A lipid budget for Antarctic krill (*Euphausia superba* Dana)

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ABSTRACT

Microplankton at five sites off South Georgia in January to February 1991 was dominated by a range of diatoms. The haptophyte *Phaeocystis* was present in three of the five sites but in low abundance only. Diatoms dominated at a more southerly site near the Antarctic Peninsula in March, whereas dinoflagellates dominated at a site near Deception Island. Multivariate analysis allowed the seven sites to be distinguished on the basis of microplankton species composition. Analysis of thirteen lipid classes present in total lipid extracted from the microplankton also demonstrated substantial differences from site to site. Multivariate analysis showed a different pattern of variation from the species ordination, with the South Georgia sites forming a distinct cluster. Outlier sites identified in the species and lipid ordinations confirmed the association between some taxonomic groups and lipid 'fingerprints'. Fatty acids extracted from total lipid in microplankton at five sites around South Georgia and two sites near the Antarctic Peninsula ranged from 37 to 195 $\mu g\ l^{-1}$, with a ratio of fatty acids in polar lipid : neutral lipid ranging from 4:1 to 1:2. A further eleven particulate samples analysed from sites around the Antarctic Peninsula had slightly lower fatty acid content with a mean of 50 $\mu g\ l^{-1}$. Fatty acids in polar lipid were rich in (n-3) polyunsaturated fatty acids, chiefly 20:5(n-3). However, 22:6(n-3) could be as abundant as 20:5(n-3) in polar lipid from microplankton less than 20 μm, and also in dinoflagellate-rich microplankton. Neutral lipid was dominated by 16:0, 16:1(n-7) and 18:1(n-9) fatty acids and contained only low levels of (n-3) polyunsaturated fatty acids. The data reveal the high nutritional quality of microplankton lipids in the Southern Ocean for filter feeding animals, including krill.

Samples of krill from eight sites around South Georgia consisted predominantly of immature animals, and females were entirely absent from samples from two of the eight sites studied. Animal wet mass varied from 0.16-1.72 g (median values of 0.47, 1.15 and 1.46 g for immatures, males and females respectively). Lipid amounts varied from 5-147 mg per animal (median values of 17.8, 21.0 and 73.3 mg for immatures, males and females respectively). Triacylglycerol (TAG) and phosphatidylcholine were the two most abundant lipid classes in all animals. Multivariate analysis of lipid composition indicated significant overlap between sex-

maturity classes, although female krill tended to be distinguished from males by higher proportions of TAG and lower proportions of phosphatidylserine plus phosphatidylinositol. Reproductive investment is implicated in the overall variability in lipid content and composition, with females containing high lipid levels as reserves for egg production, whilst males showed apparent lipid deficits resulting from short-term mobilisation of storage material for spermatophore production and attachment. Significant and systematic site-to-site variability in lipid content and composition were evident in the samples and this could not be explained by the sex ratio or animal size. Such variability might have arisen from local patterns of krill distribution but could not be ascribed simply to temporal changes in lipid during the study.

Immature Antarctic krill (length 40-45 mm) maintained in an aquarium for up to nine months were fed dense suspensions of cultures of two algal taxa, the haptophyte *Isochrysis* and the diatom *Thalassiosira*. Following acclimation to the experimental feeding regime, the animals were transferred to identical containers holding cultures of the same alga already labelled with [^{14}C]bicarbonate. Faecal pellets collected after transfer showed detectable radioactivity after 30 minutes for *Isochrysis* and 55 minutes for *Thalassiosira*, providing an estimation of gut throughput time. With both algal cultures, radioactivity in faecal pellets increased over the 4-5 hour collection period. However, whilst faecal pellets derived from *Isochrysis* showed a rapid initial increase followed by an approach to a plateau value, the radioactivity in *Thalassiosira*–derived pellets increased steadily. A first–order kinetic model fitted to these data showed a more rapid turnover time for *Isochrysis* ($k = 47$ min) than for *Thalassiosira* ($k = 256$ min).

The assimilation efficiency based on the ratio of ingested radiolabelled lipid to that egested in faeces was 86% for *Isochrysis* and 63% for *Thalassiosira*, whereas corresponding efficiencies calculated from mass lipid budgets were 75% for *Isochrysis* and 77% for *Thalassiosira*. Analysis of fatty acid content and composition of total lipid from algae, krill and faecal pellets established that all dietary fatty acids were very efficiently assimilated although there was a relatively preferential excretion of saturated fatty acids. All the assimilated fatty acids were extensively catabolised with the possible exceptions of saturated fatty acids and 18:4. Evidence was obtained for some biosynthesis of saturated fatty acids from non-lipid

dietary precursors and for a limited conversion of 18:3 to 18:4

Collating the data presented in this thesis in a budget indicates that under suitable conditions, *Euphausia superba* is capable of acquiring the lipid necessary for growth and reproduction over time scales of only a few weeks and certainly within a single summer. Hence, krill appears to be an animal capable of high energy throughput and high reproductive output.

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1. GENERAL INTRODUCTION

1.1 Antarctica: early exploration and exploitation of living marine resources.

Antarctica, the land mass centred on the South Pole, is a continent of extremes (Fig 1.1). Mostly ice-covered, it is one of the most inhospitable regions on Earth, characterised by consistently low temperatures and low precipitation. It is surrounded by the Southern Ocean, a continuous, circulating body of water. Productivity and species diversity of the Antarctic terrestrial ecosystem are low and mainly restricted to unicellular organisms, insects, and plants such as mosses and lichens, all of which are uniquely adapted both behaviourally and physiologically to survive in such a harsh environment (Walton and Bonner 1975). By contrast, the Southern Ocean supports a high diversity and high biomass of life, culminating in large populations of higher predators.

Man's interest in this inhospitable region initially began in the eighteenth century with the age of large scale national expeditions such as those led by Captain Cook (1772) and James Clark Ross (1840-43). Natural historians often accompanied these cruises and were responsible for the collection and documentation of many species new to science. From the 1820's onwards, many commercially motivated expeditions explored the Antarctic Peninsula region to exploit the prolific biological resources, particularly seals. These early expeditions collected numerous biological specimens, most of which were later characterised and classified back in Europe. At the end of the last century and during the early stages of the present, great national expeditions such as those led by Scott (1901-1904) and Shackleton (1914-1917) were undertaken in attempts to explore and map the continent itself.

During the early 20th century, after the collapse of the whaling industry in the Northern Hemisphere, attention turned rapidly to the whale stocks of the Southern Ocean. The fishery was largely uncontrolled and as early as the 1930's the stocks of many species had been drastically reduced. Continued exploitation of this fishery ultimately led to its collapse in the early 1970's with the virtual extinction of most species of baleen whales. During the last few decades, commercial fisheries in the

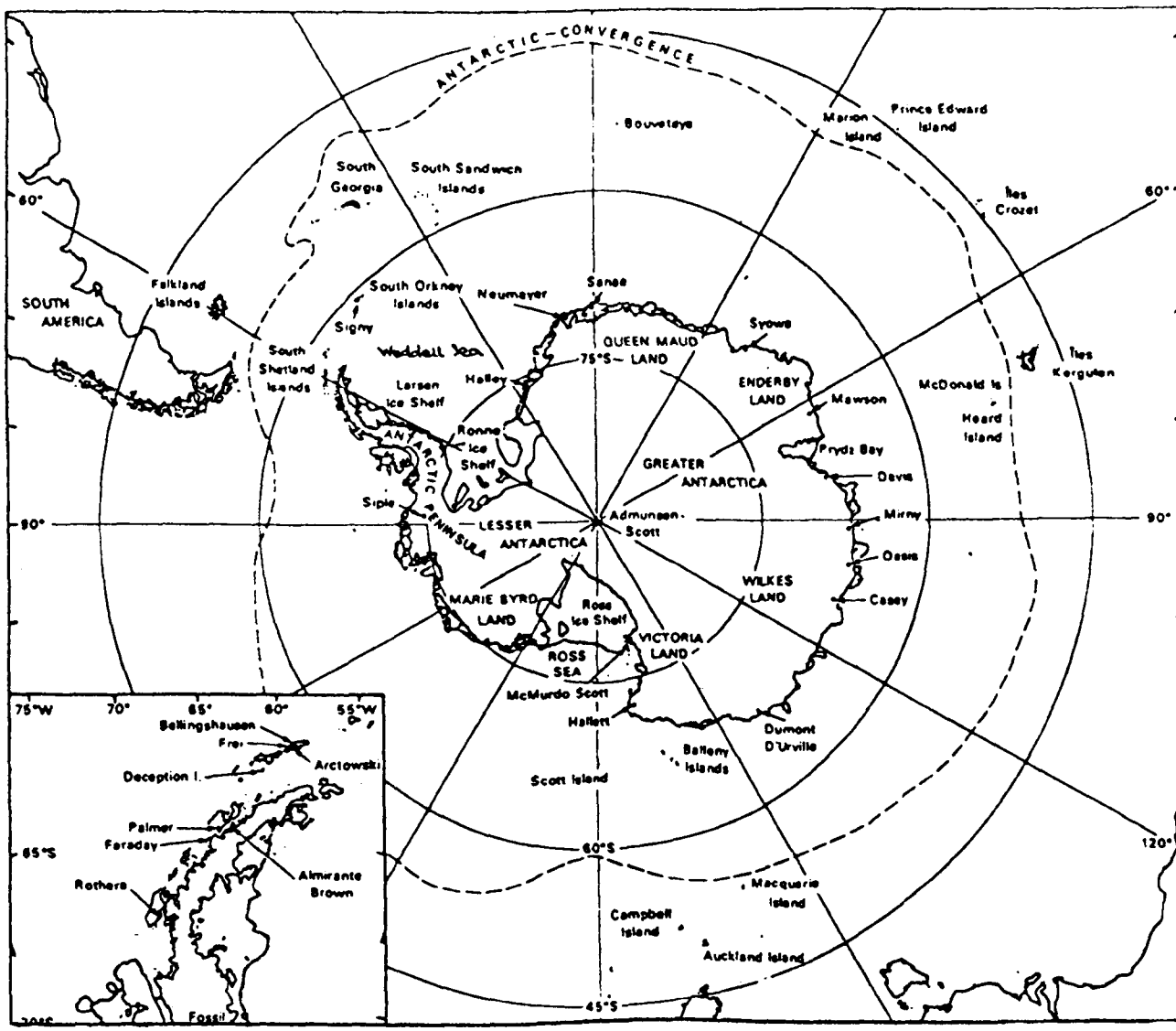


Fig 1.1 Map of Antarctica (from Bonner and Walton 1975).

Antarctic have turned their attention to various fin-fish, squid and krill.

1.2 Physical oceanography.

The Southern Ocean surrounds Antarctica, forming a continuous ring of water. The continent is an ice-covered land mass with a particularly deep continental shelf (400 - 500 m) resulting from isostatic depression by the ice mass. The Southern Ocean contrasts with that of the Arctic Ocean which comprises a relatively shallow sea bounded by the northern margins of continents. In addition, the continental shelves of the Arctic Ocean are relatively shallow, and unlike the Southern Ocean it has an extensive riverine input.

The hydrography of the Southern Ocean has been well documented. The system is dominated by an atmospherically driven, clockwise circulation called the Antarctic Circumpolar Current (ACC, Fig. 1.2) which has been reviewed by Nowlin and Klinck (1986) and Gordon (1988). Nearer mainland Antarctica, the presence of easterly winds induces an anticlockwise current system known as the Antarctic Coastal Current (Fig. 1.2). These two systems interact in the form of gyres, the most prominent of which are those in the Weddell and Ross Sea. The interaction between the ACC and the Antarctic Coastal Current induces a region of upwelling, the Antarctic Divergence, which is the major source of nutrients to the pelagic ecosystem (Holm-Hansen 1985). The ACC is delimited to the north at the Antarctic Convergence which is a complex, turbulent frontal zone where cold polar water flowing northwards descends below warmer water flowing to the south. It is here that important biogeochemical interactions take place between the cold, nutrient-rich water Antarctic water and the warmer, nutrient-poor water of the adjacent Southern Hemisphere oceans (Fig. 1.2). The Southern Ocean is characterised by low temperatures which range in summer from 3.5°C at the Antarctic convergence to only -1°C near the continental margin. In winter these temperatures drop to 0.5°C and -1.8°C respectively. With the onset of winter, the sea freezes at the surface and cold, highly saline water is expelled which sinks to the ocean floor to become the Antarctic Bottom Water (Fig. 1.2). This frigid water then flows northwards over the abyssal plain and ultimately affects the oceanography and climate as far away as the North Atlantic.

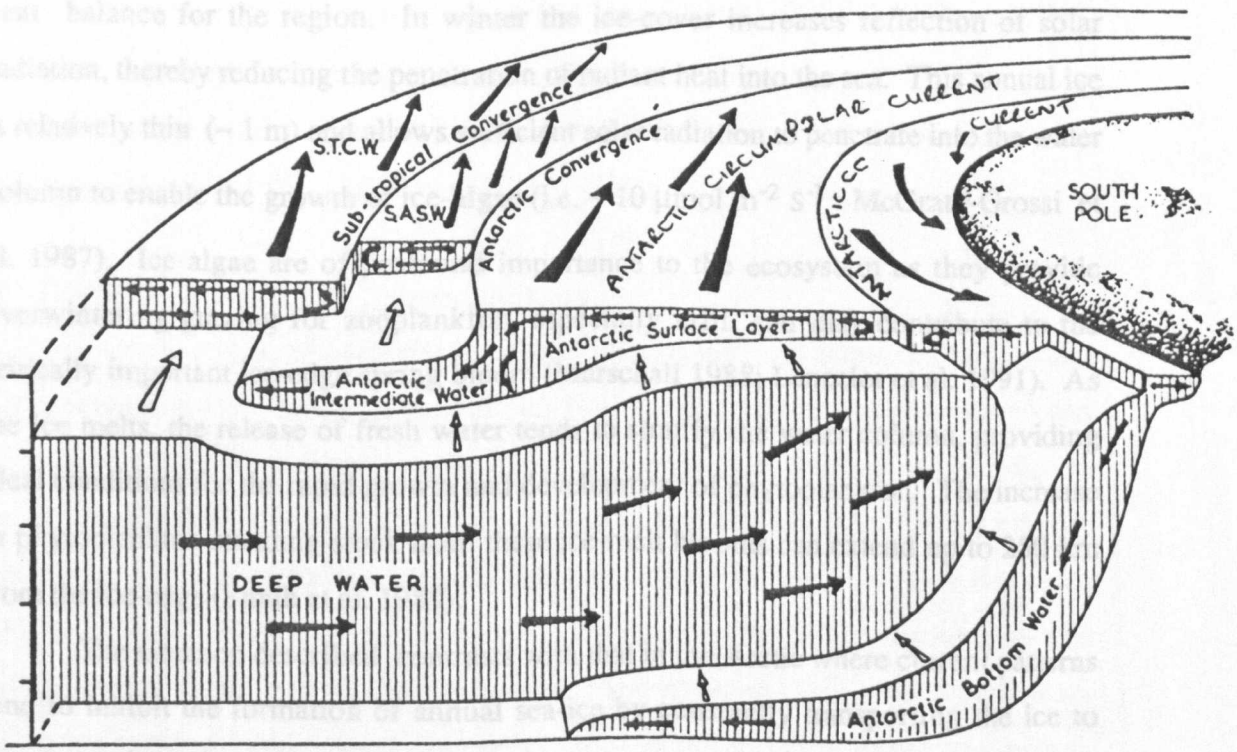


Fig 1.2 Schematic representation of the currents and water masses of the Southen Ocean (after Foxton 1956).

1.3 Sea-ice: ecological implications of ice cover and water column stratification.

One of the most important physical factors affecting the Southern Ocean ecosystem is the seasonal formation and retreat of sea-ice which covers a maximum of 20 million km^2 in winter and retreats to only 4 million km^2 in summer (Squire 1990; Stromberg 1991). This seasonal process results in massive variations in the radiation heat balance for the region. In winter the ice-cover increases reflection of solar radiation, thereby reducing the penetration of radiant heat into the sea. This annual ice is relatively thin (~ 1 m) and allows sufficient solar radiation to penetrate into the water column to enable the growth of ice-algae (i.e. ~ 10 $\mu mol\ m^{-2}\ s^{-1}$ McGrath-Grossi et al. 1987). Ice-algae are of profound importance to the ecosystem as they provide overwintering grazing for zooplankton, especially krill, and may contribute to the critically important ice-edge spring bloom (Marschall 1988; Lancelot et al. 1991). As the ice melts, the release of fresh water tends to stratify the water column, providing ideal conditions for the rapid growth and development of phytoplankton. The increase in phytoplankton standing stock from these ice-melt blooms can extend up to 250 km from the ice-edge (Smith et al. 1988).

The situation described contrasts with that in the Arctic where current patterns tend to inhibit the formation of annual sea-ice by constantly transporting the ice to warmer regions (Stromberg 1991). Also, the majority of the Arctic Ocean is at a higher latitude than that of the Antarctic which tends to promote the formation of thicker sea-ice. Therefore, most of the ice cover in the Arctic is multi-year ice, approximately 3-5 m thick, and often overlain by a deep layer of snow. This allows only limited solar radiation to penetrate the upper layers of the water column, therefore promoting only limited growth of ice-algae and hence providing only limited food for overwintering, grazing zooplankton (Stromberg 1991).

1.4 The Southern Ocean ecosystem.

The classical concept of the Southern Ocean ecosystem detailed by Fricker (1900) and Lillie (1913) is one of short food chains with few trophic interactions that efficiently transfer 'energy' from primary producers to higher predators, i.e. from

diatoms to krill to whales (Fig. 1.3). This concept persisted until the 1970's. It was assumed by these early workers that the high standing stock of both krill and higher predators was supported by a high biomass of microplankton. In recent decades this view has been increasingly challenged by research indicating that overall primary production in the Southern Ocean is only moderate compared with other oceans (Holm-Hansen et al. 1977; El-Sayed 1984; Smith 1991). However, primary production in the Antarctic can be very high locally, up to 150 μg chlorophyll *a* l^{-1} (El-Sayed 1967; Hewes et al. 1983). In addition, the importance of recycling pathways involving microheterotrophs and protozoan grazers and their enhancement of productivity is being increasingly realised (Fig. 1.3, Hewes et al. 1983).

1.5 Primary production: phytoplankton.

As the Antarctic continent is largely ice-covered and often has ice-covered margins there is a negligible input of allochthonous material into the Southern Ocean ecosystem which is, therefore, totally dependent on *in situ* primary production. In recent years studies have concentrated on understanding the factors controlling primary production and especially the apparent under-utilisation of nutrients (Lancelot et al. 1991). The Southern Ocean ecosystem is characterised by marked seasonality and fundamentally driven by a pulse of primary productivity during the austral summer with the phytoplankton, predominantly diatoms, utilising the abundant inorganic nutrients (Clarke 1988). Numerous studies have investigated the species composition and abundance of these Antarctic phytoplankton (Hewes et al. 1983; 1985; Marchant 1985; El-Sayed 1988; Graneli et al. 1993). Diatoms commonly dominate Antarctic planktonic communities, although taxa such as haptophytes (e.g. *Phaeocysytis*) and dinoflagellates can dominate in some situations, especially during the winter (Lancelot et al. 1991). As krill feeds predominantly on microplankton in the 10-200 μm size range, it is the productivity and availability of these microscopic organisms that determines to a large degree the productivity of the Antarctic pelagic food web. However, recent studies have highlighted the importance of nanoplankton (2-20 μm) in Antarctic pelagic food webs and have indicated that these small particles can, in some regions, account for up

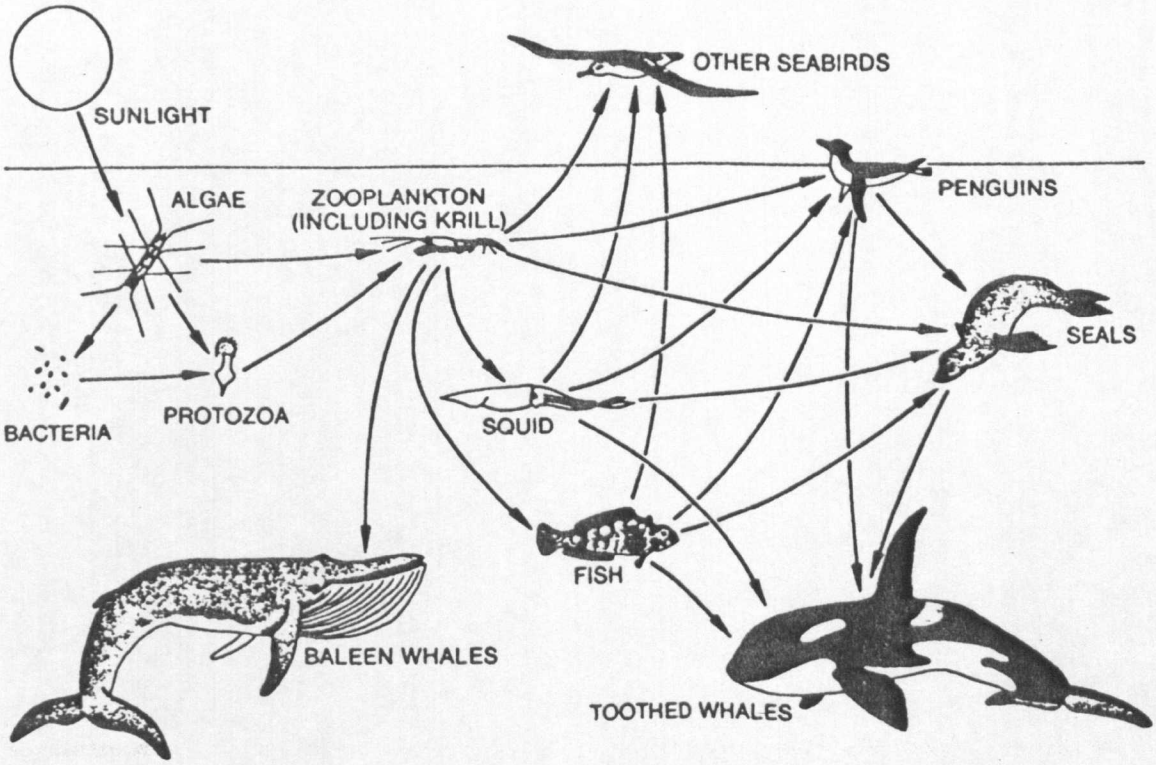


Fig 1.3 Simplified food web for the Southern Ocean pelagic ecosystem (after Priddle 1990)

to 70% of the biomass (El-Sayed and Taguchi 1981; Yamaguchi and Shibata 1982). Even smaller particles, i.e. < 2 μm (picoplankton), have been found to contain up to 70% of the chlorophyll *a* in the water column (El-Sayed and Weber 1986), although it is likely that a large proportion of these particles may be free chloroplasts released from ruptured phytoplankton (Gieskes and Elbrachter 1986). The importance of the heterotrophic community has been increasingly realised, especially since the international programme, Research on Antarctic Ecosystem rates (RACER, Huntley et al. 1991). Pelagic food webs are often dominated by pico- and nano-producers and micrograzers, resulting in the rapid and efficient remineralisation of organic carbon and nitrogen in the euphotic zone (Garrison and Buck 1989).

1.5.1 Illumination and vertical mixing.

A number of factors determine production of autotrophic organisms within the system although the most important is the highly seasonal light climate (Whitaker 1982; Clarke et al. 1988a). Submarine illumination (insolation) is highly variable and dependent locally on such factors as the day length, the angle of incidence radiation (which is usually shallow, resulting in high reflectance), the abundance of suspended particles and the extent and thickness of ice-cover (Cota and Smith 1991; Smith and Harrison 1991). Physical stability of the water column is a major determinant of primary production as it affects profoundly the light climate that algal cells experience. Deep vertical mixing is characteristic of the turbulent Southern Ocean and this tends to limit primary production by transporting algal cells beneath the critical depth, hence exposing them to highly variable irradiance regimes (Mortan-Bertrand 1988; 1989). Areas of high phytoplankton biomass tend to be associated with features that stabilise the water column such as the ice-edge and coastal embayments (Whitaker 1982; Nelson and Smith 1986; Sullivan et al. 1988). Ice-cover itself tends to reduce primary production because, although it stabilises the water column, the reduction in irradiance it causes restricts the growth of algae, except ice-algae. Indeed, the clearest sea water ever recorded was under the ice, in the Weddell Sea (Fig. 1.1), where a Secchi disc could be seen to a depth of 79 m (Gieskes et al. 1987). Stable conditions such as those found under ice tend to promote the loss of algae from the euphotic zone by passive sinking. Diatoms, and especially the colonial and chain forming species, have high

potential sinking rates (Jaworski et al. 1988) with single cells sinking at 1-10 m day^{-1}, whereas large colonies can sink up to 200 m day^{-1} (Whitaker 1982; Dunbar 1985; Johnson and Smith 1985).

1.5.2 Nutrient availability - trace limitation.

In the Southern Ocean, unlike the other world's oceans, macro-nutrient availability is unlikely to be a limiting factor as both nitrate and phosphate concentrations remain high after the phytoplankton bloom, primarily due to under-utilisation (Priddle et al. 1992). Despite these high nutrient levels, most nutrients are recycled up to 6-7 times by microbial processes before sinking to deeper water as particulate organic matter (Koike et al. 1981; Ronner et al. 1983). As macro-nutrients do not appear to limit primary productivity, in recent years the availability of trace elements in the Southern Ocean has been investigated and these have also been shown to be non-limiting for primary producers (Hayes et al.1984). However, a study of phytoplankton growth in the sub-arctic region of the Pacific has implicated a deficiency of iron as a limiting factor (Martin and Fitzwater 1988; Martin and Gordon 1988). A similar study in Antarctic waters has shown that high and low phytoplankton growth in the Gerlache Straight and the Drake Passage can be equated with high (7 µmol) and low (0.16 µmol) concentrations of iron respectively (Martin et al. 1990). The addition of Fe to bottle incubations conducted by de Baar et al. (1990) and Buma et al. (1991) did indeed stimulate algal growth, although control bottles also exhibited increased growth compared with field samples. As zooplankton were excluded from these incubations, Dugdale and Wilkerson (1990) suggested that phytoplankton productivity is mostly controlled by loss terms such as grazing.

1.5.3 Effect of zooplankton grazing on primary productivity.

Zooplankton grazing is likely to be a major factor influencing primary production in the Southern Ocean. The grazers, which range in size from microscopic phagotrophic protists to larger animals such as salps and Antarctic krill, are capable of reducing drastically the standing stock of phytoplankton, at least locally (Von Bodungen 1986; Wefer et al. 1988). The effect of particle selection by zooplankton tends to shift

the size distribution of phytoplankton to smaller sizes (Meyer and El-Sayed 1983; Quetin and Ross 1985; Kopczynska 1992) and the implications of this for primary production are largely unknown. On an oceanic scale, grazing by zooplankton has been estimated to remove only ~ 3% of daily production per day in the Southern Ocean (Miller et al. 1985). However, on a local scale the impact of krill grazing can be considerable (Lancelot et al. 1991; Priddle et al. 1992; Kopczynska 1992).

1.6 Zooplankton.

The Southern Ocean pelagic ecosystem can be divided into three main zones each characterised by distinct zooplanktonic food webs. The northern Antarctic Circumpolar Current is relatively ice-free and its biota is dominated by herbivorous copepods such as *Calanoides acutus*, *Calanus proprinquus* and *Rhincalanus gigas* (Voronina, 1966; 1968; Hopkins 1971; Atkinson 1990) and also by small euphausids and salps. Second is the Antarctic Coastal Current with its associated fronts and eddies which is dominated by krill. Krill may be advected from this region to the vicinity of South Georgia. Third is the permanent sea-ice zone near to the continental land mass where both primary productivity and zooplankton biomass are generally low.

1.7 Predators of krill.

The Southern Ocean supports a large biomass of higher predators most of which are heavily reliant on *Euphausia superba* as a source of food (Table 1.1). Krill are vitally important as their efficient filtering abilities and large size provides an efficient and short link between the microplanktonic primary producers and higher predators.

The most important invertebrate predators of krill are the Cephalapoda, which comprise mostly oceanic squid, and which contrast with most other major groups of predators of krill in that they are short lived and fast-growing (Saville 1987). Unfortunately, biological assessment of squid stocks is difficult, primarily due to sampling difficulties such as net avoidance (Laws 1985). However, squid samples are

Table 1.1 Possible changes in patterns of consumption of Antarctic krill by major groups of predators (after Laws 1985).

	Year	
Predator	**1900**	**1984**
Whales	190	40
Seals	50	130
Birds	50	130
Fish	100	70
Cephalopods	80	100
Total	**470**	**470**

* Annual consumption (tons x 10^6)

frequently encountered in the stomachs of birds, seals and whales and analyses of these have indicated that species such as *Kondakovia longimanna* and *Moroteuthis knipovitchi* feed on krill (Table 1.1; Clarke 1983; Laws 1985).

Fish are also known to be major consumers of krill and fortunately their life history and feeding preferences are more amenable to study than those of cephalapods. The Southern Ocean is unusual in that most of the fish species it contains are demersal and it does not support a significant biomass of pelagic fish, although bathypelagic myctophids are common in oceanic regions (Laws 1985). Nototheniiformes are the dominant group comprising ~75% of all species and their early years are spent in coastal waters, after which they move offshore to feed predominantly on krill (Laws 1985).

Only thirty five species of seabird breed south of the Antarctic Convergence, but their populations are considerable. Penguins and albatrosses together with petrels are the two dominant groups and, not surprisingly, their distribution closely mimics that of krill which has been estimated to comprise 78% of their diet (Croxall 1984). In winter when the ocean is largely ice-covered and food becomes scarce, many of these birds either migrate northwards, or switch to a diet of fish and squid.

Six species of seal inhabit the Southern Ocean and each is well adapted to its particular niche. Their life histories are inextricably linked to the seasonal environment. The crabeater (*Lobodon carcinophagus*) is the most abundant seal species in the world, and feeds exclusively on krill. Whale stocks, although reduced by over-exploitation, still play an important role in the Southern Ocean as they are major consumers of zooplankton, fish, squid and seals. The large baleen whales such as the southern right (*Balaena australis*) and humpback (*Megaptera novaeangliae*) feed on krill, copepods and amphipods, whereas the toothed whales such as sperm (*Physeter catodon*) feed on squid and fish. Killer whales (*Orcinus orca*) are major predators of fish and to a lesser extent of seals.

It is important to note that many of these higher predators migrate out of the Southern Ocean to lower warmer latitudes during the Austral winter. This annual migration is likely to result in a significant export of `fixed carbon' from the region.

1.8 Krill: basic biology.

Knowledge of the life cycle of Antarctic krill is still incomplete, despite several

decades of intensive international research. *Euphausisa superba* is a large, pelagic zooplankter achieving a maximum wet mass of ~ 3 g and maximum length of ~ 65 mm. Its distribution is circumpolar and ranges from the Antarctic Convergence in the north to the continental margin in the south. Sexual maturation is thought to occur after 2-3 years, although the age at which this occurs is probably dependent on food availability (Miller and Hampton 1989; Quetin et al. 1993). Breeding is thought to take place throughout the summer, depending on local environmental conditions (Ross and Quetin 1986). The most productive krill breeding areas include the Bellinghausen and Scotia Seas (Everson 1977: Priddle et al. 1988). Eggs of 5-6 μm are usually released in the surface waters, although Hempel (1986) has suggested that spawning may take place over a range of depths. The eggs then sink before hatching at depths of between 800 m and 2500 m (Marr 1962; Hempel 1979; Hempel et al. 1979; Quetin and Ross 1984). After hatching, the larvae ascend and develop through three non-feeding stages, i.e. two naupliar and one metanaupliar stage, before entering the first calyptopis phase and commencing feeding in surface waters at 30-100 m. The time for development from eggs to final larval stages is thought to be about 130 days, although this is likely to be dependent on both food availability and temperature (Ikeda 1984; 1985; Hoffman et al. 1993).

Determining the age of an animal that moults regularly and contains no statoliths is very difficult as no permanent growth record is retained in the body tissue. Euphausids are particularly problematical in this respect because they moult regularly at approximately monthly intervals (Buchholz 1983) throughout their lives (Nichol 1990). Traditional aging methods involving length frequency analysis have proved inconclusive (Rosenberg et al. 1986), undoubtedly because krill are capable of regressing/shrinking at times of low food availability, mainly during the winter (Ikeda and Dixon 1982). More recently Ettershank et al. (1984) proposed a method of aging krill by quantifying fluorescent age pigments, although Nichol (1987) has since suggested the method may be inaccurate. However, in spite of the technical difficulties it is generally accepted that krill are long-lived, with estimates of potential maximum age of 4-7 years (Rosenberg et al. 1986).

As krill are negatively buoyant and must remain in the upper water column to survive, the energetic costs of swimming are thought to be high, resulting in a high

metabolic rate and a high energy throughput estimated at >20% of their body carbon per day (Clarke and Morris 1983).

1.8.1 Feeding ecology.

Euphausia superba feeds mainly by filtering particulate matter from the water column by the rapid extension and retraction of its forward projecting peripods at a rate of 2-3 Hz. The periopods are covered with numerous fine setae spaced at ~ 2-10 μm intervals and it is the spacing of these setae that determines filtering efficiency and particle selectivity by the animals (Boyd et al. 1984). Boyd found that all particles > 12 μm were retained with equal efficiency. However, Meyer and El-Sayed (1983) found evidence for size selectivity, with larger particles with spines being filtered most efficiently. Mauchline and Fisher (1969) and Miller and Hampton (1989) have reviewed the wide range of microplankton that comprises the diet of krill, although their results are based largely on stomach analyses and may not represent the true range of the diet.

1.8.2 Swarming.

A characteristic behaviour of Antarctic krill is a tendency to aggregate into distinct swarms. These tend to be highly variable in both size and composition and can occupy areas from a few square metres to an area of several square kilometres, such as the Elephant Island "super-swarm" (Mathisen and Macaulay 1983; Macaulay et al. 1984; Watkins et al. 1986). Swarming occurs both in summer and winter, although those krill overwintering under the annual sea-ice are thought to disperse (Marschall 1988). Swarms may contain animals of a similar sex and maturity stage, implying that there are underlying segregational processes involved in swarm formation (Watkins et al. 1992).

1.8.3 Implications of swarming.

Large swarms of actively feeding krill can drastically reduce the standing stock of microplankton in the water column, having a profound local effect on primary productivity (Antezana and Ray 1984; Lancelot et al. 1991). In addition swarms of feeding krill are likely to affect the size and/or species distribution of the phytoplankton

by shifting the composition towards smaller sizes. This again has implications for productivity and phytoplankton succession (Quetin and Ross 1985; Meyer and El-sayed 1983). Swarming of course, tends to render the animals susceptible to mass predation by higher predators such as penguins, seals and especially mysticete whales. Nonetheless, it seems reasonable to assume that swarming offers krill some benefit, e.g. increased reproductive success or predator avoidance.

1.8.4 Overwintering strategies of krill.

Until recently the winter distribution and behaviour of the massive swarming summer stocks of Antarctic krill have been unresolved. Direct observations of the overwintering strategy of the krill have been scarce because of obvious logistical and environmental difficulties that restrict winter cruises (e.g. Heywood et al. 1985). Few krill have been noted in ice-covered zones (Marr 1962; Guzman 1983; Stepien 1983). The first extensive winter and early spring survey of the distribution and over-wintering biology of krill was conducted in the winter of 1986 in the Winter Weddell Sea Project (Marschall 1988). By conducting an intensive sampling programme both in open water and under the ice, Marschall (1988) concluded that the morphology and behaviour of krill are adaptions to the sea-ice environment, that seasonal sea-ice sustains the majority of the krill population over winter and that their unique ability to adapt to this annual cycle explains their dominance in the Southern Ocean ecosystem. In addition, four main overwintering strategies have been suggested: body shrinkage, lipid utilisation, alternative food sources and reduced metabolism (Quetin and Ross 1991). Body shrinkage has previously been known to occur in Arctic euphausids (Bamstedt 1976; Falk-Petersen 1981) and this was also shown to be the case in Antarctic krill when animals were maintained in the laboratory without food for up to 211 days (Ikeda and Dixon 1982). These animals were observed to shrink by 1-2 % of their body length per moult. Similarly, Quetin and Ross (1991) found that a population of krill in winter exhibited "de-growth" equivalent to -1.6 to -2.03 % of the body length per intermoult period of ~5-9 weeks. Although shrinkage may be an overwintering strategy adopted by krill, it should be appreciated that moulting is likely to incur metabolic costs, as suggested by Morris and Priddle (1984).

Lipids and especially wax-esters are known to be important over-wintering

energy reserves in polar zooplankton whose habitat is characterised by marked seasonality in food availability (Lee et al. 1971; Lee and Hirota 1973; Sargent and Henderson 1986). Antarctic krill contrasts with most polar zooplankton in that it does not accumulate wax esters (Clarke 1980; Fricke et al. 1984) and it has, therefore, been suggested that *Euphausia superba* does not utilise lipids as an energy source during winter (Mauchline and Fisher 1969; Clarke 1984). However, these conclusions were drawn from samples obtained only during the summer months. A more recent analysis of krill sampled in late autumn, winter and early spring has shown that the average lipid content of all sexes in autumn was 7.74% of their wet mass, a value which dropped to 3.86% in late winter, thus indicating that lipid is utilised as an energy reserve in winter (Quetin and Ross 1991). It is known that krill are also able to utilise a wide variety of food sources and possibly the most important of these in winter is ice-algae (Hamner et al. 1983; Spiridonov et al. 1985; Garrison 1986; O'Brien 1988; Daly and Macaulay 1988; Stretch et al. 1988). Detritus may also be an important food item (Everson 1977) as may also be copepods (Boyd et al. 1984; Price et al. 1988) but probably only locally and for limited periods. Cannibalism has also been observed under laboratory conditions although, as yet, this phenomenon has not been witnessed in the field (Ishii et al. 1985; Maihara and Endo 1986). Nonetheless, cannibalism has been suggested as a possible, albeit an implausible overwintering strategy (Miller and Hampton 1989).

Reducing metabolic rate is an obvious method by which an animal can reduce its energy requirements, although the extent to which *Euphausia superba* can adopt this strategy is limited by its need to swim constantly to remain in the upper water column. In support of a reduction of metabolic rate in winter, Quetin and Ross (1991) found that the oxygen consumption of krill in winter was only 33% its summer level. Lack of feeding could explain this reduction since Ikeda and Dixon (1984) found that the oxygen consumption of non-feeding krill was only 62.5% that of actively feeding krill. Other plausible causes of reduced oxygen consumption are behavioural modifications such as reduced swimming activity and/or biochemical modifications such as reduced enzyme activity. It is likely that krill adopt a combination of all four strategies depending on environmental conditions and food availability (Fig. 1.4; Quetin and Ross 1991).

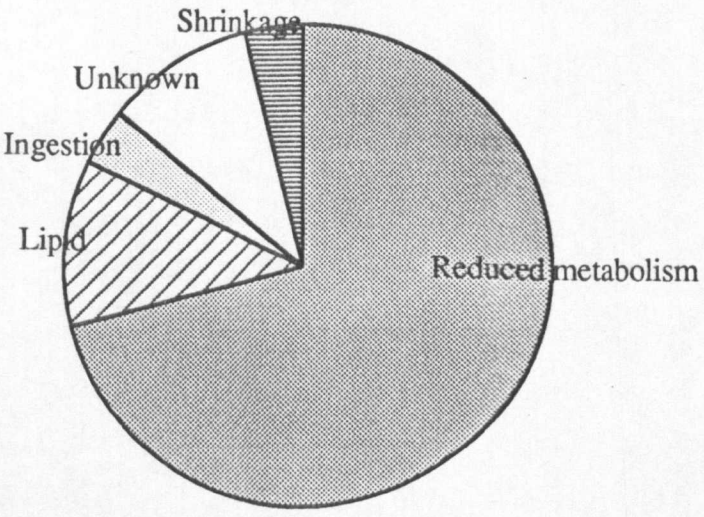


Fig 1.4 The suggested relative importance of the four major overwintering stratergies of Antarctic krill (after Quetin and Ross 1991)

1.9 Man's effect on the Southern Ocean ecosystem: the need for conservation and research.

In recent years, man has become a consumer of krill, which now forms the largest crustacean fishery in the world with a 357 x 10^3 tonne harvest in 1990/91 (Anon 1991). In order to manage such a fishery effectively, it is essential to understand the Southern Ocean ecosystem. It has been estimated that as a result of man's uncontrolled whaling activities in the Antarctic, there was effectively an annual surplus of 150 million tons of krill available for consumption by the remaining whale stocks and other higher predators (Laws 1985). However, recent evidence suggests that this surplus has already been taken up. Fin and sei whales now become sexually mature at a younger age and have exhibited increased pregnancy rates (Brown and Lockyer 1984). Similarly, the age at which minke whales reach maturity has dropped from 14 to 6 years and the population size has doubled (Brown and Lockyer 1984). The numbers of Antarctic fur seals, which were hunted almost to extinction at the turn of the century, has increased dramatically, with the South Georgia population increasing by 14 to 17% per annum (Laws 1985). This rate of increase is remarkable as populations of marine mammals recovering from over-exploitation usually increase by only 6 to 10% per annum. Although the "krill surplus hypothesis" has been challenged recently, the high rates of population increase observed for the fur seals can probably be attributed to increased abundance of krill (Laws 1985).

Historically, the exploitation of the Southern Ocean ecosystem has been largely uncontrolled with catastrophic consequences for seal and whale stocks. Hence, in the early 1970's when the commercial exploitation of Antarctic marine biological resources (especially krill) became increasingly likely, the Scientific Committee on Antarctic Research (SCAR) identified a need for considerable expansion of scientific research on the Antarctic marine ecosystem to understand the effects of fisheries exploitation. The outcome was the establishment of the international research programme, Biological Investigations of Marine Antarctic Systems and Stocks (BIOMASS). This programme was directed towards increasing scientific knowledge of the ecology and biology of the Antarctic marine ecosystem with the view to managing any future commercial exploitation of Antarctic fisheries. A major component of this research was directed

towards *E. superba* with the ultimate aim of understanding its productivity and sustainable yield. Prior to the BIOMASS programme, production estimates of krill were based either on studies of changes in the size distribution in a population over time, or on determinations of the proportion of the population consumed by predators. However, the scientific knowledge of the behaviour and biology of krill gained from the BIOMASS programme has enabled improved estimates of production, based on measures of food intake, assimilation efficiency and metabolic costs (Miller and Hampton 1989). With this improved knowledge of the biology of krill and also improved acoustic technology, pre-BIOMASS Russian estimates of krill stocks have been reduced by an order of magnitude.

The study to be detailed in this thesis attempts to extend the knowledge gained during the BIOMASS programme by concentrating on a specific dietary component of krill, namely lipid. The study of lipids, which are major constituents of most marine organisms (Sargent 1976; 1981) and especially those in high latitudes, can shed light on trophic interactions in marine ecosystems, particularly when such studies are a component part of integrated biological investigations (Falk-Petersen 1990: Hopkins et al. 1989). An example of this approach is the application of lipid analyses to ecosystems in northern Norwegian fjords where euphausids are as quantitatively important as they are in the Southern Ocean (Falk-Petersen et al. 1981; Sargent and Falk-Petersen 1988; Hopkins et al. 1989).

1.10 Lipids: Structure and function.

Numerous previous authors have described the structure and function of lipids in detail (Christie 1982; Gurr and Harwood 1991), so that only an outline of the subject needs be presented here. Lipids are a "chemically heterogeneous group of substances having in common the property of insolubility in water, but solubility in non-polar solvents such as chloroform, hydrocarbons or alcohols" (Gurr and Harwood 1991). This description is based simply on physical properties and, while there is only a loose chemical relationship between all the numerous compounds classified as lipids on this basis, it is pertinent that the majority of the quantitatively important lipids contain esterified fatty acids.

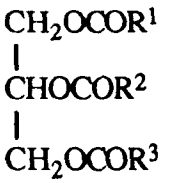
The major lipid classes and their constituent fatty acids investigated in this study

are shown in Figs 1.5 and 1.6. Lipids are conventionally separated into two groups on the basis of their solubility in organic solvents, namely the polar lipids and the neutral lipids. The polar lipids are relatively soluble in polar organic solvents, e.g. methanol, and relatively insoluble in non-polar solvents e.g. hexane. The converse holds for the neutral lipids. This difference in solubility reflects differences in the overall polarity of the two groups of lipids. Thus, polar lipids contain a substantial proportion of polar groups associated with, *inter alia*, the hydroxy groups of sugar constituents such as galactose or inositol, and the ionic groups of substituents such as amino acids and related compounds, sulphate and above all phosphate. Glycolipids, which are particularly abundant in the thylakoid membranes of photosynthetic eukaryotes, and phospholipids, which are particularly abundant in all animal cell membranes, are both catagorised as polar lipids. It is because these lipids contain polar (hydrophilic) head groups that associate naturally with water, as well as non-polar (hydrophobic) fatty acid substituents that associate naturally with each other, that they form the basic bilayer structure characteristic of most cellular membranes. In contrast, neutral lipids, have a paucity of polar groups and are instead dominated by their non-polar fatty acid substituents. Therefore, the neutral lipids, chief of which are the triacylglycerols and wax esters, self associate into droplets that comprise the bulk of adipocytes and give rise to commercial oils and fats derived from the seeds of many plants and the subcutaneous, perirenal or perivisceral adipose depots of fish and higher animals. Neutral lipids in such adipose tissue have the primary function of being metabolic energy reserves or stores.

The major building blocks of lipids are fatty acids, which vary considerably in their degree of unsaturation, i.e. their number of double bonds. In this study fatty acids will be referred to by a conventional shorthand notation, e.g. 22:6(n-3). The first number denotes the carbon chain length. The second number denotes the total number of double bonds in the chain and the (n-x) denotes the distance of the first double bond from the methyl terminus (n). Since the double bonds naturally occurring in fatty acids are invariably "methylene-interupted", i.e. 1:4 - dienoic acid structures, the nomenclature gives a full description of the chemical structure of a particular fatty acid (Fig 1.6).

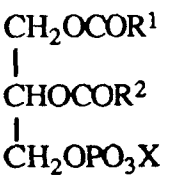
It is convenient to categorise fatty acids as saturated (containing no double

Triacylglycerols



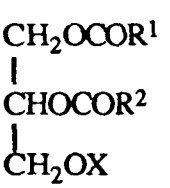
R^1, R^2, R^3 = fatty acyl glycerols

Phospholipid



R^1, R^2 = fatty acyl units; X = base (ethanolamine, serine, choline, etc)

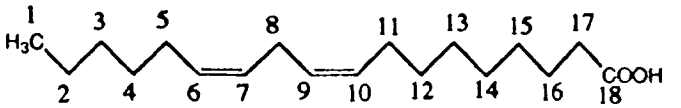
Glycolipids



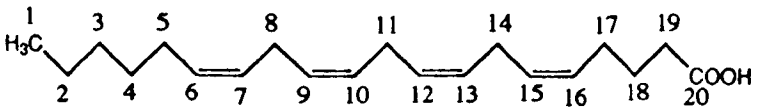
R^1, R^2, = fatty acyl units; X = carbohydrate (galactose, galactosylgalactose, sulphoquinovose etc).

Fig 1.5 Lipid class structures.

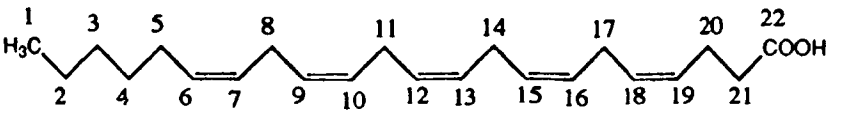
OMEGA-6 SERIES



LINOLEIC ACID 18:2(n-6)

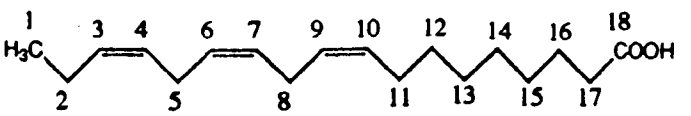


ARACHIDONIC ACID 20:4(n-6)

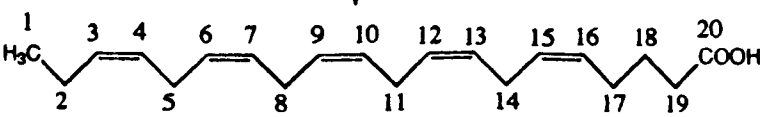


DOCOSAPENTAENOIC ACID 22:5(n-6)

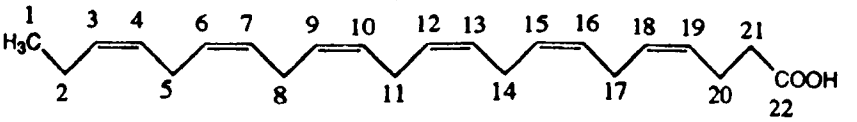
OMEGA-3 SERIES



LINOLENIC ACID 18:3(n-3)



EICOSAPENTAENOIC ACID 20:5(n-3)



DOCOSAHEXAENOIC ACID 22:6(n-3)

Fig. 1.6 fatty acid structures.

bond), monounsaturated (containing one double bond) and polyunsaturated (containing two or more double bonds). The former two tend to predominate in neutral lipids whereas the latter two tend to predominate in polar lipids. This reflects the two main functional roles of lipids. Thus, because fatty acids contain a substantially higher proportion of reduced carbon than either protein or carbohydrate, they provide the highest yield of metabolic energy per unit weight of all these three major biochemical constituents. This is why lipid is a major and important energy source in all living organisms. Moreover, because the saturated fatty acids are more reduced than the polyunsaturated fatty acids the former group tends to dominate the fatty acids in the triacylglycerols and wax esters that constitute the metabolic energy stores in animal and plant adipose tissue. The polyunsaturated fatty acids differ fundamentally from the saturated fatty acids in having much lower phase transitions (melting points), a property that is conventionally viewed as being of particular importance in maintaining the fluidity of cellular membranes at low environmental temperatures where saturated fatty acids exist as solids. In addition, the polyunsaturated fatty acids in membrane polar lipids may have quite specific structural associations with membrane proteins that can not be fulfilled by saturated fatty acids, and the polyunsaturated fatty acids in cell membrane phospholipids are also the origin in higher animals of an important class of "local hormones" known collectively as the eicosanoids that are intimately involved in membrane signal transduction processes.

Thus, the operational division of lipid into neutral and polar classes based on differential solubility in organic solvents has its origins in fundamental differences in the physical chemistry of the two classes, and these differences are exploited in the two major biological functions of lipids, energy sources in the case of neutral lipids and membrane constituents in the case of polar lipids.

1.10.1 Lipids as bio-indicators.

As lipids can be major biochemical constituents of many marine organisms, especially those from polar regions (Falk-Petersen et al. 1990) and lipid composition can be related to taxonomy, lipids can be used as bio-indicators to study trophic interactions between marine consumers and their food supply (Table 1.2; Sargent et al. 1987; Currie and Johns 1988). In animals, lipid content and composition can also be

Table 1.2 Bio-indicators (modified from Sargent et al. 1987).

Bio-indicator	Indicates	Reference
Polar glycolipids (Monogalactosyldiacylglycerol Digalactosyldiacylglycerol Sulpholipids)	Photosynthetic organisms (associated with chloroplasts)	(Sargent et al. 1987)
Hopanoids	Some bacteria	Rohmer et al. (1984)
Phytanyl ether lipid	Archaebacteria	Harwood and Russell (1984)
Phosphatidlycholine	Reproductive status (egg production)	(Sargent et al. 1987)
16:4(n-3)	Bacillariophycae (diatoms) Herbivory	(Sargent et al. 1987)
18:2(n-6)	Cyanobacteria (blue-green algae)	(Sargent et al. 1987)
18:4(n-3), 18:5(n-3)	Dinophycae (dinoflagellates), Haptophycae, Cryptophycae	(Mayzaud 1976) (Sargent et al. 1987)
20:5(n-3)	Bacillariophycae	(Sargent et al. 1987)
22:1(n-11)	Dietary input of 20:1 or 22:1 fatty alcohols	(Falk-Peterson et al. 1991)
22:6(n-3)	Haptophycae (eg *Phaeocystis*) Dinophycae	(Sargent et al. 1987)
Phospholipid 16:0	Total biomass, prokaryotes and eukaryotes	White et al (1979)
Branched chain fatty acids	Biomass of some bacteria	Leo and Parker (1966)
Cyclopropyl fatty acids	Biomass of some bacteria	Harwood and Russell (1984)
Lipopolysaccharides	Gram-negative bacteria	Rogers (1983)
Teichoic acid	Most Gram-positive bacteria	Rogers (1983)
Muramic acid	Bacteria except Archaebacteria (Note that there are different amounts in Gram-positive and Gram-negative bacteria)	Rogers (1983)
PUFA	Eukaryotes, gliding bacteria, some deep sea bacteria	Harwood and Russell (1984) Johns and Perry (1977) DeLong and Yayanos (1986)
Phytopigments	Photoautotrophs	Gillian and Johns (1983)

used as indicators of physiological and reproductive status (Table 1.2). Thus, Hakanson (1984) analysed the lipid content of *Calanus pacificus* and suggested that wax-esters and triacylglycerols could be used as indicators of long- and short-term feeding condition respectively. Similarly, as lipid is a major component involved in reproductive investment, it can be used as an indicator of state of sexual maturity (Henderson et al. 1984; Hopkins et al. 1984).

Polyunsaturated fatty acids (PUFA), i.e. fatty acids with two or more double bonds, are particularly useful as bio-indicators as they are essential for the growth and development of marine consumers as far as is known, and are synthesised *de novo* only by phytoplankton (Sargent et al. 1987). Therefore, as these PUFA a) are not synthesised in significant quantities by any heterotrophic marine organism so far studied and b) are likely to be highly conserved by marine animals, PUFA are particularly useful as tracers to investigate both qualitative and quantitative interactions within pelagic food webs. For example, Sargent et al. (1981; 1985) and Falk-Peterson et al. (1982), in a study of a northern Norwegian fjord, used fatty acids as bio-indicators to investigate qualitative food web interactions and so gained valuable insights into the feeding behaviour and life histories of many of the organisms inhabiting the fjordic ecosystem (Table 1.2). Sixteen and 18 carbon PUFA, originating in phytoplankton were traced to PUFA in zooplankton and fish lipids, and 20:1 and 22:1 fatty alcohols originating in copepods were traced to 20:1 and 22:1 fatty acids in oil-rich fish such as capelin and herring. Similarly, fatty acids were used as bio-indicators by Klungsoeyr (1989) to investigate the switch from endogenous to exogenous feeding in cod larvae. Very long-chain monounsaturated fatty acids (24:1, 26:1) have been found in the Antarctic sea-ice diatom *Nitzschia cylindrus* and these compounds may allow the input of this algae into benthic sediments and food webs to be monitored (Nichols et al. 1986). Lipids and especially fatty acids can also be useful in resolving the taxonomy or the origin of fine particulate matter unidentifiable by conventional microscopy (Harwood and Russell 1984; Currie and Johns 1988).

1.10.2 Lipid biosynthesis.

It is known that both environmental conditions and the physiological status of marine algae influence their lipid content and their lipid biosynthesis capacity (Chen

1991; Hodgson et al. 1991). Smith and Morris (1980), using incorporation of $^{14}CO_2$, found that Antarctic microplankton channelled ~80% of their fixed carbon into lipid under conditions of low light and low temperature. However Li and Platt (1982) and Sargent at al. (1985) found that Arctic phytoplankton incorporated only 20-30% of their fixed carbon into lipid. Differing species composition and differing environmental and physiological conditions are possible factors explaining the discrepancy. Thus, algae grown under conditions of nitrogen limitation tend to accumulate the end products of photosynthesis as lipid, especially triacylglycerol (Ben-Amotz et al. 1985; Parrish and Wangersky 1987; 1990; Hodgson et al. 1991). In addition, lipid biosynthesis by algae also varies throughout the growth cycle. Thus, during the early growth phase when algae are actively dividing and photosynthesising, most of the lipid biosynthesis generates polar lipids which are primarily associated with the thylakoid membrane of the chloroplasts. By contrast, as growth reduces during the lag phase with the onset of senescence, a situation often parallelled by nitrogen depletion in the environment, biosynthesis of triacylglycerols (TAG) is prevalent (Parrish et al. 1987). This could account for the finding that up to 68% of the total lipid in Antarctic sea-ice algae is triacylglycerol (Nichols et al. 1989).

1.10.3 Lipids in krill.

The lipid composition of krill has been well studied (Clarke 1980; Fricke et al. 1984). The total lipid content of the animals tends to vary both with season and sexual maturity. Clarke (1980) found that male krill contained 2-4% lipid and immature krill contained 4% lipid on a fresh mass basis, and this was seen to increase to 5-6% for both males and immatures later in the season. Females generally contained 5-6% of their fresh mass as lipid, although some gravid animals contained as much as 9% lipid. Phospholipid, free sterol, free fatty acids and triacylglycerols were the major lipid classes found in *E. superba* (Bottino 1975; Clarke 1980; Fricke et al. 1984). Wax esters are known to be synthesised and stored in very large amounts by many polar zooplankton that inhabit regions subject to large, seasonal fluctuations in food availability (Lee et al. 1973). As krill contain only trace levels of wax ester (Clarke 1980) and store only limited quantities of triacylglycerols, it can be inferred that krill

feed all year round and are, therefore, able to exploit food sources other than the summer phytoplankton bloom.

Analysis of the fatty acid composition of *E. superba* has shown krill to be relatively rich in PUFA (Clarke 1980; Fricke 1984). Phospholipid was particularly rich in PUFA containing 24% of its total fatty acids as 20:5 (n-3) and 25% as 22:6 (n-3). By contrast, the neutral storage lipids, triacylglycerols, were relatively depleted in PUFA, containing only 11% 20:5 (n-3) and 3% 22:6 (n-3) (Clarke 1980). However, 16C and 18C saturated and monounsaturated fatty acids were both abundant in the triacylglycerols. The presence of 16C and 18C PUFA, notably 16:4 (n-3) and 18:4 (n-3), and especially phytanic acid, found by Clarke (1980), and derived from the phytol moiety of chlorophyll, are all indicative of a largely herbivorous diet (Sargent and Falk-Peterson 1981; Sargent et al. 1987). The fatty acid 20:5 (n-3), a known bio-indicator of diatoms, is also common in krill (Clarke 1980; Fricke 1984; Sargent et al. 1987).

1.11 Aim.

Overall, the aim of this study was to produce a lipid budget for the trophic level microplankton - krill: first, by determining the lipid content and composition of phytoplankton; second, by determining the rate of ingestion and assimilation of this lipid by krill; third, by determining the lipid content and composition of krill. The study was based on the premise that there is a need for a detailed quantitative investigation of the flux of lipid through the trophic level of phytoplankton - krill to understand the potential productivity of krill.

2. MATERIALS AND METHODS

2.1 Sites.

Samples of microplankton and krill were collected during a cruise of RRS *John Biscoe* off South Georgia and also close to the Antarctic Peninsula, during January to March 1991 (Fig. 2.1). Environmental variables at the stations SG1-SG8, AP1 and AP2 are shown in Table 2.1.

2.2 Particulates.

2.2.1 Sampling and storage.

Microplankton, predominantly phytoplankton, was collected from five South Georgia sites (SG1, SG3, SG4, SG5 and SG8, Fig. 2.1) in 30 litre Go-Flo bottles from 40 m depth, which approximated to the chlorophyll maximum. Using a Sartorius stacked filtration system, 10 litres of seawater were fractionated by successive wet filtration under slight vacuum through 200 μm and 20 μm meshed nylon gauze (Plastok Associates Ltd, Birkenhead) and finally through ashed glass fibre filters (Whatman, GF/C, retention ~ 1.5 μm). Microplankton samples from thirteen Antarctic Peninsula sites (AP1-AP13) were collected using the ship's non-toxic pumped sea-water supply (inlet ~ 3 m depth), with 10 litres of this seawater being filtered directly on to an ashed GF/C filter. Obvious contaminating zooplankton and detritus were removed manually from the filtered microplankton using fine forceps. Nylon and glass fibre filters containing the different size fractions were stored at -40°C in 7 ml screw-capped, Teflon septum vials completely filled with absolute methanol to minimise oxidation, until lipid analyses in the U.K.

2.2.2 Microscopic analyses of particulates.

Separate 200 ml samples of seawater from the sites were preserved in 2% Lugol's fixative and stored at 4°C until microscopic examination up to eighteen months later in the U.K. Before analysis, sample bottles were carefully rotated to thoroughly resuspend the particulates. A 20 ml sub-sample was then gently decanted into a settling

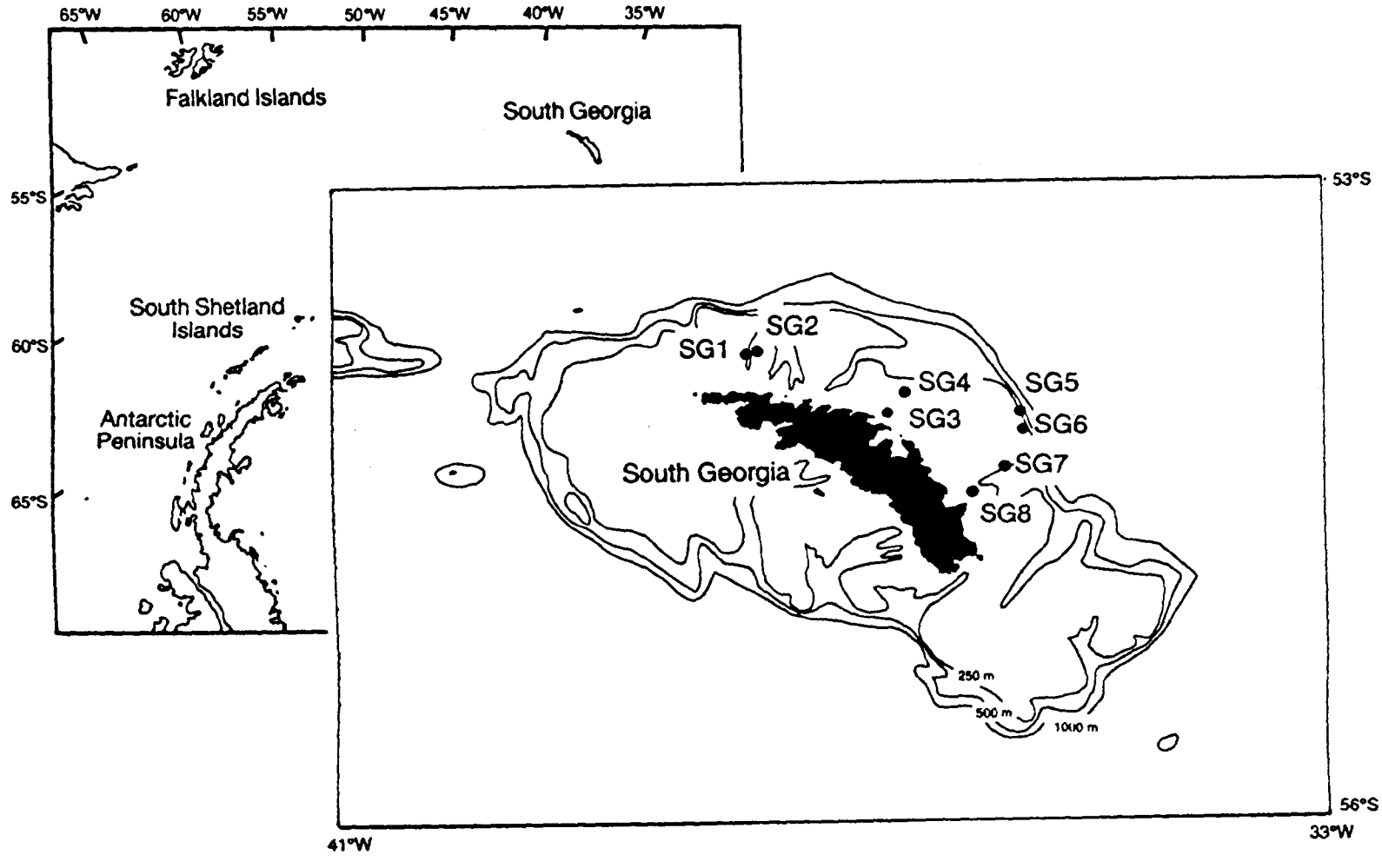


Fig 2.1 Positions of the eight sites sampled around South Georgia.

Table 2.1 Environmental variables at the eight South Georgia and two Antarctic Peninsula sites.

Site	Date	Depth range fished (m)	Temperature (°C)	Salinity (‰)	Chlorophyll *a* ($\mu g\ l^{-1}$)	Fatty acids ($\mu g\ l^{-1}$)
SG1	19,1,91	0-30	2.4	33.9	1.24	195.4
SG2	19,1,91	18 - 25	2.4	33.9	-	-
SG3	24,1,91	5 - 110	1.7	34.0	2.45	187.8
SG4	25,1,91	9 - 170	1.8	34.0	1.69	37.4
SG5	8,2,91	0 - 185	-	-	0.63	133.2
SG6	8,2,91	5 - 160	2.0	34.0	-	-
SG7	12,2,91	0 - 218	-	-	-	-
SG8	13,2,91	0 - 200	-	-	0.26	58.2
AP1	29,3,91	-	-	-	2.37	72.4
AP2	31,3,91	-	-	-	0.09	61.8

chamber which was left overnight (> 12 hours). The whole cover-slip was then scanned using an inverted microscope (Utermohl 1958) to determine microplankton species and abundance.

Representative proportions (20-30 cells) of each taxonomic group were sized using a calibrated graticule and cell volume estimations were made using approximations to simple geometric shapes (see Appendix 1).

2.3 Krill.

2.3.1 Sampling and storage.

Samples of Antarctic krill were caught at night, using a rectangular mid-water trawl (RMT8) of nominal mouth area 8 m^2 which was trawled obliquely from 200 m depth to the surface. The RMT8 comprised three nets each of which was fished sequentially for 20 minutes and the opening and closing of the nets was controlled acoustically. Krill for lipid analyses were mostly taken from the third net because, as it was fished last, the animals from it were in particularly good condition. However, due to a paucity of adult krill, some adult animals were also taken from the second net. The range of depths at which the second and third nets were fished are shown in Table 2.1. Krill were also captured at night, using a net of mouth area 1 m^2 which was deployed from the foredeck and fished immediately sub-surface for 5-15 minutes. Although only limited numbers of krill were caught using this method, i.e. 3-15 animals per haul, most were in excellent condition and suitable for use in grazing experiments. At night, whilst the RRS *John Biscoe* was moored at the Rothera jetty (Fig. 1.1), numerous zooplankton including krill were attracted to the surface by the ship's lights and approximately fifty animals were captured in excellent condition using a dip-net.

Krill were not sampled randomly from the RMT8 net hauls but were selected according to sex and maturity. Immature krill exhibited no evidence of sexual differentiation whereas males and females showed well developed secondary sexual characteristics. The krill were blotted dry on filter paper to remove excess sea water, adhering copepods were removed with forceps and individual specimens were then

frozen in pre-weighed, self-sealing plastic bags at -60°C until analyses in the U.K.

2.3.2 Krill culture

2.3.2.1 Ship deck tanks.

Krill caught by RMT8 and foredeck nets were placed immediately into 500 litre deck tanks. These tanks received a constant supply of pumped seawater which served to provide food, remove metabolic waste products and maintain a constant temperature. Dead animals were removed regularly and only healthy swimmers were selected for further experimentation.

2.3.2.2 Ship hold tanks.

Three 100 litre plastic bins, each with its own circulating biological filter system were used to transport live krill back to the U.K. The tanks were situated in the ship's cool room and maintained at a temperature of 3°C (±1°C). Initially ~150 krill were maintained in the aquarium system but, due to mortality, only ~ 60 survived transport to the UK. Whilst in Antarctic waters the animals were fed with natural particulates by regularly changing the water. North of the Antarctic Convergence, freeze dried algae (*Tetraselmis* 262 from Cell Systems, Celsys, Cambridge) were used as food for the krill. This proved to be only moderately successful because, although the krill were able to filter the algae from the water, the algal cells tended to break down after only a few hours thereby overloading the biological filter. Therefore, in Cambridge, freeze dried algae were abandoned as a source of food and cultured algae used instead. The krill were fed on most days, approximately 100 ml of dense algal culture (either *Isochrysis tahitiana*, *Thalassiosira weissflogeii* or *Phaeodactlyum tricornutum*) being added to each of the three tanks . The recirculating biological filters were switched off for ~ 2 hours after the introduction of the culture. Approximately 25% of the seawater was changed every 3-4 days to reduce the accumulation of metabolic waste and bacteria. The krill appeared to remain healthy in Cambridge using the same aquarium system as on the ship but operated at 0-1°C. They moulted regularly, with some animals surviving a further 12 months after return to the UK.

2.4 Algal culture.

2.4.1 Algal culture protocol.

Two contrasting species of algae were cultured and fed to the krill both routinely and during experiments. The haptophyte, *Isochrysis tahitiana*, with a diameter of circa 8 μm and a calculated cell volume of 295 μm^3 was obtained from cultures maintained at CCAP Oban (ref. CCAP 927/12). The diatom, *Thalassiosira weissflogeii*, was also obtained from CCAP Oban (ref. CCAP 1085/1) and had a diameter of circa 17 μm and a calculated cell volume of 2651 μm^3.

Seawater collected from the BAS supply was autoclaved in cotton-wool stoppered, five-litre conical glass flasks at 121 °C for 20 minutes and allowed to cool to room temperature (15 °C). Guillard's F2 medium for marine algae (Appendix 2) was pre-filtered through a 0.2 μm Millipore filter and 2.3 ml added to the autoclaved seawater with a 50 ml innoculum of the required algal culture. The algae were then grown under conditions of constant light (~ 25 $\mu mol\ m^{-2}\ s^{-1}$) and temperature (15°C) and harvested during the log growth phase, ~ 1-2 weeks after inoculation. All the above manipulations were carried out under a laminar flow hood to reduce the risk of bacterial or fungal infection.

2.4.2 Labelling of algae with ^{14}C bicarbonate.

Algae were cultured as described previously. Radiolabelling of cultures was performed by adding 37 MBq of $NaH^{14}CO_3$ to a 1 litre culture flask approximately 16 hours before the culture was used in a feeding experiment. This period was chosen on the basis that the algal cellular carbon pool would equilibrate with the label so that artifacts due to apparent changes in uptake rates of label during the course of the experiment would not affect the results (Peterson, 1980; Dring and Jewson, 1982).

2.5 GRAZING EXPERIMENTS

2.5.1 Grazing experiments conducted on board ship.

All shipboard experiments were carried out in a converted chest freezer at 2°C

(±1°C) situated in the hold. Between one and six krill (44-49 mm, see 2.5.2) were placed into experimental feeding chambers containing between 5 and 20 litres of unfiltered seawater (Table 6.2). To determine cell density, a 200 ml aliquot of the same water was stored in an amber glass bottle and preserved using 2 ml of Lugol's solution. Animals were allowed to graze undisturbed in constant darkness for 12-24 hours. When experiments were terminated, the animals were removed, faecal pellets were collected and between one and five litres of water were filtered onto an ashed GF/F for future lipid analysis. A further 200 ml of water from the grazing chamber was preserved in Lugol's solution. On return to the UK these Lugol's-preserved samples were counted by inverted microscopy. In one grazing experiment (Graze 3), filtration rate was also monitored using a Coulter Counter with tubes of orifice size 70 µm and 140 µm.

Filtration rates were calculated using the equation of Frost (1972)

$$F = V (\log_e C_i - \log_e C_t) / t$$

where;

F = apparent filtration rate ($l\ h^{-1}$)

V = volume of chamber (l)

C_i = particle concentration of control at termination of experiment

C_t = final particle concentration of experimental chamber

t = time interval (hours)

2.5.2 ^{14}C grazing experiment protocol.

Fifteen immature krill (44-48 mm from anterior edge eye to tip of telson) were initially fed with non-labelled *Thalassiosira weissflogeii* or *Isochrysis tahitiana* (4,000 and 20,000 cells ml^{-1} respectively) for 2 hours in a dimly lit cool room at 2°C. The animals, which were feeding actively and with full hind guts, were then divided into 3 groups of 5 and each group gently transferred to 7 litres of GF/F filtered seawater

containing the same density of [^{14}C] algae previously labelled by incubation for 16 hours in seawater containing 37 MBq of $NaH^{14}CO_3$. At 0, 4 and 12 hours for *Isochrysis* and 0, 5 and 12 hours for *Thalassiosira*, 100 ml samples of sea water were removed from the feeding chambers and preserved in Lugol's solution for subsequent determination of cell density using a haemocytometer. Similarly, at 0, 4 and 12 hours for *Isochrysis* and 0, 5 and 12 hours for *Thalassiosira*, 1 litre samples of sea water were filtered on to ashed glass fibre filters which were stored in chloroform:methanol (2:1 vol/vol) at -20°C until subsequent analysis of lipid and chlorophyll *a* and/or radioassay.

For the first four or five hours (*Isochrysis* and *Thalassiosira* respectively), faecal pellets were carefully pipetted from the three feeding chambers at three minute intervals, filtered on preweighed 100 µm nylon mesh and gently washed with distilled water to remove both adhering algal cells and inorganic bicarbonate. Excess water was removed under light vacuum. To obtain faecal pellet dry weight, filters containing the faecal pellets were first fumed over concentrated hydrochloric acid to remove inorganic bicarbonate, then dessicated over silica gel in preweighed scintillation vials for 16 hours. The animals were left to feed undisturbed for a further eight and seven hours for *Isochrysis* and *Thalassiosira* respectively, a total of 12 hours for both. At this stage, all the remaining faecal pellets from each of the 3 chambers were removed and stored as above except the faecal pellets were back-flushed from the 100 µm nylon mesh onto an ashed GF/C filter and stored in chloroform : methanol 2:1 (v/v) at -40°C. Finally, the 15 krill were removed from each of the 2 experiments, briefly washed in distilled water, blotted dry, weighed and stored in glass vials under nitrogen at -40°C until subsequent analysis.

2.6 Lipid analysis.

2.6.1 Extraction of lipid from particulates.

Total lipid was extracted using chloroform : methanol (2:1 v/v) following Folch et al. (1957). The method was applied directly to glass fibre filters by homogenising the entire sample in chloroform : methanol. The sample was left in the freezer at -20°C for 2 hours to allow complete pigment and lipid extraction. The sample was then

filtered through a N° 1 Whatman paper filter prewashed in chloroform : methanol. A one ml aliquot of the supernatant was removed to determine chlorophyll *a* and phaeopigment concentrations (section 2.6.5.1). To the remainder of the sample (now 20 ml) 5 ml of 0.88% KCl was added. The sample was shaken vigorously, centrifuged and the top, aqueous layer discarded. The organic layer was filtered through a chloroform : methanol prewashed N° 1 Whatman paper filter into a preweighed sample tube and the solvent evaporated under nitrogen. The lipid extract was then dessicated in a vacuum over NaOH pellets for at least one hour, weighed and redissolved to a concentration of 10 mg ml^{-1}. The samples were stored under nitrogen in the freezer at -20°C. Total lipid extracts were then purified and stored as above. After lipid class analysis (section 2.6) 0.01% butylated hydroxytoluene (BHT) was added to the samples as an antioxidant.

Nylon filters containing microplankton (stored in 7 ml methanol) were first homogenised only in methanol to avoid leaching plasticisers from the mesh. The nylon mesh was then removed by filtration through a prewashed (chloroform : methanol) Whatman N° 1 paper filter. Seven ml of ice-cold chloroform were added to the filtrate and the mixture thoroughly re-homogenised. The homogeniser was washed with a further 7 ml of ice-cold chloroform and this was added to the original sample (now 21 ml chloroform : methanol (2:1 v/v)). One quarter volume of 0.88% KCl was added to the sample which was then treated as before.

2.6.2 Extraction of lipid from krill.

Animals for analysis were first categorised as adult males, adult females or immatures. They were then measured to the nearest mm (tip of telson to anterior edge of eye) and weighed. The hepatopancreas and stomach was dissected out by first making an incision along the dorsal axis of the thorax and the hepatopancreas and stomach removed with a spatula. The hind gut was not dissected out and was included with the body lipid. Together, the hepatopancreas and stomach were homogenised in 4 ml of ice-cold chloroform : methanol (2:1 v/v) and the homogenate transferred to a test tube on ice. The homogeniser was washed with a further 4 ml of chloroform : methanol

and this was added to the original sample. The homogenate was left in the freezer under nitrogen at -20°C for 2 hours to allow complete pigment and lipid extraction. The homogenate was then passed through a chloroform : methanol prewashed N° 1 Whatman paper filter and the filter washed with a further 2 ml of chloroform : methanol making a total of 10 ml of supernatant. The remainder of the krill body was homogenised in 10 ml of ice-cold chloroform : methanol and the homogenate transferred to a test tube on ice. The homogeniser was washed with a further 4 ml of chloroform : methanol and this was added to the original sample and placed in the freezer as above except that the filter was washed with 4 ml of chloroform : methanol making a total of 18 ml. Both the hepatopancreas and body supernatant were washed with 1/4 volume of 0.88% KCl and treated as above.

2.6.3 Lipid class analysis.

High performance liquid chromatography plates (10 x 10 cm) were pre-developed in polar solvent (propane-2-ol / chloroform / methyl acetate / methanol / 0.25% potassium chloride, 5:5:5:2:1.8, v/v/v/v/v). The plates were dessicated under vacuum over NaOH pellets for at least 24 hours. Six 40 µg lipid samples were then applied along a baseline 10 mm from the lower edge of the plate at increments of 15 mm. The plates were initially developed in polar solvent (as above) to a point 45 mm above the baseline. The plates were again dessicated under vacuum for at least 30 minutes to remove all traces of both solvent and moisture. The second solvent system consisted of (hexane : diethyl ether : acetic acid, 90:10:1, v/v/v) and the plates were developed to 85 mm above the baseline.

2.6.4 Visualisation and identification of lipid classes.

2.6.4.1 Non-specific staining techniques:

1. Copper acetate.

Developed high performance liquid chromatography plates were first dessicated for 30 minutes to remove excess solvent, then sprayed to saturation with 3% copper acetate (w/v) in 8% phosphoric acid (v/v) (Fewster et al. 1967) followed by charring at 160°C for 15 minutes. The lipid classes, apparent as blue black spots, were quantified

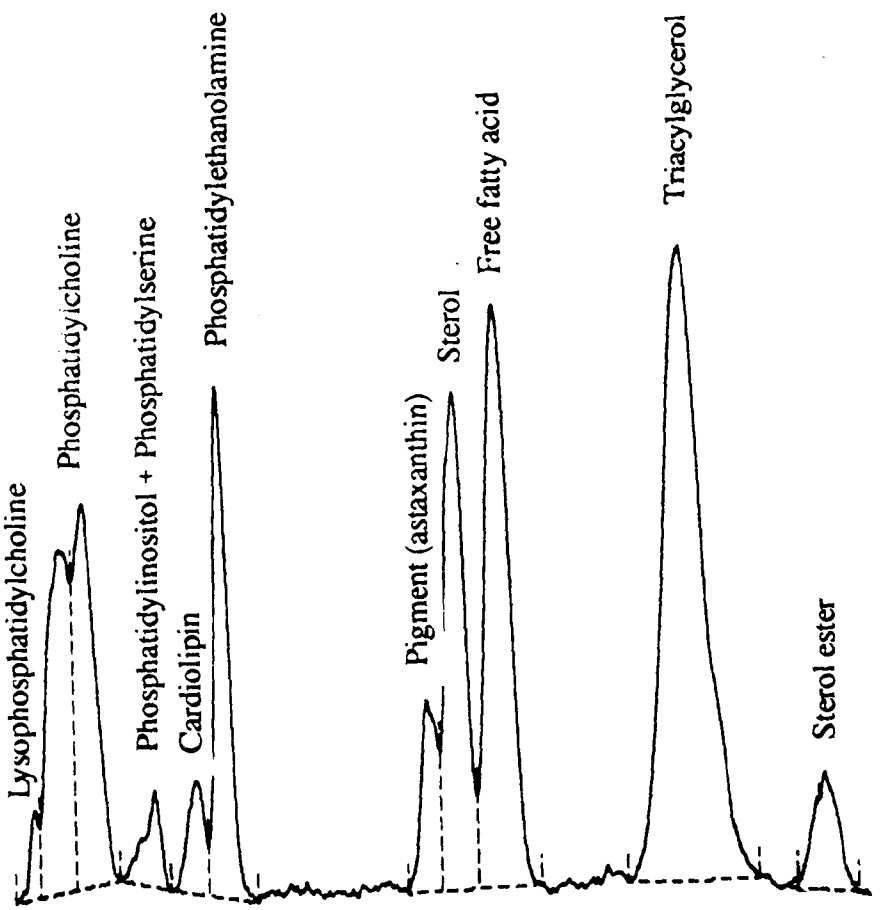


Fig 2.2 A representative scanning densitometry trace of krill lipid classes

using a Shimadzu scanning densitometer (Olsen and Henderson 1989).

2. 2,7- Dichlorofluorescein.

Developed high performance liquid chromatography plates were first dessicated for 30 minutes to remove excess solvents, then sprayed with 0.1% (w/v) 2', 7'-dichlorofluorescein in 95% methanol containing 0.05% (w/v) BHT. Plates were then examined under ultraviolet radiation, and lipids were visualised as yellow-green spots. This method is non-destructive and lipids stained in this way can be used for further analysis (Christie 1982).

2.6.4.2 Specific staining techniques:

1. Glycolipids.

Developed high performance liquid chromatography plates were lightly sprayed with 0.5% (w/v) 1-napthol in 50% methanol (v/v) and dessicated for 30 minutes. The plates were then sprayed with 95% H_2SO_4 and heated in an oven at 120°C. Glycolipids were visualised as blue-grey spots, sulphoquinovosyldiacylglycerol appeared as a pink-red spot whilst other classes appeared as faint yellow spots (Christie 1982).

2. Phosphatidylethanolamine and phosphatidylserine.

Developed high performance liquid chromatography plates were sprayed with 0.2% (w/v) ninhydrin in water-saturated butanol. Lipid classes containing amino groups were visualised as purple spots after heating at 120°C for ten minutes (Christie 1982).

2.6.5 Identification of pigments:

1. Chlorophyll *a* and phaeopigments.

An aliquot of particulate lipid extract was dissolved in 3 ml of 90% acetone. Chlorophyll *a* was then quantified fluorimetrically following Strickland and Parsons (1972) and using a purified chlorophyll *a* standard obtained from Sigma Chemicals,

Kent. Excitation and emission wavelengths were 428 and 665 nm respectively.

2. Carotenoid This pigment was tentatively identified as β Carotene by spectrophotometric comparison with a purified standard (Sigma Chemicals, Kent).

2.6.6 Fatty acid analysis.

For fatty acid analyses of microplanktonic lipids, the total lipid was first separated into neutral zones (comprising triacylglycerols, free fatty acids and sterol esters) and total polar zones by high performance thin layer chromatography. Zones corresponding to the polar and neutral lipids were scraped from the plates and, after the addition of an internal standard, (21:0 Sigma Chemicals, Kent), were transesterified overnight in a catalytic methylation reagent (2 ml 1% sulphuric acid in methanol) in nitrogen-purged, stoppered glass boiling tubes at 50°C. Methyl esters were isolated from the methylation reagent by two sequential 5 ml washes with hexane : diethyl ether (1:1 v/v) containing 0.01% BHT (w/v) followed by a final wash with 3 ml of 2% potassium carbonate (w/v). The fatty acid methyl esters (FAME's) were purified by high performance thin layer chromatography and eluted from the silica as above. Excess solvent was removed by evaporation under nitrogen. FAME's were dessicated under vacuum over NaOH pellets, weighed and redissolved in hexane containing 0.01% BHT (w/v) to a concentration of 10 mg ml^{-1}. The samples were stored in screw-topped vials under nitrogen at -20°C.

2.6.7 Identification of fatty acids.

FAME's were analysed using a Packard 436 capillary gas chromatograph (Packard Instruments Ltd) equipped with an open fused silica capillary column 50 m length x 0.32 mm i.d. and coated with CP Wax 51 liquid phase (Chrompack, Middelburg, Netherlands). The flame ionisation detector was operated at 250°C. Fatty acid methyl-esters dissolved in hexane (2 mg $ml^{-1)}$ were injected onto the capillary column via an on-column injector. The oven was programmed to hold temperature at 50°C for 0.1 minute post injection. Temperature then increased to 190°C at a rate of

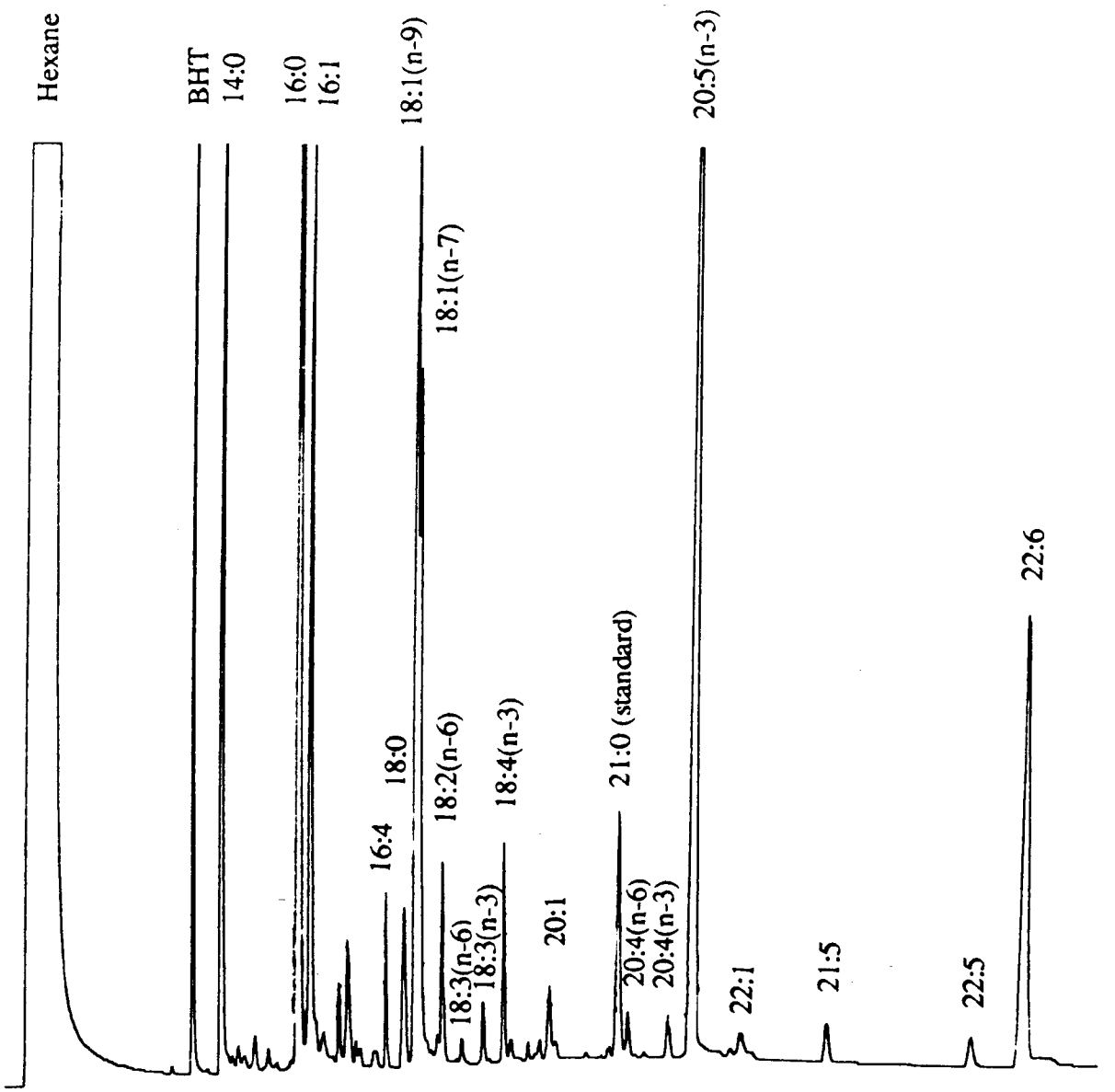


Fig 2.3 A representative gas chromatograph trace of krill fatty acids

39 °C min^{-1} and thereafter at 1.5°C min^{-1} to a final temperature of 225°C which was held for a further 18 min.

Fatty acids were identified by comparison of retention times against a GC-MS characterised marine fish oil standard (Marinol) and quantified by a Shimadzu C-R3A recording integrator coupled with the GC.

2.6.8 Hydrogenation of fatty acids.

Fifty microgrammes of the fatty acid methyl esters were dissolved in methanol (~1 ml) in a screw capped reacti-vial with a teflon septum. Adams' catalyst was added and the vial continuously stirred with a magnetic stirrer and purged with hydrogen for ~ 2 hours. The catalyst was then removed by filtration (Whatman N°1 paper filter), methanol removed by evaporation under nitrogen and the hydrogenated methyl ester redissolved in hexane.

2.7 Radioassay.

2.7.1 Radioactive determination of total lipid.

Lipids were extracted as described previously. Twenty μg of total lipid were placed in a 5 ml scintillation vial, solvent evaporated under nitrogen and the lipid redissolved in 2.5 ml of liquid scintillant (Ecoscint A, National Diagnostics, Manville, USA). Radioactivity was determined using a Packard Tricarb 2000CA liquid scintillation counter.

2.7.2 Radioactive determination of lipid classes.

Individual lipid classes were separated using double-development high performance liquid chromatography following Olsen and Henderson (1989), as detailed above, visualised by spraying with dichlorofluorescein, scraped into scintillation vials and counted as in section 2.7.1.

2.7.3 Radioactive determination of fatty acids.

After methylation of total lipid (section 2.6.6), individual fatty acids methyl esters (FAME) were separated and counted following the high resolution, argentation

TLC method of Wilson and Sargent (1992). Silica gel TLC plates (20 cm x 20 cm) were sprayed uniformly with 2 g silver nitrate dissolved in 20 ml of acetonitrile until saturated. The plates were air dried to remove excess solvent, then activated by heating at 110°C for 30 minutes. Plates were wrapped in aluminium foil and stored under *vacuo* until used. One hundred microgramme samples of FAME containing 20 000 - 100 000 DPM were applied to a silver nitrate-impregnated TLC plate over a narrow, 2 cm band using a Hamilton micro-syringe. Radioactive FAMEs standards, prepared from authentic radioactive fatty acids from Sigma Chemicals, Kent, were also applied to the plates, which were then developed with toluene : acetonitrile (97:3, v/v) to within one cm from the top of the plate. To visualise the individual FAME, the plates were subject to autoradiography at -70°C for 4-7 days using Konica A2 X-ray film. After the film was developed, radioactive FAMEs were located on the TLC plates and the corresponding silica removed for scintillation radioassay (section 2.7.1).

2.8 Data analysis.

All statistical analyses of the data were conducted using the Minitab statistical package (Ryan et al. 1988).

2.8.1 Particulates.

Principal components were generated from a correlation matrix of the percentage contributions of lipid classes and percentage contributions of species to total cell volumes at each site. Ordinations of the sites on the first three principal components were then produced. Relations between particulate lipid class and species ordination were examined by simple correlation analysis between pairs of species and lipid class principal components.

2.8.2 Krill.

Principal components were generated from a correlation matrix of the percentage contributions of lipid classes or fatty acids at each site. Ordinations on the first two principal components were then produced.

3. MICROPLANKTON: SPECIES ABUNDANCE AND LIPID COMPOSITION.

3.1 Introduction.

Euphausia superba appears to lack specific metabolic specialisations that could account for its abundance in the Southern Ocean (Quetin et al. 1993), suggesting that krill production and particularly its reproductive effort in summer could be determined largely by the quantity and quality of its food supply (Clarke and Morris 1983; Ross and Quetin 1986). However, due to obvious practical difficulties krill nutrition remains largely unstudied. The lipid content and composition of microplankton were investigated at various sites in the Southern Ocean as a component of this study of the lipid budget of Antarctic krill.

3.2 RESULTS

3.2.1 Species Composition of microplankton.

High resolution lipid analyses were conducted on size-fractionated microplankton of determined species composition from five South Georgia (SG1, SG3-SG5, SG8) and two Antarctic Peninsula sites (AP1 and AP2, Fig 2.1, Tables 3.1-3.6). A further eleven samples were analysed from the Antarctic Peninsula region to provide more information on absolute fatty acid levels (AP3-AP13, Fig 2.1, Table 3.7). For a more realistic measure of particulate biomass at the different sites than that provided by cell number, mean cell sizes and mean chain lengths were used to calculate total cell volumes for each particulate category (Table 3.2; details of the calculations are shown in Appendix 1).

Sites SG1, SG3, SG5, SG8 and AP1 were dominated by a range of diatoms of which *Coscinodiscus, Nitzschia* and *Rhizosolenia* occasionally reached concentrations of greater than 10 000 cells l^{-1} (Table 3.1). Colonies of the haptophyte *Phaeocystis* (~100 μm diameter) were present in significant quantities at SG1, SG3 and SG5 but contributed considerably less to the biomass than diatoms at these sites. Dinoflagellates, and to a lesser extent ciliates, were present at all of the sites, but again in much lower numbers than diatoms. However, at site AP2 dinoflagellates were the dominant organisms due to the relative paucity of diatoms (Table 3.1).

Table 3.1 Species contribution at the seven sites (numbers expressed as cell volumes, µl l^{-1}).

	Site						
	SG1	**SG3**	**SG4**	**SG5**	**SG8**	**AP1**	**AP2**
Pleurosigma	0.04	0.11	0.53	0.41	0.02	0.11	0.02
Nitzschia	4.50	8.00	123.05	41.50	1.50	36.00	9.50
Chaetoceros	21.76	0.39	8.10	4.78	17.88	16.99	4.40
Chaetoceros socialis	1.55	-	-	-	2.58	-	-
Chaetoceros (large cell)	7.56	0.27	1.50	12.85	12.75	-	5.25
Corethron	53.75	299.73	55.39	102.62	55.45	3.34	-
Eucampia	5.88	-	157.23	16.16	1.49	56.33	-
Odontella	-	-	-	-	-	97.97	-
Coscinodiscus >30 µm	-	65.45	14.55	36.36	-	269.08	7.27
" " <29 µm	-	95.05	2.36	17.73	-	59.11	3.78
Thalassiosira	6.05	1.18	0.95	16.32	14.54	0.95	0.95
Thalassiothrix	2.85	29.90	11.31	32.04	1.88	1.88	-
Rhizosolenia	43.56	5.94	3.96	39.60	17.82	1706.80	11.88
Dactyliosolen	-	7.83	13.42	5.59	2.24	4.47	3.35
Phaeocystis (colonies 100 µm)	1.87	6.38	-	5.70	0.34	-	-
Silicoflagellates	1.35	1.89	2.16	1.08	4.87	1.35	-
Dinoflagellates >30 µm	24.75	9.00	16.50	45.00	50.25	15.00	85.50
" " <29 µm	0.68	1.67	6.92	13.47	0.68	4.25	4.75
Ciliates >30 µm	13.50	-	7.50	17.25	18.00	4.50	15.00
" " <29 µm	0.32	-	2.08	4.66	0.45	0.45	0.90
Total cell volumes µl l^{-1}	189.97	532.79	427.51	413.51	202.74	2278.58	152.55
Total cell numbers l^{-1}	18150	43600	39050	55000	20300	82150	17200
Chlorophyll *a* µg l^{-1}	1.24	2.45	1.69	0.63	0.26	2.37	0.09

Table 3.2 Summary of the key variables contributing to the first four principal components from multivariate species and lipid class analyses.

		Loading	Variable	Proportion of variance (%)	Cumulative variance (%)
Species					
	PC1	+	*Chaetoceros* sp, *Chaetoceros socialis, Corethron, Thalassiosira*, ciliates > 30 µm	32.7	32.7
		-	*Thalassiothrix, Pleurosigma, Nitzschia, Coscinodiscus*		
	PC2	+	*Thalassiothrix, Phaeocystis*	27.5	60.2
		-	*Nitzschia*		
	PC3	+	*Rhizosolenia, Odontella*	18.3	78.5
		-	*Corethron, Thalssiothrix*		
	PC4	+	*Eucampia, Rhizosolenia, Odontella*	11.3	89.8
		-	Dinoflagellates > 30 µm		
Lipid Class					
	PC1	+	None.	52.3	52.3
		-	All 13 lipid classes		
	PC2	+	PS + PI, PG, SQDG.	25.3	77.6
		-	FFA, TAG, SE.		
	PC3	+	LPC, PC, PE	13.4	91.0
		-	PG , SQDG, unknown.		
	PC4	+	LPC, unknown, MGDG.	5.4	96.5
		-	PS + PI, pigment + S		

Abbreviations
PS, Phosphatidylserine; PI, Phosphatidylinositol; PG, Phosphatidylglycerol; SQDG, Sulphoquinovosyldiacylglycerol; FFA, Free Fatty Acids; TAG, Triacylglycerol; SE, Sterol ester; LPC, Lysophosphatidylcholine; PC, Phosphatidlycholine; PE, Phosphatidylethanolamine; MGDG, Monogalactosyldiacylglycerol; S, Sterol.

Table 3.3 Correlation analysis of the scores of the first four principal components of a species and lipid class, principal component analysis. Significant values of the product moment correlation coefficient (r) are shown in bold ($p < 0.05$).

		SPECIES			
		PC1	**PC2**	**PC3**	**PC4**
LIPID	**PC1**	-0.128	0.059	**0.755**	0.223
	PC2	-0.395	0.444	-0.049	**0.727**
	PC3	**0.572**	-0.142	-0.298	0.503
	PC4	-0.427	-0.072	**-0.564**	0.070

Table 3.4 Levels of fatty acids in lipids of size fractionated particulates.

Site	Size (μm)	Polar Lipid	Neutral Lipid ($\mu g\ l^{-1}$)	Total Lipid
SG1	<20	83.4	23.1	106.5
	20-200	20.5	30.0	50.5
	>200	17.9	20.5	38.4
	total	121.8	73.6	195.4
SG3	<20	71.1	22.7	93.8
	20-200	18.6	19.4	38.0
	>200	14.0	42.0	56.0
	total	103.7	84.1	187.8
SG4	<20	2.8	11.6	14.4
	20-200	2.3	6.1	8.4
	>200	7.7	6.9	14.6
	total	12.8	24.6	37.4
SG5	<20	27.4	13.2	40.6
	20-200	22.9	23.7	46.6
	>200	17.0	29.0	46.0
	total	67.3	55.9	133.2
SG8	<200	13.6	26.5	40.1
	>200	5.0	13.1	18.1
	total	18.6	39.6	58.2
AP1	total	35.3	37.2	72.4
AP2	total	49.6	12.2	61.8

Table 3.5 Percentage composition of the predominant fatty acids in the polar and neutral lipids in size fractionated particulates from SG5.

	< 20 μm		20-200 μm		> 200 μm	
Fatty acid	Polar	Neutral	Polar	Neutral	Polar	Neutral
16:0	11.0	19.6	10.2	20.9	8.2	20.0
16:1	6.9	12.2	8.6	11.3	10.7	12.1
16:4	2.3	2.3	8.2	0.7	9.8	1.8
18:0	3.6	7.6	0.6	7.9	2.3	4.0
18:1(n-9)	3.3	12.7	3.3	10.5	3.3	13.8
18:1(n-7)	2.6	1.7	3.2	1.5	1.2	0.8
20:4(n-6)	0.3	-	-	-	0.3	0.3
20:5(n-3)	14.7	7.6	34.1	2.6	36.6	6.4
22:6(n-3)	10.5	1.2	6.5	1.1	7.5	1.9
Saturates	21.9	36.8	16.4	39.6	15.3	34.1
Monounsaturates	16.4	27.9	16.4	25.9	16.6	28.2
PUFA	38.9	19.2	55.9	12.8	64.0	16.9

Table 3.6 Percentage composition of the predominant fatty acids in polar and neutral lipids in total particulates from AP1 and AP2.

	AP1		AP2	
	Polar	Neutral	Polar	Neutral
16:0	7.4	15.7	7.3	24.0
16:1	9.4	12.8	2.2	14.5
16:4	9.3	1.0	0.0	0.0
18:0	1.9	5.4	7.3	6.2
18:1(n-9)	3.7	7.8	10.8	16.4
18:1(n-7)	2.1	4.3	10.9	1.4
18:2(n-6)	1.4	5.0	3.3	2.3
20:4(n-6)	0.4	2.3	1.0	0.1
20:5(n-3)	31.9	6.8	11.9	1.0
22:6(n-3)	3.7	1.8	16.1	0.5
Saturates	17.0	31.5	25.6	45.2
Monounsaturates	17.6	29.7	27.1	33.8
PUFA	52.9	27.1	37.1	8.0

Table 3.7 Percentage composition of lipid classes and fatty acids in particulates from the Antarctic Peninsula region.

	AP3	AP4	AP5	AP6	AP7	AP8	AP9	AP10	AP11	AP12	AP13
Date	26.2.91	28.2.91	1.3.91	2.3.91	3.3.91	4.3.91	4.3.91	5.3.91	5.3.91	10.3.91	11.3.91
Position Lat S	62.05	62.52	64.56	65.14	64.40	63.02	62.41	61.18	60.42	54.01	54.02
Long W	57.25	61.45	63.18	64.15	62.57	59.25	58.13	51.57	45.34	38.02	36.41
Polar lipid(1)	9.8	12.4	12.0	15.1	13.3	10.0	12.3	12.9	14.4	9.0	9.4
Glycolipid(1)	25.5	31.0	24.8	20.5	22.8	30.8	24.2	24.7	24.5	26.6	28.4
Neutral lipid(1)	64.7	56.6	63.2	64.4	63.9	59.2	53.5	63.2	61.1	64.4	62.2
16:0(2)	24.3	23.3	22.8	30.1	19.2	17.5	16.4	21.0	16.2	21.8	23.4
16:1	14.8	18.8	12.4	12.5	14.4	13.1	12.2	13.3	12.5	14.7	16.4
16:4	0.4	0.0	0.0	0.1	1.2	2.5	2.3	0.8	1.5	1.0	0.6
18:0	6.6	5.4	6.4	0.1	4.9	5.1	4.7	5.7	3.9	6.1	6.7
18:1(n-9)	8.4	9.3	12.9	6.1	8.4	5.8	6.5	7.2	4.5	10.8	8.2
18:1(n-7)	4.6	3.1	2.2	9.7	2.0	2.9	1.4	3.4	4.8	4.0	5.7
18:4(n-3)	0.7	0.3	0.8	2.5	2.1	1.8	1.7	2.2	4.6	0.3	2.2
20:4(n-6)	0.2	0.0	0.3	0.1	0.2	0.7	0.3	0.0	0.4	0.9	0.0
20:5(n-3)	6.1	2.6	4.6	4.7	7.0	21.9	19.9	6.3	12.9	5.8	3.6
22:6(n-3)	3.3	2.1	4.6	5.0	6.2	6.9	6.7	5.8	6.6	4.0	3.7
Sat(2)	42.5	40.0	39.8	39.3	36.8	29.0	29.6	41.8	35.2	35.2	40.0
Mono(2)	30.9	34.3	30.9	30.3	29.9	27.2	24.8	29.5	22.8	32.7	34.7
PUFA(2)	17.2	12.3	18.3	20.4	22.9	38.9	35.1	21.5	33.6	17.8	17.1
Total fatty acid μg l^{-1}	74.5	58.8	45.7	41.5	58.2	37.5	31.5	53.6	44.8	41.9	58.9
Chlorophyll *a* ng l^{-1}	0.94	1.12	0.89	1.56	0.57	0.78	1.09	0.19	0.89	0.45	0.23

(1) % total lipid, (2) % fatty acid

Table 3.8 Correlation analysis of AP3-AP13 particulate variables. Significant values of the product moment correlation coefficient (r) are shown in bold.

	Total fatty acid	Chlorophyll *a*	Saturated fatty acid	Monounsaturated fatty acid
Chlorophyll *a*	-0.249			
Saturated fatty acid	**0.769**	-0.126		
Monounsaturated fatty acid	**0.525**	-0.239	**0.605**	
Polyunsaturated fatty acid	**-0.644**	0.103	**-0.844**	**-0.877**

Variation in species composition at SG1, SG3-SG5, SG8, AP1 and AP2 was examined using Principal Components Analysis (PCA). Insufficient data were available for AP3-AP13 to include these sites in this multivariate analysis. The first four axes of the PCA accounted for 90% of the total variation and key taxa contributing to these axes are presented in Table 3.2. Although diatoms, predictably, have a major impact on the composition of these axes, *Phaeocystis* is a key taxon on PC2 and dinoflagellates dominate PC4. It is noteworthy that an ordination of the sites using the first three axes does not segregate the South Georgia sites from those close to the Antarctic Peninsula (Fig. 3.1).

3.2.2 Particulate lipid classes.

Lipid class analyses were performed using quantitative TLC on total lipids from particulates at the seven sites i.e. SG1, SG3-SG5, SG8, AP1 and AP2 as detailed above. Variation in the lipid class compositions was explored using PCA and this identified the key lipid classes determining the variability in the dataset obtained. A single outlier AP1 is identified in PC1 (Fig. 3.2) so that no particular class or classes dominate this component (Table 3.2). Similarly, PC2 segregates SG5 and PC3 sites AP2 and SG8. A summary ordination of the sites using the first three components contrasts with the species–based ordination in that the South Georgia sites form a discrete group separate from the Antarctic Peninsula sites (Figs. 3.1, 3.2).

3.2.3 Correlation analysis between species and lipid classes.

Relations between these two multivariate analyses can be explored by simple correlation analysis. High values of the product-moment correlation coefficient (r) between pairs of species- and lipid-principal components arise only where both axes identify the same outlier site (Table 3.3). These provide the opportunity to confirm links between particular species composition and lipid classes. Thus AP2, dominated by dinoflagellates, had high triacylglycerols (TAG), free fatty acids (FFA) and sterol esters (SE), and SG8, with a large proportion of the diatom genus *Chaetoceros*, had high phosphatidylcholine (PC) and phosphatidylethanolamine (PE). SG1, SG3 and SG4 were characterised by some diatom taxa, including *Corethron*, and were discriminated by high levels of sulphoquinovosyldiacylglycerols and

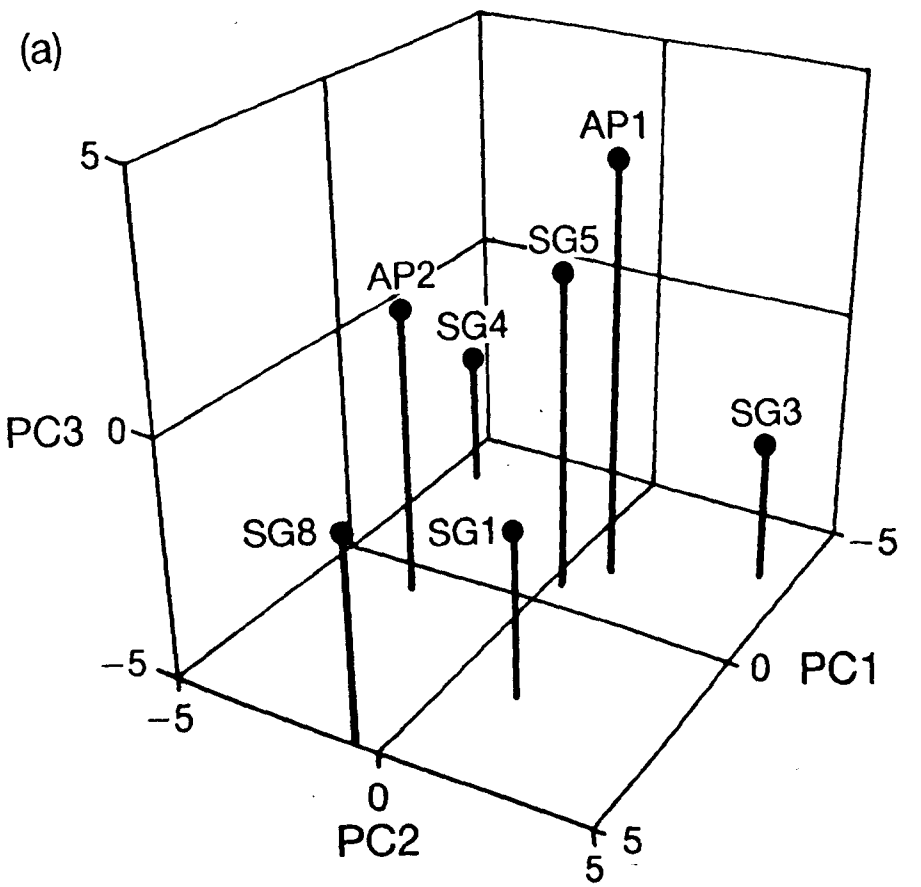


Fig 3.1 Ordination of the scores of variables derived from the percentage species compositions at SG1, SG3-SG5, SG8, AP1 and AP2.

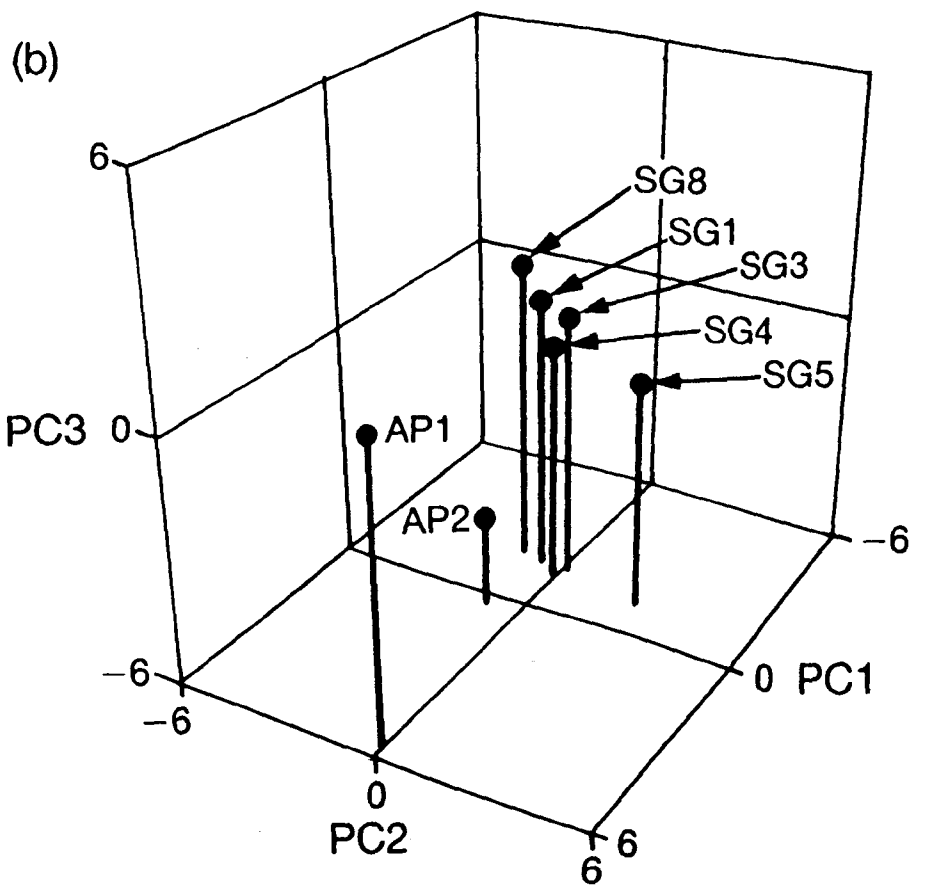


Fig 3.2 Ordinations of the scores of variables derived from the percentage lipid class compositions at SG1, SG3-SG5, SG8, AP1 and AP2.

monogalactosyldiacyl-glycerols (MGDAG). Of the lipid classes MGDAG, digalactosyldiacylglycerols (DGDAG), and sulphoquinovosyldiacylglycerols (SQDAG) are well known to be abundant in and characteristic of the thylakoid membranes of photosynthetic organisms. TAG are present in both photosynthetic and non-photosynthetic organisms alike but are known to accumulate in phytoplankton towards the end of the bloom.

3.2.4 Fatty Acid Analyses of Polar and Neutral Lipids in Particulates.

Fatty acid analyses were performed on the polar and neutral lipid fractions separated by TLC from total lipid extracted from size-fractionated particulates (> 200 μm, 20-200 μm and < 20 μm) from sites SG1, SG3, SG4, SG5 and SG8, and also on polar and neutral lipid fractions from total lipid in unfractionated particulates from sites AP1 and AP2. Since the fatty acid analyses were performed after the addition of an internal standard to the respective lipid fractions, the results can be expressed on an absolute basis of μg fatty acid in a given particulate fraction per litre of sea water (Table 3.4). Levels of total fatty acids in the microplankton from the seven sites ranged from 37 μg l^{-1} to 195 μg l^{-1} with a mean value of 107 μg l^{-1}. The ratio of polar lipid : neutral lipid varied from 4:1 at site AP2 to 1:2 at sites SG4 and SG8 (Table 3.4). Total lipid in microplankton at sites AP1 and AP2 contained substantial levels of triacylglycerols and this suggests that the organisms at these sites were in late bloom-senescence phase. In addition to differences between sites, the ratio of polar : neutral lipid varied markedly between size fractions at a given site (Table 3.4). For example, at SG4 the microplankton smaller than 20 μm was substantially richer in neutral lipid than the microplankton larger than 200 μm.

With the exception of the size fraction < 20 μm for SG5, the fatty acid compositions of all three size fractions and polar lipid and neutral lipid for SG1, SG3, SG4, SG5, SG8 and AP1 were essentially the same. Therefore, the salient features of the results are encompassed in Table 3.5, which describes only site SG5, and in Table 3.6, which contrasts sites AP1 and AP2. The neutral lipid of all three size fractions at SG5 contains small but significant amounts of polyunsaturated fatty acids, chiefly 20:5(n-3), but was dominated by the three fatty acids, 16:0, 16:1(n-7) and 18:1(n-9)

(Table 3.6). These data are consistent with the neutral lipid comprising primarily triacylglycerols. The polar lipid in all three size fractions at SG5 was dominated by (n-3) polyunsaturated fatty acids, consistent with the polar lipid being the dominant membrane glycolipid of algae (Table 3.5). The two larger size fractions contained polar lipid with 20:5(n-3) as its major polyunsaturated fatty acid and substantial levels of 16:4(n-3) (Table 3.5). This is a fatty acid profile characteristic of diatoms (Sargent et al. 1987), which dominated the microplankton at SG5 (Table 3.1). The smallest size fraction at SG5 (less than 20 μm) contained polar lipid with both 20:5(n-3) and 16:4(n-3) in its polar lipid but at much lower levels than in the two larger fractions. However, the smallest fraction contained 10% 22:6(n-3) in its polar lipid, substantially more than in the two larger fractions (Table 3.5). *Phaeocystis* was present at SG5 (Table 3.1, and 3.5) and is an organism capable of passing through a 20 μm filter as the colonies tend to disintegrate into individual cells on filtration. It is also known to be a potential source of 22:6(n-3) in marine ecosystems (Sargent et al. 1985; Nichols et al. 1991; Virtue et al. 1993).

Site AP1 (Table 3.6) generated very similar data to those for the two larger size fractions of SG5 (Table 3.5) and sites SG1, SG3, and SG4 (as previously stated in the text) for both polar and neutral lipids, being characterised by an abundance of 20:5(n-3) and 16:4(n-3) in polar lipid, consistent with the domination of this site by diatoms (Table 3.6). The neutral lipid of AP2 is not notably different from the neutral lipid of sites AP1 (Table 3.6) and SG5 (Table 3.5) (and also sites SG1, SG3 and SG4), but its polar lipid differs from all the other sites in having 22:6(n-3) as its major fatty acid. Dinoflagellates dominated AP1 and these organisms are well known to be rich in 22:6(n-3) (Sargent et al. 1987; Okuyama at al. 1993).

3.2.5. Lipid content and composition of particulates from the Antarctic Peninsula region.

Total lipid was analysed from unfractionated particulate samples from a further eleven sites, i.e. AP3-AP13 (Fig 2.1, Table 3.7). Lipid class compositions were very similar at all the sites. Neutral lipid, predominantly TAG, was the major lipid class in all cases, ranging from 53.5% at AP9 to 64.7% at AP3. Polar and glycolipid were consistently the minor components and their combined contribution to total lipid ranged

from 35.3% at AP3 to 43.4% at AP4. Fatty acid compositions of total lipid from the sites accord well with the lipid class data. Thus, the predominance of saturated and monounsaturated fatty acids is consistent with the high neutral lipid content of the particulates.

Total fatty acids at the 11 sites AP3-AP13 ranged from 31.5 to 74.5 $\mu g\ l^{-1}$ with a mean of 49.7 $\mu g\ l^{-1}$. Major fatty acids at all sites were 16:0, 16:1 and 18:1 (Table 3.7). Levels of PUFA ranged from 12.3% at AP4 to 38.9% at AP8 with an overall mean for the 11 sites of 23.2% (Table 3.7). AP8 and AP9 were notable in that they contained the highest levels of 20:5(n-3). The PUFA 22:6(n-3), although not a major component, was present in appreciable quantities (2.1-6.9%) at all sites.

Correlation analysis (Table 3.8) indicates that total fatty acid concentrations at the sites were positively correlated with both saturated and monounsaturated fatty acids, which is consistent with the observation that those sites contained the highest levels of these fatty acids. By contrast, fatty acid concentrations at the sites were negatively correlated with PUFA, which iindicates that those sites with the highest levels of fatty acids, contained the lowest levels of PUFA. The correlations between chlorophyll *a* and total, saturated or monounsaturated fatty acids were not significant (Table 3.8).

Table 3.8 Correlation analysis of AP3-AP13 particulate variables. Significant values of the product moment correlation coefficient (r) are shown in bold.

	Total fatty acid	Chlorophyll *a*	Saturated fatty acid	Monounsaturated fatty acid
Chlorophyll *a*	-0.249			
Saturated fatty acid	**0.769**	-0.126		
Monounsaturated fatty acid	**0.525**	-0.239	**0.605**	
Polyunsaturated fatty acid	**-0.644**	0.103	**-0.844**	**-0.877**

3.3 DISCUSSION.

3.3.1 The Nutritional Quality of Fatty Acids in Particulates.

It is clear that the microplankton lipid at all sites, and especially those from South Georgia was of high nutritional quality on the basis of the fatty acid compositions (Tables 3.4-3.7). It was consistently rich in (n-3) polyunsaturated fatty acids, particularly 20:5(n–3) and 22:6(n-3) which were primarily associated with polar lipid. Microplankton sampled in the vicinity of the Antarctic Peninsula generally contained lower levels of PUFA than those from South Georgia, and tended to be richer in saturated and monounsaturated fatty acids. As these samples were collected both later in the season and at higher latitudes (at low light and temperature), it is likely that the particulate communities in the Antarctic Peninsula region were in late bloom phase, when algae characteristically accumulate neutral lipid (Hodgson et al. 1991). Although the taxonomy of the Antarctic Peninsula microplankton samples was not investigated, inferences can be drawn from their fatty acid compositions. Levels of 20:5(n-3), a known bio-indicator for diatoms, were low at most Antarctic Peninsula sites possibly indicating that diatoms were scarce. However, at sites AP8 and AP9 levels of 20:5(n-3) were 21.9% and 19.9% respectively, suggesting that diatoms contributed significantly to the microplankton biomass. Levels of 22:6(n-3) were high relative to 20:5(n-3) (although both were low in absolute terms) and were probably associated with small flagellates and dinoflagellates thought to be characteristic of post bloom communities.

Taken as a whole, there appear to be ample (n-3) polyunsaturated fatty acids in the microplankton to sustain the growth of krill. This implies that there is no requirement for the animals to modify their dietary polyunsaturated fatty acids by anabolic processes required for chain elongation and further desaturation processes, e.g. in the conversion of 18:4(n-3) to 20:5(n-3) and finally to 22:6(n-3). The extent to which such processes occur in marine organisms is currently a matter of some debate and concerns the quantitative importance of the essential dietary fatty acids, particularly 22:6(n-3), for tissue growth. Given the abundance of both 20:5(n-3) and 22:6(n-3) in Southern Oceanic microplankton, it is difficult to conceive of circumstances when krill growth will be limited specifically by its dietary input of essential fatty acids. However, because 22:6(n-3) appears to be associated particularly with, in one instance

Phaeocystis (Table 3.5) and, in the other instance dinoflagellates (Table 3.6), the availability of 22:6(n-3) for krill may be dependent, first, on the availability of these algal species and second, on their being ingested and assimilated efficiently by krill. Nonetheless it can be concluded that the fatty acid composition of the microplankton analysed here would provide a good-quality diet for grazing zooplankton and hence no complex metabolic adaptations of krill to accommodate dietary inadequacies of fatty acid nutrition need be postulated.

3.3.2 Levels of Fatty Acids in Particulates.

The data in Tables 3.4-3.7 can be interpreted in terms of the quantity as well as the quality of lipid in Southern Oceanic phytoplankton available for the growth and reproduction of krill. First, it is clear from the data that the quantity and quality of lipid in Southern Ocean phytoplankton available for growth and reproduction of krill can vary quite widely. Part of this variation obviously stems from different densities of phytoplankton at the different sites. However, the stage of development of the phytoplankton will also be a factor in the variation since phytoplankton towards the end of the bloom can be enriched in triacylglycerols and, therefore, have elevated cellular lipid levels. This was especially true for the Antarctic Peninsula particulates (AP3-AP13 Table 3.7) which were sampled in late summer and had high neutral lipid contents (mean = 61.5%). Additional evidence for this is the marked variation in the ratios of polar to neutral lipid in particulates observed at SG8 (Table 3.4). It should be noted that the analytical method employed here involved the addition of an internal standard after separating total lipid into neutral and polar fractions. Therefore, although great care was taken to perform the lipid extractions as quantitatively as possible, the method feasibly underestimated the actual levels of fatty acids associated with microplanktonic lipid. Nonetheless, the values found in this study are not dissimilar from the mean value of 51 μg of total lipid per litre of seawater recorded in particulates at a range of stations in an area encompassed by 60-64°S and 30-50°E (Mayzaud et al. 1985). Similarly, values of 18-65 μg (mean 34 μg) of fatty acids per litre of seawater were recorded for microplankton in Lofoten waters in northern Norway (Klungsoeyr et al. 1989) and 81 μg of fatty acids per litre were recorded for a *Phaeocystis* bloom in the Irish Sea

(Claustre et al. 1990).

For the samples around South Georgia, a mean fatty acid concentration of 107 $\mu g\ l^{-1}$ in the microplankton particulates was associated with a mean particulate chlorophyll *a* concentration of 1.25 $\mu g\ l^{-1}$ (Table 3.1, 3.4). Similarly, the microplankton samples collected from the vicinity of the Antarctic Peninsula contained mean fatty acid concentrations of 49.7 $\mu g\ l^{-1}$ and mean chlorophyll a concentrations of 0.79 $\mu g\ l^{-1}$ (Table 3.7). The range of ratios of fatty acid : chlorophyll *a* and the overall mean obtained in the present study are as follows:

Smallest ratio (SG4)	**22:1**
Largest ratio (AP2)	**687:1**
Mean for all sites	**135:1**

Plausible previously published carbon : chlorophyll *a* ratios for healthy phytoplankton populations are in the order of 40:1-100:1 (Strickland 1960; Vollenweider 1969; Probyn and Painting 1985). Assuming that on a mass basis: a) total lipid comprises approximately 20% of total cell carbon and, b) fatty acids comprise 75% of total lipid, the published carbon : chlorophyll *a* ratios can be recalculated as fatty acid to chlorophyll ratios. The values of 6:1-15:1 so obtained differ greatly from the ratios presented in this study. However, as gas chromatography is considered a quantitatively accurate technique and both chlorophyll *a* and fatty acid levels determined in this study are not dissimilar from other published values (Mayzaud et al. 1985; Klungsoyr et al. 1989; Claustre et al. 1990) it is likely that previous studies of carbon to chlorophyll ratios have tended to underestimate the true carbon content of the microplankton as a whole.

Many previous studies in the field of plankton ecology have, after determining chlorophyll *a* concentrations, utilised literature values for carbon to chlorophyll a ratios and thence estimated `total particulate carbon' (Kato et al. 1982; Boyd et al. 1984; Ishii et al. 1985). Estimates and measures of organic carbon have also been used to estimate

assimilation efficiency. What is total organic carbon and what is its nutritional value? Field particulate samples often contain large quantities of detritus and it is likely that this detritus comprises largely organic carbon, e.g. cellulose and chitin. The validity of measurements and estimates of `total carbon' must be called into question, as it is unlikely to be an accurate indicator of either the energetic and or nutritional composition of microplankton.

In the present study, high fatty acid to chlorophyll *a* ratios indicate that heterotrophic microplankton and other organic material made a substantial contribution to the fatty acid pool at some of the sites sampled, and especially AP2 where dinoflagellates dominated. This finding accounts for the poor correlation between fatty acid levels and chlorophyll *a* (Table 3.8) and has important implications for the construction of energy budgets for zooplankton such as krill.

For a number of years, scientists working in the field of marine biology and especially zooplankton ecology, have used chlorophyll *a* as an indicator of productivity, `particulate biomass' and hence the availability of food to herbivorous zooplankton. On a global scale, the quantification of chlorophyll *a* concentrations in the upper layers of the ocean by satellite imagery has provided valuable information on the abundance and distribution of phytoplankton and hence insights into areas of high and low primary productivity. However, it has been clearly shown in this chapter that the concentration of chlorophyll *a* is a very poor indicator of both the abundance and nutritional quality of the microplankton (in terms of lipid at least). Thus, the validity of this approach, especially when applied on a much smaller scale, such as the trophic interactions within planktonic communities, has been seriously called into question. It has been shown in previous studies (Morris 1984; Quetin and Ross 1985) that the use of algal cultures in carefully controlled laboratory experiments, especially when monitored with Coulter Counters, is of great value and avoid the complications of feeding experiments utilising natural phytoplankton assemblages, such as microzooplankton grazing (Harbison and McAlister 1980). In circumstances when only the apparent filtration rates (volume of water swept per unit time) of zooplankton are being investigated, chlorophyll *a* can be a useful measure, although this still provides little information on the quantity and more importantly the nutritional quality of material consumed.

Misinterpretations arise when the high chlorophyll *a* concentrations which are

used in laboratory grazing experiments are applied directly to field situations (Kato et al. 1979; 1982 ; Price et al. 1988). Price et al. (1988) discussed the findings of Antezana (1982), Kato et al. (1982), and Boyd et al. (1984) who all monitored chlorophyll a levels in krill grazing experiments and suggested that krill feed maximally at chlorophyll *a* concentrations of 13 $\mu g\ l^{-1}$. They suggest that, as 13 $\mu g\ l^{-1}$ is rarely reached in the Southern Ocean (typical values are 0.6 $\mu g\ l^{-1}$ Holm-Hansen and Huntley, 1984; ~1 $\mu g\ l^{-1}$ the present study), therefore krill are rarely satiated in the natural environment. Log growth phase laboratory algal cultures tend to contain vigorous, actively growing cells, containing high levels of chlorophyll and hence exhibiting high levels of fluorescence. By contrast, in the Southern Ocean particulates are likely to comprise various proportions of detritus (which is readily ingested by krill), heterotrophs, which at times can dominate the particulates, and autotrophs which may be in various stages of development and senescence. To monitor laboratory grazing rates by chlorophyll *a* analysis, and to then apply these measurements directly to the field is likely to be of limited use in understanding the energetics of marine zooplankton and indeed at times may be misleading.

Therefore, the present results indicate that for some regions of the Southern Ocean and for some times of the year, when krill are feeding on particulates where phytoplankton do not represent the dominant fraction, assessment of food availability using chlorophyll *a* will provide a misleading picture of the food available to krill. Quantification of more important dietary components such as lipid, protein or carbohydrate, is clearly a better approach and, as has been established here, lipids and especially their constituent fatty acids offer a very practical and theoretically sound choice.

4. KRILL: LIPID CONTENT AND COMPOSITION

4.1 INTRODUCTION.

Studying the lipid content and composition of marine zooplankton can provide useful insights into life history strategies of the animals in relation to reproduction, overwintering and feeding behaviour (Hopkins et al. 1989; Falk-Petersen 1990). Lipids can also be used as indicators of food web dynamics and ecological niche partitioning (Sargent 1981a, b; 1988; Falk-Peterson et al. 1990). In addition, the lipid content of zooplankton is especially important when considering their nutritional value to higher predators (Clarke 1984). Zooplankters tend to segregate into two distinct categories according to their major lipid storage compounds and life history strategies. Some species, including *Euphausia superba* (Clarke 1980, 1984; Fricke 1984) and the Antarctic copepod *Calanus propinquus* (Schnack-Schiel et al. 1991; Hagen et al. 1993) tend to accumulate triacylglycerols as their primary energy reserve, although generally not in very large quantities. These triacylglycerol-storing zooplankters tend to accumulate relatively limited overwintering lipid stores which implies that they must remain relatively active and feed overwinter. Triacylglycerol-storing zooplankton also tend to spawn at intervals during the summer months and the spawning frequency is thought to be directly related to food availability that same summer (Clarke and Morris 1983; Ross and Quetin 1986). Other zooplankters accumulate considerable reserves of wax esters, e.g. up to 80% of dry body mass in *Calanus finmarchicus* (Falk-Peterson et al. 1990) and *Calanus acutus* (Schnack-Schiel et al. 1991; Hagen et al. 1993). These animals tend to overwinter in diapause, before mobilising lipid reserves for egg production and energy-intensive mating behaviour during late winter, enabling females to spawn immediately prior to the spring bloom (Hopkins et al. 1984; Smith 1990).

Numerous previous studies have investigated the fatty acid composition of *Euphausia superba,* although most of the early work was motivated by the need of commercial concerns to understand the nutritional quality of krill in fertilisers, animal feeds and human consumption (Nonaka and Koizumi 1964; Bottino 1974; Shibata 1983). More detailed, biologically motivated lipid analyses were conducted by Clarke (1980) and Fricke (1984). However, as yet no single comprehensive study has been conducted that investigates how lipid content and composition of krill varies with

season, sex and maturity, reproductive status, sampling region, feeding behaviour and food availability/quality.

The present investigation determines the lipid content and composition of male, female and immature krill from eight sites around South Georgia (Fig 2.1). The objective is to investigate the roles of lipid in krill metabolism in general and in reproduction in particular.

4.2 RESULTS.

4.2.1 Wet mass.

One hundred and sixty five individual krill from eight sites around South Georgia were analysed in the present study. There was an overall predominance of immature krill at all eight sites and a total of 76 animals of this category were analysed (Fig 4.1). Mature male and female krill were relatively scarce and only 48 and 41 of these animals were analysed respectively. Indeed, at SG7 and SG8 female krill appeared to be totally absent as none were found in the RMT8-net hauls, despite a deliberate search for representatives of all three sex and maturity classes.

Females were generally the largest animals of the three categories of krill at all sites, ranging from 0.94 g to 1.72 g wet mass (overall median value 1.46 g: Fig 4.2a). Male krill tended to be slightly smaller than the females ranging from 0.71 g to 1.68 g with a median wet mass of 1.15 g. Corresponding values for immatures were a median wet mass of 0.47 g and a range of 0.16 to 0.94 g. However, there was overlap in the ranges of body mass for the three categories, especially between males and females. At individual sites the ranking of sex and maturity classes by size was the same as that of the overall sample with the ranges of median fresh mass 1.30 - 1.68 g for females, 0.82 - 1.45 g for males and 0.37 - 0.52 g for immatures. The ranking of animal sizes was consistent for all sites, i.e. females were always larger than males which in turn were larger than immatures. There was a slight tendency for large animals of one category to co-occur with large animals of another (Kendall coefficient of rank concordance = 0.491, $P = 0.172$). However, this generalisation did not apply universally. Immatures at SG2 had high median mass but females and males were relatively small (Fig 4.2a). No obvious properties of animal size distinguished sites SG7 and SG8 which lacked

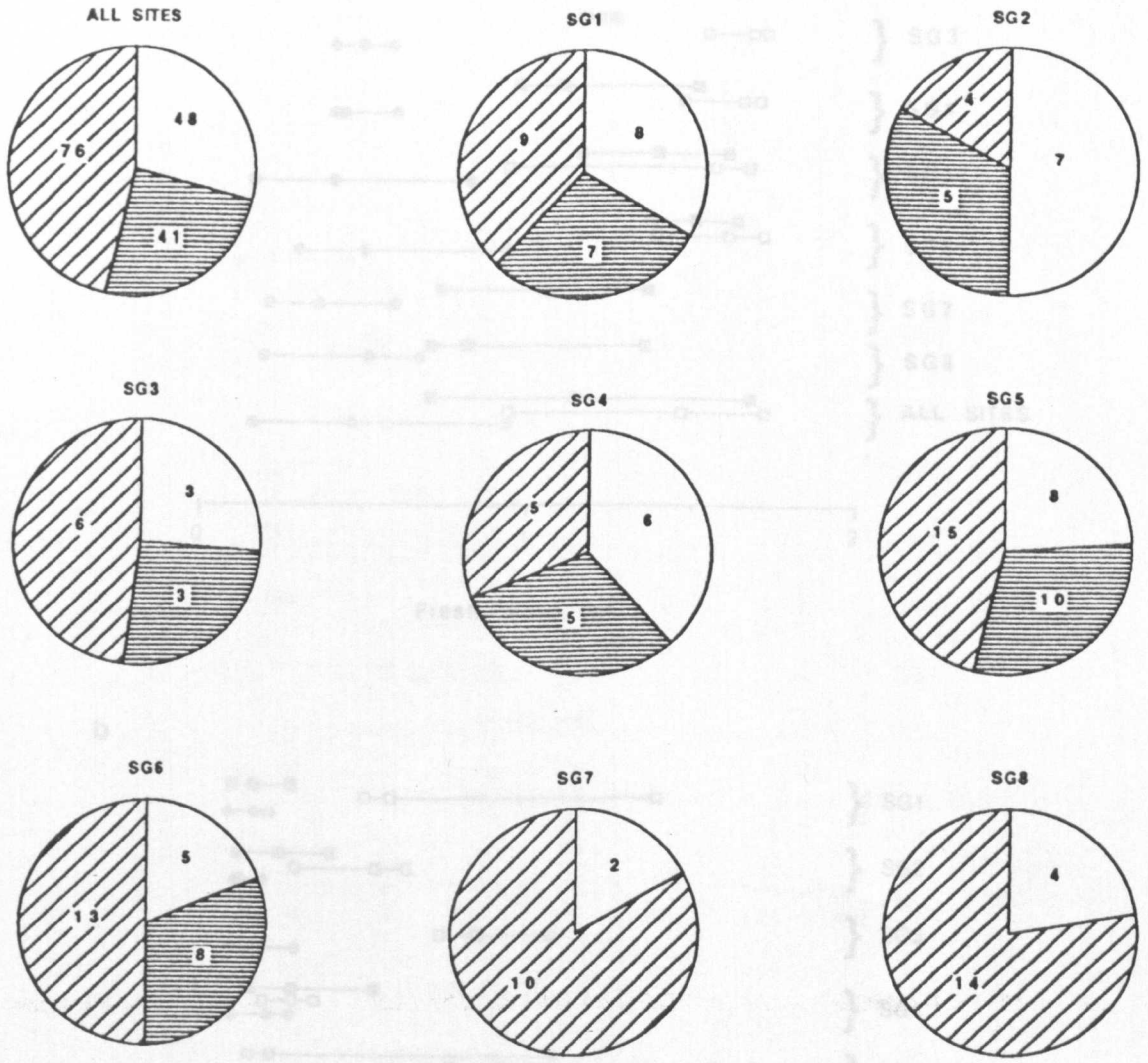
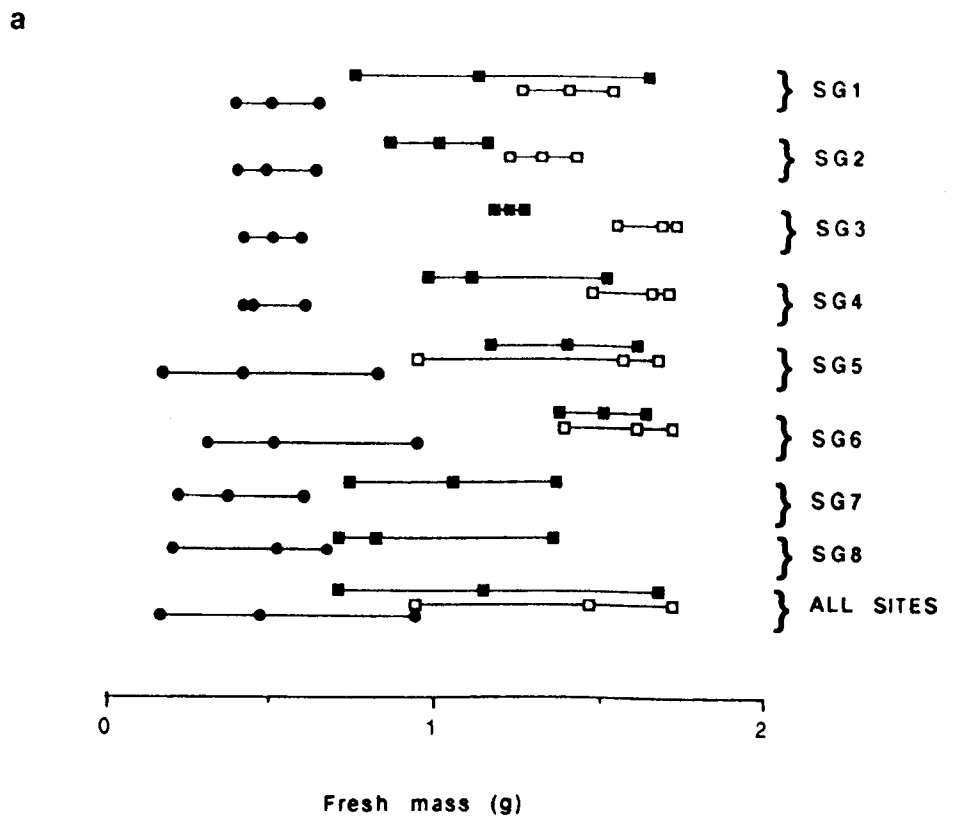


Fig 4.1 Proportions and numbers of male ☐, female ▤, and immature ▨ krill at the eight sites



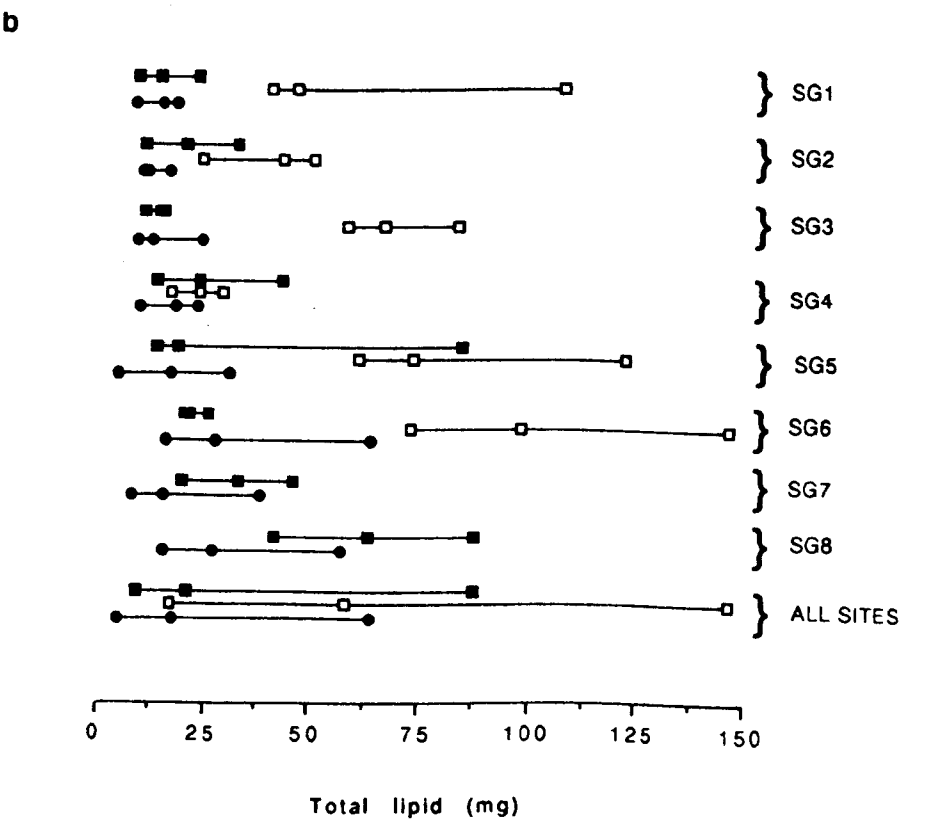
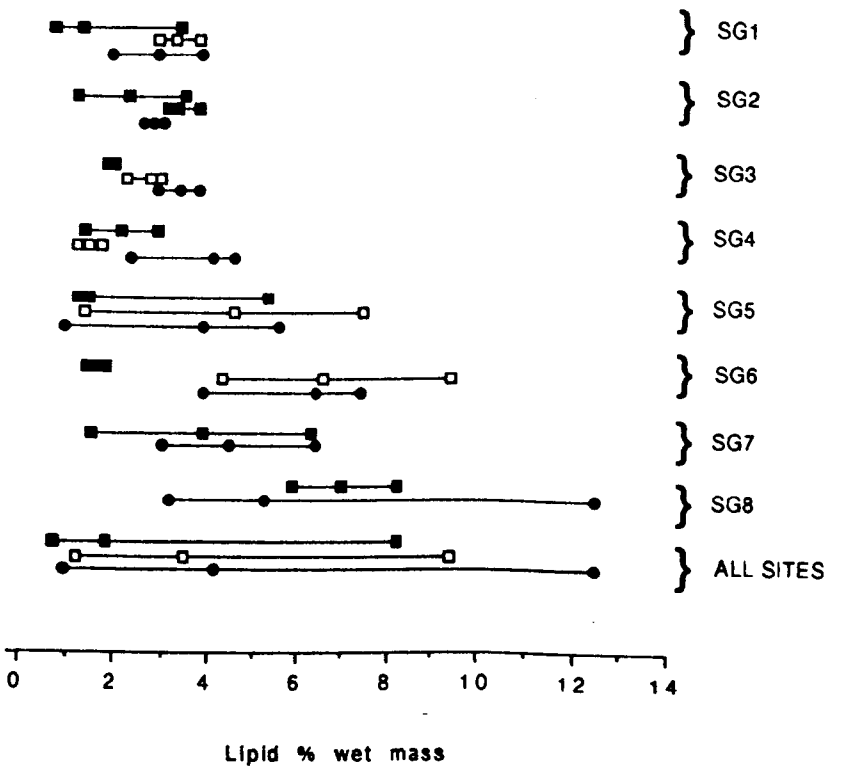
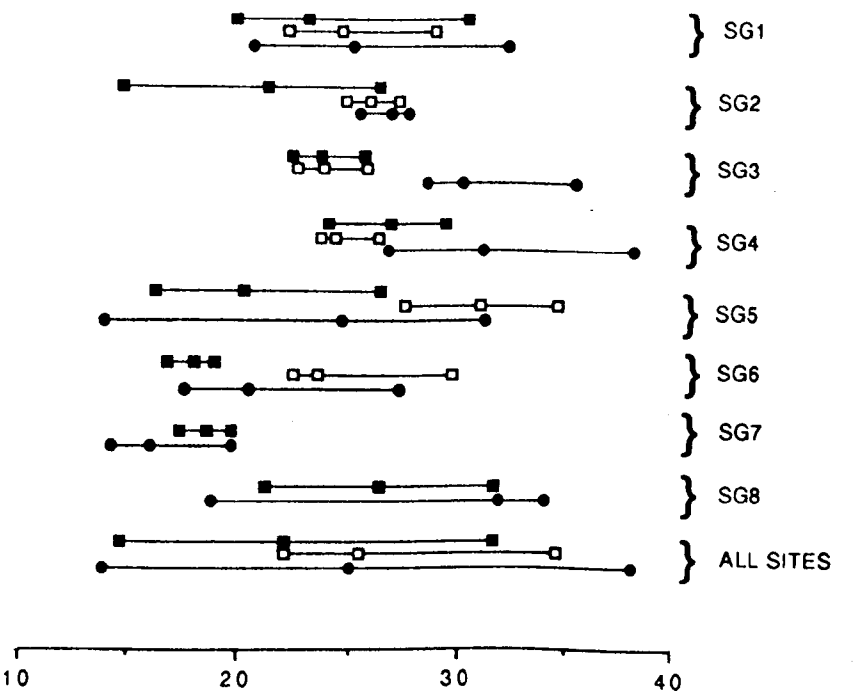


Fig 4.2 Medians and ranges of male (- - -■- -), female (·····□·····) and immature krill (—●—) for : **a** fresh mass, **b** total lipid, **c** lipid percentage fresh mass, **d** percentage PC in total lipid, **e** percentage TAG in total lipid.

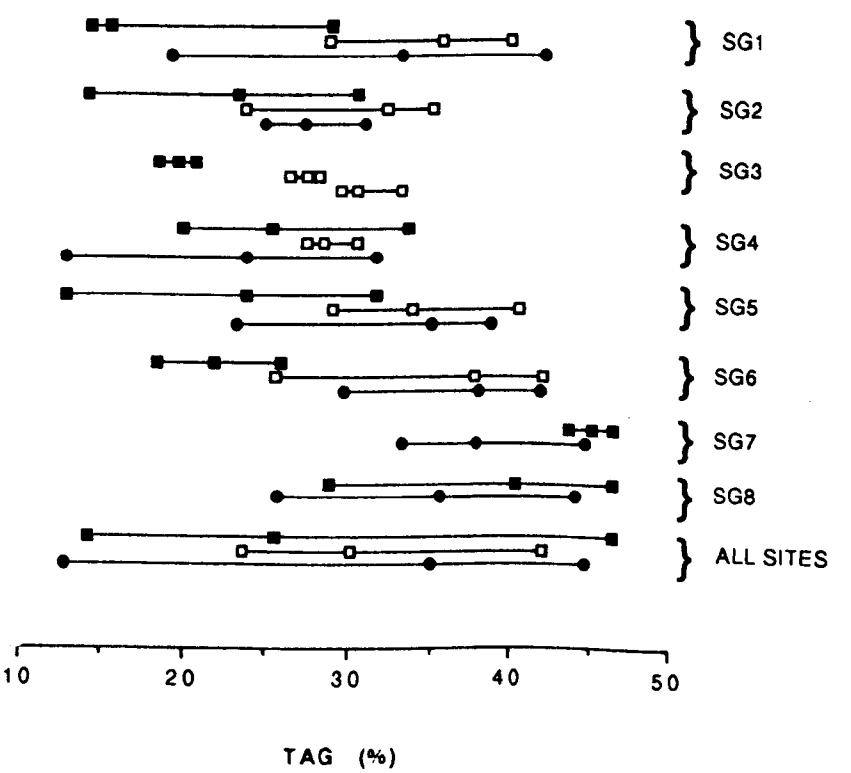
c


SG1
SG2
SG3
SG4
SG5
SG6
SG7
SG8
ALL SITES
0
2
4
6
8
10
12
14
Lipid % wet mass

d


SG1
SG2
SG3
SG4
SG5
SG6
SG7
SG8
ALL SITES
10
20
30
40
PC (%)

e


SG1
SG2
SG3
SG4
SG5
SG6
SG7
SG8
ALL SITES
10
20
30
40
50
TAG (%)

female krill. Median wet mass for immature krill was smallest of all sites at SG7 but largest at SG8 (Fig 4.2a).

4.2.2 Lipid content.

Results for the lipid content and composition of body tissue only are presented here since analysis of extracts from the gut fraction confirmed the conclusion of Bottino (1974) that the lipid composition of krill hepatopancreas and stomach did not differ significantly from that for body tissue. The hepatopancreas lipid comprised approximately 10% of the krills' overall lipid content and is included with body lipid in Figs 4.2b, c.

Females (present at SG1-SG6 only) consistently contained more total lipid (median 70 mg per individual) than both male (median 21.0 mg per individual) and immature krill (median 17.8 mg per individual) of SG1-SG6 (Fig 4.2b). However, the ranges of these three categories of krill overlapped, i.e. 17.3 - 146.9 mg for females, 9.3 - 87.7 mg for males and 5.1 - 64.2 mg for immatures. For individual sites the pattern of females containing more lipid than males, and males containing more lipid than immatures still held, although the range of median values for sites was rather more variable (23.9 - 98.7 mg for females, 14.6 - 63.6 mg for males and 11.7 - 27.7 mg for immatures).

The proportion of lipid per unit wet mass varied considerably both between sex and maturity classes, and between sites (Fig 4.2c). Overall, males contained the lowest proportion of lipid (median value of 1.81% of wet mass), immature animals the highest (4.16%) and females an intermediate value (3.48%). The variability of lipid content and wet mass meant that lipid proportion showed similar variation in the three sex and maturity classes (ranges of 0.72 - 8.20%, 1.18 - 9.40 % and 0.95 - 12.5% for males, females and immatures respectively). Individual sites did not all conform to the pattern found for the pooled sample with the median proportion of lipid for females exceeding that for immatures at four sites, that for males exceeding immatures at one site, and males exceeding females at one site.

4.2.3 Lipid composition.

Phosphatidylcholine (PC), the major membrane lipid, and triacylglycerols

(TAG), the predominant energy storage lipid, were the two major lipid classes found in krill at the eight sites (Fig 4.2 d, e). Percentage levels of PC tended to be less variable than TAG in all sex and maturity stages (median values for PC being 22.1%, 25.5% and 25.0 % for males, females and immatures respectively). The proportion of TAG in total lipid differed slightly from PC in that males had slightly lower median proportions and much greater variability between sites (overall median value 21.7%, range for sites 14.2-46.5%) than both females and immatures (overall medians 30.2 and 35.0%, and site ranges 23.6-42.0% and 22.8-44.4% respectively).

4.2.4 Multivariate analysis of lipid class composition.

The body lipid-class compositions of all 165 krill from the eight South Georgia sites were subjected to a multivariate analysis (Principal Component Analysis, PCA, Fig 4.3 a-d). The first four principal components accounted for 78.6% of the total variation within the data set (Table 4.1). Variables making a significant contribution to PC1 were TAG (positive loading) and phosphatidylserine plus phosphatidylinositol (PS+PI), cardiolipin (CL) and phosphatidylethanolamine (PE), all of which are involved in membrane structure and function. TAG, an energy storage lipid, was one of the two most abundant lipid classes in the krill, whereas the other compounds were more minor constituents. Proportion of phosphatidylcholine (PC) was contrasted with pigment (PIG) and free fatty acids (FFA) in PC2.

Ordination of all of the animals on principal components 1 and 2 produced no separation of sex-maturity classes (Fig 4.3a). Females, males and immature animals all showed significant scatter on PC1. Females tended to have more positive scores on PC1 (higher proportion of TAG) than males. PC2 tended to reflect variability amongst immature krill, and females consistently scored negative or near-zero on this axis.

There was no influence of animal size on lipid composition. Regression of the scores of the three sex and maturity classes on animal wet mass produced no significant relationship (Table 4.2).

Although the ordinations do not resolve individual sites, when the ordinations for the sex and maturity stages are separated, there is clearly a pattern in the scores of animals from different sites which is consistent for the three sex and maturity classes (Fig 4.3b-d). Sites SG1-SG4 tended to have negative scores on PC1, whilst sites SG7

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Table 4.1 Summary of the key variables contributing to the first four principal components from a multivariate, lipid class analysis of krill from eight sites around South Georgia.

PC	Loading	Variable	Proportion of variance (%)	Cumulative variance(%)
PC1	+	TAG.	35.8	35.8
	-	PS+PI, CL, PE.		
PC2	+	PIG, FFA.	20.2	56.0
	-	SM+PC.		
PC3	+	SE.	12.6	68.6
	-	S.		
PC4	+	LPC, SM+PC.	10.0	78.6
	-	S, SE.		

Abbreviations of lipid classes as used in text are: (1) Neutral lipids: TAG Triacylglycerol, FFA Free fatty acid, S Sterol ester: (2) Polar lipids: SM Sphingomyelin, PC Phosphatidylcholine, PE Phosphatidylethanolamine, CL Cardiolipin, PIG Pigment, (e.g. Astaxanthin, Carotenoids and Xanthophyll), PS Phosphatidylserine, PI Phosphatidlylinositol.

Table 4.2 Values of the coefficient of determination, r^2, for regression analyses of scores on principal components 1 and 2, versus body mass.

	Males	Females	Immatures
PC1	0.02	0.04	0.01
PC2	0.10	0.00	0.08

and SG8 were positive. SG5 and SG6 had intermediate scores. The positive scores for animals from sites SG7 and SG8 are especially noteworthy because females tended to have the most positive scores of the three categories, but this category was absent from these two sites. No further separation of sites was evident on PC2.

4.2.5 Fatty acid content of krill.

Overall, median fatty acid levels in male, female and immature krill from all eight South Georgia sites tended to reflect the levels of total lipid (Fig 4.2b) and accounted for between 69-75% of the total lipid mass. Females contained generally higher median levels of fatty acids (54.2 mg) than males (median value 14.9 mg) and males more than immature krill (median value 12.8 mg, Table 4.3). However, there was considerable overlap in the ranges of fatty acid levels between the sex and maturity stages (Table 4.3). It is noteworthy that the range of polyunsaturated fatty acid levels within the sex and maturity classes tended to be less than that for saturated and monounsaturated fatty acids, i.e. high total fatty acid levels did not correspond with high PUFA levels.

4.2.5.1 Fatty acid composition of total lipid of krill.

Fatty acid compositions of total body lipid was determined for male, female and immature krill from all eight sites (Table 4.4-4.7). Overall, males tended to show greatest variability in the percentage contribution of their constituent fatty acids, especially 20:5(n-3) and 22:6(n-3) (Table 4.4). Male krill contained lower median levels of saturated fatty acids (median 30%) than either females (median 35%) or immatures (35%), whereas the converse was true for PUFA, with males containing higher proportions (median 35%) than either females (median 26%) or immatures (median 17%). It should be noted that the described variability between the different sex and maturity stages is driven largely by the variation in the absolute levels of neutral storage lipid in the krill. The absolute levels of PUFA remain relatively constant in relation to unit krill mass (data not shown).

The percentage fatty acid compositions of the different sex and maturity stages for three sites are presented in Tables 4.5-4.7. The high overall variability of the lipid compositions of male krill can be attributed more to different fatty acid compositions

Table 4.3 Fatty acid levels (mg) in male, female and immature krill from eight sites around South Georgia.

Fatty Acid	Male		Female		Immature	
	median	range	median	range	median	range
20:5(n-3)	3.3	3.7 -8.3	7.8	2.3 - 11.5	2.3	0.8 - 5.1
22:6(n-3)	1.2	1.4 - 2.6	2.9	1.0 - 4.2	0.9	0.3 - 1.9
Saturates	4.5	1.7 - 28.0	19.2	3.4 - 44.0	4.4	1.5 - 18.5
Monounsaturates	3.9	1.2 - 21.5	18.2	3.0 - 37.4	3.7	0.9 - 17.4
Polyunsaturates	5.3	3.7 - 14.9	14.3	4.3 - 18.8	4.1	1.4 - 8.2
Total fatty acid	14.9	7.2 - 64.0	54.2	12.6 - 104.3	12.8	3.8 - 47.5

Table 4.4 Percentage fatty acid compositions of total lipid in male, female and immature krill from all eight sites.

ALL SITES

Fatty Acid	Male		Female		Immature	
	median	range	median	range	median	range
14:0	7.1	2.9 -13.5	8.1	5.8 - 13.9	10.1	6.9 - 12.2
16:0	21.1	16.0 - 24.9	22.8	18.3 - 24.5	21.9	18.1 - 24.2
16:1	7.3	3.2 - 14.3	12.4	9.5 - 15.2	8.7	6.9 - 14.1
16:4	0.4	0.2 - 1.9	0.4	0.0 - 1.3	0.7	0.2 - 1.6
18:0	1.3	1.0 - 2.3	1.3	0.5 - 1.8	1.7	0.5 - 2.2
18:1(n-9)	8.7	4.2 - 12.7	11.0	9.6 - 14.8	10.3	8.0 -14.3
18:1(n-7)	8.2	5.8 - 9.2	8.6	2.3 - 8.9	6.8	3.0 - 8.0
18:2(n-6)	1.5	1.0 - 3.3	1.7	0.9 - 2.3	1.7	1.2 - 2.1
18:4(n-3)	0.8	0.5 - 1.5	0.7	0.4 - 1.6	1.3	0.4 - 1.7
20:4(n-6)	0.6	0.4 - 1.7	0.4	0.1 - 0.8	0.5	0.2 - 0.8
20:4(n-3)	0.5	0.2 - 0.9	0.4	0.2 - 0.6	0.5	0.3 - 1.1
20:5(n-3)	22.1	13.2 - 31.2	14.3	10.9 - 22.1	18.3	11.3 - 20.9
22:6(n-3)	8.2	4.1 - 19.3	5.3	3.8 - 8.4	7.3	4.0 - 9.4
Saturates	30.2	23.2 - 44.1	35.2	30.2 - 41.8	35.2	23.4 - 39.4
Monounsaturates	26.2	17.1 - 33.6	33.4	25.1 - 36.3	28.8	24.2 - 37.2
Polyunsaturates	35.4	23.4 - 51.9	26.3	18.2 - 33.4	32.4	17.1 - 37.4

Table 4.5 Percentage fatty acid compositions of total lipid in male, female and immature krill at SG1.

	SG1					
Fatty Acid	Male		Female		Immature	
	median	range	median	range	median	range
14:0	5.2	3.8 - 8.1	7.1	5.8 - 8.2	7.9	7.5 - 9.5
16:0	19.9	18.5 - 24.1	22.4	19.5 - 24.2	23.1	22.1 - 24.2
16:1	6.9	5.8 - 14.1	13.9	13.7 - 15.2	8.6	8.4 - 14.1
16:4	0.5	0.2 - 0.6	0.5	0.5 - 0.7	0.4	0.3 - 0.6
18:0	1.2	1.0 - 1.3	1.1	0.9 - 1.3	1.2	0.5 - 1.6
18:1(n-9)	5.8	5.1 - 10.0	10.9	9.8 - 11.3	11.1	9.5 - 12.1
18:1(n-7)	8.4	6.5 - 9.2	8.1	7.1 - 9.2	6.7	5.2 - 8.3
18:2(n-6)	1.4	1.2 - 1.7	1.2	0.9 - 1.4	1.5	1.2 - 1.6
18:4(n-3)	0.9	0.6 - 1.2	1.4	1.2 - 1.6	0.8	0.6 - 1.1
20:4(n-6)	0.6	0.5 - 0.9	0.5	0.3 - 0.6	0.5	0.4 - 0.6
20:4(n-3)	0.4	0.2 - 0.5	0.4	0.3 - 0.5	0.4	0.3 - 0.5
20:5(n-3)	27.2	17.9 - 29.6	16.2	14.5 - 17.6	18.4	16.8 - 20.1
22:6(n-3)	17.5	6.1 - 19.3	6.0	4.2 - 6.9	7.9	6.4 - 8.4
Saturates	27.3	25.3 - 34.2	34.1	32.6 - 36.1	35.1	27.5 - 36.2
Monounsaturates	21.7	19.5 - 32.2	31.3	30.4 - 32.8	29.4	24.2 - 31.3
Polyunsaturates	45.8	32.8 - 50.3	30.3	24.2 - 31.2	32.4	30.9 - 34.2

Table 4.6 Percentage fatty acid compositions of total lipid in male, female and immature krill at SG5.

	SG5					
Fatty Acid	Male		Female		Immature	
	median	range	median	range	median	range
14:0	8.2	6.3 - 10.5	8.1	6.5 - 10.2	11.3	8.2 - 12.2
16:0	21.8	18.5 - 23.1	22.8	18.3 - 24.5	21.5	19.3 - 22.9
16:1	6.8	3.2 - 11.1	11.2	9.5 - 12.2	8.9	8.5 - 11.1
16:4	0.5	0.3 - 1.9	0.3	0.1 - 0.5	0.7	0.3 - 1.4
18:0	1.3	1.2 - 2.3	1.7	1.5 - 1.8	1.9	1.7 - 2.1
18:1(n-9)	7.4	4.3 - 10.5	10.9	10.1 - 12.2	9.5	8.0 - 9.9
18:1(n-7)	8.3	6.5 - 8.6	8.8	7.3 - 10.0	6.5	5.9 - 7.8
18:2(n-6)	1.6	1.0 - 1.9	1.7	1.1 - 1.9	1.8	1.3 - 2.0
18:4(n-3)	0.7	0.5 - 1.4	0.6	0.4 - 0.8	1.4	1.3 - 1.5
20:4(n-6)	0.6	0.4 - 1.1	0.5	0.3 - 0.8	0.5	0.4 - 0.7
20:4(n-3)	0.6	0.4 - 0.9	0.6	0.2 - 0.6	0.6	0.5 - 1.1
20:5(n-3)	24.1	13.2 - 31.2	13.1	11.4 - 22.1	13.6	11.3 - 15.1
22:6(n-3)	8.3	4.1 - 18.3	5.3	3.8 - 7.5	4.6	4.0 - 5.1
Saturates	30.2	23.1 - 38.2	35.7	30.2 - 40.1	36.2	34.4 - 39.2
Monounsaturates	22.7	17.1 - 32.1	33.5	25.1 - 34.8	30.1	29.3 - 37.2
Polyunsaturates	32.5	27.3 - 40.2	24.2	18.2 - 33.4	20.6	17.1 - 24.2

Table 4.7 Percentage fatty acid compositions of total lipid in male and immature krill at SG8.

	SG8			
Fatty Acid	Male		Immature	
	median	range	median	range
14:0	13.0	12.5 - 13.5	10.5	10.1 - 11.5
16:0	23.7	22.3 - 24.9	21.3	20.3 - 23.2
16:1	9.8	8.9 - 10.1	7.9	7.1 - 8.7
16:4	0.6	0.5 - 0.7	1.1	0.9 - 1.3
18:0	1.4	1.2 - 1.6	1.7	1.5 - 1.8
18:1(n-9)	10.2	9.1 - 11.3	10.4	9.0 - 12.8
18:1(n-7)	6.2	5.8 - 8.4	6.2	3.0 - 7.1
18:2(n-6)	1.6	1.4 - 1.7	1.8	1.5 - 2.1
18:4(n-3)	1.2	1.0 - 1.5	1.3	1.2 - 1.6
20:4(n-6)	0.4	0.4 -0.5	0.5	0.4 - 0.6
20:4(n-3)	0.5	0.4 - 0.5	0.4	0.3 - 0.6
20:5(n-3)	14.5	13.2 - 15.2	18.2	17.2 - 19.1
22:6(n-3)	4.9	4.5 - 5.2	7.3	6.9 - 9.4
Saturates	42.9	36.5 - 44.1	34.1	32.5 - 34.2
Monounsaturates	30.0	27.2 - 30.9	25.1	24.3 - 29.7
Polyunsaturates	24.0	23.4 - 26.1	34.3	33.2 - 35.3

between sites (i.e. SG1, SG5 and SG8) than to variability within sites. For male krill the proportions of saturated and monounsaturated fatty acids increase markedly from SG1 to SG8 with those at SG5 exhibiting an intermediate value, whereas the proportions of PUFA decreases markedly (i.e. 46% at SG1 to 24% at SG8). The fatty acid compositions of female and immature krill tended to remain relatively constant over the three sites.

4.2.5.2 Fatty acid composition of polar and triacylglycerol lipids.

Fatty acid compositions of polar lipid and triacylglycerols were determined for the sex and maturity stages at two sites, i.e. SG4 and SG8 (Tables 4.8-4.11). For all sex and maturity stages it can be seen that both saturated and monounsaturated fatty acids tend to be associated predominantly with triacylglycerols and that triacylglycerols are relatively deficient in PUFA (Tables 4.8, 4.10). However, the converse is true for the polar component, with this class being rich in PUFA, with a relative paucity of saturated and monounsaturated fatty acids (Tables 4.9, 4.11). Specifically, PUFA accounts for circa 50% of the fatty acids in the polar lipid analysed in all catagories at both sites. The ratio of 20:5(n-3) : 22:6(n-3) was circa 2:1 in all polar lipid samples analysed with the exception of male polar lipid at SG4 where the ratio was circa 1:1. At SG8, where female krill appeared to be absent, the fatty acid compositions of the polar lipid component of male and immature krill tended to be similar to those at SG4. However, the fatty acid percentage composition of the triacylglycerol component differed with a marked increase in saturated and monounsaturated fatty acids, notably 14:0, 16:0 and 18:1(n-9) from SG4 to SG8. There was a consistently higher percentage of 18:1(n-9) than 18:1(n-7) in the triacylglycerol fraction for all sex and maturity stages. By contrast, the polar lipid fraction consistently contained more 18:1(n-7) than 18:1(n-9) fatty acids.

4.2.5.3 Multivariate analysis of fatty acid composition.

The body fatty acid compositions of total lipid of all eight South Georgia sites were subject to a multivariate analysis (Principal Component Analysis). The first four principal components accounted for 73% of the total variation within the data set (Table 4.12). Variables making a significant contribution to PC1 were 14:0, 16:0, 16:1, 18:1

Table 4.8 Percentage fatty acid compositions of triacylglycerols in male, female and immature krill from SG4.

	SG4					
Fatty Acid	Male		Female		Immature	
	median	range	median	range	median	range
14:0	14.9	14.1 - 15.0	20.3	20.2 - 20.9	20.3	20.2 - 20.9
16:0	14.4	14.3 - 14.5	24.5	24.4 - 24.9	24.5	24.4 - 24.9
16:1	12.6	12.5 - 12.8	15.9	15.4 - 16.1	15.9	15.4 - 16.1
16:4	2.0	2.0 - 2.1	0.8	0.7 - 0.9	0.8	0.7 - 0.9
18:0	2.5	2.4 - 2.5	1.8	1.7 - 1.9	1.8	1.7 - 1.9
18:1(n-9)	10.5	10.1 - 10.6	17.1	16.9 - 17.3	17.1	16.9 - 17.3
18:1(n-7)	8.7	8.7 - 8.9	8.4	8.3 - 8.5	8.4	8.3 - 8.5
18:2(n-6)	2.6	2.5 - 2.7	1.8	1.5 - 1.9	1.8	1.5 - 1.9
18:4(n-3)	0.7	0.6 - 0.7	1.6	1.4 - 1.7	1.6	1.4 - 1.7
20:4(n-6)	0.5	0.5 - 0.5	0.0	0.0 - 0.1	0.0	0.0 - 0.1
20:4(n-3)	0.6	0.5 - 0.7	0.0	0.0 - 0.2	0.0	0.0 - 0.2
20:5(n-3)	7.5	7.5 - 7.9	1.3	1.2 - 1.4	1.3	1.2 - 1.4
22:6(n-3)	1.4	1.3 - 1.5	0.4	0.4 - 0.5	0.4	0.4 - 0.5
Saturates	35.8	35.5 - 35.9	47.9	47.6 - 48.2	47.9	47.6 - 48.2
Monounsaturates	33.7	33.6 - 34.3	42.4	41.2 - 42.8	42.4	41.2 - 42.8
Polyunsaturates	19.2	18.0 - 19.6	6.8	6.8 - 6.9	6.8	6.8 - 6.9

Table 4.9 Percentage fatty acid compositions of polar lipid in male, female and immature krill from SG4.

SG4

Fatty Acid	Male		Female		Immature	
	median	range	median	range	median	range
14:0	1.2	1.2 - 1.3	3.1	3.0 - 3.2	2.2	1.9 - 2.9
16:0	22.4	21.8 - 22.9	24.6	24.6 - 25.3	19.7	15.1 - 23.7
16:1	2.6	2.5 - 2.9	2.9	2.1 - 3.4	2.7	2.1 - 4.9
16:4	0.1	0.0 - 0.2	0.1	0.1 - 0.2	0.1	0.1 - 0.5
18:0	1.3	1.3 - 1.5	1.2	1.1 - 1.3	1.4	1.1 - 1.9
18:1(n-9)	6.7	6.5 - 6.7	5.6	5.4 - 5.7	4.8	4.0 - 5.8
18:1(n-7)	6.1	6.0 - 6.2	6.4	6.3 - 6.7	5.2	4.6 - 8.6
18:2(n-6)	2.7	2.6 - 2.9	1.8	1.7 - 1.9	1.4	1.3 - 1.6
18:4(n-3)	0.4	0.3 - 0.4	1.9	1.8 - 1.9	1.1	0.9 - 1.4
20:4(n-6)	0.8	0.7 - 0.9	0.7	0.6 - 0.7	1.5	0.5 - 2.3
20:4(n-3)	0.6	0.5 - 0.9	0.8	0.7 - 0.9	0.6	0.6 - 0.8
20:5(n-3)	23.6	23.3 - 24.1	29.3	29.3 - 30.0	31.7	26.0 - 32.0
22:6(n-3)	21.6	25.6 - 21.8	15.1	14.2 - 15.2	12.1	11.9 - 13.6
Saturates	26.8	25.6 - 27.6	29.3	28.9 - 29.5	24.1	20.5 - 29.0
Monounsaturates	18.2	17.1 - 18.6	18.7	18.2 - 18.7	15.6	13.1 - 21.0
Polyunsaturates	52.1	50.6 - 52.8	49.6	49.1 - 51.5	52.9	44.7 - 52.9

Table 4.10 Percentage fatty acid compositions of triacylglycerols in male and immature krill from SG8.

	SG8			
Fatty Acid	Male		Immature	
	median	range	median	range
14:0	20.2	19.0 - 21.9	17.8	17.6 - 19.2
16:0	21.8	21.5 - 22.5	21.8	20.9 - 22.8
16:1	18.2	14.6 -19.8	13.4	12.9 -14.8
16:4	1.4	1.2 - 1.5	1.9	1.5 - 2.1
18:0	1.6	1.1 - 2.0	2.5	2.0 - 2.5
18:1(n-9)	15.6	12.5 - 18.5	16.9	16.8 -17.9
18:1(n-7)	6.8	6.1 - 7.5	6.2	6.0 - 7.6
18:2(n-6)	1.5	1.3 - 1.9	1.7	1.7 - 1.9
18:4(n-3)	1.1	1.0 - 1.4	1.5	1.4 - 1.9
20:4(n-6)	0.0	0.0 -0.0	0.2	0.1 - 0.2
20:4(n-3)	0.2	0.1 - 0.2	0.2	0.2 - 0.2
20:5(n-3)	2.3	2.1 - 2.3	3.9	2.3 - 4.1
22:6(n-3)	0.5	0.4 - 0.6	0.6	0.5 - 0.9
Saturates	46.2	45.5 - 46.9	46.3	45.8 - 47.6
Monounsaturates	40.5	40.3 - 41.3	37.8	37.7 - 41.4
Polyunsaturates	9.8	9.1 - 9.9	13.1	9.9 - 13.5

Table 4.11 Percentage fatty acid compositions of polar lipid in male and immature krill from SG8.

	SG8			
Fatty Acid	Male		Immature	
	median	range	median	range
14:0	2.6	2.5 - 2.6	2.1	2.0 - 2.2
16:0	25.1	24.5 -25.6	23.6	23.1 - 23.6
16:1	2.5	0.5 - 3.6	2.5	2.4 - 3.0
16:4	0.3	0.1 - 0.4	0.1	0.1 - 0.2
18:0	1.1	1.0 - 1.2	1.3	1.2 - 1.4
18:1(n-9)	4.8	4.4 - 5.4	4.5	4.5 - 4.7
18:1(n-7)	7.2	6.2 - 7.6	6.0	5.9 - 6.4
18:2(n-6)	1.6	1.5 - 1.9	1.7	1.7 - 1.8
18:4(n-3)	1.0	0.9 - 1.1	1.1	0.8 - 1.1
20:4(n-6)	0.5	0.5 - 0.6	0.7	0.6 - 0.7
20:4(n-3)	0.6	0.6 - 0.7	0.6	0.6 - 0.7
20:5(n-3)	30.3	29.3 - 31.2	31.1	30.8 - 31.2
22:6(n-3)	13.4	12.7 - 13.4	15.9	15.8 - 16.6
Saturates	29.3	29.2 - 29.5	27.6	26.9 - 27.7
Monounsaturates	17.3	14.9 - 18.3	15.2	14.9 - 15.8
Polyunsaturates	50.5	49.8 - 51.2	54.2	53.2 - 54.4

Table 4.12 Summary of the key variables contributing to the first four principal components from a multivariate, fatty acid analysis from eight sites around South Georgia.

PC	Loading	Variable	Proportion of variance %	Cumulative variance %
PC1	+	14:0, 16:0, 16:1, 18:1, total sat's	40.7	40.7
	-	20:5, 22:6, total PUFA		
PC2	+	16:1	13.3	54.0
	-	14:0, 16:4, 18:0, 18:2, 20:4		
PC3	+	18:4	10.4	64.4
	-	18:2, total dienes		
PC4	+	18:0, 18:1	8.2	72.6
	-	16:4, 18:4		

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and total saturated fatty acids (positive loading), all of which are characteristic of neutral storage lipid. The variables 20:5, 22:6 and total polyunsaturated fatty acids also made a significant contribution to PC1 (negative loading) and these tend to be characteristic of polar membrane lipids.

Ordination of all the animals on principal components 1 and 2 produced no clear separation of males, females or immatures (Fig 4.4a). Males tended to exhibit the greatest scatter on both PC1 and PC2 with approximately 50% of the males showing negative loading on PC1, which corresponds with high PUFA levels (Table 4.12). Of all sex and maturity stages females exhibited the least scatter with predominantly positive loadings for both PC1 and PC2. Immatures showed greatest separation on PC2 (Fig 4.4a).

When the ordinations for the sex and maturity catagories are separated there is a clear pattern in the scores of male and immature animals from the different sites. However, there appears to be no clear pattern of the scores of females. although they tend to have the most positive scores of the three sex and maturity stages (Fig 4.4b-d).

4.3 DISCUSSION.

4.3.1 Importance of storage lipid for males, female and immature krill.

The contribution of the two main lipid classes - TAG and PC - to the overall lipid pool, and hence their respective contributions to lipid storage and to `structural' lipid, were investigated by adapting exploratory techniques used by Clarke (1983). The present study utilises simple linear regression to determine how the proportion of a particular lipid class varies with the total lipid content, expressed as a proportion of animal fresh mass. Simplistically, a significant linear relationship with a high value for the slope and a value of the intercept which is low compared with the median proportion of that lipid class is suggestive of storage, the baseline level is low and then increases as lipid load per animal increases. Conversely, a poorly fitting regression, with near-zero slope and an intercept close in value to the median value suggests that the lipid class makes a nearly constant contribution to the overall lipid pool and hence is unlikely to be involved in storage. Clearly, both statistical artifacts and co-variation in the major lipid classes will introduce complications to this simple picture. Nevertheless, application of

the technique to data from this study has produced an interesting picture of variation in lipid allocation between sex and maturity stages (Table 4.13). Immature animals and females typically had higher proportions of total lipid than did males. Likewise, immatures and females had high proportions of TAG in total lipid, slopes of regression were low and intercepts were similar to median proportions of TAG, conventionally considered as the main storage lipid. By contrast, males had higher slopes and lower intercepts. Using the interpretations advanced above, it can be inferred that immatures and females contained uniformly high levels of TAG in their lipid pools, largely representative of the size of that pool. However, for males the percentage of TAG in the lipid pool is more dependent on the size of the pool with larger pools containing a higher proportion of TAG than smaller pools.

The picture for PC, taken predominantly as a membrane lipid, was more uniform. Slopes of regression were near-zero (especially for immature and female krill), and intercepts were similar to the median proportions for all three sex-and-maturity stages. Thus, PC appears to have been a consistent component of the lipid pool although there was a slight tendency for it to be more abundant in those male krill with higher lipid content. In this study the population of males has been sub-divided, since two sites had no female krill and here the median total lipid and TAG content were both high (Table 4.13). Males from these sites, SG7 and SG8, represented a small sample (six animals) but all the animals contained high proportion of TAG which, despite a range of 28-46%, appeared to represent a uniform proportion of total lipid.

As can be seen from Fig 4.2b, all sex and maturity classes of krill generally exhibited an increase in lipid content over the course of the twenty eight day, late summer sampling period. This supports Hagen (1988) who compiled data from a variety of sources and found evidence for the accumulation of lipid reserves immediately prior to winter. These lipid reserves, although not extensive are likely to play an important role in the overwintering strategy of krill (Hagen 1988; Quetin and Ross 1991). This apparent increase in the lipid content of krill with the onset of winter could feasibly be related to increases in the neutral lipid content of the microplankton (Chapter 3). However it is equally possible that a 'winding down' of effort and/or

Table 4.13 Regression analysis of percentage composition of phosphatidylcholine and triacylglycerol in total lipid versus percentage of total lipid in fresh mass for male, female and immature krill from eight sites around South Georgia.

	Immature	Female	Male		
			All	SG1-6	SG7, 8
Number	76	41	48	42	6
% LIPID					
Median	4.16	3.48	1.81	1.7	6.4
Range	0.95-12.5	1.18-9.4	0.72-8.2	0.72-5.31	1.5-8.2
%TAG					
Median	35	32	22	22	44
Range	12.8-44.4	23.6-42.0	14.2-46.5	14.2-33.6	28.8-46.5
Slope	1.41	0.56	3.28	2.94	-0.89
Intercept	27	30	16	16	46
F-ratio	14.32	2.55	40.42	11.21	0.40
%PC					
Median	25	26	22	22	22
Range	13.9-38.1	22.1-34.6	14.7-31.7	14.7-30.4	17.3-31.7
Slope	-0.14	0.10	0.82	2.07	0.98
Intercept	25	26	20	18	17
F-ratio	0.11	0.16	6.45	10.0	0.73

metabolic adaptations by krill with the onset of winter also contributes to late summer lipid accumulation.

4.3.2 Fatty acid content and composition of krill.

Variability in the fatty acid content of the different sex and maturity categories of krill investigated in this study clearly reflect variability in total lipid and lipid classes. Possible explanations for this observed variability, such as temporal and spatial factors, reproduction and growth will be discussed later in this chapter and in Chapter 7 where they will be used to construct a lipid budget for *Euphausia superba*.

As with the multivariate analysis of the lipid class composition of krill, the major factor determining the separations observed in Figs 4.4a-d is the level of neutral storage lipid, and its constituent levels of saturated and monounsaturated fatty acids. These variations in the content and composition of fatty acids between the different sex and maturity stages and between the eight sites, are likely to reflect both different feeding histories of the animals and or differing rates of endogenous biosynthesis from lipid and non-lipid precursors.

Analyses of polar and neutral fractions of total lipid confirmed the findings of Clarke (1980) and Fricke (1984) that polar fraction tends to contain high proportions of polyunstaurated fatty acids, notably 20:5(n-3) and 22:6(n-3), whereas the triacylglycerol component contains high levels of 16:0, 16;1, 18:1(n-7) and 18:1(n-9). Although phytanic acid, which derives from the phytol moiety of chlorophyll, has been detected in krill lipids in previous studies (Hansen 1969; Hansen 1970; Clarke 1980; Fricke 1984), it was absent from the krill lipids in this study.

4.3.3 Site-to-site variability.

Although differences between sex and maturity classes of krill can explain much of the variability in the observed data on lipid content and composition, there remains significant and systematic site-to-site variability. Because of the deliberately biased sampling of the net haul at each site, this variability cannot be ascribed either to differences in sex ratio at the sites or to differences in animal size.

No single feature of the environment at the eight sites can explain the pattern of

variability. Differences in depth, surface salinity and temperature, phytoplankton biomass (chlorophyll *a*) and particulate fatty acid concentration (Table 2.1) did not correspond to any property of krill lipid content or composition. Rather, the site-to-site variability appears to manifest an overall pattern which follows the order of sampling. This embodies both a time series and a west to east gradient, and factors which could underlie such a pattern need to be considered. Briefly, these could be a change related to sampling time itself (i.e. something affecting all krill in the area and independent of sample site) or a geographic gradient between krill of different origins.

If there is any temporal change in the lipid content or composition of krill during the study period, it is inseparable from other sources of variability. However, spatial heterogeneity in krill distribution around South Georgia has been documented (Latogursky et al. 1990) and appears to be linked to the pattern of surface water circulation (Grelowski and Pastuszak 1984). Animals at different sites could have been derived from different sources and could also have spent different periods in the vicinity of South Georgia. Overall, variability from site to site probably arises from both spatial heterogeneity in the krill population around the island, and from temporal change.

5. GUT THROUGHPUT KINETICS, ASSIMILATION AND METABOLISM OF INGESTED ALGAL LIPID.

5.1 INTRODUCTION.

Although assimilation efficiency has been investigated in a wide range of zooplankton (Mauchline 1980), studies on *Euphausia superba* are scarce and tend to be based on measurements of `total carbon'. As yet no quantitative information on the assimilation of specific macro-nutrients by krill is available. In laboratory krill grazing experiments, Tanoue et al. (1982) investigated the qualitative relationship between the chemical composition of a cultured alga (*Dunaliella tertiolecta*) and the faecal pellets after the algae had been fed to krill. Tanoue (1985) and Tanoue and Hara (1984) conducted similar experiments utilising ambient Antarctic microplankton. In all these experiments, fatty acid content as a proportion of total carbon was seen to decrease from the algae to the faecal pellets, which implies that fatty acids were assimilated efficiently. However it should be stressed that `total carbon' is not a conservative marker and, as such, these experiments provide no quantitative information on the nutritional value of fatty acids to krill.

Faecal pellet egestion rates can be used as a measure of ingestion and hence can be used to calculate the total energy requirements of zooplankton (Hargrave 1972). If assimilation efficiency is known, then the daily energy intake, C (organic carbon), of an organism can be calculated from:

$$C = R_0 / (1-a)$$

where R_0 = loss of organic matter in faeces, and a = assimilation efficiency.

Only Clarke et al. (1988) have attempted to measure faecal egestion rates in Antarctic krill. Estimates were similar in both the field and laboratory, i.e. 0.25 - 2.35 mg dry wt h^{-1} (data corrected to standard krill of 600 mg fresh mass) indicating that high summer filtration rates are consistent with a total organic carbon intake of 17-28% of body mass per day in adults.

Because of the impact krill have on the formation and fate of particulate material

in the euphotic zone, through grazing, nutrient cycling, defaecation, moulting and 'necroflux', they play an important role in the exchange and export of carbon (Bodungen 1986; Graneli et al. 1993). It is important, therefore, to understand not only basic variables such as filtration rates and faecal pellet production, but also how these variables and assimilation are related to food quality (Emerson and Roff 1987).

Radioactive lipid can be a particularly useful tracer when investigating metabolic and biosynthetic processes in marine organisms (Sargent and Lee 1975; Henderson 1981; Clarke 1987). The technique is particularly valuable when applied quantitatively, as it can give insights into rates of lipid biosynthesis, turnover, catabolism and assimilation within organisms.

The present chapter first investigates the gut throughput kinetics of krill feeding on two contrasting algae, and second, investigates assimilation efficiency and short term incorporation of dietary fatty acids into the fatty acid pool of the krill.

5.2 RESULTS.

5.2.1 Kinetics of gut throughput and turnover time.

Krill grazing experiments were conducted in the laboratory utilising as a food source either ^{14}C labelled *Isochrysis* or ^{14}C labelled *Thalassiosira* (for further details of the experiment see Chapter 2). A primary aim of the experimental design was to maintain essentially constant feeding conditions for the duration of the experiment. Many laboratory estimates of gut throughput in zooplankton have either used previously starved animals or involve transferring actively feeding animals to filtered seawater (Dagg and Wyman 1983; Clarke et al. 1988). In the present experiments actively feeding animals were maintained in algal culture suspensions before transferring the krill to similar densities of radio-labelled algae. Athough it it probable that, on transfer to the radio-labelled algae the krill experienced some stress, they quickly resumed feeding.

The fatty acid contents and composition of *Isochrysis*, krill and faecal pellets were determined and this allowed investigation of; a, assimilation of total lipid and individual fatty acids; b, catabolism of total lipid and its individual fatty acids; c,

biosynthesis of individual fatty acids by krill. (Note that in the grazing experiment conducted using radiolabelled *Thalassiosira,* the levels of radioactivity incorporated into *Thalassiosira* lipid were too low to permit investigation of its individual fatty acids).

5.2.1.1 Data analysis.

The time course of the appearance of radioactivity in the faecal pellets was modelled by first-order kinetics on the assumption that the radiolabelled material ingested by the animals equilibrated progressively with unlabelled material already present. The statistical software package GENSTAT 5 (Payne et al. 1987) was used to fit the following equation to the data using non-linear techniques:

$$F^* = F_{max} (1-e^{-t/k})$$

The parameter t in the above equation is the time elapsed from the first appearance of radioactivity in the faecal pellets up to the time of collection of a subsequent faecal pellet. F^* is the radioactivity of a faecal pellet at time t, corrected to unit dry mass of the pellet, and F_{max} is the asymptote value of this variable (only attained at $t= \infty$). The parameter k represents the turnover time for food in the gut (usually expressed in kinetic models as the reciprocal of time). The properties of this model are as follows-

After a gut throughput time, during which no radioactivity is detected in the faecal pellets, radioactivity from egested material derived from radio-labelled algae begins to appear in the faecal pellets. Initially, only a proportion of the faecal material is derived from the labelled algae, so the radioactivity is low. As the experiment continues, the contribution of the radio-labelled algae to the food in the gut and hence to the faecal material increases, and the radioactivity of individual pellets increases towards an asymptote value. The half-life of food in the gut, t_{half}, is related to k by -

$$t_{half} = 0.693.k$$

5.2.1.2 Gut throughput and turnover times.

The time course of the appearance of radioactivity in faecal pellets was clearly different for the two species of algae (Fig. 5.1a, b). In both cases the fitted models explained a high proportion of the variance in the data (0.77 for *Isochrysis* and 0.89 for *Thalassiosira*). For krill fed with *Isochrysis*, radiolabel could be detected in faecal

material after 30 minutes (Fig. 5.1a). The fitted first-order kinetic model gives a value for the turnover time, k, of 47 min (standard error 9 min) equivalent to a half–life of food in the gut, t_{half}, of 32 min. The similarity between these values and the throughput

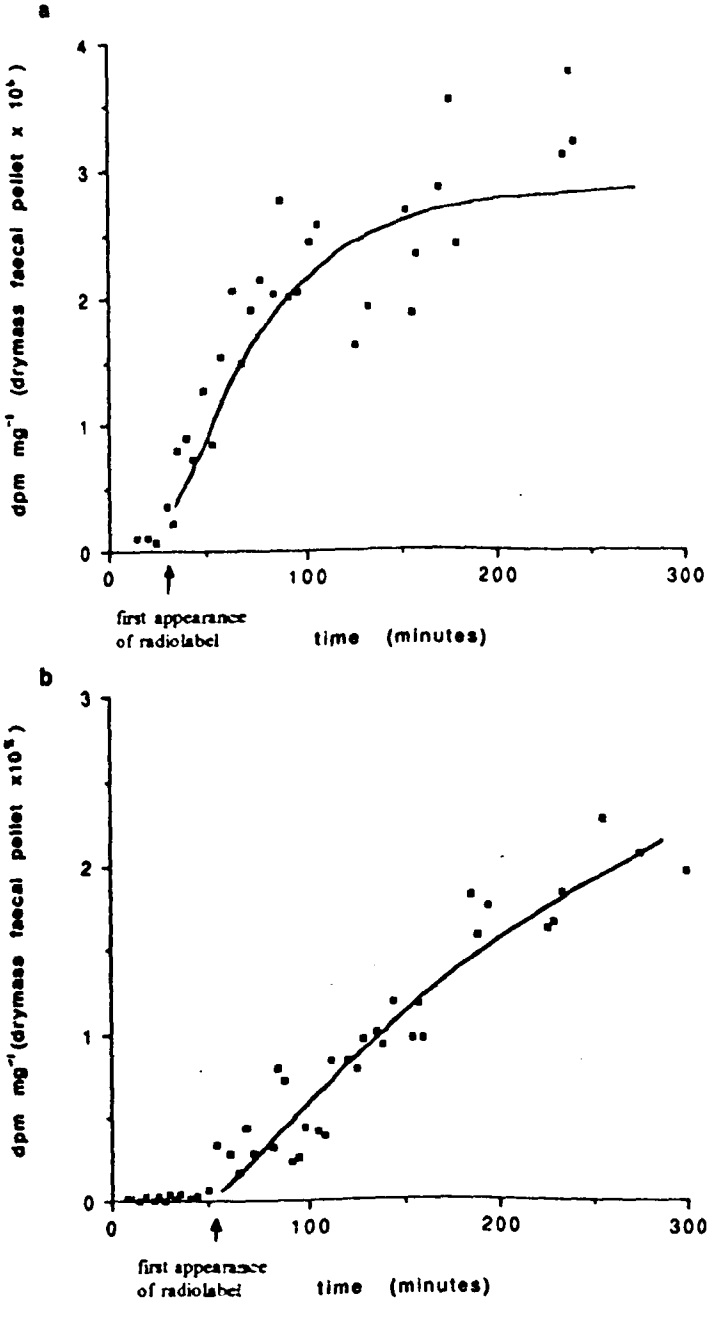

Fig 5.1 Time course of appearance of radiolabel in the faecal pellets produced by immature krill transferred from feeding on non-labelled algae at t = 0. a - *Isochrysis*, b - *Thalassiosira*.

time (33 minutes) indicates that *Isochrysis* is processed rapidly in krill guts. The asymptote value F_{max} was 2.86 x 10^6 dpm mg^{-1} (standard error 0.197 x 10^6) which falls within the range of activity detected in faecal pellets collected after 2.5 hours. By contrast, radioactivity could not be detected in the faeces of krill feeding on *Thalassiosira* until 55 min after the introduction to labelled food (Fig. 5.1b). The longer turnover time, k, of 256 min (standard error 86 min) indicates a longer residence time of food in the gut (t_{half}=177 min). Radioactivity in the faecal pellets from *Thalassiosira* did not approach an equilibrium value during the four hours of the experiment. F_{max} predicted by the model was 0.359 x 10^6 dpm mg^{-1} (standard error 0.089 x 10^6) whereas the highest value observed in the experiment was 0.217 x 10^6 dpm mg^{-1}. Thus, the two food sources differ in the timescale of their processing by krill, with differences being more marked in the turnover time, k, than the gut throughput time.

5.2.2 Incorporation of ^{14}C into algal lipid.

The carbon biomass present and the amount of [^{14}C] bicarbonate added were similar for both cultures during the initial labelling of the algae. However, the incorporation of ^{14}C into total lipid in the two cultures differed markedly (Table 5.1, 0 hour sample). The smaller-celled *Isochrysis* incorporated 5951 dpm per µg total lipid or 1.5 x 10^6 dpm in lipid per μm^3 cell volume in incubation prior to the feeding experiment. The larger-celled *Thalassiosira* incorporated 104 dpm per µg lipid or 0.1 x 10^6 dpm per μm^3 cell volume during incubation with ^{14}C bicarbonate prior to the start of the feeding experiment. These values presumably reflect the uptake of ^{14}C by the two cultures, with *Isochrysis* (295 μm^3) exhibiting size-specific uptake rates at least an order of magnitude greater than those for *Thalassiosira* (2650 μm^3). During the course of the 12 hours feeding experiment, when the algae were present in sea water at 2°C in a dimly lit cold room, the specific radioactivity in total lipid from *Isochrysis* decreased only slightly while that in *Thalassiosira* decreased by 50%. Clearly, catabolism of radioactive label during the feeding period, when photosynthesis will inevitably be decreased in the algae, is greater in *Thalassiosira* than in *Isochrysis*.

Table 5.1 Measures of removal of algal biomass during the course of the *Isochrysis* and *Thalassiosira* grazing experiments (means of three replicates with standard deviations in brackets).

		cell concentration (cells ml^{-1})	chlorophyll *a* (ng ml^{-1})	lipid ($\mu g\ ml^{-1}$)	dpm (μg lipid^{-1})
Isochrysis					
	0 hours	21400 (779)	27.21 (1.05)	1.59 (0.11)	5951 (543)
	4 hours	15633 (2739)	20.47 (1.46)	1.09 (0.06)	5687 (551)
	12 hours	10400 (990)	13.60 (0.96)	0.72 (0.09)	5335 (503)
	net 0-12 hours	11000	13.61	0.87	
Thalassiosira					
	0 hours	3716 (85)	9.28 (0.33)	0.89 (0)	104 (0)
	5 hours	1723 (95)	4.10 (0.27)	0.39 (0.03)	83 (5)
	12 hours	1147 (87)	2.65 (0.26)	0.25 (0.02)	53 (6)
	net 0-12 hours	2569	6.63	0.64	

5.2.3 Distribution of radiolabel in *Isochrysis*.

At the start of the grazing experiment, approximately 16 hours after inoculation of the *Isochrysis* with ^{14}C bicarbonate, most of the radioactivity in *Isochrysis* fatty acids has been incorporated into C18 fatty acids (18:1 > 18:3 > 18:2) (Table 5.2). The C16 polyunsaturated fatty acids (PUFA), especially 16:4, collectively accounted for 12% of the total radioactivity, but 18:4 and 20:5 contained only very small percentages.

The distribution of the radiolabel did not correspond directly to the fatty acid composition of *Isochrysis*. 16:2, 16:4, 18:2 and 18:3 were over-represented in terms of radioactivity, whilst the proportion of saturates (predominantly 16:0), 16:1, 18:4 and 20:5 were labelled to a lower extent than would be expected. Throughout the experiment both the overall fatty acid composition of the algae and the distribution of label between fatty acids remained generally constant. Correlation coefficients between total fatty acid composition for the three sampling times were all highly significant (r= 0.99 - 1.0). Similarly the distribution of radiolabel amongst fatty acids at the three incubation times were strongly correlated (r= 0.896 to 0.983). However, the correlation between the labelled and total fatty acid distribution was lower (r= 0.458 to 0.649 for equivalent samples, and r= 0.454 to 0.662 for all possible comparisons). In a few cases, changes in mass-specific radioactivity indicate changes in the distribution of label in algal fatty acids during the grazing experiment. For instance, there was significant decline in specific radioactivity in C16 PUFA during the initial 4 hours of the grazing incubation (following 16 hours exposure to ^{14}C) (Table 5.3). There was a corresponding increase in specific activity in 18:2 and 18:3, with the latter showing a very large but transient increase at 4 hours. In addition to changes in the distribution of radiolabel between individual fatty acids, there was an overall decline in fatty acid specific activity. Taken together, these observations indicate transformation of the algal fatty acid pool during the course of the grazing experiment, but the presence of label in all fatty acids throughout the grazing experiment allows further consideration of their fate in krill and faecal pellets.

5.2.4 Ingestion, egestion and assimilation of algal lipid by krill.

On the basis of cell counts 27% of the initial *Isochrysis* biomass was removed

Table 5.2 Percent distribution of radioactivity in the major fatty acids of *Isochrysis* during the course of the 12 hour grazing experiment, and of krill body and gut tissues and faecal pellets at the end of the experiment.

	Alga			Krill		
	start	4 hours	12 hours	Body tissue	Gut tissue	Faecal pellets
Hydrocarbon	2.8	3.8	1.8	nd	nd	2.7
Saturates	6.0	11.6	9.0	17.5	21.6	21.1
16:1	3.6	4.6	3.2	4.5	3.7	3.1
16:2	3.4	2.6	3.9	4.3	4.1	2.9
16:3	2.0	0.5	1.8	5.9	6.0	3.1
16:4	6.9	0.4	1.4	1.0	2.4	8.1
18:1	36.6	47.0	38.7	34.3	35.5	25.9
18:2	11.4	16.8	13.9	9.3	7.6	9.5
18:3	25.2	12.4	24.4	17.5	15.2	21.9
18:4	0.7	0.3	0.5	5.3	2.5	0.6
20:5	1.4	0.1	0.9	0.6	1.3	1.1

Table 5.3 Variations in the specific radioactivity (dpm ng fatty acid^{-1}) of fatty acids in *Isochrysis* during the course of the grazing experiment, and of krill gut and body tissues and faecal pellets at the end of the experiment.

	Alga			Krill		
	start	4 hours	12 hours	Body tissue	Gut tissue	Faecal pellets
Saturates	1.1	1.3	0.4	0.1	0.5	1.8
16:1	3.7	2.7	0.7	0.1	0.5	0.3
16:2	21.4	8.2	6.2	0.6	1.9	19.7
16:3	15.3	1.5	3.0	5.5	31.5	21.5
16:4	86.8	2.0	2.8	0.2	2.6	27.1
18:1	8.5	6.8	2.0	0.2	1.7	2.7
18:2	16.3	13.6	4.4	0.5	2.7	17.7
18:3	25.8	76.1	5.4	0.2	16.1	37.5
18:4	1.1	0.3	0.2	0.6	1.4	6.4
20:5	1.7	0.1	0.3	0.0	0.02	11.6

during the first 4 hours incubation and 51% over the full 12 hour incubation, i.e. an average grazing rate of 6.8% h^{-1} over the first 4 hours and 4.3% h^{-1} over the full 12 hours (Table 5.1). Corresponding values for *Thalassiosira* removal were 53% removed in the first 4 hours and 70% over the full 12 hour incubation, i.e. average grazing rates of 13.3% h^{-1} during the first 4 hours and 5.8% h^{-1} over the full 12 hours (Table 5.1). Thus, 51% of the *Isochrysis* cells and 70% of the *Thalassiosira* cells present initially were removed by the krill in the 12 hour feeding period, equivalent to 11,000 cells ml^{-1} and 2,569 cells ml^{-1}, or total algal volumes of $3.25x10^6$ μm^3 ml^{-1} and $6.80x10^6$ μm^3 ml^{-1} respectively. However the cellular lipid content of the two species differed markedly: 74.3 and 239.5 pg lipid $cell^{-1}$ for *Isochrysis* and *Thalassiosia* respectively (Table 5.1). In consequence, the removal of lipid for the two species were very similar, i.e. 817 and 615 ng ml^{-1} respectively.

5.2.4.1 Assimilation of total lipid and total radiolabelled lipid by krill feeding on radiolabelled algae.

Assimilation efficiencies can be calculated from the mass and the radioactivity of lipid ingested and excreted for both *Isochrysis* and *Thalassiosira*. On the basis of mass, the lipid assimilation efficiencies are similar for both algal species (Table 5.4). On the basis of radioactivity, the lipid assimilation efficiency for *Isochrysis* is somewhat higher than the value calculated from lipid mass, whereas the converse is true for *Thalassiosira* (Table 5.4). The input of radioactive lipid into krill from *Thalassiosira* was calculated from the average specific radioactivity of algal lipid over the 12 hour feeding. However, as radioactivity decreased in *Thalassiosira* over the 12 hour experiment, this approximation tends to overestimate the actual specific radioactivity of the lipid ingested and, therefore, underestimates the calculated assimilation efficiency. Nonetheless, the data in Table 5.4 point to an efficient assimilation of lipid by krill from both algal species.

Table 5.4 Percent assimilation efficiencies and percent catabolism of assimilated lipid in krill feeding on ^{14}C-labelled *Isochrysis* and *Thalassiosira*.

	Isochrysis	*Thalassiosira*
% Ingested Lipid Mass Assimilated	75	77
% Ingested Radioactive Lipid Assimilated	86	63
% Assimilated Radioactive Lipid Catabolised	61	51

5.2.4.2 Assimilation of radiolabelled fatty acids by krill feeding on *Isochrysis.*

Assimilation efficiency of radiolabelled fatty acids could only be calculated for the *Isochrysis* feeding experiment as too little ^{14}C was incorporated into the fatty acids of *Thalassiosira*. Assimilation efficiencies for fatty acids were higher than for total lipid (Table 5.5) with the exception of saturates.

5.2.4.3 Distribution of radiolabel in krill.

The percentage distributions of radioactivity in fatty acids present in both krill body tissue and gut tissue are similar to each other but different from that in the algae. In particular, both krill tissues have increased percentages of radioactivity in saturated fatty acids, 16:3 and 18:4 compared to the algae, offset by decreased percentages in the 18:2, 18:3 and 18:1. The hydrocarbons found in the alga were not detected in krill body tissue.

The extent to which the distribution of radiolabel fatty acids in krill is a reflection of tissue composition can be examined using specific radioactivity (Table 5.3). The low specific radioactivities in fatty acids in krill body tissue are obviously determined chiefly by the large endogenous levels of unlabelled fatty acids present in the animal's lipid. Gut only showed high specific activity for 16:3 and 18:3. Although both fatty acids were relatively strongly labelled in the algae, some other compounds such as 16:2 16:4 and 18:2 were equally important in the gut. Body tissue showed lower specific radioactivity, indicating that labelled algal fatty acid was even less abundant than in the gut. Only one fatty acid, 16:3, showed high specific activity.

5.2.4.4 Catabolism and biosynthesis of lipid by krill.

Comparison of the amounts of lipid assimilated by the krill with the amounts present in the krill allowed calculation of the proportion total lipid catabolised or synthesised. Calculating total lipid assimilation efficiencies revealed that the difference between the level of radioactivity in lipid ingested by the krill and the level of radioactivity appearing in faecal lipid, i.e. the level of radioactivity in lipid assimilated by the krill, was substantially less than could be accounted for by the measured level of

radioactivity in lipid extracted from krill body and gut tissue, with an apparent loss of 61% and 51% of radioactive lipid for *Isochrysis* and *Thalassiosira* respectively.

Taking all fatty acids, 69% of assimilated material was catabolised, but individual fatty acids differed markedly (Table 5.5). Although 95% of algal radiolabelled 18:4 was assimilated, this only accounted for 56% of the label in the krill body lipid and this implies that 18:4 was being biosynthesised by the krill. Similarly saturates, which had a lower assimilation efficiency of 77% accounted for only 60% of the labelled saturated fatty acids found in the body. By contrast, 16:1, 16:2, 16:4, 18:1, 18:2, 18:3 and 20:5 were all catabolised extensively (65 - 93%).

5.2.4.5 Distribution of radiolabel in faecal pellets

Fourteen percent of ingested radiolabelled lipid was egested in faecal pellets (Table 5.2). Clearly, all of this radiolabel had originated in the algal food, but could reach the faecal pellets either as unaltered algal material or following selective digestion and subsequent modification. The contribution of a peritrophic membrane to the lipid content and composition of krill faecal pellets is unknown. The distribution of radioactivity in the fatty acids of faecal pellets showed affinities to both algal and krill lipid. Thus the algal hydrocarbon was present in faecal pellets, but was absent from the krill radiolabelled lipid. However faecal pellets also contained a high proportion of label in saturates (predominantly 16:0) which were more abundant in krill than in the algae. Overall, the fatty acid composition of faecal pellets was correlated strongly with that of the algae, both on the basis of fatty acid mass ($r = 0.984$) and for distribution of radiolabel ($r = 0.835$).

5.3 DISCUSSION.

5.3.1 Kinetics of gut throughput and turnover.

Only a few studies have been conducted on the egestion rates of *Euphausia superba* and all of these previous experiments involve transferring actively feeding animals to filtered seawater so that the animals stop feeding and, therefore, gut throughput times are likely to be underestimated. The present study attempted to estimate gut throughput and gut turnover times by studying constantly feeding animals

Table 5.5 Percent assimilation of radioactive fatty acids in lipid ingested by krill feeding on *Isochrysis*, and percent of assimilated radioactive fatty acids catabolised.

	Per cent assimilated	Per cent catabolised
Sat	77	-28 *
16:1	95	65
16:2	95	65
16:3	90	8
16:4	93	93
18:1	96	71
18:2	95	77
18:3	95	81
18:4	95	-71*
20:5	95	79
Overall	94	69

* Negative catabolism = biosynthesis.

in controlled laboratory experiments using radiolabelled algae.

Gut throughput times for the two algae tested in this study - 33 minutes for *Isochrysis* and 55 minutes for *Thalassiosira* - are very similar to the 40 minutes gut throughput time suggested by Clarke et al. (1988), based on starvation experiments. However, Antezana et al. (1982) found gut throughput times to be considerably longer, i.e. ~ 3 hours, although in their experiments animals were maintained at high densities allowing coprophagy to take place.

Although the throughput time for *Isochrysis* was only slightly shorter than that for *Thalassiosira*, turnover times were substantially different (47 and 256 minutes respectively). A plausible explanation of the latter difference appeared to be associated with the time taken to digest the algal food and, therefore, reflected food quality. Thus, the small flagellate was processed more rapidly than the large silicaceous diatom. Although a larger bio-volume and, therefore, a larger biomass of *Thalassiosira* was ingested by the krill over the 12 hour feeding period compared to *Isochrysis*, a substantial proportion of *Thalassiosira* biomass is silica. Thus, neither cell number, cell volume, nor cell mass are by themselves definitive indicators of algal nutrient input to krill. The same applies to the trace constituent chlorophyll *a* where twice as much was ingested by krill from *Isochrysis* than from *Thalassiosira*. However, when the major nutrient lipid is concerned, the krill ingest similar quantities from the two algal species, the input from *Thalassiosira* being 74% that from *Isochrysis*. Therefore, in terms of their lipid nutritional value the difference between the two species is slight, underlining the importance of considering the macro nutritional composition of phytoplankton when relating krill filtration rates to krill production. Similarly, Clarke et al. (1988) found that feeding rate was governed by food quality of natural microbial communities, with krill increasing feeding rate to maintain energy intake where inorganic particulate load was high.

The factors controlling the rate of zooplankton feeding and their assimilation efficiency has been the matter of some debate. Beklemishev (1957, 1962) and Conover (1966) suggested that herbivorous zooplankton feed superfluously, i.e. as ingestion rate increases assimilation efficiency decreases. However, density-dependent changes in feeding rate may also occur. Quetin and Ross (1985) investigated the filtration rates of krill feeding on four species of algae of variable size and physical characteristics. They

found that for the diatoms *Thalassiosira eccentrica*, *Ditylum brightwellii*, and *Phaeodactylum tricornutum*, krill filtration rate increased with increasing particle concentration up to what they termed a 'critical concentration'. At this critical concentration filtration rates were seen to decrease, an observation which is incompatible with the concept of superfluous feeding. Feeding rate for a fourth algal species, *Isochrysis*, did not show any dependence on algal density, which Quetin and Ross suggested was because the krill were unable to filter these particles effectively.

However, the results of the present study and that of Clarke et al. (1988) indicate that food quality has an important effect on digestion rate. Consequently, it is possible that the ingestion rate of krill in the field is not limited by filtration but by the rate at which the gut can process food material. This implies that krill may adjust their filtering rates in relation to the nutritional quality of the food they ingest, in order to obtain amounts of major nutrients (protein, lipid and carbohydrate) compatible with their bodily requirements for maintenance and growth. The present study reinterprets the results of Quetin and Ross (1985) and suggests that the critical concentration was not determined by the mechanics of filtration but by the ability of the gut to process the food material. By calculating the total cell volumes from the dimensions as given by Quetin and Ross (1985), it is apparent that the total cell volume ingested by the krill was similar at the critical concentration for each of the three species of diatom ('nutritionally equivalent') i.e. 1.8×10^6 μm^3 ml^{-1} for *Thalassiosira*, 1.7×10^6 μm^3 ml^{-1} for *Ditylum* and 1.3×10^6 μm^3 ml^{-1} for *Phaeodactylum*. Thus, krill were taking a similar ration from each algal species albeit at different cell concentrations. It is also likely that no 'critical concentration' was reached for *Isochrysis* because of the high digestibility of this species, and hence the rapid rate at which the gut of the krill was able to process the ingested material. In another study, Price et al. (1988), investigating the predation of krill on copepods, found that clearance rates did not increase with increasing prey concentration. Although this form of feeding is different from filtering particles, it again suggests that the limiting factor in ingestion was the ability of the krill gut to process the copepod prey and not the ability of the krill to capture the copepods.

Food quality may have further implications for carbon cycling beyond the transfer between phytoplankton and krill. Krill faecal pellets are an important

component in the vertical flux of particulate material in parts of the Southern Ocean (Tanoue and Hara 1986; Emerson and Roff 1987). Cadée et al. (1992) found that krill feeding on diatoms produced large dense faecal pellets with high sinking rates. By contrast krill feeding on flagellates tended to produce small, thin faecal pellets with low sinking rates which were remineralised within the euphotic zone.

5.3.2 Lipid metabolism of krill.

Detailed study of lipid assimilation and fate has provided insight into krill metabolism. This section concentrates on the synthesis of fatty acids and on catabolic processes.

5.3.2.1 Fatty acid biosynthesis by krill.

Saturated fatty acids were the least efficiently assimilated of all the fatty acids, i.e. they were preferentially egested by the krill which accounts for their preponderance in faecal pellets. However, saturated fatty acids also appear to be biosynthesised within krill, possibly from radioactive amino acid or carbohydrate precursors, thus making it difficult to assess their role in energy production in the krill. The results here also provide evidence for the biosynthesis of 18:4 from 18:3 within krill, consistent with the animals possessing an active delta-6 fatty acid desaturase as is the case for all marine fish studied so far (Sargent et al. 1993). The fact that negligible levels of radioactivity appeared in 20:5 or in 22:6 in krill is evidence that krill do not convert significant quantities of C18 PUFA to highly unsaturated fatty acids (HUFA). However, this evidence is very weak because little or no radioactivity appeared in 20:5 in *Isochrysis* itself during the labelling period, although the alga which is relatively rich in this PUFA clearly has the ability to biosynthesise this fatty acid *de novo*. Therefore, the failure to observe significant amounts of radioactivity in 20:5 in both *Isochrysis* and krill should be interpreted much more as reflecting the relatively long time period required to biosynthesise 20:5 and 22:6, rather than being cited as evidence for the krill not being capable of converting C18 PUFA to C20 and C22 HUFA. Nevertheless, it has been argued in Chapter 3 that the abundance of 20:5 and 22:6 in the natural diet of krill is such that there would be little or no requirement for the animals to carry out these conversions routinely in nature.

5.3.2.2 Total lipid and fatty acid catabolism by krill.

A major finding in the present results is that krill catabolise circa 60% of the their assimilated lipid (Table 5.4) and circa 70% of their assimilated fatty acids (Table 5.5). It is of interest that all fatty acids, with the possible exceptions of saturated fatty acids and 18:4, are extensively catabolised by the krill. It is clear, therefore, that most of the fatty acids assimilated by the krill studied here were being utilised for energy production rather than being stored as an energy reserve. This is consistent with existing evidence that krill do not accumulate very large lipid reserves in nature (Clarke, 1980; 1984; Fricke, 1984). It could, of course, be argued that the animals studied here were in a relatively poor condition, having been maintained in captivity for some 9 months. This impression is reinforced by the observation that the algal and gut lipid were similar in terms of specific radioactivity which implies that the animals in this study did not contain significant stores of lipid in their hepatopancreas, an organ known to be lipid rich in wild krill. It is clear that assimilated fatty acids are a major source of metabolic energy in krill. This situation is entirely analogous to that well established for fish and which probably holds for marine animals in general. Some 50% of the fat ingested by actively growing trout is oxidised to provide metabolic energy (Cho and Kaushik, 1985) and about 60% of the fat mobilised for ovarian formation in the capelin, *Mallotus villosus*, is catabolised (Henderson et al. 1984).

Even more revealing were the data on percentage of individual fatty acids catabolised, calculated in the same way as for total lipid in Table 5.5. Thus, all assimilated fatty acids with the exception of saturates, 16:3 and 18:4 are very substantially catabolised by the krill (Table 5.5). The low catabolism and relative stability of 16:3 in krill is consistent with the increased percentage radioactivity observed for this fatty acid in krill body tissue relative to the algae (Table 5.2). The negative values of percentage catabolism for both saturated fatty acids and 18:4 in Table 5.5 represents a net formation (biosynthesis) of these fatty acids in krill tissue from assimilated radioactivity.

Virtue et al. (1993) examined possible PUFA biosynthesis of krill by rearing krill on two contrasting diets. One diet comprised the diatom *Phaeodactylum tricornutum* which was rich in 20:5(n-3) (~37% of total fatty acids) and the other, the haptophyte, *Phaeocystis pouchetii*, which contained only 1% of its fatty acids as

20:5(n-3). After a period of five months, no differences were detected in the levels of the total lipid of the krill in the two experiments. Hence, Virtue et al. (1993) suggested that *Euphausia superba* is capable of converting exogenous shorter chain fatty acids to 20:5(n-3) and 22:6(n-3). However, it is difficult, if not impossible to assess the ability of an organism to elongate and desaturate fatty acids solely on the basis of percentage composition data and a mass balance approach is required to answer this question definitively. Also, Virtue et al. (1993) utilised relatively mature krill, and the requirements for PUFA by these animals would be low and feasibly met by the low levels in the diet of *Phaeocystis pouchetii*.

In the present study the ability of krill to synthesise PUFA was not investigated directly, although the experiment which involved feeding radiolabelled algae to krill could have shed some light on this issue. However, as only limited quantities of 20:5(n-3) were labelled by *Isochrysis*, an organism known to synthesise high levels of PUFA, it is unlikely that krill would have been able synthesise appreciable quantities over the twelve hour time course of the experiment.

It has been previously established in other studies that the polar lipid content of an organism is much less variable than the neutral lipid content, which tends to fluctuate considerably with dietary input (Clarke 1984). When PUFA are incorporated into tissue and cell membranes it tends to be highly conserved and hence is a relatively static component of the lipid pool of an organism. The degree to which dietary PUFA are catabolised is likely to be determined by the requirements of these compounds by krill for growth and reproduction. Obviously these requirements are likely to alter with season and stage of maturity. In the present study, the krill utilised in the radiolabelling experiment had been in captivity for 6-9 months and were fed what probably only amounted to a `maintenance diet'. This was confirmed by analysis of their total lipid content which accounted for only 1-2% of the fresh mass of the animals, a reduction from 4-8% of fresh mass at capture. The low lipid content of these krill could account the high levels of radiolabelled PUFA which were efficiently assimilated from the algal diet, but then extensively catabolised (~ 80%). This probably reflects the low requirements for growth and reproduction of these krill.

The present study has indicated that the transfer of material between phytoplankton and krill and hence the magnitude and character of the vertical flux of

material to the deep ocean, is crucially dependent on the quality of the phytoplankton food. Gut throughput- and turnover-times are affected both by food quality and quantity, and extensive transformation of the lipid pool shows that calculation of filtration rates from some biomass-related variables may be misleading. Therefore, in attempting to model the flux of carbon in the Southern Ocean it is crucial to understand how variations in the availability and quality of the food of krill are related to gut processes.

In terms of the efficiency of assimilation of a major nutrient, lipid, and the extent to which it is used as a major source of metabolic energy, krill appear to conform to the general pattern already well established for marine animals (Sargent et al. 1993).

6. FIELD FILTRATION RATES, SIZE SELECTIVITY AND ASSIMILATION OF DIETARY FATTY ACIDS.

6.1 INTRODUCTION.

To understand fully both the trophic role of *E. superba* in the Southern Ocean pelagic ecosystem and its energetics, it is important to quantify accurately the filtering capabilities of the krill. Estimates of feeding rates of Antarctic krill have previously varied greatly from only a few ml per hour to tens of litres per hour (reviewed by Morris 1984, Table 6.1). However, even the highest of these filtration rates only allow krill to ingest ~ 5% of its body mass per day, although it is important to note that the energetic value of these ingested particles are usually based on estimates derived from cell volumes or chlorophyll *a* (Chapter 3). Previous studies on other euphausids (Mauchline 1980) have suggested that these animals are capable of ingesting 17-30% of their body mass per day. In a review of krill filtration rates and experimental procedure, Morris (1984) concluded that constant volume experiments were likely to lead to drastic underestimates of filtration rates. The use of relatively small containers and long incubations result in krill refiltering the same water, leading to drastic reductions in particle number. Morris (1984) suggested that 'bottle effects' (interactions of krill with the bottle walls) disrupted feeding and hence reduced filtration rate. More recently Price et al. (1988) showed that with similar densities of krill, the filtration rate of animals feeding in 50-litre containers was twice as great as those feeding in 5-litre containers. The lower filtration rates of krill in the 5-litre containers was attributed to more frequent encounters with the container walls.

In laboratory grazing experiments involving krill feeding on natural phytoplankton populations, Meyer and El-Sayed (1983), Schnack (1985) and Ishii et al. (1985) have established that krill feed on a wide variety of particle sizes and that the large and also the chain-forming species of diatoms are filtered with the greatest efficiency. Quetin and Ross (1985) were the first to utilise uni-algal cultures in laboratory feeding experiments, thereby avoiding the complications of natural phytoplankton assemblages. They concluded that *E. superba* feeds most effectively on relatively large particles and that it is physical dimensions and not the chemical

Table 6.1 Estimates of krill filtration rates, calculated for an animal ~50 mg dry mass (modified from Morris 1984).

Method	Filtration rate ($ml\ h^{-1}$)	Reference
Constant volume		
Chlorophyll *a*	20.3 ± 15.2	Kato et al. 1979
	257.6 ± 50.5	Meyer 1981
	46.3	Antenzana et al. 1982
	210	Boyd et al. 1984
	1411*	Price et al. 1988
> 3 mm particles	24.6 ± 9.5	Morris 1984
	175	Boyd et al. 1984
Through flow		
> 3 mm particles	947 ± 524.9	Morris 1984
Derived from		
Energy equivalents	1800 - 4700	Kils 1979
Filter basket	169 - 777	Morris 1984
Chlorophyll *a* required	320 - 5120	Morris 1984
	1000 - 20000	Ikeda and Bruce 1986
Oxygen consumption	1893	Rakusa-Suszczewski and Opalinski 1978

* animal dwt not known.

composition of a particle that determine clearance rates. The present chapter investigates the filtration rates and size selectivity of krill feeding on natural microbial populations at six sites in the Southern Ocean together with ingestion and assimilation of microbial lipid by krill at two of the sites.

6.2 RESULTS

6.2.1 Filtration rates.

Feeding behaviour of immature Antarctic krill was investigated at six sites around South Georgia and the Antarctic Peninsula using cell counts by inverted microscopy, and in one experiment a Coulter Counter was also used. Constant volume experiments (5-20 litres) were conducted using 1-6 krill which were fed natural phytoplankton assemblages (Table 6.2). Filtration rates were calculated from the equations of Frost (1972) (Chapter 2) and varied from a maximum of 446 ml krill^{-1} h^{-1} in `Graze 1' to only 30 ml krill$^{-1}$ h$^{-1}$ in `Graze 5' (Table 6.2). Apparent filtration rates were notably higher in Graze 1 and Graze 2, in which each replicate contained a single krill per 5 litres of seawater. Apparent filtration rates were much lower in Graze 3-6 and this corresponded both to higher densities of krill, i.e. 5-6 per replicate, and to lower seawater volumes per krill, i.e. only 2.5-3.3 litres. In Graze 6, seawater was sampled in time series after 0, 2, 4, 6 and 22 hours. Particulate counts increased in the first two hours and this was probably caused by the swimming krill disrupting chains of cells which resulted in a negative apparent filtration rate of -149 ml krill^{-1} h^{-1}. A maximum filtration of 458 ml krill^{-1} h^{-1} was measured between 4 and 6 hours and this reduced dramatically to only 40 ml krill^{-1} h^{-1} between 6 and 22 hours (Table 6.2). In this experiment, filtration rates were monitored using a Coulter Counter (as well as inverted microscopy) and the calculated filtration rates exhibit a similar pattern over the 22 hour incubation period to those determined by microscopy (Table 6.2). However, total particle number as determined by the Coulter Counter was approximately 5 times greater than the microscopic cell counts (data not shown), indicating that detritus was a significant component of the particulate pool and that detritus was readily ingested by

Table 6.2 Filtration rates of immature krill (~ 140 mg dwt) feeding on natural phytoplankton from South Georgia and the Antarctic Peninsula. Note, rates in brackets were obtained using a Coulter Counter.

Expt	Median (ml krill^{-1} h^{-1})	Range (ml krill^{-1} h^{-1})	Duration (hours)	Nº krill	Replicates	Vol (litres)
Graze 1	**410**	270 - 446	16	1	4	5
Graze 2	**323**	302 - 341	24	1	4	5
Graze 3	**110**	103 - 116	22	6	2	15
Graze 4	**146**	137 - 155	24	5	2	15
Graze 5	**76**	30 - 121	22	5	2	15
Graze 6	**-149**(-20)	-	0-2	6	1	20
	458 (517)	-	2-4			
	277 (240)	-	4-6			
	4 (17)	-	6-22			
	82 (80)	-	0-22			

the krill. Microscopic analysis involved counting only viable cells, with detritus and unrecognisable fragments being ignored.

6.2.2 Size selectivity.

Size selectivity, expressed as percentage of original particles grazed during six grazing experiments, is presented in Figs 6.1, 6.2. Most taxa appear to be grazed with high efficiency during the course of the experiments, although there tends to be high variation in percentage grazed both between taxa within experiments and within taxa between experiments. Overall there appears to be no conclusive evidence for selectivity of particles over the range of particles investigated, at least on the basis of their maximum linear dimensions (Figs 6.1, 6.2). However, smaller fractions, i.e. < 20 μm, are generally grazed less efficiently than larger particles > 20 μm. In Graze 3 and Graze 6, *Chaetoceros* chains of more than four cells are filtered more effectively than *Chaetoceros* chains containing three or less cells, although the opposite was true for Graze 2. Similarly *Nitzschia* > 2 cells were removed more efficiently than single *Nitzschia* at Graze 1, Graze 3 and Graze 4, although again there was a contrasting experiment, Graze 6. In Graze 3, Graze 4 and Graze 5, the rate of removal of *Thalassiothrix* did not differ from the other taxa present even though its maximum linear dimension was between one and two orders of magnitude greater than most other taxa present (Figs 6.1, 6.2).

6.2.3 Assimilation of fatty acids by krill feeding on natural particulates.

Grazing experiments were conducted on board ship using ambient microplankton concentrations at sites AP1 and AP2 (i.e. Graze 1 and Graze 2,). By collecting carefully all faecal pellet material produced during these grazing experiments and by knowing the lipid content and composition of the microplankton (Chapter 3), it was possible to calculate assimilation efficiencies for the krill in terms of total fatty acids (Table 6.3). In both shipboard grazing experiments the assimilation of total fatty acids was high, i.e ~86%. However, the quantity of fatty acids assimilated per hour was approximately two times higher for those krill feeding on a dense food supply of diatoms (*Proboscia inermis*, Chapter 3) in Graze 1 (conducted at AP1), than krill

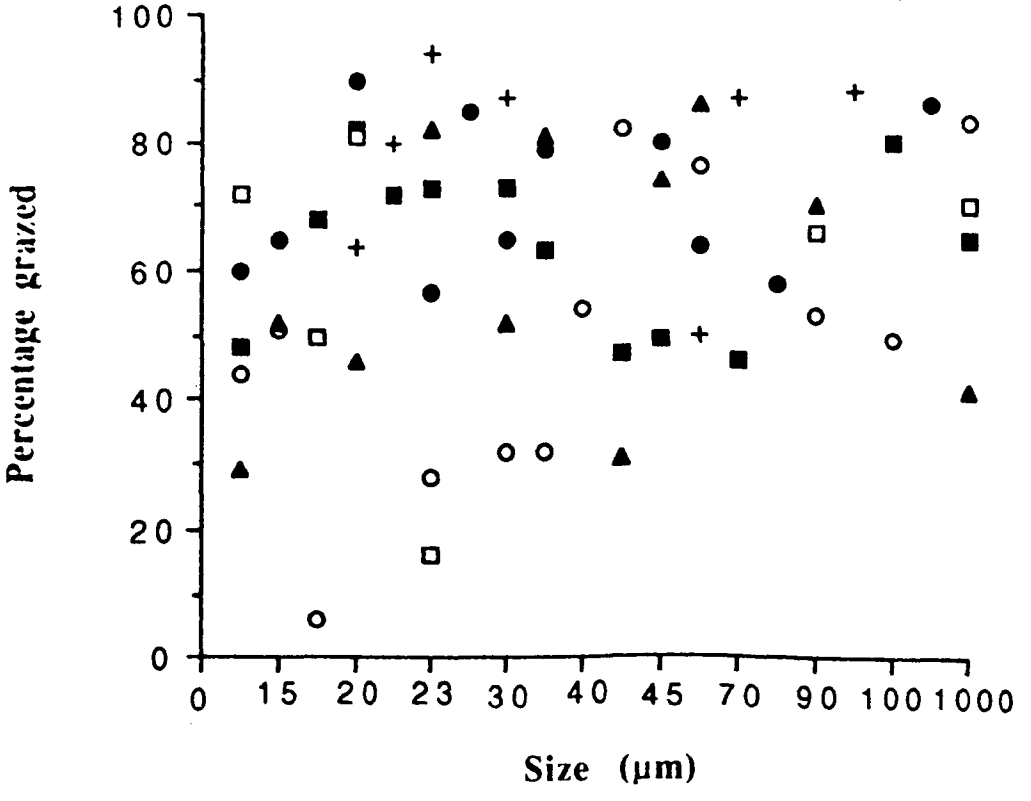

Fig 6.1 Percentage of cells grazed by immature krill feeding on a range of particulate size catagories. (+ Graze 1, ● Graze 2, o Graze 3, ▲ Graze 4, ■ Graze 5, □ Graze 6).

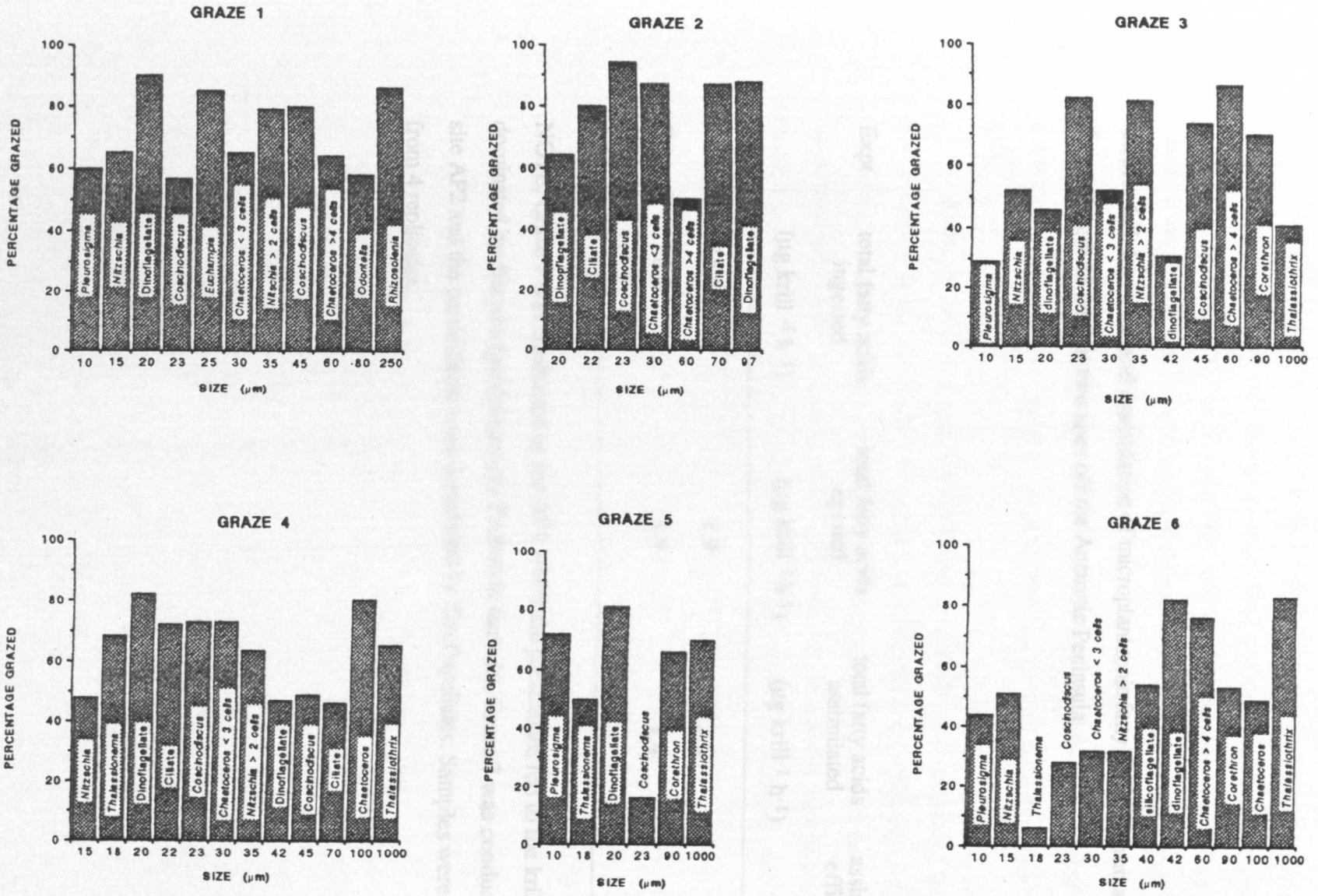

Fig 6.2 Percentage of cells grazed by immature krill feeding on a range of microplankton.

Table 6.3 Ingestion and assimilation of microplanktonic fatty acids by immature krill (mean length 45 mm) at two sites off the Antarctic Peninsula.

Expt	total fatty acids ingested (μg krill $^{-1}$ h^{-1})	total fatty acids egested (μg krill^{-1} h^{-1})	total fatty acids assimilated (μg krill^{-1} h^{-1})	assimilation efficiency (%)
Graze 1	15.5	1.9	13.6	88.2
Graze 2	8.8	1.4	7.4	84.0

NOTE; Graze 1 was conducted at site AP1 where the particulates fed to the krill were dominated by diatoms (predominantly *Proboscia inermis*). Graze 2 was conducted at site AP2 and the particulates were dominated by dinoflagellates. Samples were pooled from 4 replicates.

feeding on dinoflagellates in Graze 2 (conducted at AP2).

In both Graze 1 (AP1) and Graze 2 (AP2) the proportion of saturated fatty acids increased markedly between the particulate food and the resulting faecal pellets, i.e. from 25% to 58% for Graze 1 and from 30% to 54% for Graze 2 (Table 6.4). By contrast, the proportions of polyunsaturated fatty acids decreased considerably between the particulates and the faecal pellets (i.e. from 40% to 11% for Graze 1 and from 31% to 8% for Graze 2) and the proportions of monounsaturated fatty acids tended to remain constant (~25%) between particulates and faecal pellets for both experiments (Table 6.4).

Assimilation efficiencies of individual fatty acids were calculated for Graze 1 and 2 (Table 6.4). Overall, PUFA were assimilated more efficiently than either monounsaturated or saturated fatty acids. However, it is of note that all fatty acids were assimilated efficiently, from a minimum of 64% for the saturated fatty acid 14:0 in Graze 1 to 99% for the polyunsaturated fatty acids; 20:4(n-3), 20:5(n-3) and 22:6(n-3) for both Graze 1 and 2 (Table 6.4).

Table 6.4 Percentage compositions of fatty acids in the total lipids of particulates fed to immature krill in two grazing experiments (i.e. Graze 1 and Graze 2 conducted at sites AP1 and AP2 respectively). Also shown are the lipid compositions of the resulting faecal pellets and the calculated assimilation efficiencies of ingested fatty acids.

	Graze 1 (AP1)			Graze 2 (AP2)		
	Particulates	Faecal pellets	Assimilation efficiency	Particulates	Faecal pellets	Assimilation efficiency
	(%)	(%)	(%)	(%)	(%)	(%)
14:0	2.5	7.3	64	1.1	6.4	70
16:0	11.7	22.9	76	10.6	21.9	67
16:1	11.1	7.6	92	4.6	8.6	70
16:4	5.0	3.7	91	0.0	0.0	-
18:0	3.7	14.8	51	7.1	15.7	65
18:1(n-9)	5.8	12.0	75	12.0	12.4	84
18:1(n-7)	3.2	2.1	92	9.0	3.1	95
18:2(n-6)	3.2	2.2	92	3.1	2.6	85
20:4(n-6)	1.4	0.0	99	0.8	0.0	99
20:5(n-3)	19.0	0.3	99	9.8	0.2	99
22:6(n-3)	1.3	0.3	99	13.0	0.2	99
Saturates	24.5	57.6	71	29.5	53.7	71
Monounsat	23.8	22.1	89	28.5	27.5	84
PUFA	39.6	10.6	97	31.3	7.5	96

6.3 DISCUSSION

6.3.1 Filtration rates.

In the six shipboard experiments utilising natural phytoplankton assemblages, filtration rates were variable ranging from a maximum of 446 ml krill^{-1} h^{-1} in Graze 1 to a minimum of 30 ml krill^{-1} h^{-1} in Graze 5. This variation can probably be attributed more to variation in experimental conditions than to differences in the behaviour of the krill or their supplied food ration (Table 6.2), confirming the findings of Price et al. (1988) that filtration rates are influenced greatly by krill density and chamber volume. The present study necessitated a compromise between obtaining valid filtration rates and providing sufficient faecal pellet material for lipid analysis, hence relatively high krill densities and long incubation times were used. Although the experiments have not improved on previous constant volume estimates of filtration, they do indicate that the krill were feeding actively and ingesting particulate material. Indeed the filtration rates measured in this study are comparable with previous measures of the filtration capabilities of krill as presented in Table 6.2, although it should be noted that the literature values are calculated for animals of ~ 50 mg dry mass, approximately three times smaller than the dry mass of animals used in the present study. The validity of converting the filtration rates of animals to a standard dry mass is questionable, especially as different sized animals are likely to have different metabolic costs as well as size-specific differences in filtration capabilities. Hence in this study, filtration rates and animal dry mass are both given as measured.

It is noteworthy that particulate counts using a Coulter Counter were approximately five times higher than those for microscopic counts. As apparent filtration rates were similar for both viable cells as determined by microscopy and total `particle' counts as determined by Coulter Counter, the implication is that the krill were ingesting significant quantities of detritus, the origin and nutritional value of which was unknown.

6.3.2 Size selectivity.

Despite long incubation times and drastic particle reduction, some features are

evident from the size selectivity data presented in Figs 6.1, 6.2. No obvious particle size selection appears to be taking place and this is not surprising considering the filtration mechanism of krill. Krill filter particles from the water by rapid expansion and contraction of a feeding basket formed with their thoracic setae (Boyd et al. 1984). It is difficult to envisage a basket mechanism that, when expanded, contains ~ 0.2 ml of seawater (Boyd et al. 1984) and whose mesh diameter of ~2-3 μm is capable of 'selecting' particles. The energetic cost of forcing water through such a fine mesh is likely to be very high. Also the low Reynolds number at this scale is likely to cause water and hence particles to flow over the mesh rather than through it (Gerritsen and Porter 1982). It is likely that above ~10 μm, all particles are retained with similar efficiency, as suggested by Boyd et al. (1984) who found that particles > 6 μm were retained with 50% efficiency, whereas particles > 12 μm were retained with 100% efficiency. Size selectivity has been observed in the feeding behaviour of copepods (Frost 1972; Boyd 1976; Schnack 1985), although it should be emphasised that these animals tend to use different filtration mechanisms (Gerritsen and Porter 1982). It should also be noted that copepods are considerably smaller than krill, and often only slightly larger than their particulate food.

6.3.3 Assimilation of fatty acids by krill feeding on natural particulates.

The assimilation of fatty acids from natural particulates by immature krill was very efficient, i.e. 88% and 84% for krill feeding on diatoms and dinoflagellates respectively. These high assimilation efficiencies are not surprising considering the energetic and biochemical value of these compounds to krill. Polyunsaturated fatty acids are assimilated particularly efficiently (~99%), and this probably reflects the critical functions of these essential nutrients in krill metabolism and development. It should be stressed that both saturated and monounsaturated fatty acids, although not assimilated as efficiently as PUFA, are still assimilated efficiently (70-90%). As the lipid content of the faecal pellets of krill comprises largely saturated and monounsaturated fatty acids, this has important implications for the downward flux of particulate material which is likely to be of poor nutritional quality to deep sea plankton and benthos.

7 GENERAL DISCUSSION.

This chapter seeks to collate the findings presented in the previous chapters and to discuss the major findings in a wider context. As a framework it attempts to construct a lipid budget for *Euphausia superba* by utilising the various measures of lipid pools and fluxes quantified in this study and supplementing these with literature values where necessary

7.1 Lipid budget for immature krill.

For the immature krill analysed during this project, an average animal with a total length of 39 mm and 0.47 g fresh mass contains ~ 12.8 mg of fatty acid. At the time of the present study, microplankton around South Georgia contained mean fatty acid levels of 122 $\mu g\ l^{-1}$. Grazing experiments conducted in bottle incubations around South Georgia and the Antarctic Peninsula indicated filtration rates of ~ 300 ml krill h^{-1} (Chapter 6). However, it is now widely accepted that bottle incubations tend to underestimate the true filtration capabilities of krill and hence a filtration rate of 1 l h^{-1} is likely to be more realistic (Morris 1984; Quetin et al. 1993). Therefore, a plausible filtration rate of one litre per hour would allow an immature krill in the present study to ingest 2.9 mg of microplanktonic fatty acid per day which equates to ~ 23% of the animal's fatty acid content (Fig 7.1). This is consistent with the suggested daily food requirement for krill (17-28% of body carbon per day - summarised by Quetin et al. 1993), although it assumes that krill filter particles non-selectively (as indicated in Chapter 6) because the microplanktonic lipid was distributed throughout a range of particle sizes. Assimilation by krill of fatty acids in ingested microplankton was approximately 90% for both field and laboratory grazing experiments (Chapter 5 and 6) and this accords well with literature assimilation efficiencies of 60-95% for total carbon for other species of zooplankton (Conover 1966; Mauchline 1980; Landry 1984). Therefore, of the 2.9 mg fatty acid ingested $krill^{-1}\ day^{-1}$, 2.6 mg is assimilated and thus available for metabolism, storage and growth (Fig 7.1). In Chapter 5 it was shown that ~70% of assimilated fatty acids were rapidly catabolised which implies that 0.8 mg fatty

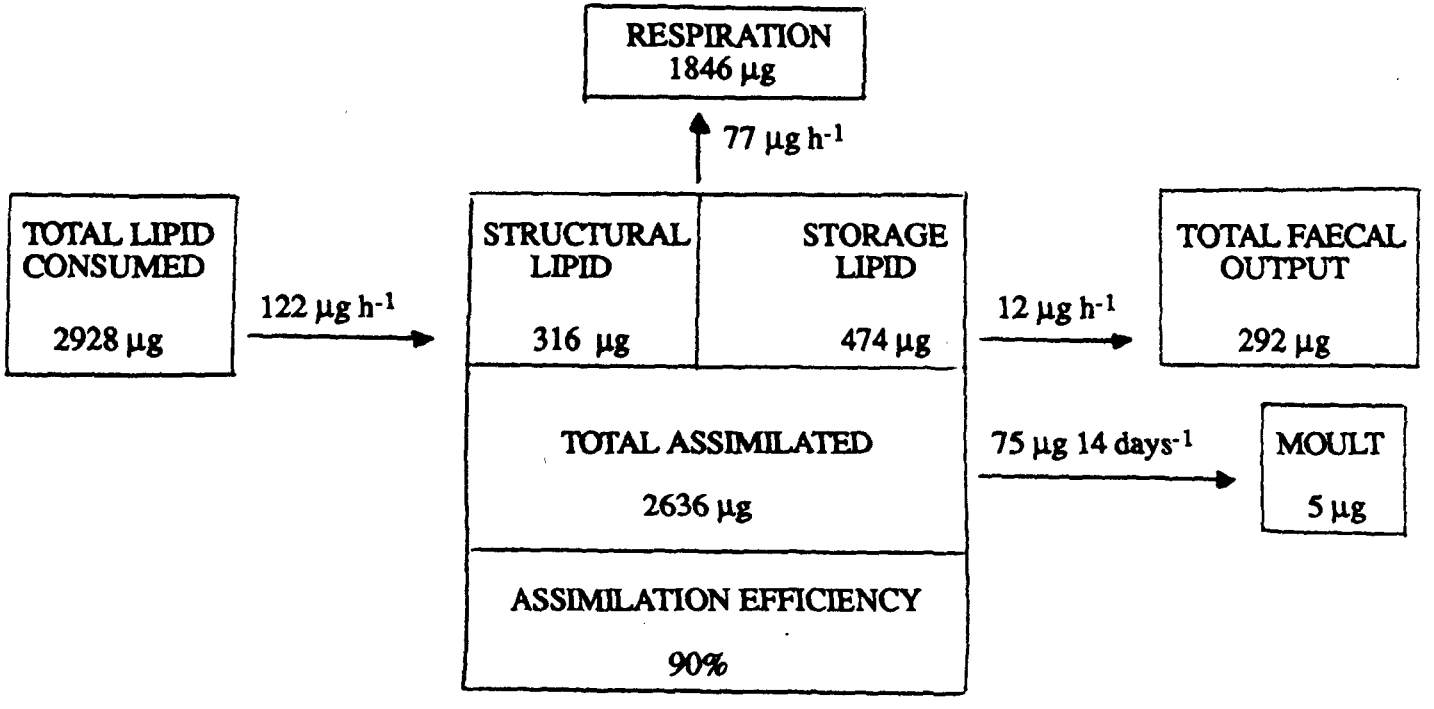

Fig 7.1. Lipid budget calculated for immature *Euphausia superba* (~ 39 mm length) for 24 hour period.

Assumptions.

1. Animal constantly filters at rate of 1 litre h^{-1} for 24 hours.
2. 70% of assimilated lipid is respired.
3. That 40% and 60% of assimilated, non-catabolised lipid enters structural and storage lipid pools respectively.
4. The animal moults every two weeks.

acid day^{-1} is available for growth and storage (Fig 7.1). Analyses of the lipid class composition of immature krill (Chapter 4) indicated that polar and neutral lipid comprised approximately 40% and 60% respectively. For the purpose of this budget, it is assumed that 40% (0.3 mg fatty acid) of the fatty acids are channelled into growth, whereas the remaining 60% (0.5 mg fatty acid) are stored for future metabolic requirements (Fig 7.1). Thus, if an immature krill with a lipid content of 12.8 mg channels each day 0.3 mg of its dietary fatty acid into growth, this implies a growth rate in terms of lipid at least of ~ 2.3% day^{-1}.

Faecal pellet production amounts to ~ 0.3 mg fatty acid krill^{-1} day $^{-1}$ and this may contribute greatly to the flux of organic carbon to the deep ocean sediments (Chapter 6; Fig 7.1; Clarke et al. 1988; Wefer at al. 1988). In terms of lipid, moulting appears to incur the relatively small cost of ~ 5.3 μg fatty acid day^{-1} and though moults do not appear contain substantial quantities of fatty acids, i.e ~ 75 μg for 45 mm krill, they are comparatively rich in PUFA (Appendix 3) which confirms the findings of Tanoue and Hara (1986). Hence, the high PUFA content coupled with the high sinking rates of moults (Nichol and Stolp 1988), is likely to contribute greatly to the downward flux of fatty acids and in particular PUFA. This may be of considerable importance to the nutrition of deep sea organisms.

7.2 Reproductive investment by female krill.

To investigate the roles of lipid in the reproductive strategy of female krill, it is possible to predict the lipid content for a non-reproducing animal, as large as the median female krill, by extrapolating from the median fatty acid content of immature animals. On this basis an immature krill of the same wet mass as the median value for females (1.5 g) would contain 39 mg fatty acid. Thus, the female krill in this study tended to contain ~ 15 mg more fatty acid (observed median value 54 mg) than extrapolated from the composition of immature animals.

Therefore, taking an extrapolated value of 39 mg fatty acid for a non-reproducing female and assuming that female krill are able to ingest 23% of their body fatty acids per day (as has been shown to be the case for immature krill) this would imply a daily requirement of 9 mg of fatty acids per day. Assuming assimilation of

fatty acid to be ~ 90% and the fatty acid content of the microplankton to be 122 $\mu g\ l^{-1}$, a daily intake of 9 mg fatty acid day^{-1}, requires a filtration rate of 3.4 litres female krill $^{-1}$ h^{-1}. When considering the large size of these adult females, this seems perfectly feasible, especially when compared with published filtration rates (Morris 1984, Quetin et al. 1993). Furthermore, by taking into account the 70% catabolism of dietary fatty acids (Chapter 5) it can be calculated that an `extrapolated' female krill could feasibly ingest a surplus of 2.7 mg of fatty acid per day.

Thus, it can be surmised that the apparent surplus of fatty acid in female krill around South Georgia, compared to the fatty acid content of female krill predicted from immature animals, may be associated with egg production. It is possible to estimate the lipid cost of egg production using the published data and assumptions of Clarke (1980), namely that a gravid female krill immediately prior to spawning contains 6.3% of lipid fresh mass, that 54% of this lipid is lost during spawning and that a single egg contains ~ 7.1 µg fatty acid. Thus, a female krill of 1.5 g, the median fresh mass of female krill in the present study, would require 36 mg of fatty acid to produce 5400 eggs in a season. The assumptions of Clarke (1980) were based on the observation that krill spawn once per year around South Georgia (Everson 1976). However, repeat spawning is documented for other areas (Ross and Quetin 1982; 1983; Quetin et al. 1993) and may also be the case at South Georgia under some circumstances. The amount of fatty acid estimated to be required for a single spawning event, i.e. 36 mg is substantially higher than the difference - ca. 15 mg – between the observed median fatty acid content in female krill in this study and the predicted somatic fatty acid content. However, in this context it is of note that gravid females were not encountered in any of the samples.

Taking the previously suggested fatty acid surplus of 2.7 mg day^{-1}, female krill around South Georgia at the time of this study could feasibly accumulate the required 36 mg of fatty acids for a season's egg production in 13 days. This of course assumes that : a, the female krill feed constantly at a high rate, and b, none of the dietary fatty acid is utilised for growth. As neither of these assumptions are likely to be correct, the true time-scale over which female krill accumulate the lipid necessary for reproduction is likely to be longer. Even so, it is apparent from this study that the female krill are

capable of acquiring the lipid associated with egg production in a relatively short time and certainly within a single summer.

7.3 Reproductive investment by male krill.

Using the same approach as was adopted for female krill, it is possible to estimate a lipid content for 'non-reproducing' male animals as large as the median-sized krill by extrapolating from data for immature animals. This predicts a fatty acid content of 31 mg for the median male of 1.2 g fresh body mass, whereas the observed median fatty acid content for male krill in this study was only 15 mg. Thus, male krill tended to have lower lipid content than predicted, the converse of the situation for females. The analysis of lipid class composition, including the multivariate analysis, suggests that there was a deficiency of TAG, ie. the animals had utilised reserve material (Chapter 4).

Again, if it is assumed that male krill require to assimilate the equivalent of 23% of their body fatty acids per day, this implies a daily fatty acid requirement of 6.2 mg fatty acids (i.e. 23% of body fatty acids). Given microplankton fatty acid concentrations of 122 $\mu g\ l^{-1}$, male krill could fulfil its predicted requirements with filtration rate of 2.6 $l\ h^{-1}$. The observed fatty acid content of the males at SG1-SG6 was only ~ 15 mg fatty acids, i.e. a deficit of 16 mg from the predicted fatty acid content. With a daily ration of 6.2 mg fatty acid per day and assuming 70% catabolism of dietary fatty acids, a male krill could achieve the predicted lipid content for a non-reproducing male in only 8 days, although again for reasons given previously, this is likely to be a minimum estimate.

At first sight, it is hard to account for the lipid deficit in male krill in terms of reproductive cost. Compared with female krill, with their very large investment of lipid towards reproduction (around twice the somatic lipid content), the costs of male reproduction i.e spermatophore production, would appear to be low. However, it is emerging that males of a wide range of taxa may incur a similar reproductive cost to that of females in a range of species. Thus, Van Voorhies (1992) suggested that sperm production by nematodes was more energetically costly that previously thought. Hopkins et al. (1984) noted that male copepods engage in sustained physical activity

during reproduction and established that the utilisation of lipid by male copepods during reproduction is very substantial and similar to that of females. For male krill, successful reproduction also requires sustained physical effort since females shed spermatophores when they moult and hence male krill may need to produce two spermatophores per week (Watkins et al. 1992). Thus, the energetic costs of searching for females and affixing spermatophores, could account for the lipid expenditure by male krill as indicated in this study. Circumstantial support for this hypothesis is provided by the composition of male krill from sites SG7 and SG8 (median value 37 mg of fatty acid per animal), where female krill were absent from net hauls and the lipid content of males was similar to the amount predicted for a non-reproductive animal of similar size. It can be surmised that these animals had not been breeding. Their lipid content was significantly higher than the median value for animals from sites SG1-SG6 (median value 16 mg) where females were present and where presumably most of the males were breeding.

Thus, the present data are consistent with the conclusion that the reproductive costs to males and females may account for their different lipid contents and compositions (Chapter 4). These costs also differ in the timescale over which lipid is expended. Females accumulate large amounts of lipid for spawning during the summer season (Clarke and Morris 1983) showing elevated TAG levels and probably building up a lipid reserve which is itself approximately twice the lipid content of a non-reproductive animal of similar size. In contrast, males invest their reproductive effort in a continuous series of events over the season, each of which has a smaller lipid cost but which collectively represent a substantial depletion of reserve lipid.

7.4 Conclusion

The over-riding conclusion from the present study, that *Euphausia superba* is not the "enigma" it once was, at least as revealed by its lipid metabolism, should not obscure the fact that much remains to be done in this field. In particular, it is obviously now extremely important to study krill overwintering under sea ice to resolve once and for all whether the animals fed significantly under these conditions and, if so, on what? In addition, we know nothing of the growth and survival rates of early nauplii and juvenile krill, and how these rates may be influenced by environmental variables.

Application of the lipid technology as used in the present study has obvious potential in advancing knowledge in these areas.

Formidable and frequently severe difficulties will always exist in studying krill in the field, particularly during the two key phases in their life history mentioned above. Therefore, every opportunity should be taken, even opportunistically, to accumulate relevant field evidence. However, experiences during the present study point to an alternative approach that can complement field studies, namely the relative ease with which krill could be transported from the Antarctic to the U.K. and kept alive in apparently good condition in the aquarium for up to a year. Aquaria and mariculture technologies have advanced rapidly in recent years and this is one obvious area where great potential exists for advancing knowledge of krill biology in the immediate future, particularly in studying early life stages. Mariculture of krill, whether on Antarctic bases or in the U.K. can never of course substitute completely for field studies but the approach can provide clear cut answers in certain areas and, as important, help formulate exact questions that may be answerable during limited opportunities available for field study.

Finally, perhaps the most important area of all for future research is to extend approaches such as used in the present research to undersatnd more fully the interactions between krill and its predators, and thereby advance knowledge of how the Antarctic marine ecosystem operates as an integral whole. Central to such studies must be the continuing realisation that production in the Southern Ocean operates efficiently and effectively at sea temperatures very close to freezing point. The special biochemical adaptations that enable both autotrophic and heterotrophic organisms to be productive at such temperatures remain largely unknown and, whilst this is the case, we remain ill equipped to explain, far less predict reliably, how the Antarctic ecosystem is likely to respond to both short and long term climate change.

For these and many other basic and applied scientific reasons, the Antarctic ecosystem will remain a particularly challenging and intriguing area of study for at least as many years in the future as in the past.

SUMMARY

1. Microplankton at five sites off South Georgia was dominated by a range of diatoms. *Phaeocystis* was present at three of the sites but in low abundance only. Diatoms dominated a site near the Antarctic Peninsula whereas dinoflagellates dominated at a site near Deception Island.

2. At five sites around South Georgia particulate fatty acid levels ranged from 37 to 195 $\mu g\ l^{-1}$ with an overall mean of 107 $\mu g\ l^{-1}$. At thirteen sites around the Antarctic Peninsula, particulate fatty acid levels were generally lower, ranging from 32 to 76 mg l^{-1} with a mean of 50 $\mu g\ l^{-1}$.

3. A wide variety of natural microplankton populations provided an adequate diet for krill.

4. Chlorophyll *a* biomass did not provide a reliable indicator of food availability because it was not correlated with the lipid content and composition of the microplankton.

5. Given the abundance of polyunsaturated fatty acids in the Antarctic microplankton, it is unlikely that krill require to elongate and desaturate dietary fatty acids.

6. Over the eight sites around South Georgia, immature krill contained higher median proportions of total lipid i.e 4.2% of fresh body mass compared with 3.7% for females and 2.1% for males. However, female krill generally contained the highest absolute levels of total lipid with an overall median of 70 mg. Male and immature krill contained median total lipid contents of 21 mg and 18 mg respectively. Triacylglycerol and phosphatidylcholine were the major lipid classes found in all sex and maturity categories of krill.

7. Site to site variability in lipid composition of male, female and immature krill

(primarily the neutral storage lipid TAG) is likely to result from both spatial and temporal factors

8. Gut throughput and gut turnover of food was determined by the composition of the food supply.

9. Assimilation of dietary fatty acids was very high, i.e 90-95%.

10. Approximately 70% of assimilated dietary fatty acids were rapidly catabolised.

11. The construction of a lipid budget confirms the high energy requirements/throughput of krill.

12. Differing lipid contents of males and females in relation to the values predicted from immatures reflected the differing time-scales of their reproductive strategies.

Appendix 1 Equations for the calculation of cell volumes based on standard geometric configurations.

Cylinder $V = \frac{\pi \times L \times B^2}{4}$

Cone $V = \frac{\pi \times L \times B^2}{12}$

Ellipsoid/sphere $V = \frac{\pi \times L \times B^2}{6}$

Appendix 2 (Reproduced from Culture Collection of Algae and Protozoa, CCAP manual 1988) Guillards F2 media for marine algae (+ Si for diatoms)

$NaNO_3$	0.075 g
$NaH_2PO_4 . 2H_2O$	0.00565 g
Trace elements stock solution (1)	1.0 ml
Vitamin mix stock solution (2)	1.0 ml
Make up to 1000 ml with autoclaved seawater	
Adjust pH to 3.0 - 4.0 with HCL	
Add Sodium Metasilicate stock solution (3)	0.3 ml
while stirring	
Adjust pH to 8.0 with 1N NaOH or 1N HCL	

Stock solutions:-

(1) Trace elements	Amounts per 1000 ml
Na_2 EDTA	4.360 g
$FeCl_3 . 6H_2O$	3.150 g
$CuSO_4 . 5H_2O$	0.010 g
$ZnSO_4 . 7H_2O$	0.022 g
$CoCl_2 . 6H_2O$	0.010 g
$MnCl_2 . 4H_2O$	0.180 g
$Na_2MO . 2H_2O$	0.006 g

(2) Vitamin mix	Amounts per 1000 ml
Cyanocobalamin (Vitamin B12)	0.0005 g
Thiamine HCL (Vitamin B1)	0.1 g
Biotin	0.0005 g

(3) Sodium metasilicate	Amount per 1000 ml
$Na_2SiO_3 . 5H_2O$	100.0 g

Appendix 3 Fatty acid composition of 4 immature krill moults (length 39 - 45 mm). Mean fatty acid content per moult = 75 μg.

Fatty acid	(%)
14:0	6.9
16:0	23.4
16:1	12.5
17:0	0.9
18:0	2.2
18:1	18.5
18:2	2.4
20:5	11.6
22:0	2.1
22:6	7.5

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