetacek V, Reinke M (2000) The expeditions ANTARKTIS XVI/3–4 of the research vessel "Polarstern" in 1999. Ber Polarforsch 364:1–139

Clarke A (1984) The lipid content and composition of some Antarctic macrozooplankton. Br Antarct Surv Bull 63:57–70

Cripps GC, Atkinson A (2000) Fatty acid composition as an indicator of carnivory in Antarctic krill, *Euphausia superba*. Can J Fish Aquat Sci 57:31–37

Cripps GC, Watkins JL, Hill HJ, Atkinson A (1999) Fatty acid content of Antarctic krill, *Euphausia superba*, at South Georgia related to regional populations and variations in diet. Mar Ecol Prog Ser 181:177–188

Dalsgaard J, St. John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment: a review. Adv Mar Biol (in press)

Dunstan GA, Volkman JK, Barrett SM, Leroi J-M, Jeffrey SW (1994) Essential polyunsaturated fatty acids from 14 species of diatom (Bacillariophyceae). Phytochemistry 35:155–161

Falk-Petersen S, Hopkins CCE, Sargent JR (1990) Trophic relationships in the pelagic arctic food web. In: Barnes M, Gibson RN (eds) Proceedings of the 24th European Marine Biology Symposium, Oban, Argyll, Scotland. University Press, Aberdeen, pp 315–333

Falk-Petersen S, Hagen W, Kattner G, Clarke A, Sargent J (2000) Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. Can J Fish Aquat Sci 57:178–191

Graeve M, Kattner G, Hagen W (1994) Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: experimental evidence of trophic markers. J Exp Mar Biol Ecol 182:97–110

Graeve M, Kattner G, Piepenburg D (1997) Lipids in Arctic benthos: does the fatty acid and alcohol composition reflect feeding and trophic interactions? Polar Biol 18:53–61

Graeve M, Dauby P, Scailteur Y (2001) Combined lipid, fatty acid and digestive tract content analyses: a penetrating approach to estimate feeding modes of Antarctic amphipods. Polar Biol 24:853–862

Hagen W (2000) Lipids. In: Harris R, Wiebe P, Lenz J, Skjoldal H, Huntley M (eds) ICES zooplankton methodology manual. Academic, San Diego, pp 113–119

Hagen W, Kattner G (1998) Lipid metabolism of the Antarctic euphausiid *Thysanoessa macrura* and its ecological implications. Limnol Oceanogr 43:1894–1901

Hagen W, Van Vleet ES, Kattner G (1996) Seasonal lipid storage as overwintering strategy of Antarctic krill. Mar Ecol Prog Ser 134:85–89

Hagen W, Kattner G, Terbrüggen A, Van Vleet ES (2001) Lipid metabolism of the Antarctic krill *Euphausia superba* and its ecological implications. Mar Biol 139:95–104

Kattner G, Fricke HSG (1986) Simple gas-liquid chromatography method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. J Chromatogr 361:263–268

Kattner G, Hagen W (1998) Lipid metabolism of the Antarctic euphausiid *Euphausia crystallorophias* and its ecological implications. Mar Ecol Prog Ser 170:203–213

Kirkwood JM (1982) A guide to the Euphausiacea of the Southern Ocean. ANARE Res Notes 1:1–45

Lipsky J (2001) AMLR 1999/2000 field season report. NOAA-TM-NMFS-SWFSC 302:1–139

Mayzaud P (1997) Spatial and life-cycle changes in lipid and fatty acid structure of the Antarctic euphausiid *Euphausia superba*. In: Battaglia B, Valencia J, Walton DWH (eds) Antarctic communities. Species, structure and survival. Cambridge University Press, Cambridge, pp 284–294

Mayzaud P, Virtue P, Albessard E (1999) Seasonal variations in the lipid and fatty acid composition of the euphausiid *Meganyctiphanes norvegica* from the Ligurian Sea. Mar Ecol Prog Ser 186:199–210

Mayzaud P, Albessard E, Virtue P, Boutoute M (2000) Environmental constraints on the lipid composition and metabolism of euphausiids: the case of *Euphausia superba* and *Meganyctiphanes norvegica*. Can J Fish Aquat Sci 57:91–103

Meyer B, Atkinson A, Stübing D, Oettl B, Hagen W, Bathmann UV (2002) Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter. I. Furcilia III larvae. Limnol Oceanogr 47:943–952

Nelson MM, Mooney BD, Nichols PD, Phleger CF (2001) Lipids of Antarctic Ocean amphipods: food chain interactions and the occurrence of novel biomarkers. Mar Chem 73:53–64

Nichols DS, Nichols PD, Sullivan CW (1993) Fatty acid, sterol and hydrocarbon composition of Antarctic sea ice diatom communities during the spring bloom in McMurdo Sound. Antarct Sci 5:271–278

Phleger CF, Nichols PD, Virtue P (1998) Lipids and trophodynamics of Antarctic zooplankton. Comp Biochem Physiol 120 B:311–323

Phleger CF, Nelson MM, Mooney BD, Nichols PD (2001) Interannual variations in the lipids of the Antarctic pteropods *Clione limacina* and *Clio pyramidata*. Comp Biochem Physiol 128 B:553–564

Phleger CF, Nelson MM, Mooney BD, Nichols PD (2002) Interannual and between species comparison of the lipids, fatty acids and sterols of Antarctic krill from the US AMLR Elephant Island survey area. Comp Biochem Physiol 131 B:733–747

Saether O, Ellingsen TE, Mohr V (1986) Lipids of North Atlantic krill. J Lipid Res 27:274–285

Sargent JR, Henderson RJ (1995) Marine (n-3) polyunsaturated fatty acids. In: Hamilton RJ (ed) Developments in oils and fats. Blackie, London, pp 32–65

Sargent JR, Whittle KJ (1981) Lipids and hydrocarbons in the marine food web. In: Longhurst AR (ed) Analysis of marine ecosystems. Academic, London, pp 491–533

Sargent JR, Parkes RJ, Mueller-Harvey I, Henderson RJ (1987) Lipid biomarkers in marine ecology. In: Sleigh MA (ed) Microbes in the sea. Ellis Horwood, Chichester, pp 119–138

Skerratt JH, Nichols PD, McMeekin TA, Burton HR (1997) Identification of dominant taxa in coastal Antarctic water and ice core samples using lipid signatures. In: Battaglia B, Valencia J, Walton DWH (eds) Antarctic communities. Species, structure and survival. Cambridge University Press, Cambridge, pp 79–85

Soudant P, Moal J, Marty Y, Samain JF (1996) Impact of the quality of dietary fatty acids on metabolism and the composition of polar lipid classes in female gonads of *Pecten maximus* (L.). J Exp Mar Biol Ecol 205:149–163

Soudant P, Moal J, Marty Y, Samain JF (1997) Composition of polar lipid classes in male gonads of *Pecten maximus* (L.). Effect of nutrition. J Exp Mar Biol Ecol 215:103–114

St. John MA, Lund T (1996) Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. Mar Ecol Prog Ser 131:75–85

Stübing D, Hagen W, Schmidt K (2003) On the use of lipid biomarkers in marine food web analyses: an experimental case study on the Antarctic krill, *Euphausia superba*. Limnol Oceanogr 48:1685–1700

Virtue P, Nichols PD, Nicol S, McMinn A, Sikes EL (1993) The lipid composition of *Euphausia superba* Dana in relation to the nutritional value of *Phaeocystis pouchetii* (Hariot) Lagerheim. Antarct Sci 5:169–177

Virtue P, Nichols PD, Nicol S, Hosie G (1996) Reproductive tradeoff in male Antarctic krill, *Euphausia superba*. Mar Biol 126:521–527

Virtue P, Mayzaud P, Albessard E, Nichols PD (2000) Use of fatty acids as dietary indicators in northern krill, *Meganyctiphanes norvegica*, from northeastern Atlantic, Kattegat, and Mediterranean waters. Can J Fish Aquat Sci 57:104–114

Chapter 3

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

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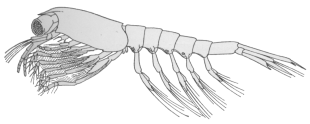

Euphausia superba Furcilia III (from Fraser 1936)

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

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Abstract

Physiological condition and feeding behavior of furcilia larvae were investigated in autumn (April 1999) in the southwestern Lazarev Sea prior to the critical overwintering period. Furcilia stage III (FIII) larvae were most abundant, so only these were used for all analyses (dry mass [DM], elemental and biochemical composition, gut content) and experiments (metabolic and ingestion rates, selective feeding behavior). Chlorophyll *a* (Chl *a*) concentrations in the mixed layer were <0.1 μg L^{-1}. Respiration rates of freshly caught FIII larvae were between 0.4 and 1.2 μl O_2 mg^{-1} DM h^{-1}, similar to larvae fed for 7 d on high food concentrations (4 μg Chl *a* L^{-1}). Excretion rates ranged between 0.01 and 0.02 μg NH_4 mg^{-1} DM h^{-1}. Their atomic O:N ratio of 72 indicated that lipids were the main metabolic substrate of FIII larvae in the field. The daily C ration ranged from 0.4% at the lowest food concentration of 3 μg C L^{-1} to 28% at the highest enriched food concentration of 216 μg C L^{-1}, whereas clearance rates decreased with increasing food concentrations. In natural seawater, 115 ml mg^{-1} C h^{-1}, and in natural seawater enriched with ice biota, 24 ml mg^{-1} C h^{-1}, the clearance rates on specific phytoplankton taxa revealed no significant difference across a food size range of 12–220 μm. The study suggests that during periods of low food supply in the water column, larvae have to exploit ice biota to cover their metabolic demands.

Antarctic krill (*Euphausia superba,* hereafter ''krill'') is a key species in the Antarctic ecosystem, being a major food item for a large number of top predators such as whales, seals, and sea birds. Krill are very successful in the extreme environment of the Southern Ocean because they are capable of exploiting a food supply that is both patchy and seasonal (Quetin and Ross 1991). However, various aspects of their biology are still poorly known. Most of the available information is from spring and summer and is based on data from juvenile and adult krill. Comparatively little is known about their larval ecology. Most data on krill larvae concern their distribution patterns in the Southern Ocean (e.g., Fraser 1936; Siegel 2000), and for both larvae and adults, very little is known about their overwintering strategies (Daly 1990; Hagen et al. 2001). However, these data are essential for modelling population dynamics and for estimating krill production (Hofmann and Lascara 2000).

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

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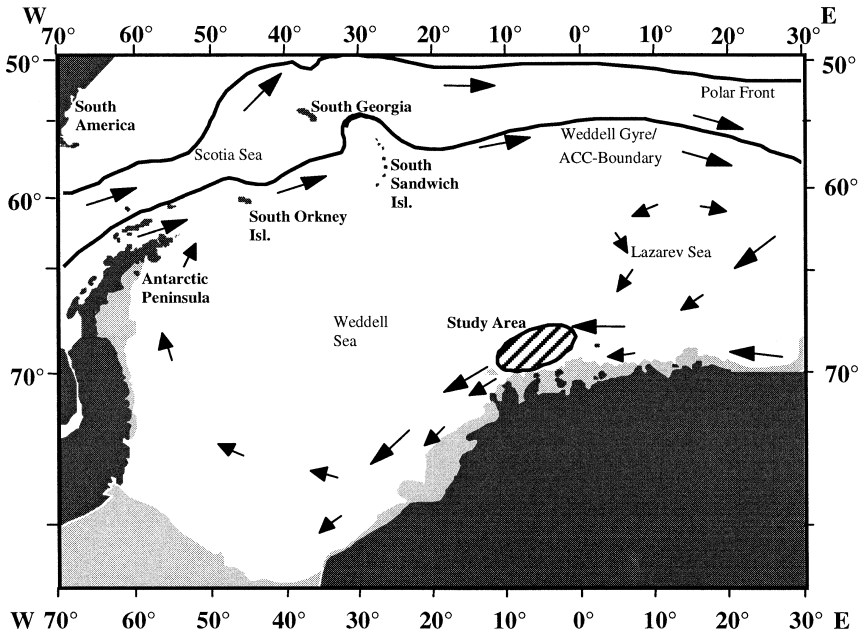

Fig. 1. Investigation area in the SW Lazarev Sea (ACC, Antarctic Circumpolar Current).

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

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

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

Methods

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

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

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

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

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

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

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

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

For measuring oxygen uptake and ammonium production, 4 FIII larvae were incubated in sealed 100-ml bottles filled with filtered seawater (0.45 μm pore size) for 15 h at in situ temperature (−1°C). Respiration and excretion rates of larvae were measured after 7 d at high food concentration and in filtered seawater, respectively (*see above*). Each respiration and excretion measurement comprised seven to eight replicates with larvae and five controls without larvae. Dissolved oxygen was determined by Winkler titration using a 716 DMS Titrino (METROHM). The decrease of oxygen concentration in the experiments was <10%, which is believed not to affect larval respiration (Johnson et al. 1984). Ammonium was measured using a Technicon Autoanalyzer II system (Bran and Lübbe) according to Koroleff (1983). All samples were analyzed in duplicate. Four standards at the beginning and two standards at the end of each run were used. The analytical precision of replicates was about 0.05 μmol.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Results

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

Metabolic rates: Respiration rates of freshly caught FIII larvae ranged from 0.4 to 1.2 μl O_2 mg^{-1} DM h^{-1}, similar to those of the well-fed larvae (0.9 to 1.5 O_2 mg^{-1} DM h^{-1}). The lowest rates were measured after 7 d of starvation, with a mean value of 0.5 μl O_2 mg^{-1} DM h^{-1} (Fig. 2). Using a respiratory quotient of 0.97 (after Ikeda et al. 2000), the oxygen uptake rates of freshly caught FIII larvae correspond to a C loss of 1.4–4.2% body C d^{-1}, whereas after 7 d of starvation, FIII larvae lost 0.2% body C d^{-1}.

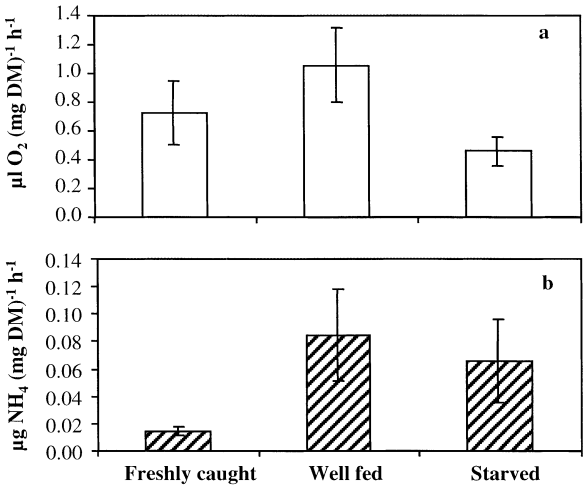

Fig. 2. *Euphausia superba*: furcilia III larvae. (a) Oxygen uptake and (b) ammonium production rates of freshly caught larvae, larvae incubated for 7 d in high food concentrations (well fed) and those that were starved for 7 d. Bars indicate standard deviations of the mean of 16 measurements with freshly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

Ammonium excretion rates were lowest in freshly caught FIII larvae, ranging between 0.01 and 0.02 μg NH_4 mg^{-1} DM h^{-1} (Fig. 2). They correspond to a N loss of 0.7–1.3% body N d^{-1}. Well-fed and starved FIII larvae showed similar values between 0.02 and 0.14 μg NH_4 mg^{-1} DM h^{-1}, corresponding to a N loss of 1.3–9.3% body N d^{-1}. The atomic O : N ratios, calculated from the mean of the measured respiration and excretion rates for freshly caught, well-fed, and starved FIII larvae, were 72, 18, and 10, respectively.

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

Trophic marker fatty acids: No clear pattern from the diatom marker fatty acids 16 : 1(n-7) and 20 : 5(n-3) in freshly caught larvae could be detected. The polyunsaturated fatty acid 20 : 5(n-3) was present in high amounts, whereas 16 : 1(n-7) played only a minor role (Table 4). The dinoflagellate marker fatty acid 18 : 4(n-3) and the major components of calanoid copepod lipids, 20 : 1 and 22 : 1 fatty acid isomers (Kattner and Hagen 1995) were found in small quantities (Table 4). However, in four of the samples, the portion of fatty acid isomers from calanoid copepods rose to 4% of total fatty acids. The 18 : 1(n-9)/18 : 1(n-7) ratio, the total 20 : 1 plus 22 : 1 (Falk-Petersen et al. 2000), and the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) (Cripps and Atkinson 2000) have all been suggested to reflect carnivory. The mean values of these indicators are 1.0, 2.4, and 3.0, respectively (Table 4).

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

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

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

Discussion

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

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

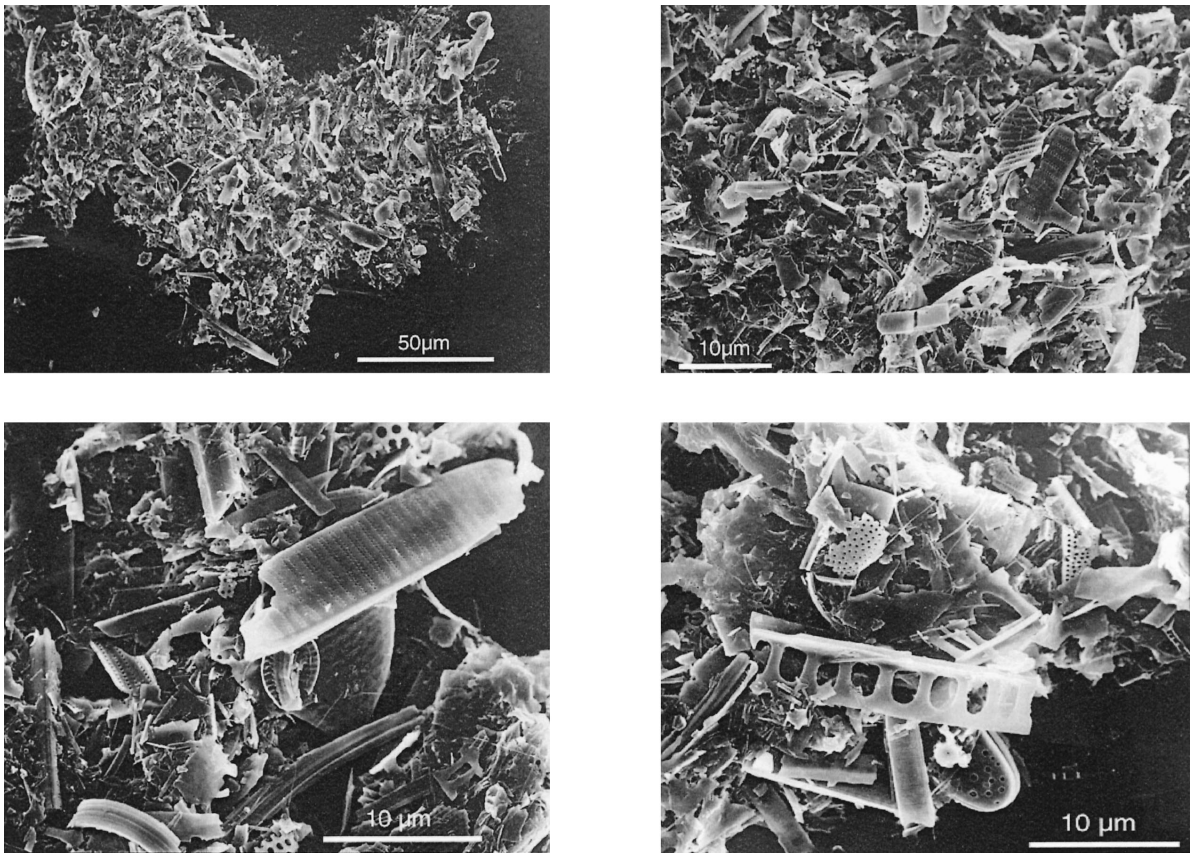

Fig. 3. *Euphausia superba*: furcilia III larvae. Gut contents of furcilia III larvae using scanning electron microscopy. Top left: overview of a gut content; top right, bottom left, and right: detailed pictures of gut contents.

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

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

Many studies have focused on food selection in adult krill (e.g., Habermann et al. 1993 and reference therein), but there is little information on larvae (Daly 1990). FIII larvae can feed across a large size spectrum, which includes protozoans and nauplii of metazoans. Indeed, the maximum size of potential food is probably even larger than our largest food items (Table 4; Fig. 5). This wide range in food items mirrors the findings for adults, which adopt several methods (Hamner and Hamner 2000) to capture planktonic food ranging from ~5 μm up to large calanoid copepods of several millimeters (e.g., Atkinson and Snÿder 1997 and references therein). The larvae had no clear preference toward a specific size or motility of prey. Unselective feeding was also found for juveniles from the same study site, whereas adults fed mainly carnivorously (Atkinson et al. 2002). The unselective feeding behavior over a wide size range might be a response to maximize intake in a suspension of generally suitable food items (Huntley 1981).

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

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

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

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

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

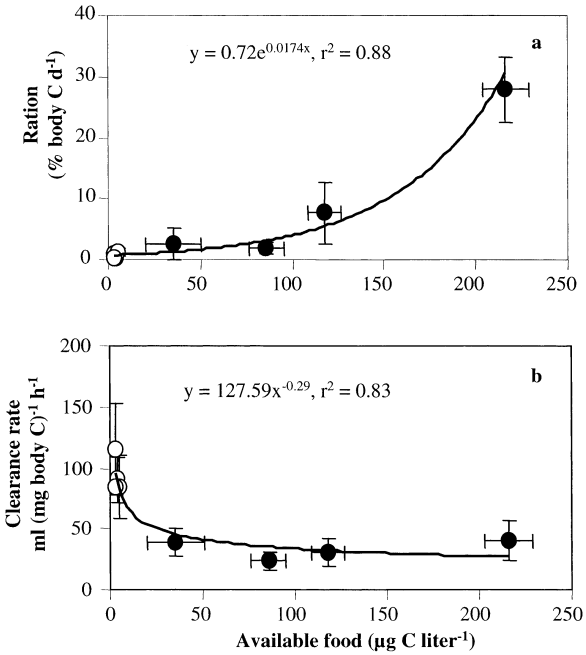

Fig. 4. *Euphausia superba*: furcilia III larvae. (a) Mean daily ration and (b) mean clearance rate versus food concentration. Open circles indicate natural seawater and filled circles natural seawater enriched with ice biota. Bars indicate standard deviation. Eight measurements of each food concentration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

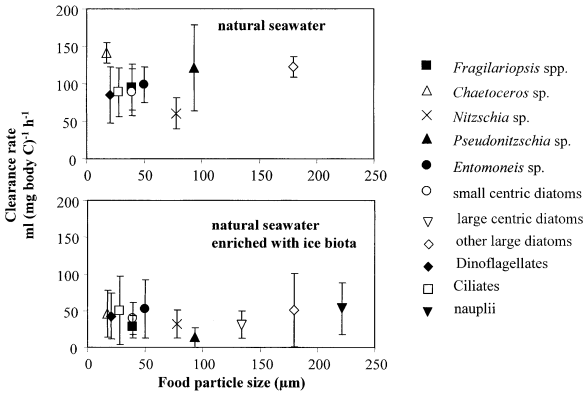

Fig. 5. *Euphausia superba*: furcilia III larvae. Clearance rates on the various prey categories in experiments with natural seawater and natural seawater enriched with ice biota (*see Table 3* for length range of food items). Bars indicate standard deviations of the means of all experiments (ambient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

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

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

understanding of trophic relationships via biochemical markers.

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

The low lipid content of furcilia contrast with that of the adults, which averaged 44% of DM (Atkinson et al. 2002). This suggests that furcilia have a much lower resistance to starvation. Hagen (1988) calculated that the lipid reserves of furcilia may last for about 9–18 d. During our study, after 7 d of starvation, metabolic substrate (as indicated by the O : N ratio) shifted dramatically from a lipid-based metabolism (O : N ratios >50) to one based on protein (O : N ratios <20). Daily C and N losses were high: 1.6% body C d^{-1} and 4.7% body N d^{-1}. Unlike the adults, which can withstand >200 d of starvation (Ikeda and Dixon 1982), the metabolic losses of FIII larvae determined in our study indicate that their lipid reserves are depleted in only 24 d, taking into account that 3–5% of lipids are essential (Hagen et al. 1996). This provides further evidence that winter feeding is essential for the furcilia, but its importance decreases with ontogeny (Quetin et al. 1994; Hofmann and Lascara 2000). Although the lipid stores were low in freshly caught furcilia from this study, their high O : N ratio of 72 suggested rapid lipid turnover.

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

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

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

References

ATKINSON, A., AND R. SNŸDER. 1997. Krill–copepod interactions at South Georgia, Antarctica, I. Omnivory by *Euphausia superba*. Mar. Ecol. Prog. Ser. **160:** 63–76.

———, B. MEYER, U. V. BATHMANN, D. STÜBING, W. HAGEN, AND K. SCHMIDT. 2002. Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter. II. Juveniles and adults. Limnol. Oceanogr. **47:** 953–966.

BRINTON, E., AND A. W. TOWNSEND. 1984. Regional relationships between development and growth in larvae of Antarctic krill, *Euphausia superba* from field samples. J. Crustac. Biol. (spec. 1) **4:** 224–246.

CRIPPS, G. C., AND A. ATKINSON. 2000. Fatty acid composition as an indicator of carnivory in Antarctic krill, *Euphausia superba*. Can. J. Fish. Aquat. Sci. **57:** 31–37.

DALY, K. L. 1990. Overwintering development, growth and feeding of larval *Euphausia superba* in the Antarctic marginal ice zone. Limnol. Oceanogr. **35:** 1564–1576.

DONNELLY, J., J. J. TORRES, T. L. HOPKINS, AND T. M. LANCRAFT. 1990. Proximate composition of Antarctic mesopelagic fishes. Mar. Biol. **106:** 13–23.

FALK-PETERSEN, S., J. R. SARGENT, J. HENDERSON, E. N. HEGSETH, H. HOP, AND Y. B. OKOLODKOV. 1998. Lipids and fatty acids in ice algae and phytoplankton from the Marginal Ice Zone in the Barents Sea. Polar Biol. **20:** 41–47.

———, W. HAGEN, G. KATTNER, A. CLARKE, AND J. SARGENT. 2000. Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. Can. J. Fish. Aquat. Sci. **57:** 178–191.

FRASER, A. J., D. R. TOCHER, AND J. R. SARGENT. 1985. Thin-layer chromatography–flame ionization detection and the quantitation of marine neutral lipids and phospholipids. J. Exp. Mar. Biol. Ecol. **88:** 91–99.

FRASER, F. C. 1936. On the development and distribution of the young stages of krill (*Euphausia superba*). Disc. Rep. **14:** 1–192.

GARRISON, D. L., AND K. R. BUCK. 1986. The biota of Antarctic pack ice in the Weddel Sea and Antarctic Peninsula regions. Polar Biol. **10:** 211–219.

HABERMAN, K. L., R. M. ROSS, AND L. B. QUETIN. 1993. Grazing by the Antarctic krill *Euphausia superba* on *Nitzschia* sp. and *Phaeocystis* sp. monocultures. Antarct. J. U.S. **28:** 217–219.

HAGEN, W. 1988. On the significance of lipids in the Antarctic zooplankton. Rep. Polar Res. **49:** 1–129.

———. 2000. Lipids, p. 113–119. *In* R. Harris, P. Wiebe, J. Lenz, H. R. Skjodal, and M. Huntley [eds.], ICES zooplankton methodological manual. Academic Press.

———, E. S. VAN VLEET, AND G. KATTNER. 1996. Seasonal lipid storage as overwintering strategy of Antarctic krill. Mar. Ecol. Prog. Ser. **134:** 85–89.

———, G. KATTNER, A. TERBRÜGGEN, AND E. S. VAN VLEET. 2001. Lipid metabolism of the Antarctic krill *Euphausia superba* and its ecological implications. Mar. Biol. **139:** 95–104.

HAMNER, W. M., AND P. P. HAMNER. 2000. Behavior of Antarctic krill (*Euphausia superba*): Schooling, foraging, and antipredatory behavior. Can. J. Fish. Aquat. Sci. **57:** 192–202.

HOFMANN, E. E., AND C. M. LASCARA. 2000. Modelling the growth dynamics of Antarctic krill *Euphausia superba*. Mar. Ecol. Prog. Ser. **194:** 219–231.

HOLLAND, D. L., AND P. A. GABBOTT. 1971. A micro-analytical scheme for the determination of protein, carbohydrate, lipid and RNA levels in marine invertebrate larvae. J. Mar. Biol. Ass. U.K. **51:** 659–668.

HOPKINS, T. L., AND J. J. TORRES. 1989. Midwater food web in the vicinity of a marginal ice zone in the western Weddel Sea. Deep-Sea Res. **36:** 543–560.

———, T. M. LANCRAFT, J. J. TORRES, AND J. DONNELLY. 1993. Community structure and trophic ecology of zooplankton in the Scotia Sea marginal ice zone in winter (1988). Deep-Sea Res. **40:** 81–105.

HUNTLEY, M. E. 1981. Nonselective, nonsaturated feeding by three calanoid copepod species in the Labrador Sea. Limnol. Oceanogr. **26:** 831–842.

———, AND E. BRINTON. 1991. Mesoscale variation in growth and early development of *Euphausia superba* Dana in the western Bransfield Strait region. Deep-Sea Res. **38:** 1213–1240.

IKEDA, T. 1981. Metabolic activity of larval stages of Antarctic Krill. Antarct. J. U.S. **16:** 161–162.

———. 1984. Sequences in metabolic rates and elemental composition (C, N, P) during the development of *Euphausia superba* Dana and estimated food requirements during its life span. J. Crustac. Biol. (spec. 1) **4:** 273–284.

———, AND P. DIXON. 1982. Body shrinkage as a possible overwintering mechanism of the Antarctic krill, *Euphausia superba* Dana. J. Exp. Mar. Biol. Ecol. **62:** 143–151.

———, AND R. KIRKWOOD. 1989. Metabolism and body composition of two euphausiids (*Euphausia superba* and *E. crystallorophias*) collected from under the pack ice off Enderby Land, Antarctica. Mar. Biol. **100:** 301–308.

———, AND A. W. MITCHELL. 1982. Oxygen uptake, ammonia excretion and phosphate excretion by krill and other Antarctic zooplankton in relation to their body size and chemical composition. Mar. Biol. **71:** 283–298.

———, J. J. TORRES, S. HERNÁNDEZ-LEÓN, AND S. P. GEIGER. 2000. Metabolism, p. 455–520. *In* R. Harris, P. Wiebe, J. Lenz, H. R. Skjodal, and M. Huntley [eds.], ICES zooplankton methodological manual. Academic Press.

JOHNSON, M. A., M. C., MACAULAY, AND D. C. BIGGS. 1984. Respiration and excretion within a mass aggregation of *Euphausia superba*: Implications for krill distribution. J. Crustac. Biol. **4:** 174–184.

KATTNER, G., AND H. S. G. FRICKE. 1986. Simple gas–liquid chromatography method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. J. Chromatogr. **361:** 263–268.

———, AND W. HAGEN. 1995. Polar herbivorous copepods—different pathways in lipid biosynthesis. ICES J. Mar. Sci. **52:** 329–335.

KAWAGUSHI, K., S. ICHIKAWA, AND O. MATSUDA. 1986. The overwintering strategy of Antarctic krill (*Euphausia superba* Dana) under the coastal fast ice off the Ongul Islands in Lutzow-Holm Bay Antarctica. Mem. Natl. Inst. Polar Res. Tokyo (Special Issue) **44:** 67–85.

KOROLEFF, F. 1983. Determination of ammonia, p. 150–157. *In* K. Grasshoff, M. Ehrhardt, and K. Kremling [eds.], Methods of seawater analysis. Verlag Chemie, Weinheim.

LE FÈVRE, J., L. LEGENDRE, AND R. B. RIVKIN. 1998. Fluxes of biogenic carbon in the Southern Ocean: Roles of large microphagous zooplankton. J. Mar. Syst. **17:** 325–345.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265–275.

MCCLATCHIE, S. 1988. Food-limited growth of *Euphausia superba* in Admiralty Bay, South Shetland Island, Antarctica. Cont. Shelf Res. **8:** 329–345.

MENDEN-DEUER, S., AND E. J. LESSARD. 2000. Carbon to volume relationships for dinoflagellates, diatoms and other protist plankton. Limnol. Oceanogr. **45:** 569–579.

NICOL, S., M. STOLP, T. COCHRAN, P. GEIJSEL, AND J. MARSHALL. 1992. Growth and shrinkage of Antarctic krill *Euphausia superba* from the Indian Ocean sector of the Southern Ocean during summer. Mar. Ecol. Prog. Ser. **89:** 175–181.

QUETIN, L. B., AND R. M. ROSS. 1991. Behavioral and physiological characteristics of the Antarctic krill, *Euphausia superba*. Am. Zool. **31:** 49–63.

———, ———, AND A. CLARKE. 1994. Krill energetics: Seasonal and environmental aspects of the physiology of *Euphausia superba*, p. 165–184. *In* S. El-Sayed [ed.], Southern Ocean ecology: The BIOMASS perspective. Cambridge Univ. Press.

SAETHER, O., T. E. ELLINGSEN, AND V. MOHR. 1986. Lipid biochemistry of zooplankton from high latitudes. Océanis **7:** 623–632.

SCHNACK, S. B. 1985. Feeding by *Euphausia superba* and copepod species in response to varying concentrations of phytoplankton, p. 311–323. *In* W. R. Siegfried, P. R. Condy, and R. M. Laws [eds.], Antarctic nutrient cycles and food webs. Springer-Verlag.

SIEGEL, V. 2000. Krill (Euphausiacea) demography and variability in abundance and distribution. Can. J. Fish. Aquat. Sci. **57:** 151–167.

STRASS, V., A. MACRANDER, C. DIETRICH, P. ROTH, S. GONZALES, AND H. LEACH. 2000. CTD stations and water bottle sampling. Rep. Polar Res. **364:** 23–30.

THOMAS, C. R., G. R. HASLE, K. A. STEIDINGER, E. E. SYVERTSEN, AND K. TANGEN. 1996. Identifying marine diatoms and dinoflagellates. Academic Press.

TORRES, J. J., J. DONNELLY, T. L. HOPKINS, T. M. LANCRAFT, A. V. AARSET, AND D. G. AINLEY. 1994. Proximate composition and overwintering strategies of Antarctic micronektonic crustacea. Mar. Ecol. Prog. Ser. **113:** 221–232.

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Chapter 4

Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter II. Juveniles and adults

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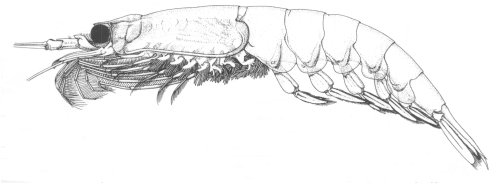

Euphausia superba adult male (from Baker et al. 1990)

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Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter—II. Juveniles and adults

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Abstract

The overwintering success of *Euphausia superba* is a key factor that dictates population size, but there is uncertainty over how they cope with the scarcity of pelagic food. Both nonfeeding strategies (reduced metabolism, lipid use, or shrinkage in size) and switching to other foods (carnivory, ice algae, or detritus) have been suggested. We examined these alternatives in the southwest Lazarev Sea in autumn (April 1999), when sea ice was forming and phytoplankton was at winter concentrations. Both juveniles and adults had a very high lipid content (36% and 44% of dry mass, respectively) of which >40% was phospholipid. However, their low O : N ratios suggested that these reserves were not being used. Results from gut contents analysis and large volume incubations agreed that juveniles fed mainly on phytoplankton and adults fed on small (<3 mm) copepods. This dietary difference was supported possibly by elevated concentrations of 20 : 1 and 22 : 1 fatty acids in the adults. The feeding methods also confirmed that feeding rates were low compared with those in summer. Even when acclimated to high food concentrations, clearance and ingestion rates were <30% of summer rates. Respiration and ammonium excretion rates of freshly caught krill were 60%–80% of those in summer and declined significantly during 18 d of starvation. These findings suggest both switch feeding and energy conservation strategies, with a trend of reduced and more carnivorous feeding with ontogeny. This points to a ''compromise'' strategy for postlarvae, but there are alternative explanations. First, the krill may have reduced their feeding in an autumn transition to a nonfeeding mode, and, second, some of the population may have maintained a high feeding effort whereas the remainder was not feeding.

The importance of krill (*Euphausia superba*) in the Southern Ocean has implications for food web dynamics (Hopkins 1985), biogeochemistry (von Bodungen et al. 1986), and commercial exploitation (Everson 1992). Sea ice extent and overwintering success are major factors dictating their condition, survival, recruitment, and population size (Ross and Quetin 1991; Loeb et al. 1997; Siegel 2000; Quetin and Ross 2001). However, the mechanisms for overwintering in krill are still poorly known, and this topic is characterized by much speculation, few data, and some controversy.

Much of the krill habitat is ice-covered in winter, and pelagic phytoplankton, a major food source during summer, is in short supply. Suggested survival mechanisms fall into two categories: first, nonfeeding strategies and second, switching to alternative foods. Possible nonfeeding strategies include the use of stored lipids (Hagen et al. 1996, 2001), reduction in the metabolic rate (Kawaguchi et al. 1986; Quetin and Ross 1991; Torres et al. 1994*b*), and shrinkage in size (Ikeda and Dixon 1982; Quetin and Ross 1991). Feeding strategies involve switching to ice biota (Marschall 1988; Stretch et al. 1988; Daly 1990), zooplankton (e.g., Hopkins

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et al. 1993; Huntley et al. 1994), or seabed detritus (Kawaguchi et al. 1986).

All of these overwintering mechanisms have been observed at different times and places, but their relative importance remains unclear. Four factors have probably contributed to this. First, the strategies can differ with ontogeny, with furcilia larvae having a greater need to feed than adults (Quetin et al. 1994, 1996). Second, regional differences may be important; for example, the ice types in the Weddell Sea have been suggested to be a better winter habitat than those west of the Antarctic Peninsula (Quetin et al. 1994). Third, there are severe practical problems of covering all strategies simultaneously; both the species and its icy habitat are hard to study. The fourth factor is that krill seems to have flexible behavior. For example, west of the Antarctic Peninsula, Quetin and Ross (1991) found low metabolic rates of postlarvae, whereas, in another winter, Huntley et al. (1994) found them feeding and excreting at summer rates.

Indeed, behavioral flexibility provides a convenient explanation for all the opposing findings on krill overwintering. However, this is inadequate: a feeding strategy involves enzyme synthesis and energy expenditure for foraging, which may preclude a close-down ''hibernation.'' Switching rapidly and flexibly between such opposing modes seems unlikely. It is still unclear whether starvation and shrinkage or feeding and growing are more typical during the long winter period, but the position along this spectrum has clear implications for population dynamics. Most studies have focused on the Weddell Sea–Antarctic Peninsula, neither of which may be typical of their whole circumpolar range. Better regional coverage is one prerequisite for an appreciation of krill overwintering.

In this study, we sampled krill from the shelf break in the southwest Lazarev Sea in austral autumn. Ice cover was locally extensive and in the process of formation, with water column phytoplankton at relatively low winter concentrations. Therefore, this study allows an appraisal of feeding versus nonfeeding strategies at the onset of winter. It is hard to obtain meaningful rate measurements for this large, active animal, so our rationale was to use multiple methods to examine feeding, to use methods comparable to those used in summer, and to compare furcilia, juveniles, and adults caught from the same place at the same time. Measurements include morphometrics, elemental, proximate, and fatty acid composition, plus rates of feeding, excretion, and respiration, with these being compared between freshly caught krill and those acclimated to a range of feeding and starvation regimes. This article addresses juveniles and adults, whereas its companion, Part I (Meyer et al. this volume) covers furcilia.

Materials and methods

Krill were located by use of an Acoustic Doppler Current Profiler (ADCP) on transects of the RV *Polarstern* across the shelf break north of Neumayer station (southwest Lazarev Sea). The study area was in the region 69°43′–69°70′S and 4°38′–6°44′W (see map in Meyer et al. this volume). Krill swarms were found at night at depths of 50–100 m and were sampled between 14 and 20 April 1999 from the top 150 m with vertical tows of a Bongo net (0.5 m diameter, 350 μm mesh). This net had a 5-liter closed cod end, and the slow tows with this comparatively fine-meshed net-collected krill in excellent physiological condition. The drawback, however, was that too few krill were caught to allow large sample sizes across our whole suite of measurements. The krill were either frozen immediately for gut content and biochemical analysis or were used in a series of experiments on feeding, excretion, and respiration.

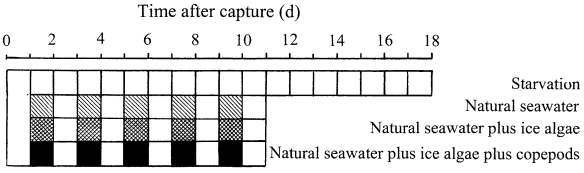

Fig. 1. Schematic representation of the krill experiments in the four feeding regimes. Each box represents a 1-d period in either filtered seawater (open box), natural seawater (single hatch), natural seawater plus ice algae (double hatch), or natural seawater plus ice algae plus copepods (filled box). Each of these four treatments was composed of a single batch of juvenile krill plus a control and a single batch of adults with a control.

Experimental design—There are three main problems in measuring feeding and metabolism of postlarval krill. First, they are stressed by capture, so rates measured on freshly caught animals may not reflect those in the wild. Second, after adequate acclimation, feeding rates obtained can depend partly on the size of their container, which suggests some effect of confinement (Price et al. 1988; Quetin et al. 1994). Third, the feeding rate (and thus respiration and excretion rates) further depends on the food concentrations offered, and these are difficult to relate to those actually located by foraging krill (Price et al. 1988).

To counteract these problems, our experiments were made up of two stages. The first was to measure metabolic rates, gut contents, and biochemical composition of freshly caught krill for comparison with such data from other seasons. The second stage was to acclimate them to various feeding regimes and monitor their feeding and metabolic response. A large suite of measurements (e.g., three methods to examine feeding) was used to reduce possible bias from each method. Because the rates reflect partly the experimental setup, this was standardized with summer studies published elsewhere (Atkinson and Snÿder 1997; Atkinson and Whitehouse 2000), to enable comparison. To follow ontogenetic differences, furcilia, juveniles, and adults were measured concurrently but separately.

Figure 1 summarizes the experiments to measure feeding, excretion, and respiration. Excretion and respiration rates were first measured for freshly caught krill, after which they were divided into batches and exposed to one of four regimes for the next 1–2 weeks. These regimes were (1) starvation in filtered seawater, (2) natural seawater, (3) natural seawater plus ice algae, and (4) natural seawater plus ice algae plus copepods. For the three feeding regimes, 24-h periods of feeding were alternated with 24-h periods in fil-

Table 1. Summary of the five sequential feeding experiments on batches of juvenile and adult krill. Values are means for the second, third, fourth and fifth experiments. Grazing rates during the first experiment were lower than in the subsequent four and were thus treated as an acclimation period, the results not being presented.

Treatment	Container	Volume of container (liters)	Mean krill size (mg DM ind^{-1})	Number of krill incubated	Stocking density (mg DM $liter^{-1}$)
NSW	Juveniles	51	54	7	7.4
NSW	Control	6			
NSW	Adults	51	253	4	20
NSW	Control	6			
NSW + IA	Juveniles	52	45	8	6.9
NSW + IA	Control	6			
NSW + IA	Adults	52	257	4	20
NSW + IA	Control	6			
NSW + IA + C	Juveniles	60	50	7	5.8
NSW + IA + C	Control	60			
NSW + IA + C	Adults	60	207	5	17
NSW + IA + C	Control	60			

NSW; natural seawater; IA, ice algae; C, copepods.

tered seawater to monitor excretion and respiration rates (Fig. 1). In the starvation experiment, we changed their filtered seawater every day and measured respiration and excretion over the next 18 d.

Feeding experiments—The feeding experiments are summarized in Table 1, and the food sources are detailed in Table 2. Natural seawater was obtained from a 10-m cast with 30-liter water bottles. These were drained through silicon tubing into eight experimental 60-liter aspirators, homogenizing across them by filling 10 liters at a time from each bottle. Two aspirators were left with natural seawater, whereas ice algae were added in equal quantities to the remaining six. This was obtained by scooping up brown discolored chunks of multiyear ice with the ship's crane, slowly thawing lumps in seawater at 2°C, and sieving out large particles through 100-μm gauze. A copepod food source was added to four of the aspirators with ice algae. These were taken from nighttime 0–150 m hauls from the krill collection area by use of the Bongo net and supplemented with 0–50 m catches

Table 2. Food items enumerated for the three feeding regimes and their mean contribution to available C across experiments 2, 3, 4, and 5 in each regime. The mean lengths of the copepods refer to prosome lengths.

Food group	Group enumerated	Length class (μm)	Mean length	Total C in food source (mg C m^{-3})		
				NSW	NSW+IA	NSW+IA+C
Diatoms	Unidentified small diatom sp.	All sizes	14	0.017	0.085	0.066
	Small centric diatoms	<50	17	0.042	0.078	0.17
	Fragilariopsis spp.	All sizes	23	4.0	9.7	22
	Entomoneis spp.	All sizes	44	0.0016	1.1	0.96
	Nitzschia spp.	All sizes	76	0.33	1.1	1.6
	Large pennate diatoms	>50	88	0.75	2.0	3.1
	Chaetoceros spp.	All sizes	92	0.066	1.0	1.0
	Large centric diatoms	>50	142	NA	NA	0.16
Motile taxa	Small dinoflagellate sp.	All sizes	17	0.24	0.28	0.46
	Dinoflagellates and ciliates	All sizes	22	0.39	1.1	1.4
	Distephanus sp.	All sizes	56	0.13	0.42	1.4
Copepods	Copepod nauplii	All sizes	181	NA	NA	1.4
	Small *Oithona* spp.	<350	423	NA	NA	2.3
	Large *Oithona* spp.	>350	776	NA	NA	4.1
	Very small calanoids	<750	425	NA	NA	1.6
	Small calanoids	750–1,250	962	NA	NA	9.5
	Medium calanoids	1,250–3,000	2,850	NA	NA	54
	Large calanoids	3,000–5,000	4,230	NA	NA	155
Total for available food	**Counted diatom taxa**			**1.8**	**15**	**29**
	Counted motile taxa			**0.67**	**1.8**	**3.3**
	Autotrophs*			**3.9**	**35**	**54**
	Copepods			**0**	**0**	**228**
	Autotrophs plus copepods			**3.9**	**35**	**282**

* Based on Chl *a* assay and converted to C with a C : Chl *a* ratio of 50.
NA, not analyzed; NSW, natural seawater; NSW+IA, natural seawater enriched with ice algae; NSW+IA+C, natural seawater plus ice algae and copepods.

from a hand-hauled Apstein net. These catches were sorted under a microscope to select only the actively swimming copepods with intact setae. *Pareuchaeta* spp. were discarded, because their predation on other copepods could have led to artifacts in the estimates of predation by krill. The copepod food assemblage was mixed gently and decanted equally into four of the aspirators with ice algae.

The controls were set up in the same way as the experimentals, and these were either of the same volume (for the treatment with enriched copepods; see Table 1) or were of 6 liters obtained by decanting mixed experimental water just before the addition of the krill.

At the start of the experiment, two subsamples of 250 ml for microscopic analysis and two of 1 liter for chlorophyll *a* analysis were siphoned from the mixed contents of the incubation water. The 250-ml samples were fixed in 2% acid Lugol's solution, and Chl *a* subsamples were filtered under gentle vacuum onto 25-mm GF/F filters and frozen at −80°C.

Experimental and control containers were incubated in dim light in the cold room (1–2°C) and stirred gently with a plunger every 1–2 h for the experimental duration (mean, 27 h). After the final mixing at the end of the experiment, 250 ml Lugol's subsamples were taken from each container, and the krill were transferred to the next excretion and respiration experiment. Then, for experimental and controls with copepods, the whole of the aspirator contents were filtered onto a 53-μm sieve and preserved in 10% Lugol's solution. After the alternating experiments on feeding and metabolism (Fig. 1), the krill were measured to the nearest 1 mm from the front of the eye to the tip of the telson and frozen.

Analysis of feeding experiments—The protocols for sample enumeration were standardized to those in a summer study (Atkinson and Snÿder 1997), which should be consulted for further details. From the enriched incubations, uneaten copepods were counted under a binocular microscope. These were enumerated in the same size and taxonomic groups as in the Atkinson and Snÿder (1997) study to allow comparison (see Table 2).

Dry masses (DMs) of copepodite stages of the dominant copepods (*Calanus propinquus, Metridia gerlachei, Calanoides acutus,* and *Ctenocalanus* spp.) were determined directly from the frozen 0–150-m catches of the Bongo net. These were thawed, rinsed, and their prosome lengths measured before being dried at 50°C for >24 h, cooled in a desiccator, and weighed immediately to the nearest microgram. The masses of calanoid copepods in the small size fractions were determined from length-mass regressions constructed from these data. C contents of *Oithona* spp. and copepod nauplii were calculated from their lengths according to the equations in Fransz and Gonzalez (1995, 1997).

Microplankton were enumerated by use of the Utermöhl (1958) technique. Two to three replicate 50-ml aliquots per container were analyzed under an inverted microscope. We selected only the prominent diatoms and motile cells that could be identified consistently (Table 2). However, visual inspection of the settled samples showed that the taxa selected, in particular *Fragilariopsis* spp., were dominant in terms of biolovolume. For each experiment, the dimensions of 30–50 individuals of each category were measured, and their volumes and C concentrations were calculated according to the methods of Eppley et al. (1970) and Putt and Stoecker (1989). Across all experiments and food taxa, the mean reduction due to krill grazing in counted cells was 18%.

The initial Chl *a* values provide independent estimates of available food. These were obtained by use of a Turner 700D fluorometer as described in Meyer et al. (this volume) and have been converted to C under the assumption of a C : Chl *a* ratio of 50. This ratio is in the middle of the range (36–61) for ambient and enriched experiments with the same incubation water (Meyer et al. 2002). The prominent diatoms that were counted comprised ~50% of the total C estimated from the Chl *a* analysis (Table 2).

Feeding rate calculations—Clearance rates on each food group in Table 2 were calculated as

$$F = \ln(C_c/C_k) \times V/(m_k \times t)$$

where F is the clearance rate in ml (mg krill DM)$^{-1}$ h^{-1}, C_c is the final concentration in the control, C_k is the final concentration in the carboy grazed by krill, V is the experimental volume (ml), m_k is the krill DM (mg), and t is the experimental duration (h). This equation allows for changes in prey abundance unrelated to krill grazing (Båmstedt et al. 2000). These were in any case minor, because the total concentrations of counted food items at the end of the control runs were within 8% of initial values. Ingestion rates of the various food categories were calculated as the product of the clearance rate of that category and its C concentration in the final control.

Excretion and respiration experiments—These were run in seawater filtered through 0.45-μm mesh and equilibrated to cold room temperature. The six glass flasks for the krill were 12 liters, and six control flasks without krill were 6 liters. Each batch of krill from a feeding aspirator (*see Table 1*) was used together in a single flask, which thus allowed us to keep track of the feeding and metabolic history of each batch. The krill were rinsed and added to the flasks, which were then topped-up and sealed with Parafilm. Subsampling was done by rapidly inserting a glass tube and siphoning the mixed contents of the flasks into bottles, flushing them to purge air. For both the oxygen and ammonium determinations, three replicate subsamples of 20 ml were used.

Oxygen concentrations were measured after immediate fixing for Winkler titrations, as described in Meyer et al. (this volume), by use of a 716 DMS Titrino (METROHM). The decrease in oxygen concentration in all experiments was <20%. Ammonium excretion rates were measured colorimetrically by use of a Technicon Autoanalyzer II (Bran and Lübbe, Norderstedt, Germany). Four standards at the beginning of each run and two at the end were used, and the analytical precision of replicates was ~0.05 μmol.

Metabolic rate calculations—Excretion rates were calculated by use of the general formula in Atkinson and White-

house (2000). Respiration rates were calculated by use of the formula

$$R = (O_c - O_k)\ V/(m_k \times t)$$

where R is the respiration rate in μl O_2 (mg krill DM)$^{-1}$ h^{-1}, O_c is the O_2 concentration in the control flask at the end of the experiment (μl O_2 liter^{-1}), O_k is the corresponding value in the experimental flask, V is the volume before subsampling (liters), and t is the experimental duration (h).

Unfortunately, heavy use of the cold room meant that we were unable to maintain its temperatures at that of the sea, and incubation temperatures were in the range 1–2°C. This is within the normal winter limits of this species, because it is a characteristic temperature of the warm deep layer. However, we have corrected our results to −1°C, a midrange ambient value, by use of a Q_{10} value of 2.5 (Ikeda 1985). This effectively multiplies our measured rates by a factor of 0.75.

Morphometric, elemental, and biochemical analysis—Lengths of fresh krill were measured before they were frozen individually at −80°C. Their DMs were obtained by thawing, rinsing briefly in deionsed water, drying (at 60°C for 48 h), cooling in a dessicator, and weighing on a Mettler UM3 microbalance. All biochemical analyses were on freeze-dried individual krill that had been homogenized to a powder over ice. C and N contents were analyzed in a Carlo Erba Elemental analyzer against an acetanilide standard. Carbohydrate and protein were measured by placing single krill in 5% trichloroacetic acid in an ice bath, homogenizing with a Branson Sonifer B15 cell disrupter, and centrifuging (at 5,000 × *g*) for 10 min. The supernatant was used for carbohydrate analysis and the pellet for protein (*see* Meyer et al. this volume).

Total lipid was measured gravimetrically by extracting whole krill in a 2:1 mixture of dichloromethane and methanol, as described by Hagen (2000). To analyze the component fatty acids (FAs) in the total lipids, they were hydrolyzed and converted to fatty acid methyl ester (FAME) derivatives in methanol that contained 3% concentrated sulphuric acid at 80°C for 4 h (Meyer et al. this volume). FAMEs were then extracted with hexane, analyzed in a gas chromatograph (HP 6890A), and identified by comparing retention times with those obtained from standard mixtures.

Gut-content analysis—Frozen krill were thawed, measured, and dissected to remove their stomachs and guts. These were rinsed and placed separately in counting chambers. Fullness was scored from 0 (empty) to 10 (full) The whole samples were examined for crustacean fragments under an inverted microscope (×64), and copepod mandibles were measured and counted. Numbers of crustacean fragments were scored as 0 (none), 1 (between 1 and 10), or 2 (>10). *Fragilariopsis* spp. was the dominant identifiable diatom, so whole frustules of these were counted at ×160.

Results

Environment—Krill were found over the shelf break in open water between floes of first-year and multiyear ice. The upper mixed layer varied between 40 and 220 m in depth, with salinities of 33.9–34.6 and temperatures of −1.8–0°C (Strass et al. 2000). This area was characterized by very low water column phytoplankton biomass, with five water-bottle profiles showing Chl *a* concentrations in the upper mixed layer ranging from 0.06 to 0.09 mg Chl *a* m^{-3}. The potential food source from sea ice is hard to assess in such an environment, but we saw ice algae as brown discolorations in many of the large, perforated blocks of multiyear ice.

Table 3. Mesozooplankton community composition, as indicated by three 0–150-m tows with a 350-μm Bongo net.

Species, stage	Abundance (number of individuals m^{-3}) Mean	Range
Calanus propinquus		
CI	0.53	0.4–0.8
CII	2.1	2.0–2.2
CIII	11	7.9–16
CIV	8.1	5.6–11
CV	4.2	2.1–7.2
CVI female	0.07	0–0.2
Metridia gerlachei		
CIV	0.83	0–1.6
CV	8.3	6.4–10
CVI female	4.9	0.4–14
Calanoides acutus:		
CIV	1.0	0.5–1.2
Paraeucheta antarctica		
CIV	0.16	0–0.4
CV	0.09	0–0.28
Ctenocalanus spp.:		
CIV–CVI	10	5.7–14
Oithona spp.:		
CIV–CVI	2.5	1–5.3
Euphausia superba:		
Furcilia	9	1.9–15
Ostracoda:		
All stages	0.50	0–1.1
Amphipoda:		
All stages	0.09	0–0.40
Total for copepods:		
All stages	**54**	**40–62**
Total for metazooplankton:		
All stages	**64**	**48–78**

The mesozooplankton assemblage was sparse (Table 3) and was dominated numerically (84%) by copepods. The biomass-dominant zooplankters (*C. propinquus*, *M. gerlachei*, and *E. superba* furcilia) were retained by the 350-μm net but not the smallest species. The mean biomass of mesozooplankton was 1.37 g DM m^{-2}, within the normal range of values for the East-Wind Drift and the Weddell Sea (e.g., Hopkins and Torres 1989). This biomass was dominated by copepods (60%) and stage III furcilia of *E. superba* (24%).

Distribution and condition of krill—Our direct observations of the underside of the ice were from two daytime observations, each of ~2 h, with a video camera. No furcilia or postlarvae were seen, and none were seen on the under-

Table 4. Biochemical composition of freshly caught juvenile and adult krill.

Measurement	Juvenile krill (n = 34)			Adult krill (n = 21)		
	Mean	Standard deviation	Range	Mean	Standard deviation	Range
Dry mass, DM (mg)	54	20	23–96	249	49	179–384
Carbon content (% of DM)	51	1.3	41–54	54	1.5	46–57
Nitrogen content (% of DM)	8.6	0.59	7.0	7.9	0.48	7.1–8.8
C : N ratio	6.0	0.48	4.3–6.9	6.9	0.59	5.4–8.3
Lipid (% of DM)	36	5.2	26–44	44	8.1	30–58
Protein (% of DM)	35	4.3	28–45	32	1.9	24–38

n, number of krill analyzed. Carbohydrate values were <1% of DM and so not presented.

side of ice floes turned over by the ship. The only krill detected were swarms at 50–100 m depth, located during nighttime ADCP transects across the shelf break. The ADCP transducer was at 8 m depth, so swarms above this would not have been seen.

The postlarval krill were composed of two distinct size groups, 28–38 and 48–58 mm, hereafter termed juveniles and adults. Freshly caught animals had pale yellowish-green hepatopancreases, and food was seen in the guts of some of them.

Morphometrics and body composition—The regression of DM (mg) on length (mm) was

$$\log_{10}(\text{DM, mg}) = 3.25 \log_{10}(\text{length, mm}) - 3.18$$

$$(n = 31 \text{ krill}, r^2 = 0.978)$$

Lipid reserves were very large, reaching 58% of DM (Table 4). These C-rich stores meant that C comprised >50% of DM, as reflected in C : N ratios >6. Triacylglycerols (TAGs) and phospholipids dominated the lipids, with minor contributions from the others (Table 5). TAG is recognized as an energy store for krill, but phospholipids are considered to be primarily structural membrane lipids. However, the quantities of these in both juveniles and adults are in excess of those required for structural purposes, which supports suggestions that polar lipids are an unusual energy store in *E. superba* (e.g., Hagen et al. 1996, 2001).

FA composition—The FA compositions of the experimental krill were similar to the freshly caught specimens and so are not presented here. The latter contained saturated fatty acid (SFAs) and polyunsaturated fatty acids (PUFAs) in similar amounts (Table 6). Three indices have been suggested to reflect the degree of carnivory in krill. These are the content of 20 : 1 and 22 : 1 FAs, the 18 : 1(n-9)/18 : 1(n-7) ratio (Falk-Petersen et al. 2000; Virtue et al. 2000), and the PUFA : SFA ratio (Cripps and Atkinson 2000). The latter two were similar for adults and juveniles (Table 6), whereas the former was greater for adults, which suggests more reliance on carnivory.

Table 5. Lipid class composition (% by mass) of four freshly caught juvenile krill and four freshly caught adults.

Lipid class	Juveniles mean % (SD)	Adults mean % (SD)
Triacylglycerol	54 (1.6)	51 (2.5)
Phospholipid	42 (1.5)	44 (2.6)
Sterol	3.3 (0.53)	4.4 (0.20)
Sterolester	1.2 (0.45)	0.65 (0.12)
Free fatty acid	0.02 (0.01)	0.04 (0.03)

SD, standard deviation.

Gut contents—Although only 12 krill were available for analysis, the adults and juveniles differed clearly in diet (Table 7, Fig. 2) Juveniles had fuller stomachs and guts and contained more phytoplankton than the adults, which contained crustacean fragments. The sizes of the copepod mandibles, m (mm), were used to estimate their prosome lengths, l, from Båmstedt et al. (2000):

$$l = 0.0431 + 0.0112m$$

This equation is for north Atlantic copepods, and individual species differ, so results are presented in broad size categories to show the general picture (Fig. 3). The sample size is small, but the results support those from the feeding incubations (see next section), which suggests that adult krill ate mainly the small copepods (<3 mm).

In situ ingestion rates—The numbers of *Fragilariopsis* spp. in the guts of juvenile krill could not be used to calculate ingestion because most were fragmented and not countable. However, for adults we used the paired mandible method (Båmstedt et al. 2000) to estimate ingestion rates of copepods. Not all of its component measurements were made in this study, which forced some assumptions; nevertheless, this is an independent check on whether feeding rates at our site were indeed low.

In this method, each matched pair of mandibles represents the ingestion of one copepod. Prosome lengths of these were calculated from mandible width (previous section) and then to C by use of regressions constructed from this study site. Literature values of gut passage times of freshly caught postlarvae, albeit from spring and summer (Clarke et al. 1988; Perissinotto and Pakhomov 1996; Atkinson and Snÿder 1997; Daly 1998 and references therein) yielded a value of ~4 h, which we used herein. The final assumption was that feeding rates at the time of capture are representative of daily rates. Daily C rations based on these assumptions were low (mean 0.3%) and variable (range 0–1.0%). Our last two as-

Table 6. Fatty acid composition of freshly caught *Euphausia superba.* Values are percentages by mass of total fatty acids in storage plus structural lipids. Data are for 10 juveniles (mean DM 51 mg) and 8 adults (mean DM 262 mg). Summary data in bold type are for fatty acid characteristics that have been used in previous studies as an index of carnivory (see Results section).

	Juvenile krill		Adult krill	
Fatty acids	Mean	Range	Mean	Range
14:0	11.9	11.1–13.5	12.5	10.5–13.7
15:0	0.5	0.3–0.6	0.4	0.2–0.6
16:0	19.3	17.5–21.4	18.2	15.4–20.4
16:1(n-7)	6.6	1.4–8.2	8.1	5.5–10.5
16:1(n-5)	1.0	0.3–5.5	0.4	0.2–0.5
16:2(n-7)	1.7	1.5–1.8	1.5	1.2–1.7
16:3(n-4)	0.4	0.2–0.4	0.3	0.2–0.4
16:4(n-1)	0.7	0.4-1.0	0.6	0.3–0.7
17:0	0.1	0.05–0.2	0.1	0.05–0.2
18:0	1.7	1.0–1.9	1.4	0.7–1.7
18:1(n-9)	11.7	9.9–16.1	12.8	9.3–15.8
18:1(n-7)	6.5	5.9–7.1	7.1	6.0–8.0
18:2(n-6)	2.6	2.04–2.94	2.3	1.7–2.6
18:3(n-3)	0.7	0.69–0.8	0.7	0.6–0.7
18:4(n-3)	2.3	1.9–2.78	2.2	1.4–2.9
20:1(n-9)	1.1	0.8–1.7	1.4	1.2–1.8
20:1(n-7)	0.4	0.2–1.8	0.4	0.3–0.4
20:4(n-6)	0.7	0.5–0.8	0.7	0.5–1.0
20:4(n-3)	0.6	0.4–0.8	0.6	0.4–0.8
20:5(n-3)	15.2	13.0–16.3	15.3	12.5–16.0
22:1(n-13)	0.2	0.02–0.3	0.08	0.01–0.2
22:1(n-11)	0.7	0.6–1.2	1.0	0.7–1.2
22:1(n-9)	0.1	0.07–0.2	0.2	0.1–0.2
22:4(n-3)	0.4	0.3–0.5	0.4	0.3–0.5
22:5(n-3)	0.5	0.3–0.9	0.5	0.3–0.7
22:6(n-3)	8.2	5.6–9.9	9.1	6.3–10.7
Unknown	3.4	1.5–4.7	2.2	1.2–3.7
Total lipid (% of DM)	37	35–48	44	30–60
Total PUFA (% of fatty acids)	33.9	27.4–37.6	33.1	27.8–40.2
Total SFA (% of fatty acids)	34.4	30.9–36.9	33.2	28.9–36.9
Sum of 20:1 and 22:1 (% of total FA)	**1.8**	**1.4–2.9**	**2.4**	**1.9–3.0**
18:1 (n-9)/18:1(n-7) ratio	**1.81**	**1.6–2.6**	**1.8**	**1.37–2.6**
PUFA/SFA ratio	**0.99**	**0.77–1.1**	**1.0**	**0.78–1.3**

sumptions were probably generous, so the in situ ingestion rate of the adult krill was probably low.

Experiments: clearance rates—Compared with juveniles, the mass-specific clearance rates of adults were low, and they fed more carnivorously (Figs. 4, 5). Adult clearance rates on algae and protozoans were negligible, and the only consistently positive rates were on copepods. In contrast, the juveniles had similar clearance rates on algae, protozoans, and copepods.

The same methods have been used in a summer study at South Georgia (Atkinson and Snÿder 1997), enabling a valid comparison of juvenile feeding behavior (Figs. 4, 5). In the summer, postbloom study, they cleared motile foods (protozoans, ciliates, and copepods) faster than diatoms, even when autotrophs dominated available C. In the present study, however, raptorial feeding was seen only in adults. Juveniles fed at similar rates across the whole size range (Fig. 4), even though copepods made up ~80% of C in the enriched food (Table 2). Also, clearance rates on motile cells were similar to those on diatoms of similar size.

There are two possibilities for artifacts in these interpretations. The first is that krill excretion caused higher growth rates of autotrophs in the grazed containers than in the controls, leading to an underestimation of their clearance rates. However, Fig. 4 shows adult clearance rates consistently around zero for all autotrophs except *Fragilariopsis* spp., which were consistently negative. The use of median clearance rates to summarize these data (Fig. 5) is therefore robust.

The second possible artifact is for ''food chain effects'' to occur in mixed prey assemblages. If krill ingested the higher trophic levels (e.g., copepods) that were feeding on diatoms, then the measured clearance rates of krill on the latter would be artificially low. However, for the copepod-enriched incubations, a paired *t* test showed no significant change in the counted taxa within the controls ($P < 0.05$), which suggests that the copepods had little grazing effect at the con-

Table 7. Analysis of stomach and gut contents of krill, arranged in order of increasing body length. See Materials and Methods: gut content analysis section for explanation of indices. Dashes indicate that data are not available.

	Analysis of stomach contents				Analysis of gut contents		
Krill size, mm	Fullness index (0–10)	No. whole frustules of *Fragilariopsis* spp. per stomach	No. crustacean fragments (score 0, 1, 2)	No. mandibles	No. whole frustules of *Fragilariopsis* spp. per gut	No. crustacean fragments (score 0, 1, 2)	No. mandibles
Juvenile, 28	5	1,678	0	0	433	0	0
Juvenile, 30	7	3,114	1	0	241	0	0
Juvenile, 30	5	986	1	0	234	1	0
Juvenile, 31	5	1,125	0	0	65	0	0
Juvenile, 32	3	35	2	0	0	0	0
Juvenile, 33	6	6,005	0	0	433	0	0
Juvenile mean	**5.2**	**2,157**	**0.67**	**0**	**234**	**0.17**	**0**
Adult, 43	3	69	2	2	0	0	0
Adult, 47	2	440	2	1	—	—	—
Adult, 48	2	880	1	0	—	—	—
Adult, 50	3	346	2	3	17	2	1
Adult, 56	3	1,371	2	5	104	1	0
Adult, 57	2	0	2	1	0	0	0
Adult mean	**2.5**	**518**	**1.8**	**2.0**	**30**	**0.75**	**0.25**

centrations used. Thus, any food chain artifacts are small compared with krill grazing, and the contrasts in Fig. 5 are too large to be explainable by container artifacts.

Experiments: ingestion rates and functional response—Even after a week of acclimation to high food concentrations (~300 μg C liter^{-1}), juvenile rations were <1% of body C d^{-1} (Fig. 6). On the basis of the regressions in Fig. 6, this autumn ration at 300 μg C liter^{-1} is only 17% of that in the comparable South Georgia study, which used the same methods and container sizes but at summer temperatures of 2°C.

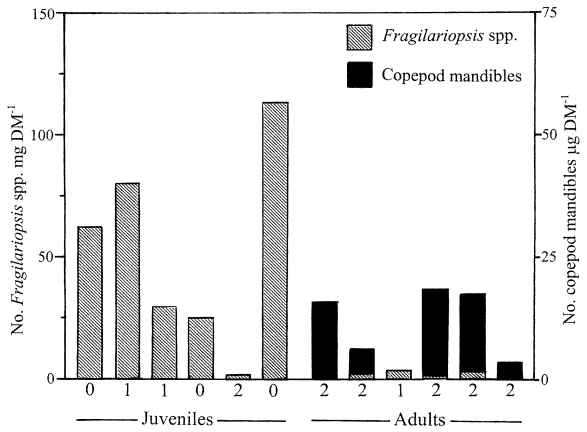

Fig. 2. *E. superba.* Gut contents of the six adults and six juveniles. Hatched bars, left axis: numbers of whole frustules of *Fragilariopsis* spp. mg^{-1} Krill DM. Solid bars, right axis: numbers of copepod mandibles μg^{-1} krill DM. The numbers below the bar for each krill denote the scores (0, 1, or 2) for the number of crustacean fragments found in the stomach (*see Table 7*).

Respiration rates—Mean respiration rates of freshly caught krill were 18 (juveniles, mean DM 44 mg) and 39 (adults, mean DM 148 mg) μl O_2 ind h^{-1}. On the basis of a compilation of equivalent summer data (Rakusa-Suszczewski and Opalinski 1978; Segawa et al. 1979; Ikeda and Hing-Fay 1981; Kils 1981; Ikeda and Mitchell 1982; Ikeda 1984; Ikeda and Bruce 1986; Ishii et al. 1987 in fig. 7 of Quetin et al. 1994), our autumn values for −1°C are 60% (adults) and 77% (juveniles) of summer rates. Starved krill had much lower respiration rates than when freshly caught, and they continued to decline during the experiment (Fig. 7).

Gains and losses of C due to feeding and respiration are

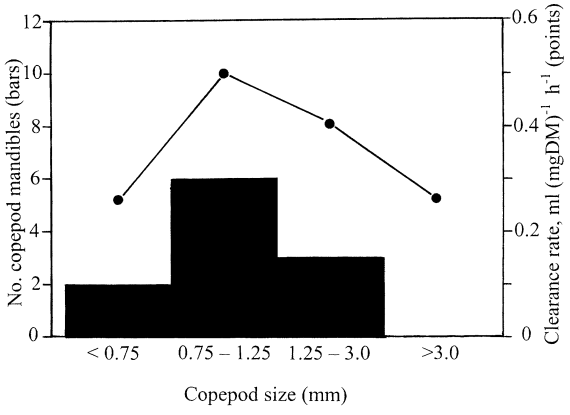

Fig. 3. Adult *E. superba.* Size spectrum of copepods in the diets of adults. Left axis and bars: frequency distribution of prosome lengths of copepods in krill guts as based on mandible widths. Right axis and points: mass specific clearance rates on the same size categories as based on the incubations (*see Fig. 4*).

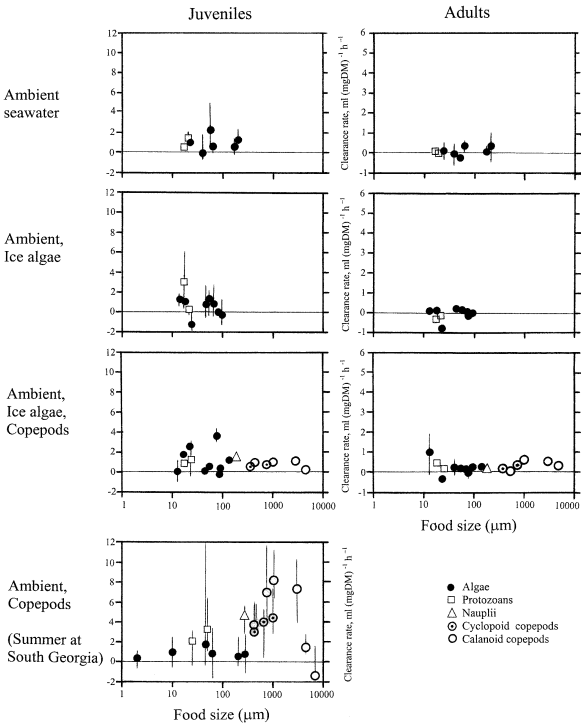

Fig. 4. *E. superba.* Mean and range of mass-specific clearance rates on the various prey categories in relation to prey length. Results are presented in relation to median and interquartile ranges from a comparable summer study at South Georgia.

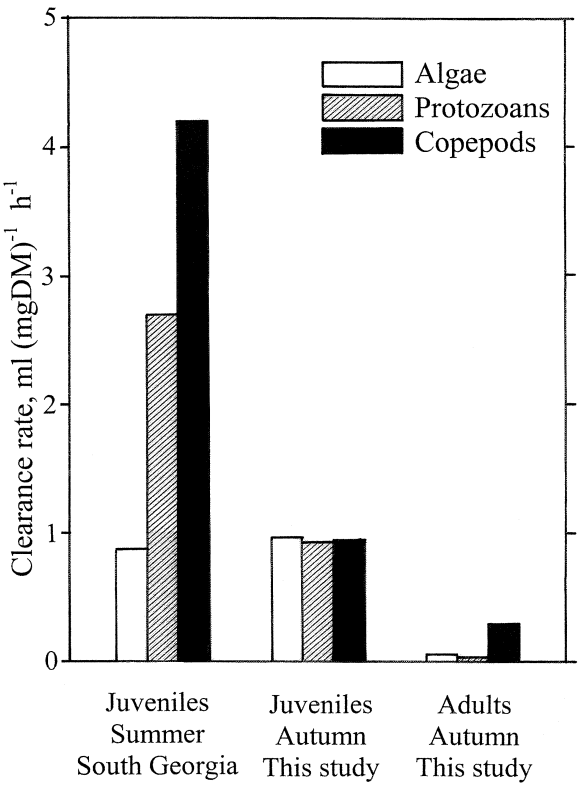

Fig. 5. *E. superba.* Median clearance rates of juveniles and adults on algae, protozoans, and copepods, calculated across all feeding regimes. The food category "algae" is composed of mainly diatom taxa, "protozoans" here includes the counted ciliate and dinoflagellate taxa, which have been assumed for simplicity as mainly heterotrophic, and "copepods" are composed of nauplii and copepod size groups (Table 2) up to a prosome length of 5 mm.

compared in Fig. 8. Respiration rates are expressed as daily C losses by use of a respiratory quotient of 0.97 (Ikeda and Kirkwood 1989). These values are plotted against food availability in the preceding feeding periods. For freshly caught krill, in situ food concentration is plotted as the mean copepod and autotroph biomass from the net samples and water bottle profiles. This is almost certainly an underestimate of the mean, because krill can forage on patches. Indeed, the C gains and losses from freshly caught krill are relatively high compared with those in acclimated krill, being similar to those at the highest food concentrations. At lower values of food availability, respiration rates declined to 0.18% body C d^{-1}, which was observed at the end of the 18-d starvation. Respiratory C losses exceeded the gains from ingestion at all food concentrations measured, and this was also true for freshly caught krill. This discrepancy is particularly marked at low food concentrations.

Excretion rates—Mean ammonium excretion rates of freshly caught adult krill of mean DM 148 mg were 183 nmol NH_4^+ ind^{-1} h^{-1}. This is 81% of that predicted from a regression of summer data at 0°C (fig. 5a in Atkinson and Whitehouse 2000, compiling data of Biggs 1982; Ikeda and Mitchell 1982; Segawa et al. 1982; Hirche 1983; Ikeda and Bruce 1986; Ikeda and Kirkwood 1989). Another comparison with summer data is possible for krill acclimated to feeding in the lab at high food concentrations (the regression in fig. 5b of Atkinson and Whitehouse 2000). Our values for the krill in the treatment with added copepods are only 33% of the corresponding summer values.

In common with the respiratory losses of C, daily losses of NH_4^+ ind^{-1}-N increased with feeding rate (Fig. 9). Also in common was the fact that the daily N loss of freshly caught krill was at a rate nearer that found in the copepod-enriched incubations than that of starving krill.

O : N ratios—Ikeda et al. (2000) calculated that O : N ratios lower than ~21 indicate the dominance of proteins as a metabolic substrate, and values greater than this meant the dominance of lipids. The highest ratios we found were in krill either starving or subjected to low food concentrations (Table 8), which suggests that they were relying on body lipids more than those in situ or at higher food concentrations.

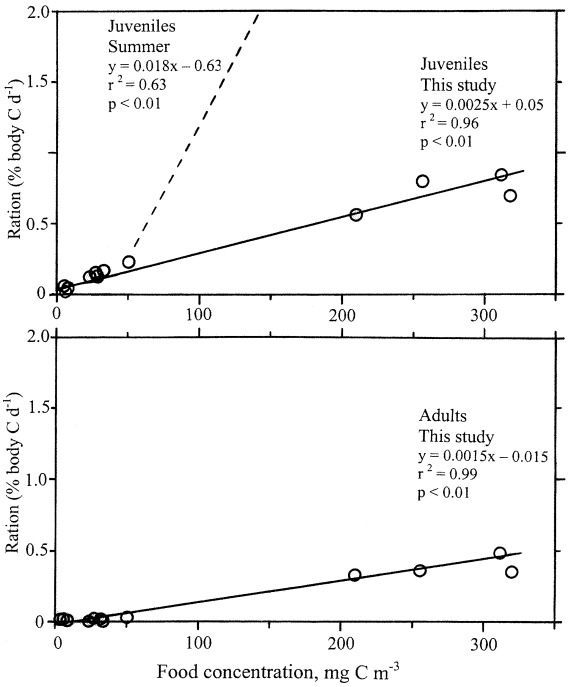

Fig. 6. *E. superba.* Daily C ration as a function of food availability. Data points represent individual experiments for the three feeding regimes. Functional responses are approximated by simple, least-squares linear regressions, because the data were insufficient to define the shape of a functional response curve. The broken regression line describes the functional response for juveniles from Atkinson and Snÿder (1997) on the basis of 43 data points (food concentration 25–1,164 mg C m^{-3}, ration up to 36% body C d^{-1}).

Discussion

In this April study, phytoplankton biomass was at winter levels and there was partial ice cover, so krill had to cope with low phytoplankton concentrations at the onset of winter. Despite having lipid reserves among the highest recorded, their low O : N ratios suggested that they had not resorted to burning these reserves. Feeding rates were lower than those in summer; concomitantly, respiration and excretion rates were lower. Adults had switched to feeding on copepods, but clearance rates could not increase quickly to take advantage of abundant food. Our results thus suggest that both switch feeding and energy conservation mechanisms were being adopted. Below, we outline the evidence for each and examine some of the tradeoffs involved in such a ''compromise'' strategy.

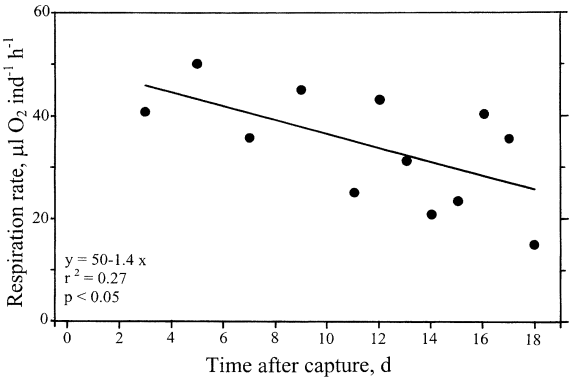

Fig. 7. Adult *E. superba.* Time course of respiration rates of starving krill (mean DM 194 mg) maintained in daily changes of filtered seawater.

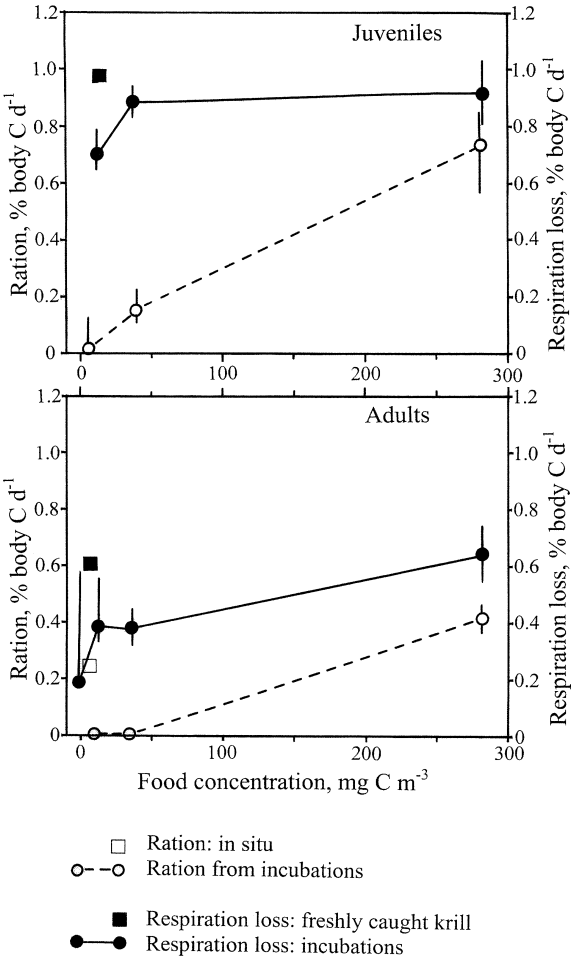

Fig. 8. *E. superba.* Left axis: daily C ration from ingestion. Right axis: daily percentage of body C losses from respiration of freshly caught krill, after 18 d starvation and in the three food regimens. The points represent a mean across experiments, with bars denoting the range. Food concentrations are the means of the four experimental periods (*see Table 2*), with a value of zero for the starved animals.

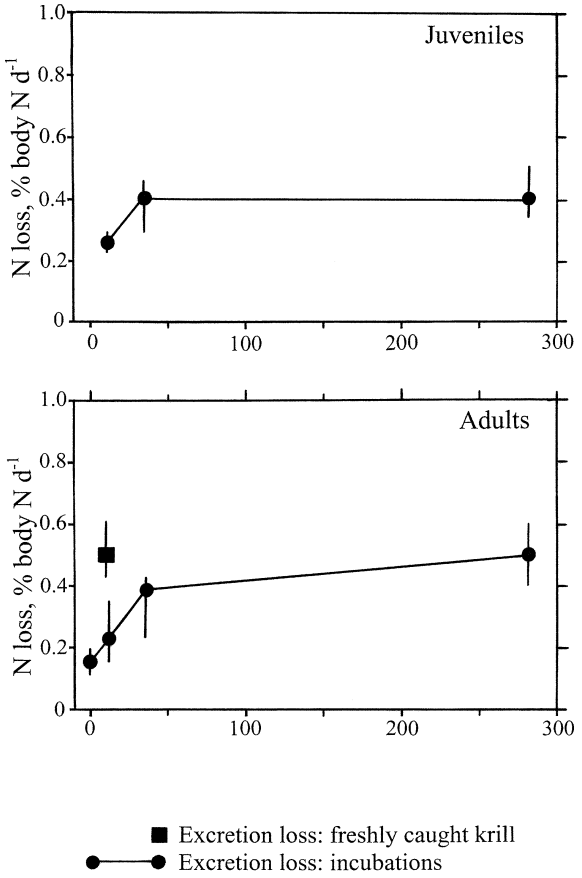

Fig. 9. *E. superba.* Excretion rates of freshly caught and starving krill and those in the three food regimens. The open circle represents freshly caught krill. The points represent a mean across the experiments with bars denoting the range. Food concentrations are the means of the four experimental periods (*see Table 2*), with a value of zero for the starved animals.

Feeding—Because quantifying feeding in krill is difficult yet critical in elucidating overwintering mechanisms, we used three approaches—namely, FA biomarkers, gut content analyses, and incubations. One of the three FA indices in Table 6 suggests that the adults were more carnivorous than juveniles, but low levels of 20:1 and 22:1 FAs imply less carnivory than in the northern krill, *Meganyctiphanes norvegica* (*see* Virtue et al. 2000). However, without measuring the signatures of food and pathways of biosynthesis, the degree of carnivory at the time of lipid deposition is uncertain (Falk-Petersen et al. 2000; Virtue et al. 2000; Hagen et al. 2001). Future ''calibration'' experiments will be needed to place measurements such as those in Tables 5 and 6 into a trophic context.

Gut content analysis is more direct but also has problems, such as feeding occurring in the net. However, the presence of crustaceans in the hind guts as well as in the stomachs suggests that they had not been eaten recently. Furthermore, the juveniles contained diatoms that were not retained by the 350-μm mesh net, whereas adults contained copepods too small to be caught in abundance. These points are hard to explain in terms of net feeding and imply that adults were more carnivorous than juveniles.

Table 8. Atomic O:N ratios of juvenile and adult krill, both freshly caught and in the various food regimes. Dashes indicate no data. Data are ratios of mean values of experiments 2, 3, 4, and 5, plus mean values for freshly caught krill and at the end of 18 d of starvation.

Treatment	Juveniles	Adults
Freshly caught	—	10
Starved	—	23
Natural seawater	22	15
Natural seawater plus ice algae	16	8
Natural seawater, ice algae; plus copepods	17	11

Incubations provide insights into feeding behavior but could suffer from container artifacts. Against the notion that krill just eat anything and everything in captivity, the juveniles fed differently from those in a comparable study (Atkinson and Snÿder 1997). In the present study, they had similar clearance rates across most of the food spectrum, which suggests a ''filtration'' type of behavior. During the summer, postbloom study at South Georgia, juveniles cleared motile food, especially small copepods, most rapidly. Only the adults fed like this in the present study, and this contrast supports the gut content analysis, pointing to a different feeding behavior.

The analyses of gut contents and the incubations provide insights into the spectrum of food available to krill. Feeding rates on copepods >3 mm were low in this study (Figs. 3, 4) and in that by Atkinson and Snÿder (1997). Because most of the winter copepod biomass is in these large, lipid-rich species, many of which are at depth, total biomass values may overplay the importance of this alternative food source. The availability of suitable size copepods varies regionally as well, which perhaps explains why the juveniles were less carnivorous than those at South Georgia. There, the mean copepod biomass was higher (26 mg DM m^{-3}; Atkinson et al. 1999) than in the seasonal ice zone of this study (4.3 mg DM m^{-3}). Microplankton biomass was low in the South Georgia study, and, because ice was not present, an alternative was copepods. In our study, the very limited observations did not reveal krill feeding under sea ice, but it is possible that they did so and fed across a wide size spectrum.

The incubations and gut content analyses also converge on the conclusion that feeding rates of krill were <25% of their capabilities in summer. They were not feeding slowly simply because food was scarce and temperatures were lower; clearance rates failed to increase even after 5 d in abundant food. However, the rations are probably underestimates: even 60-liter containers might constrain feeding (Quetin et al. 1994). In parallel experiments in similar maximum food concentrations but in 180-liter containers, rations averaged 1.3% of body C d^{-1} (K. Schmidt unpubl. data), which is still well below summer values (e.g., Clarke et al. 1988; Pakhomov et al. 1997; Perissinotto et al. 1997).

Carbon and nitrogen conservation—By autumn, these krill were already relying to some extent on energy conservation strategies, because excretion and respiration rates were 60%–80% of summer values. This percentage reduction is not so great as that observed during midwinter (Kawaguchi et al. 1986; Quetin and Ross 1991; Torres et al. 1994*a*) and may be explained simply by lower feeding rates and lower water temperatures rather than by prolonged starvation or ''dormancy.'' Nevertheless, it is still a significant saving. The only clear evidence for a major physiological change is that clearance rates were low and did not respond to increased food.

The potential for more radical energy conservation measures was already available to the krill. They had large lipid depots and reduced their metabolic losses greatly during the starvation experiment. However, the freshly caught animals had low O:N ratios and high metabolic losses compared with these starved animals, which suggests that they had not yet progressed fully into energy conservation.

A myth still persists that krill do not accumulate large lipid depots. Their stores in this study were very high: 36% of DM for juveniles and 44% of DM for adults. These are at the top end of the seasonal range (Hagen et al. 1996, 2001) and indeed would be respectable for diapausing Antarctic copepods (Hagen and Schnack-Schiel 1996). Coupled to this are the very low metabolic losses of adults after only 18 d of starvation: 0.18% of body C d^{-1} and 0.15% of body N d^{-1}. These losses are similar to those during longer term starvation (Ikeda and Dixon 1982) and translate to only 30% of body C and 25% of body N lost over a 200-d winter. Thus, the adults at least would be able to use body lipids and protein and survive an entire winter without feeding.

Central to these arguments is the actual amount of energy krill expend in the sea compared with that in a respirometer. Our flasks were large (12 liters), to minimize stress, but some problems remain. During summer, when krill have low lipid levels, they need to expend energy merely to avoid sinking. However, during early winter, their large lipid reserves add significant buoyancy (Falk-Petersen et al. 2000). Although the krill swam freely in our respirometers, a buoyancy advantage would be hard to measure. Furthermore, Ritz (2000) suggested that wild, schooling krill have lower swimming costs than nonaggregated individuals—for example, those in respirometers. Because winter may last 6 months, the subtleties of these small gains and losses could dictate the condition of krill emerging the following spring (Hofmann and Lascara 2000).

Feeding and energy conservation: A viable compromise strategy for overwintering?—Torres et al. (1994*b*) classed overwintering strategies of Antarctic zooplankton and micronekton into three types. ''Business as usual'' involves continued feeding and growth, whereas at the other extreme was ''dormancy'' with cessation of feeding and energy conservation. A third strategy, ''compromise,'' involves a mixture of reduced, opportunistic feeding and reduced metabolism (*see also* Hagen 1999). Most studies of postlarval krill have found low or zero feeding rates during winter (e.g., Morris and Priddle 1984; Daly and Macaulay 1991; Quetin and Ross 1991; Hopkins et al. 1993). Our autumn study also suggests a compromise.

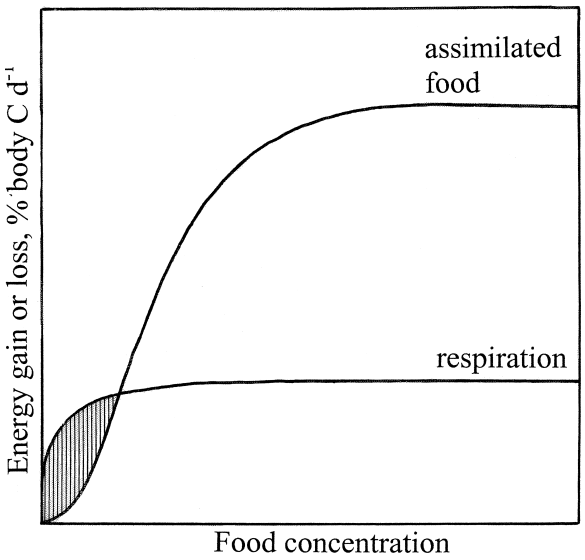

Fig. 10. Schematic representation of the C loss from respiration and gain from assimilated food, expressed in the same units of percentage of body C gained or lost d^{-1}. The horizontal axis represents food concentration. The hatched range of food concentrations represents those in which the animal gained no net energy from feeding.

This strategy sounds plausible: with a reduced metabolism, low food intake might suffice to balance the energy budget. Although this ''bet hedging'' sounds appealing, our results suggest some problems. For it to pay, food intake must cover the costs of feeding and evading predators. At the low food levels of winter, intake increases slowly with food concentration, but metabolic loss increases sharply (Figs. 8, 9). This sharp initial increase may reflect the large difference between nonfeeding and the energy costs of foraging, ingesting, and digesting food. Thus, at low food concentrations, the high basic cost of feeding provides little payback. This tradeoff is shown schematically in Fig. 10. At low winter food concentrations, a low clearance rate might even lead to greater loss of energy than what would result from a nonfeeding strategy. This deficit is probably exaggerated by the constrained conditions in our 60-liter feeding experiments. Nevertheless, if food is indeed scarce during winter, it is hard to see how reduced clearance rates are beneficial.

This point suggests some risk in ''compromise'' overwintering strategies and a paradox: why don't krill simply stop feeding altogether in winter? Possibly, net energy losses are the rule, but feeding is maintained to benefit from brief contacts with dense food patches. This might explain why Huntley et al. (1994) found krill feeding at high rates during midwinter, whereas Quetin and Ross (1991) found very low rates in the same region. Another explanation is that our autumn study was in a transition period, when the krill were

still physiologically active but feeding was slowing to a winter close down. A further alternative is that ''compromise'' is a population-level strategy, not an individual-level one. Our measurements were averages from pooled krill; if only some of them stopped feeding and the remainder had summer clearance rates, the impression would be of a compromise strategy for individuals.

Several authors (Torres et al. 1994*b;* Quetin et al. 1996) have suggested that postlarval krill use a compromise overwintering strategy. Such a strategy now needs to be better defined and characterized. After allowing for temperature differences, are clearance rates similar to those during summer, with the lower metabolic losses resulting simply from lower ingestion rates? Or do they reduce their foraging activity and clearance rate more substantially to achieve energy savings? It is still not clear whether the observed ''flexibility'' of krill is real or is a reflection of the methodologies used. The full implications of the various overwintering strategies will require integrated models of behavior, physiology, growth, predation, and mortality risk.

Ontogenetic differences—In this study and its companion (Meyer et al. this volume), furcilia, juvenile, and adult krill were sampled from the same place at the same time. Insufficient krill were caught to allow large samples sizes across our broad suite of measurements. Alone, these provide circumstantial evidence, but together they demonstrate that furcilia, juveniles, and adults adopt strategies so different that they could almost have been different species.

The furcilia had high feeding and metabolic rates (Meyer et al. this volume), in contrast to the reduced rates for the postlarvae. This supports suggestions that the importance of winter feeding decreases with ontogeny and implies that the furcilia were feeding on ice algae (Daly 1990; Ross and Quetin 1991). But if furcilia could feed at such high rates, why not the postlarvae? Either the under-ice habitat was partitioned to reduce competition or predation risk or simply the adults simply could not enter the narrow ice crevices to feed. Although these general differences between larvae and postlarvae have hitherto been suggested, our study adds to a growing appreciation that the behaviors of juvenile and adult krill are also fundamentally different.

References

ATKINSON, A., AND R. SNŸDER. 1997. Krill-copepod interactions at South Georgia, Antarctica, I. Omnivory by *Euphausia superba.* Mar. Ecol. Prog. Ser. **160:** 63–76.

———, P. WARD, A. HILL, A. S. BRIERLEY, AND G. C. CRIPPS. 1999. Krill-copepod interactions at South Georgia, Antarctica, II. *Euphausia superba* as a major control on copepod abundance. Mar. Ecol. Prog. Ser. **176:** 63–79.

———, AND M. J. WHITEHOUSE. 2000. Ammonium excretion by Antarctic krill *Euphausia superba* at South Georgia. Limnol. Oceanogr. **45:** 55–63.

BAMSTEDT, U., D. J. GIFFORD, X. IRIGOIEN, A. ATKINSON, AND M. ROMAN. 2000. Feeding, p. 297–399. *In* R. P. Harris, P. H. Wiebe, J. Lenz, H. R. Skjoldal, and M. Huntley [eds], ICES Zooplankton methodology manual. Academic.

BIGGS, D. C. 1982. Zooplankton excretion and ammonium cycling in near surface waters of the Southern Ocean. I. Ross Sea, austral summer 1977–1978. Polar Biol. **1:** 55–67.

CLARKE, A., L. B. QUETIN, AND R. M. ROSS. 1988. Laboratory and field estimates of the rate of faecal pellet production by Antarctic krill, *Euphausia superba.* Mar. Biol. **98:** 557–563.

CRIPPS, G. C., AND A. ATKINSON. 2000. Fatty acid composition as an indicator of carnivory in Antarctic krill. Can. J. Fish. Aquat. Sci. **57:** 31–37.

DALY, K. L. 1990. Overwintering development, growth, and feeding of larval *Euphausia superba* in the Antarctic marginal ice zone. Limnol. Oceanogr. **35:** 1564–1576.

———. 1998. Physioecology of juvenile Antarctic krill (*Euphausia superba*) during spring in ice-covered seas. Antarct. Res. Ser. **73:** 183–198.

EPPLEY, R. W., F. M. H. REID, AND J. D. H. STRICKLAND. 1970. Estimates of crop size, growth rate, and primary production. Bull. Scripps Inst. Oceanogr. Univ. Calif. **17:** 33–42.

EVERSON, I. 1992. Managing Southern Ocean krill and fish stocks in a changing environment. Phil. Trans. R. Soc. Lond. B. **338:** 311–317.

FALK-PETERSEN, S., W. HAGEN, G. KATTNER, A. CLARKE, AND J. P. SARGENT. 2000. Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. Can. J. Fish. Aquat. Sci. **57:** 178–191.

FRANSZ, H. G., AND S. R. GONZALEZ. 1995. The production of *Oithona similis* (Copepoda: Cyclopoida) in the Southern Ocean. ICES J. Mar. Sci. **52:** 549–555.

———, AND ———. 1997. Latitudinal metazoan plankton zones in the Antarctic Circumpolar current along 6°W during austral spring 1992. Deep-Sea Res. II **44:** 395–414.

HAGEN, W. 1999. Reproductive strategies and energetic adaptations of polar zooplankton. Invertebr. Reprod. Dev. **36:** 24–34.

———. 2000. Lipids, p. 113–119. *In* R. P. Harris, P. H. Wiebe, J. Lenz, H. R. Skjoldal, and M. Huntley [eds], ICES Zooplankton Methodolgy Manual. Academic.

———, G. KATTNER, A. TERBRÜGGEN, AND E. S. VAN VLEET. 2001. Lipid metabolism of the Antarctic krill *Euphausia superba* and its ecological implications. Mar. Biol. **139:** 95–104.

———, AND S. B. SCHNACK-SCHIEL. 1996. Seasonal lipid dynamics in dominant Antarctic copepods: Energy for overwintering or reproduction? Deep-Sea Res. **43:** 139–158.

———, E. S. VAN VLEET, AND G. KATTNER. 1996. Seasonal lipid storage as overwintering strategy of Antarctic krill. Mar. Ecol. Prog. Ser. **134:** 85–89.

HIRCHE, H.-J. 1983. Excretion and respiration of the Antarctic krill *Euphausia superba.* Polar Biol. **1:** 205–209.

HOFMANN, E. E., AND C. M. LASCARA. 2000. Modeling the growth dynamics of Antarctic krill *Euphausia superba.* Mar. Ecol. Prog. Ser. **194:** 219–231.

HOPKINS, T. L. 1985. Food web of an Antarctic midwater ecosystem. Mar. Biol. **89:** 197–212.

———, T. M. LANCRAFT, J. J. TORRES, AND J. DONNELLY. 1993. Community structure and trophic ecology of zooplankton in the Scotia Sea marginal ice zone in winter (1988). Deep-Sea Res. **40:** 81–105.

———, AND J. J. TORRES. 1989. Midwater food web in the vicinity of a marginal ice zone in the western Weddell Sea. Deep-Sea Res. **36:** 543–560.

HUNTLEY, M. E., W. NORDHAUSEN, AND M. D. G. LOPEZ. 1994. Elemental composition, metabolic activity and growth of Antarctic krill *Euphausia superba* during winter. Mar. Ecol. Prog. Ser. **107:** 23–40.

IKEDA, T. 1984. Sequences in metabolic rates and elemental composition (C, N, P) during the development of *Euphausia superba* Dana and estimated food requirements during it lifespan. J. Crustac. Biol. (Spec No. 1) **4:** 273–284.

———. 1985. Metabolic rates of epipelagic marine zooplankton as a function of body mass and temperature. Mar. Biol. **85:** 1–11.

———, AND B. BRUCE. 1986. Metabolic activity and elemental composition of krill and other zooplankton from Prydz Bay, Antarctica, during early summer (November–December). Mar. Biol. **92:** 545–555.

———, AND P. DIXON. 1982. Body shrinkage as a possible overwintering mechanism of the Antarctic krill, *Euphausia superba.* Dana. J. Exp. Mar. Biol. Ecol. **62:** 143–151.

———, AND E. HING-FAY. 1981. Metabolic activity of zooplankton from the Antarctic Ocean. Aust. J. Mar. Freshw. Res. **32:** 921–930.

———, AND R. KIRKWOOD. 1989. Metabolism and body composition of two euphausiids (*Euphausia superba* and *E. crystallorophias*) collected from under the pack-ice off Enderby Land, Antarctica. Mar. Biol. **100:** 301–308.

———, AND A. W. MITCHELL. 1982. Oxygen uptake, ammonia excretion and phosphate excretion by krill and other Antarctic zooplankton in relation to their body size and chemical composition. Mar. Biol. **71:** 283–298.

———, J. J. TORRES, S. HERNANDEZ-LEON, AND S. P. GEIGER. 2000. Metabolism, p. 455–532. *In* R. Harris, P. H. Wiebe, J. Lenz, H. R. Skjoldal, and M. Huntley [eds], ICES Zooplankton methodolgy manual. Academic.

ISHII, H., M. OMORI, M. MAEDA, AND Y. WATANABE. 1987. Metabolic rates and elemental composition of the Antarctic krill *Euphausia superba* Dana. Polar Biol. **7:** 379–382.

KAWAGUCHI, K., S. ISHIKAWA, AND O. MATSUDA. 1986. The overwintering strategy of Antarctic krill (*Euphausia superba* Dana) under the coastal fast ice off the Ongul Islands in Lützow-Holm Bay, Antarctica. Mem. Nat. Inst. Polar Res. Spec Iss. **44:** 67–85.

KILS, U. 1981. Swimming behaviour, swimming performance and energy balance of Antarctic krill *Euphausia superba.* BIOMASS Sci. Ser. **3:** 1–233.

LOEB, V., V. SIEGEL, O. HOLM-HANSEN, R. HEWITT, W. FRASER, W. TRIVELPIECE, AND S. TRIVELPIECE. 1997. Effects of sea-ice extent and krill or salp dominance on the Antarctic food web. Nature **387:** 897–900.

MARSCHALL, H.-P. 1988. The overwintering strategy of Antarctic krill under pack-ice of the Weddell Sea. Polar Biol. **9:** 245–250.

MEYER, B., A. ATKINSON, D. STÜBING, B. OETTL, W. HAGEN, AND U. V. BATHMANN. 2002. Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter. I. Furcilia III larvae. Limnol. Oceanogr. **47:** 943–952.

MORRIS, D. J., AND J. PRIDDLE. 1984. Observations on the feeding and moulting of the Antarctic krill, *Euphausia superba* Dana, in winter. Br. Antarct. Surv. Bull. **65:** 57–63.

PAKHOMOV, E. A., R. PERISSINOTTO, P. W. FRONEMAN, AND D. G. M. MILLER. 1997. Energetics and feeding dynamics of *Euphausia superba* in the South Georgia region during the summer of 1994. J. Plankton Res. **19:** 399–423.

PERISSINOTTO, R., AND E. A. PAKHOMOV. 1996. Gut evacuation rates and pigment destruction in the Antarctic krill *Euphausia superba.* Mar. Biol. **125:** 47–54.

———, ———, C. D. MCQUAID, AND P. W. FRONEMAN. 1997. *In situ* grazing rates and daily ration of Antarctic krill *Euphausia superba* feeding on phytoplankton at the Antarctic Polar Front and the Marginal Ice Zone. Mar. Ecol. Prog. Ser. **160:** 77–91.

PRICE, H. J., K. R. BOYD, AND C. M. BOYD. 1988. Omnivorous feeding behavior of the Antarctic krill *Euphausia superba.* Mar. Biol. **97:** 67–77.

PUTT, M., AND D. K. STOECKER. 1989. An experimentally determined carbon : volume ratio for marine ''oligotrichous'' ciliates from estuarine and coastal waters. Limnol. Oceanogr **34:** 1097–1103.

QUETIN, L. B., AND R. M. ROSS. 1991. Behavioural and physiological characteristics of the Antarctic krill, *Euphausia superba.* Am. Zool. **31:** 49–63.

———, AND ———. 2001. Environmental variability and its impact on the reproductive cycle of Antarctic krill. Am. Zool. **41:** 74–89.

———, ———, AND A. CLARKE. 1994. Krill energetics: Seasonal and environmental aspects of the physiology of *Euphausia superba,* p. 165–184. *In* S. Z. El-Sayed [ed], Southern Ocean ecology: The BIOMASS perspective. Cambridge Univ. Press.

———, ———, T. K. FRAZER, AND K. L. HABERMAN. 1996. Factors affecting distribution and abundance of zooplankton, with an emphasis on Antarctic krill, *Euphausia superba,* p. 357–371. *In* R. M. Ross, E. E. Hofmann, and L. B. Quetin [eds], Antarctic Research Series 70. American Geophysical Union.

RAKUSA-SUSZCZEWSKI, S., AND K. W. OPALINSKI. 1978. Oxygen consumption in *Euphausia superba.* Polsk. Arch. Hydrobiol. 25: 633–641.

RITZ, D. A. 2000. Is social aggregation in aquatic organisms a strategy to conserve energy? Can. J. Fish. Aquat. Sci. **57:** 59–67.

ROSS, R. M., AND L. B. QUETIN. 1991. Ecological physiology of larval euphausiids, *Euphausia superba* (Euphausiacea) Mem. Queensl. Mus. **31:** 321–333.

SEGAWA, S., M. KATO, AND M. MURANO. 1979. Oxygen consumption of the Antarctic krill *Euphausia superba.* Trans. Tokyo Univ. Fish. **3:** 113–120.

———, ———, AND ———. 1982. Respiration and ammonia excretion rates of the Antarctic krill, *Euphausia superba* Dana. Trans. Tokyo Univ. Fish. **5:** 177–187.

SIEGEL, V. 2000. Krill (Euphausiacea) life history and aspects of population dynamics. Can. J. Fish. Aquat. Sci. **57:** 130–150.

STRASS, V., A. MACRANDER, C. DIETRICH, P. ROTH, S. GONZALEZ, AND H. LEACH. 2000. CTD stations and water bottle sampling. Rep. Polar Res. **364:** 23–30.

STRETCH, J. J., P. HAMNER, W. HAMNER, W. C. MITCHELL, J. COOK, AND C. W. SULLIVAN. 1988. Foraging behaviour of Antarctic krill *Euphausia superba* on sea ice macroalgae. Mar. Ecol. Prog. Ser. **44:** 131–139.

TORRES, J. J., A. V. AARSET, J. DONNELLY, T. L. HOPKINS, T. M. LANCRAFT, AND D. G. AINLEY. 1994*a.* Metabolism of Antarctic micronektonic Crustacea as a function of depth of occurrence and season. Mar. Ecol. Prog. Ser. **113:** 207–219.

———, J. DONNELLY, T. L. HOPKINS, T. M. LANCRAFT, A. V. AARSET, AND D. G. AINLEY. 1994*b.* Proximate composition and overwintering strategies of Antarctic micronektonic Crustacea. Mar. Ecol. Prog. Ser. **113:** 221–232.

UTERMÖHL, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Verh. Int. Limnol. **9:** 1–38.

VIRTUE, P. P., MAYZAUD, E. ALBESSARD, AND P. NICHOLS. 2000. Use of fatty acids as dietory indicators in northern krill, *Meganyctiphanes norvegica,* from the northeastern Atlantic, Kattegat, and Mediterranean waters. Can. J. Fish. Aquat. Sci. **57:** 104–114.

VON BOGUNGEN, B., V. SMETACEK, M. M. TILZER, AND B. ZEITZSCHEL. 1986. Primary production and sedimentation during spring in the Antarctic Peninsula region. Deep-Sea Res. **33:** 177–194.

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Chapter 5

Energy storage via phosphatidylcholine (lecithine) in Antarctic euphausiids – preliminary biochemical and histochemical evidence

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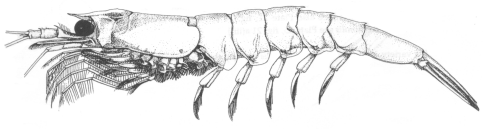

Euphausia triacantha adult male (from Baker et al. 1990)

Energy storage via phosphatidylcholine (lecithine) in Antarctic euphausiids – preliminary biochemical and histochemical evidence of an unusual phenomenon

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Abstract

Phosphatidylcholine (PC), a typical component of the lipid bilayer of biomembranes, has been reported to linearly accumulate with increasing lipid levels in many polar euphausiids, indicating the function of PC as an energy reserve, which is very unusual among marine zooplankton and micronekton. We investigated the role of PC in three Antarctic species, *Euphausia superba*, *E. triacantha*, and *Thysanoessa macrura*. Transverse sections of the cephalothorax were performed by the freeze–sectioning technique and stained alternatively for neutral lipids and phospholipids. All tissues/organs rich in neutral lipids were also stained by the phospholipid dye. Droplets of neutral lipids were detected in "storage tissue" of lipid–rich male *E. superba*. Muscles were stained dark blue indicating high concentrations of phospholipids, but they were entirely devoid of neutral lipids. The hepatopancreas was also predominantly stained for phospholipids, consistent with lipid class analyses of the excised organ. Free lipids were separated from membrane bound lipids by means of sub–cellular fractionation. In *E. superba*, up to 83% of the phospholipids were not bound to membranes and the distribution of neutral and phospholipids in the various fractions was similar in *T. macrura*. In contrast, *E. triacantha* had lower levels of non–membrane bound phospholipids (65% of total phospholipids). Comparative studies were carried out on an Arctic copepod, *Calanus* sp., since copepods do not accumulate PC with increasing lipid content. Accordingly, no free phospholipids were detected in this species. The data presented here provide clear evidence that PC functions as an energy reserve and the potential ecophysiological advantages of PC storage are discussed in relation to the life history adaptations of the various species.

KEY WORDS: Antarctic euphausiids, lipid classes, phosphatidylcholine, biomembranes, sub–cellular fractionation, energy reserves, energetic adaptations

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Introduction

The accumulation of large lipid depots is a common phenomenon in polar zooplankton (reviewed by Clarke & Peck 1991). It is one of the most important adaptations particularly of herbivorous species to the extremely seasonal food availability at high latitudes (Sargent et al. 1981, Clarke 1983, Hagen 1988). Calanoid copepods from polar oceans usually contain very high lipid levels with major portions (up to 90%) being wax esters, or in some cases also triacylglycerols (e.g. Hagen et al. 1993, Kattner et al. 1994, Hagen & Schnack-Schiel 1996). In contrast, neutral lipid levels of polar euphausiids never reach such high percentages, despite their comparably high total lipid contents (see Falk-Petersen et al. 2000, for review). Their lipid compositions show an interesting peculiarity with exceptionally high polar lipid concentrations (e.g. Saether et al. 1985). Expressed as percent of dry mass, polar lipids exhibit a linear increase with increasing total lipid levels, similar to neutral lipids. Based on this unusual relationship, Ellingsen & Mohr (1981) as well as Saether et al. (1986) proposed that polar lipids, besides their structural functions in membranes, serve as additional energy reserves in the investigated euphausiids. Hagen (1988) could demonstrate that only phosphatidylcholine (PC) is responsible for the increase in polar lipids, whereas the other dominant phospholipid, phosphatidylethanolamine, remains at a constant level on a % dry mass basis.

This phenomenon is very extraordinary, since phospholipids are not utilised as energy reserve in any other pelagic species except for the postlarvae of spiny lobsters (*Jasus* sp., Jeffs et al. 2001, 2002). Phospholipids are the basic structural units of all biomembranes forming semipermeable bilayers that are essential to the complex operations of cells. Due to their amphipathic nature, phospholipid molecules spontaneously aggregate to bilayers in aqueous media and are therefore supposed to exclusively occur as integral components of membranes. Hence, the original hypothesis that these high amounts of PC are used as an additional energy reserve has been doubted (Sargent & Henderson 1995), especially since there was no evident energetic and ecological advantages connected to phospholipid deposition.

Therefore, one major objective of this study was to investigate the ecophysiological role of PC accumulation for selected Antarctic euphausiids and to shed new light on the controversy whether PC is deposited as an additional energy reserve. Cell and lipid biochemical techniques were combined to differentiate between free and membrane bound PC, and in comparison with histochemical methods phospholipid depots could be localised. The results are discussed in the light of potentially elevated requirements for this specific lipid class related to the life histories of these highly active euphausiid species.

Material and Methods

Sampling

Euphausia superba specimens were sampled during a summer cruise with R.V. Yuzhmorgeologiya off the South Shetland Islands from 18 February – 12 March 2000. *E. superba*, *E. triacantha*, and *Thysanoessa macrura* were obtained during an autumn cruise with R.V. Polarstern (18 April – 1 May 2001) west of the Antarctic Peninsula and in the Bellingshausen Sea. Detailed information about the programmes and the station data have been published by Lipsky (2001) and Bathmann (2002). In 2000, double oblique IKMT tows (mesh size 505 μm)

were performed in the top 170 m. In 2001, euphausiids were sampled by double oblique RMT 1+8 hauls (mesh size 325 and 4500 μm, respectively, 20 L closed cod end) or vertical bongo net tows (mesh size 335 μm, 5 L closed cod end). Euphausiids were thus obtained in excellent condition and immediately transferred to the cool lab for sorting. Prior to freezing at −80 °C, developmental stage or sex (after Kirkwood 1982), and length (tip of rostrum to end of telson) were recorded, the animals were briefly rinsed with deionised water and blotted dry.

Sub–cellular fractionation

Deep–frozen euphausiid specimens were homogenised in approx. the three–fold volume of ice–cold buffer (50 mM Tris-HCl, 5 mM EDTA, 250 mM D(+)-sucrose, pH 7.4) by three brief strokes of an ultra–turrax. The samples were then subjected to a discontinuous density gradient centrifugation: 1.1 M, 0.9 M, and 0.7 M sucrose solutions (3 ml each), prepared in Tris–buffer, were layered in a 12 ml centrifuge tube (Nalgene PA). The top layer consisted of 3 ml sample homogenate. The gradients were centrifuged at 100,000 g (35,000 rpm) and 4° C for 16 h (Optima LE-80 K Ultracentrifuge, Beckman Coulter) using a 6 x 12 ml swing–out rotor (SW 40, Beckman). The separated materials were collected at each interface. The first fraction was transferred to a 10 ml centrifuge tube (Beckman) and diluted 1:1 (by volume) with ice–cold sucrose–free buffer containing 150 mM NaCl and 10 mM Tris-HCl (pH 7.4). This suspension was centrifuged at 100,000 g (45,000 rpm) and 4° C for 2 h (Optima MAX-E Ultracentrifuge, Beckman) using an angle rotor (MLA-80, Beckman). The resulting supernatant was separated from the pellet. Thus, five fractions were obtained in total (see Fig. 1 for the fractionation protocol).

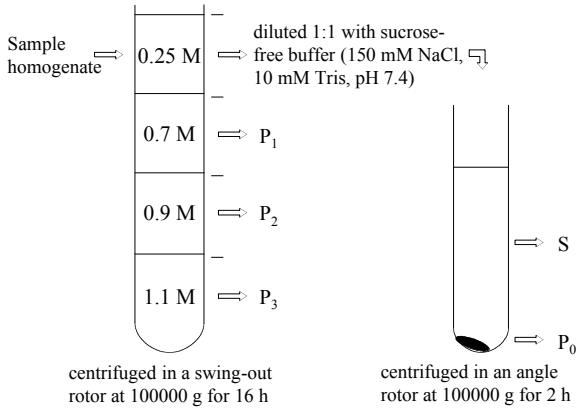


Figure 1: Protocol for the sub–cellular fractionation

Lipid analyses

Total lipid was extracted from the entire volumes of the density layers with 8 ml dichloromethane/ methanol (2:1, by volume) (modified after Hagen 2000). P_0 was resuspended in extraction solvent using a Potter teflon homogeniser. All extracts were treated with ultrasound for 30 s. Extracts were purified by shaking with 2 ml of a 0.88% KCl solution, centrifuged at 1,500 g and 2° C for 10 min. The lower phase was transferred to pre–weighed teflon–capped glass vials and the solvent evaporated to dryness under a stream of nitrogen. The vials were further dried in

an evacuated desiccator for 30 min. Lipid was weighed on a microbalance (Sartorius R200D), redissolved in dichloromethane/methanol (2:1) and stored under nitrogen atmosphere at −80° C until further analysis.

Neutral lipid classes were measured in duplicate by thin–layer chromatography (TLC)–flame ionisation detection (FID) on an Iatroscan Mk V according to Fraser et al. (1985). Since different lipid classes give different FID responses (e.g. Parrish 1987), two mixtures of commercial standards (Sigma), which approximated the lipid class compositions of the analysed samples, were prepared for calibration: Phosphatidylcholine:triolein:cholesterol:cholesteryl oleate:oleic acid = 49:35:8:5:3 (V:V) for lipid–rich samples and = 64:25:6:4:1 (V:V) for lipid–poor samples.

As the separation of polar lipids remained unsatisfactory by TLC–FID, the phospholipid composition was determined by high performance (HP) TLC–scanning densitometry (modified after Olsen & Henderson 1989). Five μl of the total lipid extracts were applied by means of a CAMAG Linomat IV in duplicate on pre–developed HPTLC–plates (silica gel 60, Merck). The plates were developed in a horizontal chamber with isopropanol:methylacetate:chloroform:methanol:0.25% KCl (25:25:25:10:9, V:V) for 17 min and dried for 30 min in an evacuated desiccator. The plates were then immersed for 5 s in a post–chromatographic derivatisation reagent with a CAMAG Chromatogram Immersion Device III and charred at 200° C for 20 min. The derivatisation reagent was prepared by dissolving 1.2 mg manganese(II)–chloride in 180 ml of deionised water and adding 180 ml methanol and 12 ml concentrated sulphuric acid. The lipid bands were quantified with a CAMAG TLC–Scanner 3 at 550 nm wavelength and calibrated using commercial standards for each detected lipid class.

Histochemistry

Cryosections were performed with a Leica CM3050 S cryomicrotome. The cryostat, containing all instruments used, was cooled to −25° C. The cephalothorax was dissected from the deep–frozen specimens, trimmed to remove the cuticle, and frozen to the cutting–block. The knife was orientated towards the sample in an angle of $20-25°$. The sample blocks were trimmed for a few millimetres and then a series of cross–sections of $8-9\,\mu$m thickness was made. The cryosections were then transferred to the slides that were kept at room temperature and immersed in coplin jars containing the different dyes. Neutral lipids were stained red using "Oil red O" and sections mounted in glycine, phospholipids were stained blue with acid haematein and mounted in Euparal (according to Chayen and Bitensky 1991).

Results

Figure 2 shows the relationship between the three dominant lipid classes and the total lipid content in the three euphausiid species investigated. Phosphatidylcholine (PC) exhibits a linear increase with increasing lipid content similar to that of the typical neutral storage lipids triacylglycerols (TAG, in *Euphausia superba* and *E. triacantha*) or wax esters (WE) in *Thysanoessa macrura*. Phosphatidylethanolamine (PE) on the other hand, remains at a fairly constant level irrespective of the total lipid content in all three species. However, the PC increase is less steep in *E. triacantha* than in *E. superba* and *T. macrura*, indicating a lower accumulation rate and hence a weaker dependence of *E. triacantha* on this lipid class as compared to the other species examined.

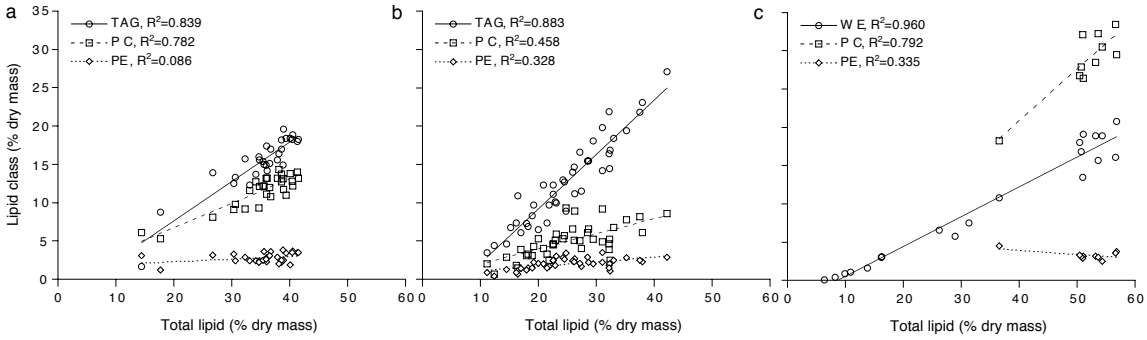


Figure 2: Relationship between total lipids (in % dry mass) and the three dominant lipid classes triacylglycerol (TAG) or wax ester (WE), phosphatidylcholine (PC), and phosphatidylethanolamine (PE) in (a) *E. superba* (n=29), (b) *E. triacantha* (n=41), and (c) *T. macrura* (n=20)

Figure 3 shows the distribution of lipid classes among the various fractions obtained by density gradient centrifugation. According to their specific weights, membrane phospholipids (PL) should be confined to the fractions P_1–P_3, and inversely these fractions should not contain any lighter lipids such as wax esters (WE), TAG, or free PL. The observed patterns largely match this expected distribution: neutral lipids prevail in the supernatant and constitute only smaller portions of the denser fractions P_1–P_3, which are clearly dominated by membrane components (PL and sterols). However, in the three euphausiids studied, PL comprise a significant portion of total lipids also in the supernatant. This is in clear contrast to *Calanus* sp., which did not show any non–membrane bound PL (Fig. 3). The increase of free fatty acids in the fractions P_1–P_3 was probably due to autolytic activity during the time–intensive collection of the lower phases in the centrifuge tube.

Since the relative lipid class composition does not allow inference on which is the most important fraction in terms of absolute PL abundance, the mass distribution of PL among the various fractions was plotted in absolute terms (Fig. 4a) and in % of dry mass (Fig. 4b). In *E. superba*, total lipids, and correspondingly also PL, varied considerably with differences in size and maturity stage. Sample number Es64 was a gravid female (Table 1), which contained on average 28% lipid of dry mass (DM), Es207 was a postspawn female (mean lipid content 15% DM), Es65 and Es66 were subadults, and Es5 was a postmated male. Such males were characterised by extremely low lipid contents of 3.5–7.8% DM.

Considerable portions of PL (50–90%) were recovered in the upper low–density fractions (S and P_0) in all but two of the samples analysed. One of these exceptions was the postmated male *E. superba* (Es5), which was entirely devoid of neutral lipids and just contained structural PL in the dense fractions P_2 and P_3. The other exception was *Calanus* sp.. Although being lipid–rich (about 62% DM), PL comprised only a minor portion of its total lipids, which essentially occurred in the membrane fractions, as was already evident from Figure 3. Because of the variations of absolute lipid amounts with size and stage, general trends emerge more clearly when expressed as relative levels in % dry mass, as shown in Figure 4b. Since the sub–cellular fractionation requires frozen fresh samples, direct dry mass data were not available. Therefore, these calculations refer to mean values derived from comparable samples (same expedition, maturity stage, and size). Saether et al. (1985) estimated that the portion of PL serving structural functions in membranes of *E. superba* is about 3.5% DM. In the present samples,

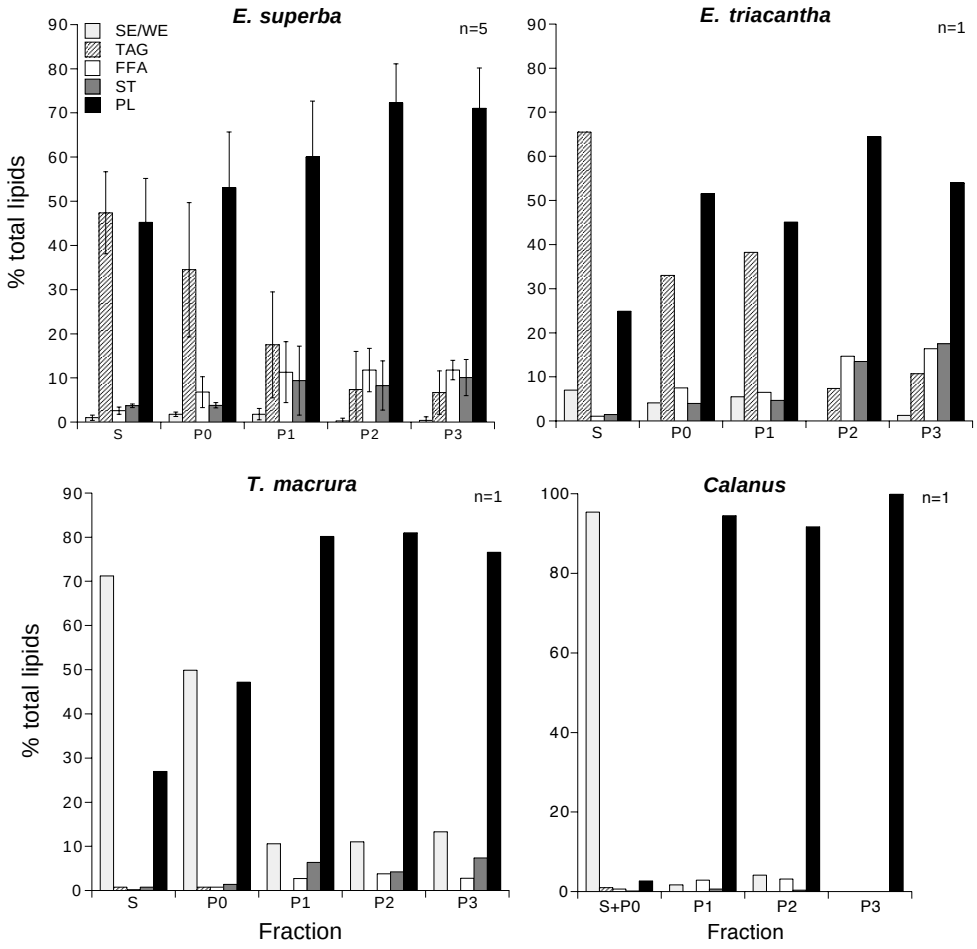


Figure 3: Lipid class composition of the five fractions obtained by density gradient centrifugation from the euphausiids *E. superba*, *E. triacantha*, *T. macrura*, and the copepod *Calanus* sp.. SE/WE sterol or wax esters, TAG triacylglycerols, FFA free fatty acids, ST sterols, PL phospholipids, S supernatant, P_0–P_3 pellets 0–3, see Fig. 1

PL in the dense fractions P_1–P_3 range between 2 and 3% DM. The homogenisation procedure and the collection of the isolated fractions is inevitably connected with a loss of lipids. This is most evident in the low total lipid levels in Es5, which were well below the values present in comparable specimens (3.4 mg and 8.1±1.0 mg, respectively), but it also applies to the other samples.

In order to obtain an estimate for the energy content of the storage lipid classes, the average carbon chain length of PC and TAG fatty acids were calculated from 36 lipid–rich (35% of dry mass) juvenile and adult *E. superba* sampled in autumn. On average, PC fatty acids consisted of 18.4±0.2 carbon atoms, two more than those of TAG (16.5±0.2). Based on the amount of energy released during fatty acid catabolism (β-oxidation) the calorific content was calculated, expressed as kilojoules per mole fatty acid. The corresponding values were 4618 and 4061 kJ per mole for PC and TAG fatty acids, respectively.

Table 1: Maturity stage, length, and total lipids of the samples used for sub–cellular fractionation. CV copepodite V, f female, subA subadult, m male, Es *Euphausia superba*, Et *E. triacantha*, Tm *Thysanoessa macrura*, n.d. not determined

Sample #	Stage	Length (mm)	Total lipids (mg)
Calanus	CV + f	n.d.	6.4
Es64	f3D	52	60.7
Es65	subA	44	49.8
Es66	subA	39	19.6
Es5	m3B	50	3.4
Es207	f3E	50	11.5
Et35	f3E	29	7.4
Tm7	f	24	17.6

Figure 5 shows transverse–sections through the anterior part of the cephalothorax of a gravid female (maturity stage 3D, according to Kirkwood 1982) stained with acid haematein for PL (Fig. 5a, c) or with oil red O for neutral lipids (NL, Fig. 5b, d). The anterior lobes of the ovary, containing mature oocytes (type 4, according to Cuzin-Roudy 1987, Cuzin-Roudy & Amsler 1991), dorsally and laterally surrounded the hepatopancreas. The oocytes had a granular appearance and were rich both in NL and PL. This is in line with the lipid class analysis from the isolated mature ovary, which revealed the dominance of TAG and PL in similar amounts (Table 2). The hepatopancreas was hardly stained by the NL dye, this was true for females as well as for males. The relatively small portions of TAG in combination with high PL levels in the isolated hepatopancreas samples (Table 2) agreed with these histological findings. In *E. superba* males sampled in autumn, the anterior part of the cephalothorax was filled by a vast, uniform appearing tissue, which was rich both in PL and NL and contained oil droplets (Fig. 6). This tissue was also observed in male *T. macrura*, where it occupied virtually the entire cephalothorax posterior to the hepatopancreas (Fig. 7c, d). Figure 7a, b show transverse–sections performed through the stomach–region of *E. triacantha*. The stomach is the cavity in the upper middle, which is surrounded by neutral lipid–poor lobes of the digestive gland. The sections of *E. triacantha* generally showed a weaker coloration as compared to those of *E. superba* and *T. macrura* in accordance with their lower total lipid content. Furthermore, regions of NL and PL deposition hardly coincided and the tissue, that was stained both for NL and PL in *E. superba* and *T. macrura*, is missing in this species.

Discussion

Neutral lipid reserves are accumulated by krill, and indeed by all animals, whether marine or terrestrial, fundamentally as metabolic energy reserves for future use. For various Antarctic euphausiids, an unusual accumulation of phosphatidylcholine (PC) additional to neutral lipids has been reported (Hagen 1988, Hagen et al. 1996, Mayzaud 1997). This holds probably also true for several Arctic species which exhibit a corresponding increase in phospholipids (PL), although their composition has not been determined in detail (Saether et al. 1986). However, the metabolic physiology involved in the accumulation of this amphipathic lipid molecule and

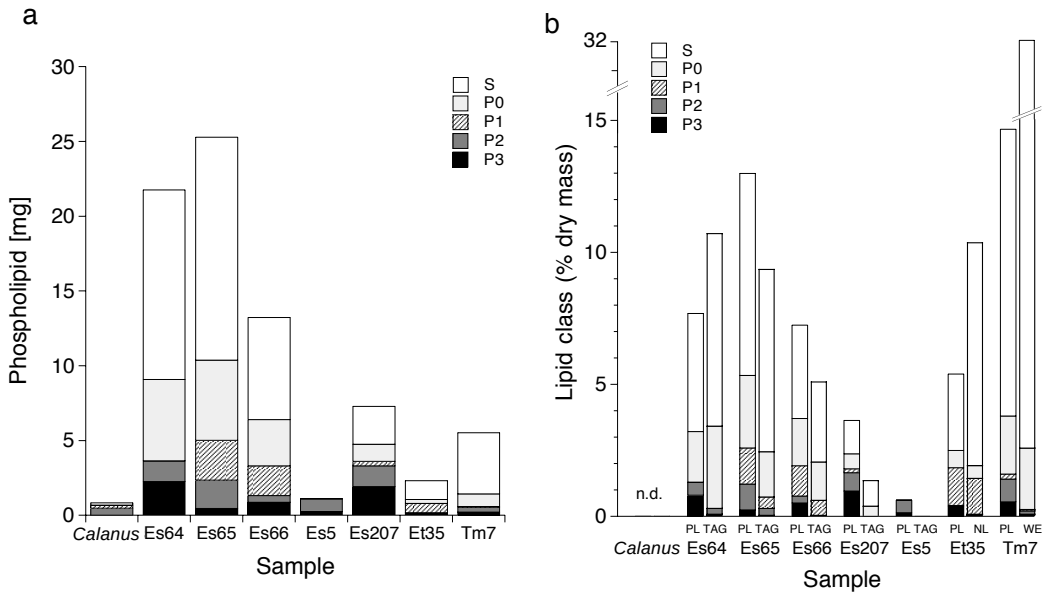
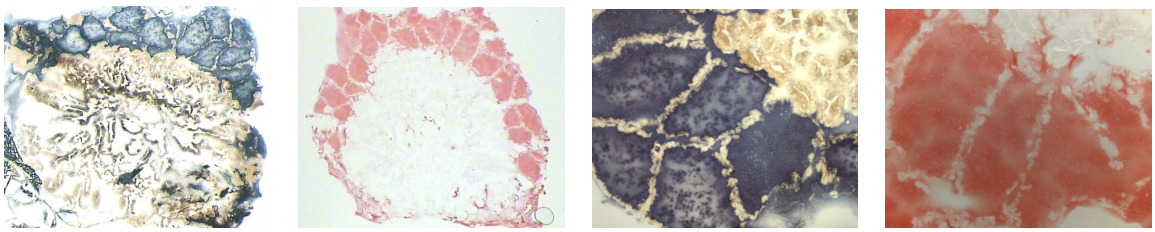


Figure 4: (a) Distribution of phospholipids (PL, in mg) and (b) phospholipids and neutral lipids (NL, TAG = triacylglycerols, WE = wax esters, TAG+WE = NL, in % dry mass) among the five fractions obtained by density gradient centrifugation. Es *Euphausia superba*, Et *E. triacantha*, Tm *Thysanoessa macrura*, n.d. not determined

its ecological implications are still poorly understood and this topic is characterised by few data, some controversy, and much speculation.

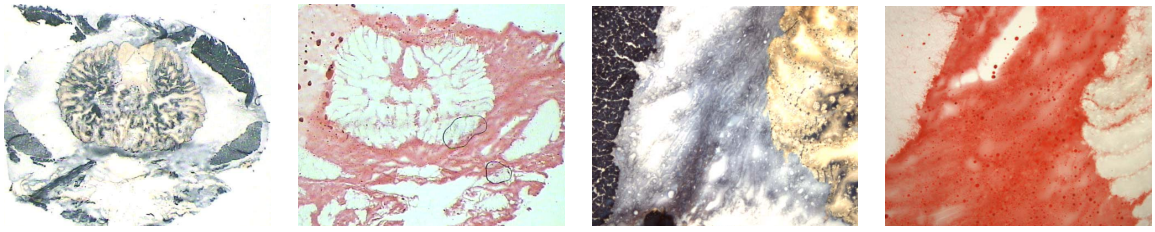
The linear increase of PL, specifically PC, with total lipid content (expressed as % of dry mass) analogous to the increase of the typical storage lipids triacylglycerols (TAG) or wax esters (WE) suggests its function as an energy reserve (Ellingsen & Mohr 1981, Saether et al. 1986, Hagen 1988, Hagen et al. 1996). However, this conclusion has been questioned by Sargent & Henderson (1995). They considered the voluminous, PL–rich hepatopancreas responsible for the high PL levels in *E. superba*. It is true, that the digestive gland of polar euphausiids is dominated by PL (Table 1 and Figs. 5–7, see also Virtue et al. 1993, Mayzaud et al. 1998, Albessard et al. 2001, Alonzo et al. 2003). However, in contrast to many benthic decapod crustaceans, in which the digestive gland is the most important site of lipid storage (e.g. Chandumpai et al. 1991), the contribution of the hepatopancreas to total *E. superba* lipids is less than 2% in gravid females (Clarke 1980).

Saether et al. (1985) conducted lipid histochemical analyses on male and female *E. superba*. In accordance with our results, they reported an essentially similar distribution of NL and PL among krill tissues. NL–rich tissue comprised mainly the ovary, while muscles and hepatopancreas were only weakly stained for NL. These analyses were conducted on summer samples, when especially male *E. superba* had low lipid levels. Therefore, they did not reveal the extensive storage tissues present in our samples collected in autumn (late April). The histochemical findings are supported by the lipid class analyses of isolated organs, which showed fairly equal amounts of NL and PL in the ovary and eggs, higher levels of PL than NL in the digestive gland and negligible amounts of NL in muscular tissue. Previous studies on the distribution of lipids among different organs of *E. superba* (Clarke 1980, Mayzaud et al. 1998) agree principally



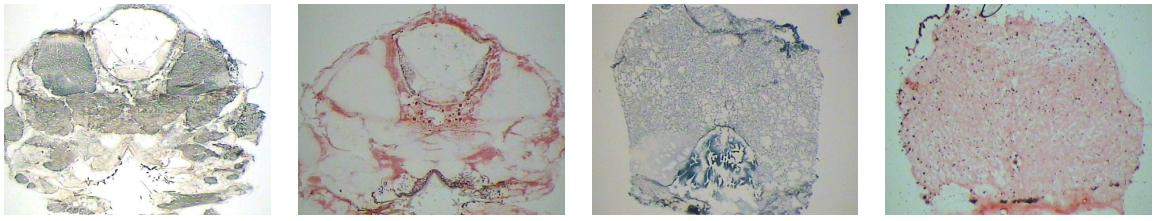
(a) Polar lipids (b) Neutral lipids (c) Polar lipids (d) Neutral lipids

Figure 5: *Euphausia superba*: (a, b) overview of transverse–sections through the anterior part of the cephalothorax of a gravid female (scale 1:6). (c, d) details of ovary (center and lower left) and hepatopancreas (upper right) (scale 1:24)



(a) Polar lipids (b) Neutral lipids (c) Polar lipids (d) Neutral lipids

Figure 6: *Euphausia superba*: (a, b) overview of transverse–sections through the anterior part of the cephalothorax of a lipid–rich male (scale 1:4.6). (c, d) details of muscle tissue (left) and hepatopancreas (right), in between "storage tissue" (scale 1:24)



(a) Polar lipids (b) Neutral lipids (c) Polar lipids (d) Neutral lipids

Figure 7: Overviews of transverse–sections through the cephalothorax of male (a, b) *Euphausia triacantha* and (c, d) *Thysanoessa macrura* (scale 1:10)

Table 2: *Euphausia superba*. Distribution of total lipids (TL), triacylglycerols (TAG), and phospholipids (PL) among various organs / body parts of adult specimens. DM dry mass, n.d. not determined, n number of samples

	TL [% DM]	TAG [% TL]	PL [% TL]	n
Hepatopancreas	n.d.	16.9±1.4	59.5±4.9	3
Ovary	n.d.	42.2	47.6	1
Eggs	53.5±9.5	33.0±4.6	53.5±9.5	3
Abdominal muscle	5.7±0.7	4.8±2.9	88.4±5.1	3

with our results. However, under exceptionally favourable feeding conditions TAG levels in the hepatopancreas of *E. superba* can markedly increase and constitute the major lipid class in this organ (Alonzo et al. 2003). In starving *E. superba*, on the other hand, the lipid content of the digestive gland is decreasing again, albeit PL was metabolised in preference of TAG (Virtue et al. 1993). Also in starving *E. superba* furciliae PL levels decreased more strongly than those of TAG (Stübing et al. 2003). While these results point towards a preferential mobilisation of PL over TAG, findings by Clarke (1987) indicate the reverse. He measured the incorporation of radio–labelled 16:0 in different tissues and lipid classes of adult *E. superba*. Turnover of this fatty acid was higher in TAG than in PL, indicating that the latter is rather maintained as a longer–term energy reserve. These contradictory results illustrate the difficulties inherent in such experimental studies and a complex approach based on a coherent sample set is needed to clarify the patterns of differential lipid class utilisation.

The lower slope of PC accumulation with increasing lipid content in *E. triacantha* as compared to *E. superba* and *T. macrura* (Hagen et al. 1996) indicates a weaker dependence on this alternative storage lipid in this species with a more northerly distribution. Apparently, there is a general trend of decreasing utilisation of phospholipids towards lower geographical latitudes. While the truly Arctic species *Thysanoessa inermis*, *T. longicaudata*, and *T. raschii* as well as the Antarctic euphausiids *E. superba*, *E. crystallorophias*, and *T. macrura* are all known to accumulate also PL (Saether et al. 1986, Hagen 1988, Hagen et al. 1996, Mayzaud 1997), the sub–Arctic *Meganyctiphanes norvegica* does not show this phenomenon (Saether et al. 1986).

The mobilisation of PL for energy production has also been reported for several other marine invertebrates, especially during embryonic and larval development. There is clear such evidence for two species of spiny lobster (*Jasus*) (Jeffs et al. 2001, 2002). The development from the non–feeding nektonic puerulus postlarva to the first instar juvenile is connected with a considerable horizontal migration and therefore associated with high metabolic costs. These are primarily satisfied by the mobilisation of internal lipid reserves (Jeffs et al. 1999), specifically PL (Jeffs et al. 2001, 2002). Another case is known for sea urchin spermatozoa, where PC is catabolised to produce energy for swimming, while the other polar lipid classes are conserved (Mita & Ueta 1990). No satisfactory explanation has been presented so far, why specifically PC is utilised as energy reserve. Jeffs et al. (2001) speculate that the transparency of PL as opposed to the opaque nature of NL accumulations might be a reason since total transparency is of vital importance to the *Jasus* pueruli in order to be indiscernible to visual predators. According to Olsen et al. (1991), the amphipathic PL molecules are more easily emulsified and transferred across the plasma membranes than the larger hydrophobic TAG, which have to be hydrolysed first. Furthermore, the primary transport lipids in many crustacean species are phospholipids (reviewed by Chang & O'Connor 1983, Chapelle 1986).

Our data confirm that PL, specifically PC, are stored in significant amounts in addition to neutral lipids in several Antarctic euphausiids. But what ecological implications are linked to the accumulation of PC? What are the specific advantages over pure neutral lipid reserves? Is the primary role of PC to provide additional energy or do these depots rather serve for quickly supplying structural components upon enhanced demand? From an energetic point of view PC storage is not more efficient, since the calorific value of PC is not markedly higher as compared to TAG or WE. An advantage might be its direct availability without prior hydrolysis as for TAG and resynthesis into PL.

There are several aspects specific to the life history of krill that would make PC a convenient lipid store for this crustacean group. Firstly, the eggs of oceanic euphausiids sink to depths of up to 2000 m (Marr 1962, Makarov 1977, cited in Makarov 1979, Ross & Quetin 1989, 1991, Siegel 2000), depending on the species. In most marine invertebrates lipids are the principal energy reserve for developing eggs (Herring 1974, Holland 1978). Due to their low density in relation to seawater, neutral lipids, and particularly wax esters, generate considerable upthrust. Hence, the specifically heavier phospholipids may play an important role in regulating the sinking behaviour of euphausiid eggs beside providing energy and structural material for the developing embryo. However, apart from *E. superba* data on the lipid class composition of euphausiid eggs are scarce. The eggs of the neritic *E. crystallorophias* are buoyant and remain suspended in the surface water layers (Makarov 1979, Harrington & Thomas 1987). Corroborating the hypothesis of the regulation of sinking behaviour via lipid class composition, the lipid–rich eggs (54% DM) of this species contain high levels of WE (62% of total lipids, Kattner & Hagen 1998) as compared to only 33% TAG and 50–60% PL at similar total lipid levels in *E. superba* (Table 2, see also Clarke & Morris 1983). However, as long as direct measurements of species–specific egg density combined with the determination of their proximate composition (including proteins and carbohydrates) are lacking, such conclusions remain speculatory.

Secondly, the long non–feeding larval development (up to 40 days in *E. superba*, strongly depending on water temperature, Ross et al. 1988) in conjunction with a considerable increase in length involves a substantial demand for structural cell components such as PL. In particular, the moult from metanauplius to calyptopis, the first feeding stage (Marschall 1985), is associated with a pronounced increase in length, from 0.95 to 1.71 mm in *E. superba* (Bargmann 1945) and this near doubling in size is also known from other Antarctic species (Kirkwood 1982). However, extended larval developmental times are not unusual among polar crustaceans, particularly in breeding benthic decapod crustaceans (e.g. Clarke 1983, Lovrich et al. 2003). However, PL are not as extensively incorporated into the eggs as in *E. superba* (Kattner et al. 2003). Apparently, these breeding decapods follow a different strategy, producing comparatively few, large and neutral lipid–rich eggs. Thus, the energy demanding resynthesis of TAG into structural PL is affordable.

Another potential advantage of PL over NL might be their higher percentages of PUFAs ($\sim$50% versus $<$10% of total fatty acids in *E. superba*, Hagen et al. 2001). The degree of unsaturation of the fatty acid moieties of PL influences the flexibility of plasma membranes (Shinitzky 1984, Chapelle 1986). Specifically PC contains higher levels of PUFAs as compared to PE (Mayzaud 1997, Stübing et al. 2003). However, even irrespective of the unsaturation degree of their fatty acids, PE causes higher membrane rigidity as compared to PC due to interactions of its head group with neighbouring phosphate groups (Shinitzky 1984). Whether the PC deposits lead to an enhanced body flexibility of *E. superba* via the above described molecular characteristics and thus allow quick reactions (e.g. tail–flipping to escape from predators) at near freezing temperatures, still needs to be clarified.

And lastly, shrinkage in size resulting from mobilisation of body protein has been observed in *E. superba* as well as in other krill species as response to poor feeding conditions (Ikeda & Dixon 1982, Hosie & Ritz 1989, Quetin & Ross 1991, Dalpadado & Skjoldal 1996, Marinovic & Mangel 1999). Assuming that shrinkage is a common mechanism also in the field (e.g. Nicol et al. 1992), PL requirements will be elevated during regrowth. During times of food plenty,

these costly compounds can be synthesised and deposited and upon demand, they are quickly available as essential structural components.

The present results confirmed the accumulation of PC in various Antarctic euphausiids. Furthermore, we have elucidated body sites of particularly high lipid deposition and demonstrated that in lipid–rich individuals the majority of PL occurs in a free, non–membrane bound form, strongly suggesting its function as energy reserve. However, further comparative studies including species from Arctic and subpolar regions combined with experimental evidence are required to consolidate the conclusions drawn here and to improve our understanding of the ecophysiological role of PC storage in the complex patterns of energetic and life history adaptations in polar euphausiids.

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References

Albessard E, Mayzaud P, Cuzin-Roudy J (2001) Variation of lipid classes among organs of the Northern krill *Meganyctiphanes norvegica*, with respect to reproduction. Comp Biochem Physiol 129A:373–390

Alonzo F, Nicol S, Virtue P, Nichols PD (2003) Lipids as trophic markers in Antarctic krill, I. Validation under controlled laboratory conditions. In: Huiskes AHL, Gieskes WWC, Rozema J, Schorno RML, van der Vies SM, Wolff WJ (eds) Antarctic Biology in a Global Context, Backhuys Publishers, Leiden, p 121–128

Bargmann HE (1945) The development and life history of adolescent krill *Euphausia superba*. Discovery Rep 23:103–176

Bathmann U (2002) The expedition ANTARKTIS XVIII/5b of the research vessel "Polarstern" in 2001. Ber Polarforsch 407:1–98

Chandumpai A, Dall W, Smith DM (1991) Lipid-class composition of organs and tissues of the tiger prawn *Penaeus esculentus* during the moulting cycle and during starvation. Mar Biol 108:235–245

Chang ES, O'Connor JD (1983) Metabolism and transport of carbohydrates and lipids. In: Mantel LH (ed) The Biology of Crustacea, Academic Press, New York, vol 5, p 263–287

Chapelle S (1986) Aspects of phospholipid metabolism in crustaceans as related to changes in environmental temperatures and salinities. Comp Biochem Physiol 84B:423–439

Chayen J, Bitensky L (1991) Practical Histochemistry. Wiley, Chichester, 321 pp

Clarke A (1980) The biochemical composition of krill, *Euphausia superba* Dana, from South Georgia. J Exp Mar Biol Ecol 43:221–236

Clarke A (1983) Life in cold water: the physiological ecology of polar marine ectotherms. Oceanogr Mar Biol Annu Rev 21:341–453

Clarke A (1987) A preliminary radioisotope study of the fate of ingested palmitic acid in the Antarctic krill, *Euphausia superba* Dana. British Antarctic Survey Bull 77:99–107

Clarke A, Morris DJ (1983) Towards an energy budget for krill: The physiology and biochemistry of *Euphausia superba* Dana. Polar Biol 2:69–86

Clarke A, Peck L (1991) The physiology of polar marine zooplankton. Polar Res 10:355–369

Cuzin-Roudy J (1987) Gonad history of the Antarctic krill *Euphausia superba* Dana during its breeding season. Polar Biol 7:237–244

Cuzin-Roudy J, Amsler MO (1991) Ovarian development and sexual maturity staging in Antarctic krill, *Euphausia superba* Dana (Euphausiacea). J Crust Biol 11:236–249

Dalpadado P, Skjoldal HR (1996) Abundance, maturity and growth of the krill species *Thysanoessa inermis* and *T. longicaudata* in the Barents Sea. Mar Ecol Prog Ser 144:175–183

Ellingsen TE, Mohr V (1981) Lipids in Antarctic krill. Composition and *post mortem* changes. In: Marcuse R (ed) Proc 11th Scand Symp on Lipids, SIK, Gothenburg, Sweden, p 110–116

Falk-Petersen S, Hagen W, Kattner G, Clarke A, Sargent J (2000) Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. Can J Fish Aquat Sci 57:178–191

Fraser AJ, Tocher DR, Sargent JR (1985) Thin-layer chromatography-flame ionization detection and the quantitation of marine neutral lipids and phospholipids. J Exp Mar Biol Ecol 88:91–99

Hagen W (1988) Zur Bedeutung der Lipide im antarktischen Zooplankton. Ber Polarforsch 49:1–129

Hagen W (2000) Lipids. In: Harris R, Wiebe P, Lenz J, Skjoldal H, Huntley M (eds) ICES Zooplankton Methodology Manual, Academic Press, San Diego, p 113–119

Hagen W, Schnack-Schiel SB (1996) Seasonal lipid dynamics in dominant Antarctic copepods: Energy for overwintering or reproduction? Deep-Sea Res 43:139–158

Hagen W, Kattner G, Graeve M (1993) *Calanoides acutus* and *Calanus propinquus*, Antarctic copepods with different lipid storage modes via wax esters or triacylglycerols. Mar Ecol Prog Ser 97:135–142

Hagen W, Van Vleet ES, Kattner G (1996) Seasonal lipid storage as overwintering strategy of Antarctic krill. Mar Ecol Prog Ser 134:85–89

Hagen W, Kattner G, Terbrüggen A, Van Vleet ES (2001) Lipid metabolism of the Antarctic krill *Euphausia superba* and its ecological implications. Mar Biol 139:95–104

Harrington SA, Thomas PG (1987) Observations on spawning by *Euphausia crystallorophias* from waters adjacent to Enderby Island (East Antarctica) and speculation on the early ontogenetic ecology of neritic euphausiids. Polar Biol 7:93–95

Herring PJ (1974) Size, density and lipid content of some decapod eggs. Deep-Sea Res 21:91–94

Holland DL (1978) Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. In: Malins DC, Sargent JR (eds) Biochemical and biophysical perspectives in marine biology, Academic Press, London, p 85–123

Hosie GW, Ritz DA (1989) Body shrinkage in the subtropical euphausiid *Nyctiphanes australis*. J Plank Res 11:595–598

Ikeda T, Dixon P (1982) Body shrinkage as a possible over-wintering mechanism of the Antarctic krill, *Euphausia superba* Dana. J Exp Mar Biol Ecol 62:143–151

Jeffs AG, Willmott ME, Wells RMG (1999) The use of energy stores in the puerulus of the spiny lobster *Jasus edwardsii* across the continental shelf of New Zealand. J Plankton Res 11:595–598

Jeffs AG, Nichols PD, Bruce MP (2001) Lipid reserves used by pueruli of the spiny lobster *Jasus edwardsii* in crossing the continental shelf of New Zealand. Comp Biochem Physiol 129A:305–311

Jeffs AG, Phleger CF, Nelson MM, Mooney BD, Nichols PD (2002) Marked depletion of polar lipid and non-essential fatty acids following settlement by post-larvae of the spiny lobster *Jasus verreauxi*. Comp Biochem Physiol 131A:305–311

Kattner G, Hagen W (1998) Lipid metabolism of the Antarctic euphausiid *Euphausia crystallorophias* and its ecological implications. Mar Ecol Prog Ser 170:203–213

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

Kattner G, Graeve M, Calcagno JA, Lovrich GA, Thatje S, Anger K (2003) Lipid, fatty acid and protein utilization during lecithotrophic development of *Lithodes santolla* (Molina) and *Paralomis granulosa* (Jacquinot). J Exp Mar Biol Ecol 292:61–74

Kirkwood JM (1982) A guide to the Euphausiacea of the Southern Ocean. ANARE Res Notes 1:1–45

Lipsky J (2001) AMLR 1999/2000 field season report. NOAA-TM-NMFS-SWFSC 302:1–139

Lovrich GA, Thatje S, Calcagno JA, Anger K, Kaffenberger A (2003) Changes in biomass and chemical composition during lecithotrophic larval development of the southern king crab, *Lithodes santolla* (Molina). J Exp Mar Biol Ecol 288:65–79

Makarov RR (1979) Larval distribution and reproductive ecology of *Thysanoessa macrura* (Crustacea: Euphausiacea) in the Scotia Sea. Mar Biol 52:377–386

Marinovic B, Mangel M (1999) Krill can shrink as an ecological adaptation to temporarily unfavourable environments. Ecol Lett 2:338–343

Marr JWS (1962) The natural history and geography of the Antarctic krill (*Euphausia superba* Dana). Discovery Rep 32:33–464

Marschall HP (1985) Structural and functional analyses on the feeding appendages of krill larvae. In: WR Siegfried, PR Condy, RM Laws, eds., Antarctic nutrient cycles and food webs, Springer, Berlin, p 346–354

Mayzaud P (1997) Spatial and life-cycle changes in lipid and fatty acid structure of the Antarctic euphausiid *Euphausia superba*. In: Battaglia B, Valencia J, Walton DWH (eds) Antarctic Communities: Species, Structure and Survival, Cambridge University Press, Cambridge, p 284–294

Mayzaud P, Albessard E, Cuzin-Roudy J (1998) Changes in lipid composition of the Antarctic krill *Euphausia superba* in the Indian sector of the Antarctic Ocean: influence of geographical location, sexual maturity stage and distribution among organs. Mar Ecol Prog Ser 173:149–162

Mita M, Ueta N (1990) Phosphatidylcholine metabolism for energy production in sea urchin spermatozoa. Biochim Biophys Acta 1047:175–179

Nicol S, Stolp M, Cochran T, Geijsel P, Marshall J (1992) Growth and shrinkage of Antarctic krill *Euphausia superba* from the Indian Ocean sector of the Southern Ocean during summer. Mar Ecol Prog Ser 89:175–181

Olsen RE, Henderson RJ (1989) The rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densitometry. J Exp Mar Biol Ecol 129:189–197

Olsen RE, Henderson RJ, Pedersen T (1991) The influence of dietary lipid classes on the fatty acid composition of small cod *Gadus morhua* L. juveniles reared in an enclosure in northern Norway. J Exp Mar Biol Ecol 148:59–76

Parrish CC (1987) Separation of aquatic lipid classes by chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. Can J Fish Aquat Sci 44:722–730

Quetin LB, Ross RM (1991) Behavioral and physiological characteristics of the Antarctic krill, *Euphausia superba*. Am Zool 31:49–63

Ross RM, Quetin LB (1989) Energetic cost to develop to the first feeding stage of *Euphausia superba* Dana and the effect of delays in food availability. J Exp Mar Biol Ecol 133:103–127

Ross RM, Quetin LB (1991) Ecological physiology of larval euphausiids, *Euphausia superba* (Euphausiacea). Mem Queensl Mus 31:321–333

Ross RM, Quetin LB, Kirsch E (1988) Effect of temperature on developmental times and survival of early larval stages of *Euphausia superba* Dana. J Exp Mar Biol Ecol 121:55–71

Saether O, Ellingsen TE, Mohr V (1985) The distribution of lipid in the tissues of Antarctic krill *Euphausia superba*. Comp Biochem Physiol 81B:609–614

Saether O, Ellingsen TE, Mohr V (1986) Lipids of North Atlantic krill. J Lipid Res 27:274–285

Sargent JR, Henderson RJ (1995) Marine (n-3) polyunsaturated fatty acids. In: Hamilton RJ (ed) Developments in oils and fats, Blackie Academic and Professional, London, p 32–65

Sargent JR, Gatten RR, Henderson RJ (1981) Lipid biochemistry of zooplankton from high latitudes. Océanis 7:623–632

Shinitzky M (1984) Physiology of membrane fluidity, vol I, II. CRC Inc, Boca Raton, 331 pp

Siegel V (2000) Krill (Euphausiacea) life history and aspects of population dynamics. Can J Fish Aquat Sci 57:130–150

Stübing D, Hagen W, Schmidt K (2003) On the use of lipid biomarkers in marine food web analyses: An experimental case study on the Antarctic krill, *Euphausia superba*. Limnol Oceanogr 48:1685–1700

Virtue P, Nicol S, Nichols PD (1993) Changes in the digestive gland of *Euphausia superba* during short-term starvation: lipid class, fatty acid and sterol content and composition. Mar Biol 117:441–448

Chapter 6

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

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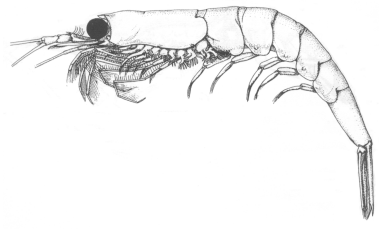

Euphausia frigida adult male (from Baker et al. 1990)

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

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Abstract

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

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

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

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

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

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

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

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

In this study, we examined regional, seasonal, and ontogenetic differences in the isotope ratios of zooplankton. The results are compared with those from previous gut content analysis, feeding experiments, and fatty acid analysis. A month-long incubation experiment was done to follow changes in the isotope ratios of larval and postlarval *Euphausia superba* while feeding on ice biota or copepods. This ''calibration'' experiment helped interpretation of the in situ results and provided information on the timescales of isotopic change in various body organs and metabolic products. The present study aimed to provide indications on the trophic position of Southern Ocean zooplankton and to identify potential problems in the application of the stable isotope method.

Materials and Methods

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

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

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

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

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

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

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

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

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

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

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

Results

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

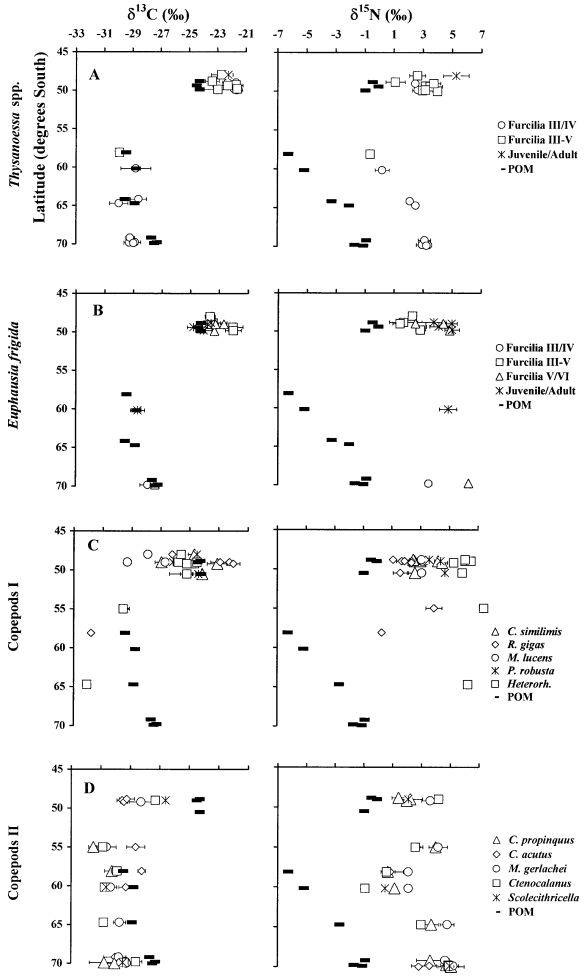

Fig. 1. Regional and species differences in δ^{13}C and δ^{15}N values (‰, ±1 SD) in autumn 1999 (data for the POM were supplied by K.-U. Richter). Symbols represent the mean value on a single sampling date, where usually between two and six replicate measurements were made (*see also Table 1*). Δ^{13}C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

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

The Lazarev Sea and Marguerite Bay were both sampled in late summer/early autumn. Although the Lazarev Sea was characterized by very low phytoplankton abundances (~0.5 μg chlorophyll *a* L^{-1}), diatoms had been blooming in the Marguerite Bay for several months (7–10 μg Chl *a* L^{-1}, A. Clarke, unpubl. data). Correspondingly, the δ^{15}N and δ^{13}C ratios of POM in the Lazarev Sea were ~5‰–6‰ lower than those in Marguerite Bay (Fig. 2). Zooplankton again reflected these differences in the baseline of the food web, showing clearly higher stable isotope ratios in Marguerite Bay (Table 2, Fig. 2). For *E. superba* furcilia larvae and the copepods *Metridia gerlachei* and *Calanoides acutus* the δ^{15}N values differed by 4‰–5‰ and the δ^{13}C by 3‰–5‰ within the two locations.

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

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

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

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

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

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

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

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

Differences in stable isotope ratios among development stages—The relationship between zooplankton δ^{15}N and their nitrogen mass was investigated throughout the survey period (Table 4). For some species (e.g., *M. gerlachei*), the relationship was variable between regions and sampling dates, whereas for others (e.g., *C. propinquus*) it was fairly constant (Table 4). Overall, the δ^{15}N values of *C. propinquus*

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

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

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

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

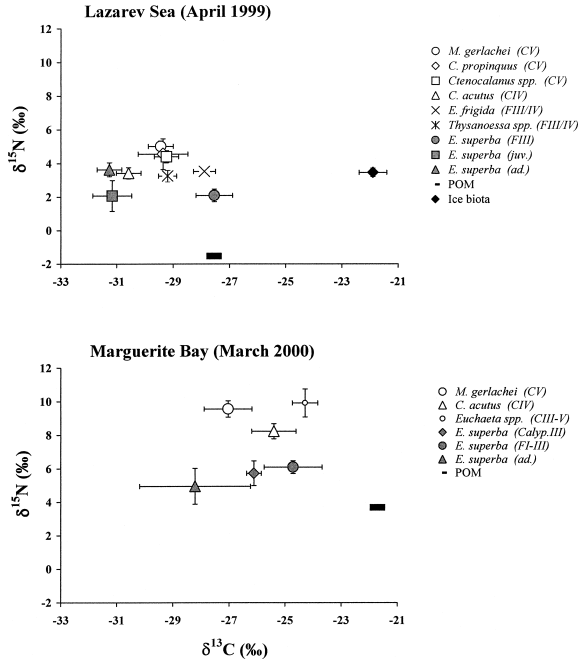

Fig. 2. Stable isotope values (‰, ±1 SD) of POM, ice biota, and zooplankton species at two different locations/seasons. Symbols represent the mean value of all measurements conducted in that area.

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

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

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

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

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

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

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

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

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

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

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

Discussion

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

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

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

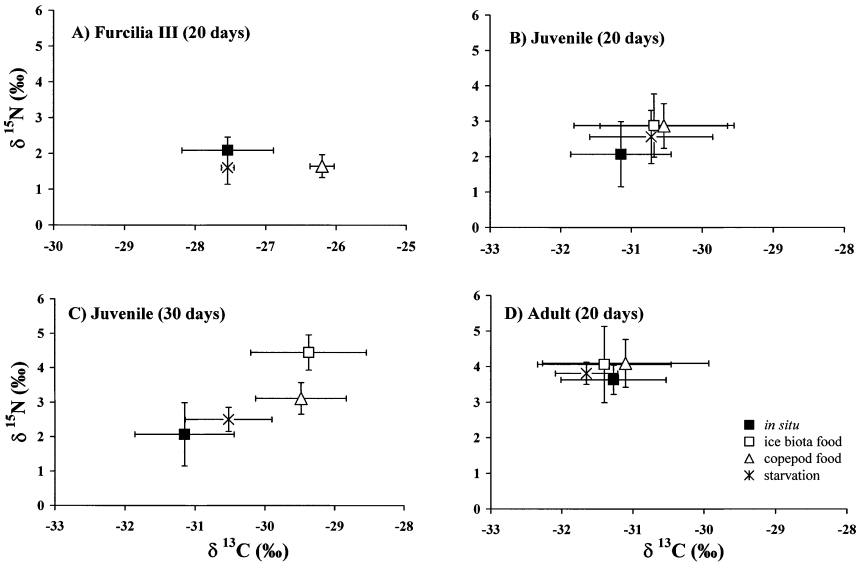

Fig. 3. Long-term incubation experiment. Stable isotope values of *E. superba* (‰, ±1 SD) collected in the Lazarev Sea during autumn 2000 and after incubation in different food regimes for 20 or 30 d. The number of replicate measurements for furcilia was 4–6, each being of three individuals, whereas,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

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

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

Uses of the stable isotope approach: trophic ecology of Southern Ocean mesozoolankton—Because of the balance of sampling in the Southern Ocean, Antarctic copepods are much better known than the inhabitants of the Sub-Antarctic water ring. Among the former, the known carnivores, *Euchaeta* spp. and *Heterorhabdus* spp., had the highest δ^{15}N values, whereas suspension feeders like *C. acutus* had low values. Acknowledged omnivores, *C. propinquus* and *M. gerlachei*, were intermediate in position. Of the lower latitude copepods, *R. gigas* and *C. simillimus* dominate the biomass, but their diets are debated (Graeve et al. 1994; Atkinson 1996). In the present study, *R. gigas* had the lowest δ^{15}N of all copepods sampled at the Polar Front, ~3‰ above the POM baseline, which implies feeding on algae. Higher δ^{15}N values of *C. simillimus* suggest omnivory. We are unaware of any food data for larvae of *Thysanoessa* spp. and *E. frigida.* However, their δ^{15}N values were higher than those of the mainly herbivorous *E. superba* larvae (Quetin et al. 1994; Meyer et al. 2002), which indicates a more omnivorous diet.

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

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

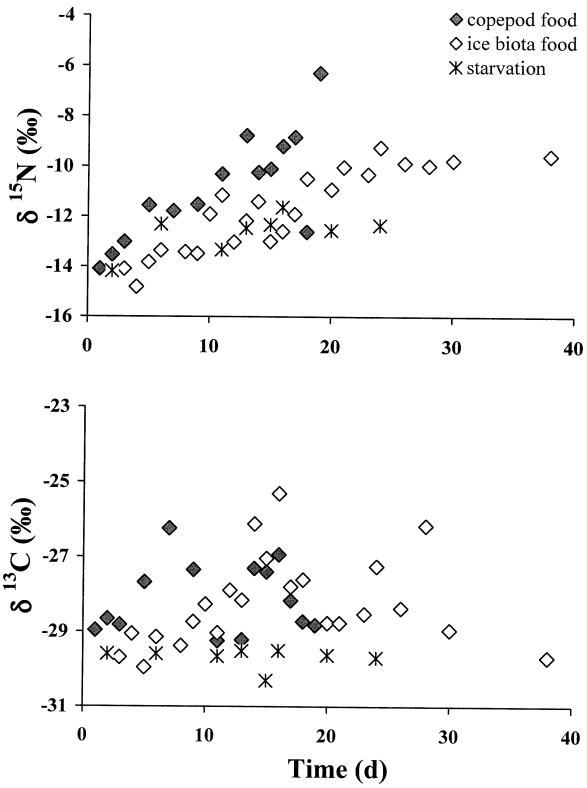

Fig. 4. Long-term incubation experiment. Changes in the stable isotope values of *E. superba* (juvenile/adult) molts during incubation in different food regimes. All molts of juveniles and adults shed between two sampling dates were pooled for a single analysis. After 20 d, most of the krill had been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

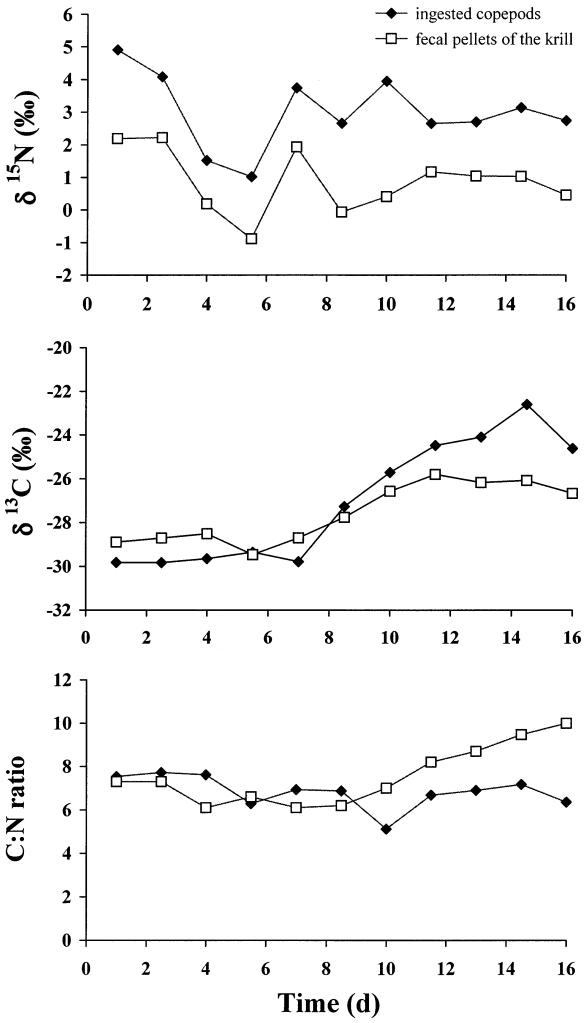

Fig. 5. Long-term incubation experiment. Stable isotope and C : N values of *E. superba* (juvenile/adult) fecal pellets in relation to their copepod food. For each sampling date one filter of concentrated fecal pellets was measured. The mean isotope signal of the ingested copepods was calculated from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

ice-dwelling harpacticoid copepod relative to pelagic copepods.

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

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

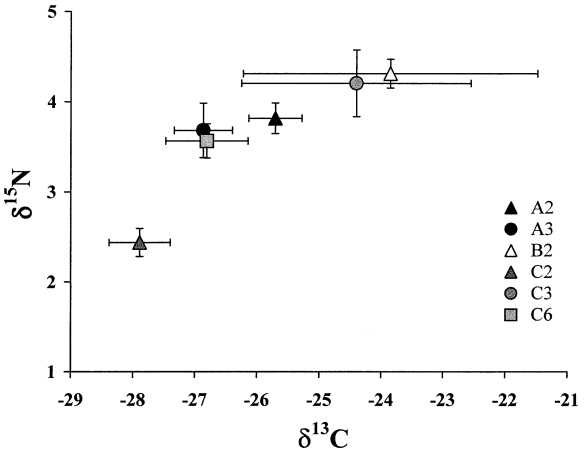

Fig. 6. Stable isotope values of *E. superba* juveniles (‰ ±1 SD, $n = 4$) collected at six stations around South Georgia in summer 1996. Sampling dates and station positions are in Cripps et al. (1999). Station prefixes A, B, and C were designated by Cripps et al. (1999) from fatty acid analysis and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

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

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

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

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

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

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

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

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

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

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

References

ADAMS, T. S., AND R. W. STERNER. 2000. The effect of dietary nitrogen content on trophic level ^{15}N enrichment. Limnol. Oceanogr. **45:** 601–607.

ATKINSON, A. 1995. Omnivory and feeding selectivity in five copepod species during spring in the Bellingshausen Sea, Antarctica. ICES J. Mar. Sci. **52:** 385–396.

———. 1996. Subantarctic copepods in an oceanic, low chlorophyll environment: Ciliate predation, food selectivity and impact on prey populations. Mar. Ecol. Prog. Ser. **130:** 85–96.

———, B. MEYER, D. STÜBING, W. HAGEN, K. SCHMIDT, AND U. V. BATHMANN. 2002. Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter—II. Juveniles and adults. Limnol. Oceanogr. **47:** 953–966.

BÅMSTEDT, U., D. J. GIFFORD, X. IRIGOIEN, A. ATKINSON, AND M. ROMAN. 2000. Feeding, p. 297–399. *In* R. Harris, P. Wiebe, J. Lenz, H. R. Skjødal, and M. Huntley [eds.], ICES zooplankton methodological manual. Academic.

BIGGS, D. C., AND OTHERS. 1989. Cooperative study of upper-ocean particulate fluxes. Prog. Ocean Drilling Prog. **119:** 109–120.

BOYD, C. M., M. HEYRAUD, AND C. N. BOYD. 1984. Feeding of the Antarctic krill *Euphausia superba*. J. Crustac. Biol. **4:** 123–141.

BURNS, J. M., S. J. TRUMBLE, M. A. CASTELLINI, AND J. W. TESTA. 1998. The diet of Weddell seals in McMurdo Sound, Antarctica as determined from scat collections and stable isotope analysis. Polar Biol. **19:** 272–282.

CRIPPS, G. C., J. L. WATKINS, H. J. HILL, AND A. ATKINSON. 1999. Fatty acid content of Antarctic krill, *Euphausia superba*, at South Georgia related to regional populations and variations in diet. Mar. Ecol. Prog. Ser. **181:** 177–188.

DAHMS, H.-U., M. BERGMANS, AND H. K. SCHMINKE. 1990. Distribution and adaptations of sea ice inhabiting Harpacticoida (Crustacea, Copepoda) of the Weddell Sea (Antarctica). Mar. Ecol. **11:** 207–226.

DALY, K. L., AND M. C. MACAULY. 1991. Influence of physical and biological mesoscale dynamics on the seasonal distribution and behavior of *Euphausia superba* in the Antarctic marginal ice zone. Mar. Ecol. Prog. Ser. **79:** 37–66.

DENIRO, M. J., AND S. EPSTEIN. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta **42:** 495–506.

———, AND ———. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta **45:** 341–351.

DITTEL, A. I., C. E. EPIFANIO, S. M. SCHWALM, M. S. FANTLE, AND M. L. FOGEL. 2000. Carbon and nitrogen sources for juvenile blue crabs *Callinectes sapidus* in coastal wetlands. Mar. Ecol. Prog. Ser. **194:** 103–112.

FANTLE, M. S., A. I. DITTEL, S. M. SCHWALM, C. E. EPIFANIO, AND M. L. FOGEL. 1999. A food web analysis of the juvenile blue crab, *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. Oecologia **120:** 416–426.

FISCHER, G. 1991. Stable carbon isotope ratios of plankton carbon and sinking organic matter from the Atlantic sector of the Southern Ocean. Mar. Chem. **35:** 581–596.

FRAZER, T. K. 1996. Stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$) of larval krill, *Euphausia superba*, and two of its potential food sources in winter. J. Plankton Res. **18:** 1413–1426.

———, R. M. ROSS, L. B. QUETIN, AND J. P. MONTOYA. 1997. Turnover of carbon and nitrogen during growth of larval krill, *Euphausia superba* Dana: A stable isotope approach. J. Exp. Mar. Biol. Ecol. **212:** 259–275.

FRY, B. 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnol. Oceanogr. **33:** 1182–1190.

———, AND C. ARNOLD. 1982. Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). Oecologia **54:** 200–204.

———, R. S. SCALAN, AND P. L. PARKER. 1983. $^{13}C/^{12}C$ ratios in marine food webs of the Torres Strait, Queensland. Aust. J. Mar. Freshw. Res. **34:** 707–716.

GOROKHOVA, E., AND S. HANSSON. 1999. An experimental study on variation in stable carbon and nitrogen isotope fractionation during growth of *Mysis mixta* and *Neomysis integer*. Can. J. Fish. Aquat. Sci. **56:** 2203–2210.

GRAEVE, M., G. KATTNER, AND W. HAGEN. 1994. Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: Experimental evidence of trophic markers. J. Exp. Mar. Biol. Ecol. **182:** 97–110.

HANSSON, S., J. E. HOBBIE, R. ELMGREN, U. LARSSON, B. FRY, AND S. JOHANSSON. 1997. The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration. Ecology **78:** 2249–2257.

HOBSON, K. A. 1993. Trophic relationships among High Arctic seabirds: Insights from tissue-dependent stable-isotope models. Mar. Ecol. Prog. Ser. **95:** 7–18.

HODUM, P. J., AND K. A. HOBSON. 2000. Trophic relationships among Antarctic fulmarine petrels: Insights into dietary overlap and chick provisioning strategies inferred from stable-isotope ($\delta^{15}N$ and $\delta^{13}C$) analyses. Mar. Ecol. Prog. Ser. **198:** 273–281.

HOPKINS, T. L., D. G. AINLEY, J. J. TORRES, AND T. L. LANCRAFT. 1993*a*. Trophic structure in open waters of the marginal ice zone in the Weddell-Scotia confluence region during spring (1983). Polar Biol. **13:** 389–397.

———, T. M. LANCRAFT, J. J. TORRES, AND J. DONELLEY. 1993*b*. Community structure and trophic ecology of zooplankton in the Scotia Sea marginal ice zone in winter (1988). Deep-Sea Res. **40:** 81–105.

IKEDA, T. 1974. Nutritional ecology of marine zooplankton. Mem. Fac. Fish. Hokkaido Univ. **22:** 1–97.

KIYASHKO, S. I., T. NARITA, AND E. WADA. 2001. Contribution of methanotrophs to freshwater macroinvertebrates: Evidence from stable isotope ratios. Aquat. Microb. Ecol. **24:** 203–207.

LESAGE, V., M. O. HAMMILL, AND K. M. KOVACS. 2001. Marine mammals and the community structure of the Estuary and Gulf of St Lawrence, Canada: Evidence from stable isotope analysis. Mar. Ecol. Prog. Ser. **210:** 203–221.

MARIOTTI, A., J. C. GERMON, P. HUBERT, P. KAISER, R. LETOLLE, A. TARDIEUX, AND P. TARDIEUX. 1981. Experimental determination of nitrogen kinetic isotope fractionation: Some principles; illustration for the denitrification and nitrification processes. Plant Soil **62:** 413–460.

MAYZAUD, P., E. ALBESSARD, AND J. CUZIN-ROUDY. 1998. Changes in lipid composition of the Antarctic krill *Euphausia superba* in the Indian sector of the Antarctic Ocean: Influence of geographical location, sexual maturity stage and distribution among organs. Mar. Ecol. Prog. Ser. **173:** 149–162.

MCCONNAUGHEY, T., AND C. P. MCROY. 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Mar. Biol. **53:** 257–262.

MEYER, B., A. ATKINSON, D. STÜBING, B. OETTL, W. HAGEN, AND U. V. BATHMANN. 2002. Feeding and energy budgets of Antarctic krill *Euphausia superba* at the onset of winter—I. Furcilia III larvae. Limnol. Oceanogr. **47:** 943–952.

MICHENER, R. H., AND D. M. SCHELL. 1994. Stable isotope ratios as tracers in marine aquatic food webs, p. 138–158. *In* K. Lajtha and R. H. Michener [eds.], Stable isotopes in ecology and enviromental science. Blackwell Scientific.

MONTOYA, J. P. 1994. Nitrogen isotope fractionation in the Modern Ocean: Implications for the sedimentary record, p. 259–277. *In* R. E. A. Zahn [ed.], Carbon cycling in the glacial ocean: Constraints on the ocean's role in the global change. Springer-Verlag

———, AND J. J. MCCARTHY. 1995. Isotopic fractionation during nitrate uptake by phytoplankton grown in continuous culture. J. Plankton Res. **17:** 439–464.

O'REILLY, C. M., AND R. E. HECKY. 2002. Interpreting stable isotopes in food webs: Recognizing the role of time averaging at different trophic levels. Limnol. Oceanogr. **47:** 306–309.

PERISSINOTTO R., L. GURNEY, AND E. A. PAKHOMOV. 2000. Contribution of heterotrophic material to diet and energy budget of Antarctic krill, *Euphausia superba*. Mar. Biol. **136:** 129–135.

PETERSON, B. J., AND B. FRY. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. **18:** 293–320.

PINNEGAR, J. K., AND N. V. C. POLUNIN. 1999. Differential fractionations of δ^{13}C and δ^{15}N among fish tissues: Implications for the study of trophic interactions. Funct. Ecol. **13:** 225–231.

QUETIN, L.B., R. ROSS, AND A. CLARKE. 1994. Krill energetics: Seasonal and environmental aspects of the physiology of *Euphausia superba*, p. 165–184. *In* S. Z. El-Sayed [ed.] Southern Ocean ecology: The BIOMASS perspective. Cambridge University Press.

———, ———, T. K. FRAZER, AND K. L. HABERMAN. 1996. Factors affecting distribution and abundance of zooplankton, with an emphasis on Antarctic krill, *Euphausia superba*. Ant. Res. Ser. **70:** 357–371.

RAU, G. H., T. L. HOPKINS, AND J. J. TORRES. 1991*a*. ^{15}N/^{14}N and ^{13}C/^{12}C in Weddell Sea invertebrates: Implications for feeding diversity. Mar. Ecol. Prog. Ser. **77:** 1–6.

———, C. W. SULLIVAN, AND L. I. GORDON. 1991*b*. δ^{13}C and δ^{15}N variations in Weddell Sea particulate organic matter. Mar. Chem. **35:** 355–369.

ROLFF, C. 2000. Seasonal variation in δ^{13}C and δ^{15}N of size-fractionated plankton at a coastal station in the northern Baltic proper. Mar. Ecol. Prog. Ser. **203:** 47–65.

SCHNACK, S. B., V. SMETACEK, B. VON BODUNGEN, AND P. STEGMANN. 1985. Utilization of phytoplankton by copepods in Antarctic waters during spring, p. 65–81. *In* J. S. Gray and M. E. Christiansen [eds.], Marine biology of polar regions and the effects of stress on marine organisms. Wiley.

SCHELL, D. M., S. M. SAUPE, AND N. HAUBENSTOCK. 1989. Bowhead whale (*Balaena mysticetus*) growth and feeding as estimated by δ^{13}C techniques. Mar. Biol. **103:** 433–443.

SHREEVE, R. S., AND P. WARD. 1998. Moulting and growth of the early stages of two species of Antarctic calanoid copepod in relation to differences in food supply. Mar. Ecol. Prog. Ser. **175:** 109–119.

SIMENSTAD, C. A., AND R. C. WISSMAR. 1985. δ^{13}C evidence of the origins and fates of organic carbon in estuarine and nearshore food webs. Mar. Ecol. Prog. Ser. **22:** 141–152.

TIESZEN, L. L., T. W. BOUTTON, K. G. TESDAHL, AND N. A. SLADE. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implication for δ^{13}C analysis of diet. Oecologia **57:** 32–37.

VERVOORT, W. 1957. Copepods from Antarctic and Sub-Antarctic plankton samples. Rep. B.A.N.Z. Antarct. Res. Exp. Ser. B. **3:** 1–160.

WADA, E., M. TERAZAKI, Y. KABAYA, AND T. NEMOTO. 1987. ^{15}N and ^{13}C abundances in the Antarctic Ocean with emphasis on the biogeochemical structure of the food web. Deep-Sea Res. **34:** 829–841.

WASER, N. A. D., P. J. HARRISON, B. NIELSEN, S. E. CALVERT, AND D. H. TURPIN. 1998. Nitrogen isotope fractionation during the uptake and assimilation of nitrate, nitrite, ammonium, and urea by a marine diatom. Limnol. Oceanogr. **43:** 215–224.

WHISTLER, R. L., AND J. N. BEMILLER. 1962. Alkaline disintegration of amino sugars. J. Org. Chem. **27:** 1161–1164.

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