THE ROLE OF MICROZOOPLANKTON IN CARBON CYCLING IN THE SOUTHERN OCEAN

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by

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PREFACE

This thesis comprises a series of chapters organised as scientific papers, some of which have been published, others which are in press or have been submitted to journals. Each chapter appears in paper format and as a consequence, there is some degree of repetition, particularly in the introduction of each chapter and reference sections.

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DECLARATION

The work described in this thesis was carried out in the Department of Zoology and Entomology, Rhodes University under the supervision of Christopher McQuaid and Renzo Perissinotto. These studies represent an original work by the author and have not been submitted in any form to another university.

ABSTRACT

A 3-year study was carried out on the role of microzooplankton in carbon cycling in the south Atlantic and the Atlantic sector of the Southern Ocean. Microzooplankton grazing impact on phytoplankton was estimated during austral summer and winter employing the dilution technique. Carnivory by larger zooplankton on microzooplankton during summer was estimated using *in vitro* incubations. Microzooplankton assemblages were always dominated by protozoans comprising ciliates and dinoflagellates. Densities in winter (< 1000 cells l^{-1}) were, however, approximately 50% lower than summer densities (> 1500 cells l^{-1}). During summer, when larger microphytoplankton cells (> 20 μm) dominated total chlorophyll, microzooplankton removed ≈ 15% of the initial standing stock or < 25% of the daily potential phytoplankton production. Size selectivity experiments showed that microzooplankton preferentially feed on the nano- (20 - 2.0 μm) and picophytoplankton (< 2.0 μm) chlorophyll fractions. Indeed, during summer the grazing impact of microzooplankton was significantly correlated with the contribution of the < 20 μm fraction to total chlorophyll ($P < 0.05$). In the < 20 μm chlorophyll fraction, microzooplankton grazing was sufficient to control the growth of the nano- and picophytoplankton suggesting that, where larger microphytoplankton cells dominate, microzooplankton maintain the background concentrations of the nano- and picophytoplankton. During winter, when small nano- and picophytoplankton cells dominate total chlorophyll concentrations, the microzooplankton grazing impact on phytoplankton is dramatically increased. Microzooplankton removed on average 37% of the initial phytoplankton stock or ≈ 70% of the daily phytoplankton production. These results suggest that in winter, microzooplankton are the main sink for phytoplankton production. Carnivory experiments conducted with selected meso- (copepods) and macrozooplankton (euphausiids and tunicates) showed that all species examined consumed microzooplankton in the presence of substantial chlorophyll concentrations. Microzooplankton can, therefore, be regarded as trophic intermediates between bacterioplankton, small phytoplankton cells and larger zooplankton species in the Southern Ocean. The results of this investigation suggest a spatio-temporal shift in efficiency of the biological pump mediated by changes in the size composition of the phytoplankton assemblages. South of the Antarctic Polar Front (APF) large

microphytoplankton cells dominate the summer chlorophyll biomass, suggesting that larger zooplankton grazers represent the main sink for phytoplankton production. Under these conditions, carbon flux to the interior of the ocean will be high due to diel vertical migrations by grazers and the production of large, fast sinking faecal pellets. The sedimentation of large phytoplankton cells also contributes to flux. In the permanently open waters south of the APF and throughout the Southern Ocean during winter, small phytoplankton cells dominate total chlorophyll, resulting in the microbial loop being the main sink for phytoplankton production. The close coupling between the microzooplankton and the microbial loop dramatically reduces the transfer of organic carbon from the surface layers to depth. Carnivory by metazoans on microzooplankton may reduce the high grazing impact of microzooplankton and, may also represent an important source of carbon flux originating from the microbial loop.

CHAPTER 1

GENERAL INTRODUCTION

Human activities have led to considerable anthropogenic emissions of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (Siegenthaler & Sarmiento, 1993; Kerr, 1995). Greenhouse gases trap longwave infra-red reradiation from the earth, thereby, reducing heat losses. Increased emmissions of CO_2 from burning of fossil fuels and reduced uptake through tropical deforestation have increased atmospheric CO_2 concentrations from the pre-industrial values of 280 p.p.m. to present day concentrations of 355 p.p.m. (Siegenthaler & Sarmiento, 1993). Based on these rates of increase in CO_2 levels, the International Panel on Climatic Change (IPCC) concluded that the mean global increase in temperature would be about 0.3 ± 0.2-0.5°C per decade, with the greatest increases occurring at the polar regions (Watson *et al.*, 1990). More recently, however, the estimates of temperature increase have been revised in the light of recent findings which suggest that aerosols induce atmospheric cooling (Kerr, 1995). The latest model incorporating both aerosols and the increases in atmospheric CO_2 levels predict a 0.08 to 0.30°C per decade. These increases in temperature are likely to result in the melting of the ice caps and consequent sea level rise (Mikalajewicz *et al.*, 1990; Nerem 1995). Changes in climatic conditions such as rainfall patterns are also expected to occur (Lindesay, 1990; Rosenwig & Parry, 1994).

Despite the production of ≈ 7 Giga (G) t C y^{-1}, atmospheric increases of anthropogenic CO_2 account for only half the emissions (Siegenthaler & Sarmiento, 1993). Models of global carbon uptake by the sea yield a mean uptake of 2 ± 0.6 G t y^{-1}, leaving 1.8 ± 1.3 G t C unaccounted for. Tans *et al.* (1990) have proposed the existence of a large "missing sink", probably located in the terrestrial biosphere. The uncertainties on the role of the sources and sinks of CO_2 arise largely because of the incomplete understanding of the terrestrial and oceanic sources and sinks for atmospheric CO_2 (Denning *et al.*, 1995; Francey *et al.*, 1995).

The world's oceans are the largest of global carbon reservoirs and the major sink for anthropogenic carbon (Longhurst, 1991; Williamson & Gribbin, 1991). The oceans contain ≈ 95% of the total circulating carbon within the biosphere and, therefore, control atmospheric CO_2 concentrations (Siegenthaler & Sarmiento, 1993). The sequestration of atmospheric carbon by the oceans is mediated by numerous processes operating over several temporal and spatial scales including physical (solubility pump, see Siegenthaler & Sarmiento, 1993) and biological processes. The biological processes which sequester CO_2 are collectively termed the **biological pump** (Longhurst & Harrison 1989; Longhurst, 1991; Siegenthaler & Sarmiento, 1993). This discussion will focus on the role of biological processes in sequestering atmospheric CO_2. It should be noted, however, that the biologically mediated flux is small when compared with the transfer of CO_2 across the surface waters driven by diffusion and solubility (Siegenthaler & Sarmiento, 1993).

The marine biota acts as a carbon pump by producing particulate (POC) and dissolved organic carbon (DOC) through the processes of photosynthesis by phytoplankton cells, sinking of dead or senescent plant cells, animal debris, and the feeding and migratory activities of zooplankton (Longhurst, 1991; Karl *et al.*, 1991; Mann & Lazier, 1991). The net effect of these processes is a reduction in the partial pressure of CO_2 in the surface waters and a resulting drawdown of atmospheric carbon (Longhurst & Harrison 1989; Longhurst, 1991; Siegenthaler & Sarmiento, 1993). In addition, it also provides a source of carbon to the deep ocean (Siegenthaler & Sarmiento, 1993). It should be noted, however, that the properties of the sea surface are not uniform over a range of spatial and temporal scales suggesting variability in the efficiency of the biological pump. At steady state, however, the biogenic flux to the deep waters is largely offset by an equally large transport of organic carbon via upwelling, respiratory losses associated with all biological activity, and diffusion processes across the surface layer (Huntley *et al.*, 1991; Longhurst, 1991). Thus, with the exception of a relatively small fraction of organic carbon that accumulates in the ocean sediments, there is no net uptake of atmospheric CO_2 by the biological pump. The organic carbon which accumulates in the oceanic sediments remains inactive for between 100 and 1000 years and can therefore be regarded as removed from the carbon cycle (Siegenthaler & Sarmiento, 1993). The dynamics of carbon uptake mediated by the biological pump in the ocean are, therefore,

determined by the rate and magnitude of the downward transport of DOC and POC from the surface waters to the oceans depths (Longhurst & Harrison, 1989; Longhurst, 1991).

The two major biological pathways for the transfer of particulate carbon from the surface waters to the deep ocean are the sinking of dead or senescent phytoplankton cells (von Bodungen *et al.*, 1986) and the grazing activity of zooplankton (Longhurst & Harrison, 1989; Longhurst, 1991). Generally, the contribution of dead or senescent plant cells to vertical flux is considered minor. Exceptions include periods when dense phytoplankton blooms occur such as those found at oceanic fronts or during ice melt in summer where sinking cells contribute significantly to carbon flux (Fischer *et al.*, 1988). For example, a recent study conducted during the ice retreat in the Scotia-Weddell Sea showed that ≈ 12% of the total phytoplankton standing stock was transported from the surface waters to depth through sedimentation (Cadee, 1992).

It is widely accepted that the community structure of the consumers dramatically affects the transfer of carbon within the pelagic subsystem (Michaels & Silver, 1988; Roman *et al.*, 1993). In particular, the partitioning of phytogenic carbon between the "**classical food web**" and the "**microbial loop**" determines the magnitude of carbon flux to depth (Sherr & Sherr, 1988; Longhurst, 1991; Fortier *et al.*, 1994). Thus, the partitioning of phytoplankton production between the two food webs dramatically affects the efficiency of the biological pump.

In the classical food web, large metazoan grazers (macrozooplankton such as tunicates and euphausiids) represent the dominant grazers. In areas where metazoans dominate, carbon flux to the interior of the ocean will be high due to the production of large, compact, fast-sinking faecal pellets which have a relatively high carbon content (Fortier *et al.*, 1994). For example, tunicates such as salps produce faecal pellets which can sink up to 2700 m d^{-1} and have a carbon content of up to 37% (Fortier *et al.*, 1994). Diel migrations, both within the euphotic zone and between the euphotic zone and the mesopelagic region, further contribute to the vertical flux of carbon through respiratory losses of carbon and egestion at depth (Longhurst, 1991; Fortier *et al.*, 1994). Therefore, in areas where the larger grazers represent the sink for phytoplankton production, biogenic flux to the interior of the ocean can be expected to be

high (see Fortier *et al.*, 1994). The contribution of the smaller metazoan herbivores (e.g. copepods) to carbon flux is, however, less than that of larger grazers largely due to the relatively low sinking rates of their small faecal pellets. Also, copepods often feed on their own faecal material (Fortier *et al.*, 1994). The resultant re-ingestion (coprophagy) or disintegration (coprorhexy) of faecal pellets greatly reduces the proportion of material that is transported to depth (Fortier *et al.*, 1994).

In contrast, phytoplankton production consumed by the microbial loop, which is broadly defined as a system of prokaryote and eukaryote unicellular organisms including bacteria and protozoans (Azam *et al.*, 1983; Sherr & Sherr, 1988), contributes little to organic flux to depth on the basis of the following observations. Protozoans produce minifaecal pellets which remain in suspension for long periods. The close coupling between protozoans and bacterioplankton results in most of the small buoyant protozoan minifaecal pellets being decomposed in the euphotic zone (Azam *et al.*, 1983). Carbon is therefore, recycled in the surface waters. Also, protozoans do not appear to undergo extensive diel migrations thus, the nutrients contained in the protozoans and respiratory losses of carbon are restricted to the surface waters. Finally, recent studies have shown that many protozoans either sequester chloroplasts (Stoecker *et al.*, 1987a) or have algal symbionts (Taylor, 1982) which results in regenerated nutrients being recycled within the symbiosis. Thus, in ecosystems dominated by microbial food webs, most of the biogenic carbon is retained and recycled within the upper surface layer. A recent study in the Gulf of Mexico found that < 1% of the total production was transferred to depth when the microbial food web represented the main sink for phytoplankton production (Fahnenstiel *et al.*, 1995)

Microzooplankton is the term used to describe the heterotrophic phagotrophic component of the plankton of < 200 µm and consisting mainly of protozoans and metazoan larvae of < 200 µm (Beers & Stewart, 1967). Generally, protozoans comprise ciliates and dinoflagellates and are the most abundant component of the microzooplankton assemblages (Porter *et al.*, 1985; Pierce & Turner, 1992). Field studies have shown that microzooplankton often form up to 75% of the total heterotrophic plankton biomass (Porter *et al.*, 1985; Hansen, 1991; Smetacek, 1991). Consequently, the role of these organisms in pelagic systems has received considerable attention in recent years (Porter *et al.*, 1985; Smetacek, 1991). Since protozoans

span several orders of magnitude in size, they exhibit considerable trophic diversity including bacterivory (Capriulo, 1991; Bernard & Rassoulzadegan, 1993), carnivory (Verity *et al.*, 1993) and herbivory (Pierce & Turner, 1992). In addition, microzooplankton are the major agents of nutrient remineralization in the ocean (Goldman *et al.*, 1987; Probyn, 1987), and are thought to act as important trophic intermediates between bacterioplankton and the larger zooplankton (Stoecker & Capuzzo, 1990; Pierce & Turner, 1992). Microzooplankton can, therefore, be regarded as a key component in pelagic systems. The role of these organisms as consumers of phytoplankton production in marine environments has been the subject of some recent investigation.

Quantitative microzooplankton grazing studies conducted in various marine environments in the northern hemisphere are well documented (see Table 1.1). The results of these studies show that microzooplankton play an important role in determining the fate of phytogenic carbon and that they may even constitute the dominant grazers (Table 1.1). Size selectivity grazing studies show that microzooplankton preferentially graze on particles < 20 µm (Rassoulzadegan *et al.*, 1988; Hansen *et al.*, 1994). Indeed, studies conducted in various marine environments have shown that grazing by microzooplankton is sufficient to control the growth of phytoplankton in regions where nano- (20 - 2.0 µm) and picophytoplankton (2 - 0.2 µm) cells dominate (Verity & Vernet, 1992; Verity *et al.*, 1993). Recently, it has been recognised that dinoflagellates are able to consume phytoplankton cells up to four times their size, implying that microzooplankton may be able to feed efficiently on particles in the microphytoplankton (> 20 µm) size range (Hansen *et al.*, 1994). The microzooplankton grazing impact may, therefore, be significant within all chlorophyll size fractions (Hansen *et al.*, 1994).

Table 1.1 Comparative results of microzooplankton grazing studies conducted in various marine environments using the dilution technique.

Study	Location	% Primary production grazed
Landry & Hassett, 1982.	Coastal waters, Washington	17-52
Campell & Carpenter, 1986.	Northwest Atlantic	37-52
Burkill *et al.*, 1987.	Celtic Sea	13-42
Paranjape, 1987.	Arctic Sea	13-114
Paranjape, 1990.	Grand Bank	50-70
Strom & Welschmeyer, 1991	Subarctic	40-50
Verity & Vernet, 1992.	Norwegian Sea	20-100
Burkill *et al.*, 1993.	northwest Atlantic	39-115
Verity *et al.*, 1993.	north Atlantic	37-100
Fahnenstiel *et al.*, 1995.	Gulf of Mexico	42-214

Microzooplankton are now recognised as important consumers of phytoplankton production in pelagic systems (Pierce & Turner, 1992). More recently, it has been shown that many metazoan zooplankton consume microzooplankton (Stoecker & Capuzzo, 1990; Gifford & Dagg, 1988; 1991; Pierce & Turner, 1992; Porter *et al.*, 1992). Recent studies of gut content and faecal pellet analyses (Stoecker *et al.*, 1987b; Gifford & Dagg, 1991; Tiselius, 1989; Jeong, 1994) have shown that planktonic protozoans are consumed by several metazoan taxa (Stoecker & Capuzzo, 1990; Pierce & Turner, 1992). Quantitative grazing studies show that the rates at which microzooplankton are consumed are of physiological significance to the consumer organism (Stoecker & Capuzzo, 1990; Gifford & Dagg, 1988; 1991). For example, studies conducted in the subarctic northern Pacific Ocean have shown that the copepod *Neocalanus plumchrus* may obtain up to 59% of its total daily carbon requirements from the ingestion of protozoan prey (Gifford & Dagg, 1991). Microzooplankton can, therefore, be regarded as important trophic intermediates between bacterioplankton, small phytoplankton cells and larger zooplankton. The consumption of protozoans by larger metazoans may reduce the high grazing impact of microzooplankton within the pelagic system and represent an important source of carbon flux originating from the microbial loop.

The role of the oceans in the carbon cycle has been the subject of extensive investigations (Longhurst, 1991; Siegenthaler & Sarmiento, 1993). Several studies have focused on the role of the Southern Ocean in the global carbon cycle. The Southern Ocean is a circumpolar water mass delimited to the north by the Subtropical Convergence (corresponding to $\approx 40^0$S) and by the Antarctic continent to the south (Tomczak & Godfrey, 1994). This ocean comprises several water masses including the South Atlantic, Indian and Pacific Ocean. In addition, several distinct but smaller water masses are found around the Antarctic continent. The surface area encompassed by the Southern Ocean represents roughly 77 x 10^6 km^2, or approximately 22% of the surface of the world's oceans (Tomczak & Godfrey, 1994). The large geographic extension of the Southern Ocean suggests that it may play an important role in the global carbon cycle.

Studies in the Southern Ocean have largely been restricted to primary production studies (see reviews of El-Sayed, 1988; Jacques, 1989; Laubscher *et al.*, 1993) and the trophic role of the larger zooplankton. In particular, extensive investigations have been carried out on the distribution and ecology of the principal herbivore, the Antarctic krill *Euphausia superba* (e.g. Schnack, 1985). Recently, the distribution, abundance and the role of other larger zooplankton including copepods (Conover & Huntley, 1991; Bathmann *et al.*, 1993; Pakhomov *et al.*, 1994; Voronina *et al.*, 1995) and tunicates (Perissinotto & Pakhomov, in prep) in carbon cycling have also been examined. In contrast, the role of microzooplankton in carbon cycling in the Southern Ocean is poorly documented. Although recent models of the Antarctic food web incorporate a microzooplankton/microbial loop component (Hempel, 1985; Garrison, 1991, Moloney & Ryan, 1995), quantitative aspects of their feeding ecology are poorly studied. In particular, the grazing impact of microzooplankton and its role in carbon cycling in the Southern Ocean are not known. Thus the fate of phytogenic carbon and the partitioning of carbon between the protozooplankton and metazoan taxa in the Southern Ocean is poorly understood.

Presently, two opposing models are available to account for the fate of photosynthetic carbon production in Antarctic waters. The first, proposed by Huntley *et al.* (1991) assume that the majority of the net carbon production (at least 80%) is directly channelled into the macrozooplankton, with the remaining 20% of the production entering the microbial loop. On

the basis of this model, the biological pump would be relatively efficient in the sequestration of biogenic carbon to depth (Longhurst & Harrison, 1989; Longhurst, 1991). In contrast, a subsequent model of the Antarctic food web proposes that the microbial loop, rather than macrozooplankton, represents the main sink for net production in the Southern Ocean (Moloney, 1992). Biogenic flux into the interior of the ocean would, therefore, be dramatically reduced due to the close coupling of the microzooplankton and the microbial loop, which results in the recycling of carbon in the euphotic zone. The partitioning of carbon between the metazoan and microzooplankton grazers is, therefore, of particular importance in determining the efficiency of the biological pump.

Studies of microzooplankton in the Southern Ocean began at the beginning of the twentieth century (Garrison, 1991). These studies have, however, largely focused on examining the abundance, distribution and taxonomy of the dominant component of the microzooplankton assemblages, namely protozooplankton. The results of these studies have indicated that microzooplankton are dominated by ciliates and dinoflagellates and the densities are in the same range as those recorded in oligotrophic and mesotrophic lower latitude areas, comprising between 10 and 75% of the heterotrophic biomass (Garrison, 1991; Gowing & Garrison, 1992; Garrison *et al.*, 1993). The distribution of microzooplankton typically mirrors that of phytoplankton with highest biomasses found in the euphotic zone, suggesting a close coupling with the sources of primary production. Geographically, maximum abundances are typically encountered in areas of elevated phytoplankton production such as oceanic fronts (Jacques, 1989), in the waters surrounding oceanic islands, in the marginal ice zone (MIZ) and in the neritic waters of Antarctica (El-Sayed, 1988; Jacques, 1989). Few seasonal studies of microzooplankton abundance, species composition and distribution have ben carried out. Recent studies suggest summer maxima in microzooplankton biomass with densities decreasing by an order of magnitude during winter (Garrison, 1991). While considerable data on the abundance, distribution and species composition of microzooplankton in the Southern Ocean are available, little is known about the role of microzooplankton grazing in carbon cycling.

There is only indirect evidence for the importance of microzooplankton grazing in the trophic dynamics of the plankton communities in the Southern Ocean. Faecal material and sediment

trap studies provide evidence of the importance of microzooplankton grazing on phytoplankton (Nothig & von Bodungen, 1989; Gowing & Garrison, 1992). However, they do not provide any estimates of grazing impact of microzooplankton on phytoplankton. Garrison & Buck (1989) estimated the grazing impact of microzooplankton by extrapolating published feeding rates of protozoans from the northern hemisphere to community feeding rates based on biomass estimates. Using this approach, they estimated that microzooplankton removed up to 41% of the daily phytoplankton production. In a subsequent study, Bjornsen and Kuparinen (1991) estimated the grazing losses of phytoplankton due to dinoflagellates by measuring changes in phytoplankton and dinoflagellates biomass. The results of this study suggested that grazing by microzooplankton may be sufficient to account for the low chlorophyll concentrations generally found in the Southern Ocean. Similarly using grazing data obtained in subarctic regions, Frost (1991) suggested that grazing by microzooplankton was sufficient to control the growth of phytoplankton in the Southern Ocean.

Direct quantitative estimates of microzooplankton grazing impact on phytoplankton in the Southern Ocean are, however, very few. Studies conducted in the Bransfield Strait during austral summer have shown that grazing losses of phytoplankton may be equivalent to < 48% of the daily potential phytoplankton production (Taylor & Haberstroh, 1988). However, this study was restricted to only 4 grazing stations. A more recent study, conducted at the ice-edge of the Weddell Sea during winter, has demonstrated that in regions where small phytoplankton cells dominate, microzooplankton are the most important grazers (Garrison *et al.*, 1993). As with the previous study, few grazing stations were occupied within a limited region. Thus, only tentative conclusions could be made about the importance of microzooplankton grazing in the trophic dynamics of the plankton assemblages, and their role in carbon cycling in the Southern Ocean as a whole. Presently, the high contribution of microzooplankton to the total microplankton biomass provides the most compelling argument for the importance of protozoans in the Antarctic pelagic system (Garrison, 1991; Kivi & Kuosa, 1994).

Microzooplankton may represent important grazers of phytoplankton in the Southern Ocean. Studies conducted in the northern hemisphere have shown that many metazoan taxa feed on microzooplankton (Gifford & Dagg, 1989; Pierce & Turner, 1992). Zooplankton feeding on microzooplankton may, therefore, reduce the grazing impact of microzooplankton on

phytoplankton which will increase the efficiency of the biological pump in the Southern Ocean. Evidence for the role of microzooplankton in the diets of larger zooplankton in the Southern Ocean is primarily derived from microscopic examinations of gut contents (Hopkins & Torres, 1989; Hopkins *et al.*, 1993). These studies have shown that all the principal components of the meso- (copepods) and macrozooplankton (euphausiids and tunicates) of the Southern Ocean consume protozoans. In particular, protozoans constitute a significant component of the diets of copepods (Hopkins & Torres, 1989). More recently, gut content analysis of the two dominant euphausiids of the Southern Ocean, *Euphausia superba* and *E. crystallorophias* has shown that microzooplankton comprise ≈ 25% of the total identifiable items in their gut contents (Perissinotto *et al.*, in press; Pakhomov *et al.*, in press). These results, however, do not provide estimates of the daily rations of these organisms or their grazing impact on the microzooplankton assemblages. The contribution of protozoans to the natural diets of zooplankton is also likely to be underestimated due to the fragility of many of the microzooplankton cells (Gifford & Dagg; 1988; Hopkins & Torres, 1989).

Quantitative grazing estimates of larger zooplankton feeding on microzooplankton are poorly documented. It has been suggested that copepods may be responsible for up to 75% of the total zooplankton production (Conover & Huntley, 1991 cited in Huntley & Nordhausen, 1995). This indicates that the consumption of microzooplankton by copepods may represent an important trophic route in the Southern Ocean. Recent studies conducted by Atkinson (1994; 1995) and Atkinson & Shreeve (1995), have shown that the consumption of microzooplankton (ciliates and dinoflagellates) by the dominant copepods of the Southern Ocean contribute on average 43% of the total daily carbon intake. Furthermore, these studies suggest that, while large copepods consume microzooplankton at the same rate as phytoplankton of similar size, small copepods appear to consume preferentially non-motile taxa such as protozoans. Small copepods feeding on microzooplankton may, therefore, represent an important trophic route in the Southern Ocean pelagic system. At present, no quantitative grazing data for larger macrozooplankton feeding on microzooplankton are available.

The Southern Ocean is the region with the greatest uncertainty in CO_2 sink and source behaviour (Attwood & Monteiro, 1994). It is apparent that microzooplankton may play an

important role in the trophic dynamics of plankton communities in the Southern Ocean. To improve our understanding of the biogeochemical processes that determine carbon flux to the interior of the ocean, a 5-year investigation on the microzooplankton of the Southern Ocean was conducted.

The main aim of this work is to address the general question, "What is the role of microzooplankton in carbon cycling in the Southern Ocean ?". This is defined more clearly as 3 specific research objectives aimed at investigating spatio-temporal variations in the grazing impact of microzooplankton on phytoplankton and the role of microzooplankton as trophic intermediates between small phytoplankton cells and larger zooplankton. The major research objectives of this investigation are:

1. To investigate spatial variations in microzooplankton grazing impact on phytoplankton in the Southern Ocean, including the open waters and the regions of elevated biological activity such as the Subtropical Convergence and the Marginal Ice Zone (MIZ).
2. To examine temporal variations in microzooplankton grazing impact, more specifically in the region of the Subtropical Convergence.
3. To examine the role of microzooplankton as trophic intermediates between bacterioplankton, small phytoplankton cells and larger metazoan zooplankton.

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CHAPTER 2

MICROZOOPLANKTON GRAZING AND PROTOZOOPLANKTON COMMUNITY STRUCTURE IN THE SOUTH ATLANTIC AND IN THE ATLANTIC SECTOR OF THE SOUTHERN OCEAN

Abstract

Microzooplankton grazing and protozooplankton community structure in the South Atlantic and Atlantic sector of the Southern Ocean were investigated along a transect during late austral summer (Jan./Feb.) 1993. Grazing rates and numerical abundances of protozooplankton were estimated in the surface waters and at the subsurface chlorophyll maximum (SCM) by employing the dilution technique and epifluorescent microscopy. Protozooplankton abundance co-varied with chlorophyll concentrations at both depths. Nanoheteroflagellates (< 20 µm) dominated numerically at all stations while the > 20 µm component was dominated by ciliates, comprising aloricate ciliates and tintinnids. Instantaneous growth rates of algae along the transect ranged between 0.24 and 1.86 d^{-1} in surface waters and between 0.06 and 1.87 d^{-1} at the SCM. Instantaneous grazing rates of microzooplankton on phytoplankton varied from 0 to 0.33 d^{-1} in the surface waters and between 0 and 0.58 d^{-1} at the SCM. This level of grazing corresponds to a daily loss of 0 - 23% of the initial standing stock (0 - 46% of potential production) in the surface waters and between 0 and 44% (0 - 60% of potential production) of the initial standing stock at the SCM. Analysis of variance and multiple range tests indicate that both the initial standing stock and potential production removed were not significantly different between depths ($F = 0.84$; $F = 0.29$: $P < 0.05$). Indirect evidence suggests that microzooplankton grazed preferentially on the nano- and picophytoplankton size fractions. The spatial distribution of phytoplankton size classes in the different regions of the Southern Ocean has important ecological implications for the oceanic carbon flux. South of the Antarctic Polar Front (APF), where larger netphytoplankton dominate chlorophyll concentration, the bulk of the photosynthetically fixed carbon appears to be channelled into the meso/macrozooplankton component or lost by sedimentation. However, north of the APF, the contribution of the smaller fractions to total chlorophyll increases, suggesting a relative increase in the amount of carbon channelled into the smaller grazing fractions.

2.1 Introduction

Microzooplankton constitute a significant proportion of total zooplankton biomass in a variety of neritic and oceanic environments and have consequently been the subject of numerous studies (Gast, 1985; Porter *et al.*, 1985; Goldman *et al.*, 1987; Mazumder *et al.*, 1990). Theoretical studies on oceanic food-web dynamics have suggested that microzooplankton are capable of consuming a significant proportion of primary production (Frost, 1991). Indeed, field studies have demonstrated that microzooplankton consume between 10 and 75% of daily primary production (Bjornsen & Kuparinen, 1991; Garrison, 1991; see review by Pierce & Turner, 1992). Furthermore these studies have shown that microzooplankton grazing can play an important role in regulating bacterial populations (Andersen & Fenchel, 1984; Andersen & Sorensen, 1986; Albright *et al.*, 1987; McManus & Fuhrman, 1988; Reid & Karl, 1990) and regenerating nutrients (Goldman *et al.*, 1987; Probyn, 1987). Microscopic examinations of consumer gut contents, feeding structures and faecal material show that a number of invertebrate and vertebrate larvae consume microzooplankton. Microzooplankton thus act as trophic intermediates between the small bacteria, nanoplankton and the larger mesozooplankton (Gifford, 1991; Gifford & Dagg, 1988; 1991).

Recent field studies suggest that microzooplankton not only control the size of phytoplankton populations but may also control the growth of certain species by selective grazing (Reynolds *et al.*, 1982; Corliss & Snyder, 1986; Burkill *et al.*, 1987; Strom & Welschmeyer, 1989). This size-selective grazing depends on the feeding mechanisms employed (Haas & Webb, 1979; Peters, 1994). For example, tintinnids typically consume phytoplankton which are ≈ 45 % of their lorica diameter (Graziano, 1989), while dinoflagellates, which feed by means of pseudopodia, are able to consume prey even larger than themselves (Goldman & Dennett, 1990; Peters, 1994).

Microzooplankton studies in the Southern Ocean have primarily been limited to species description and estimates of abundance and distribution in Antarctic waters, especially in the Weddell and Scotia Seas (Buck & Garrison, 1983; Hara & Tanoue, 1985; Buck & Garrison, 1983; Boltovskoy *et al.*, 1989; Gowing & Garrison, 1991; 1992; Ishiyama *et al.*, 1993; Stoecker *et al.*, 1993) and in waters surrounding oceanic islands (Dodge & Priddle, 1987). The few grazing studies that have been undertaken have been restricted to waters close to the

continental margins (Taylor & Haberstroh, 1988; Bird & Karl, 1990; Bjornsen & Kuparinen, 1991; Gowing & Garrison, 1992). The results of these studies suggest that microzooplankton grazing may be sufficiently high to account for the generally low phytoplankton biomass in the Southern Ocean (Bjornsen & Kuparinen, 1991).

In this paper we estimate microzooplankton grazing and protozooplankton community structure in the South Atlantic and in the Atlantic sector of the Southern Ocean during late austral summer 1993. Grazing rates were determined at 22 stations along a transect from SANÅE (Antarctica) to Cape Town in conjunction with epifluorescence microscopy studies to determine protozooplankton community structure.

2.2 Materials and methods

Microzooplankton grazing experiments were conducted during the second cruise of the South African Antarctic Marine Ecosystem Study (SAAMES) aboard the *MV. S.A. Agulhas* during late austral summer (Jan. - Feb.) 1993 (Figure 2.1). Grazing experiments were carried out using water from two depths, the surface layer (5 m) and the subsurface chlorophyll maximum (SCM), by the sequential dilution technique (Landry & Hassett, 1982). Water samples were obtained with a submersible pump (Flygt-Kyokuto model L 40- 25S), operated at a flow rate of $\approx$ 15 l min^{-1} and supplied to 25 l polyethylene containers through PVC piping. The water was then filtered through a 200 µm mesh to separate the microzooplankton fraction. Particle-free water was obtained by passing surface water collected with the shipboard pump (Iwaki Magnetic pump), operated at a flow rate of $\approx$ 1.5 l min^{-1} through a 0.2 µm Milli Q system (Millipore). Dilution series of 1:0 3:1; 1:1; 1:3 filtered to particle-free water were made in 2 l acid washed polyethylene bottles. Three replicas for each dilution series were prepared. The dilution series were then incubated on deck for 24 hours in perspex incubators cooled with running surface water and screened with shade cloth (neutral density) to simulate the light intensity at the depth of collection.

Before incubation was begun, a water sample (250 ml) for initial chlorophyll-*a* concentration was taken from each bottle of the dilution series. The corresponding bottles were sampled again (250 ml) at the end of the incubation period to determine final chlorophyll-*a* concentration.

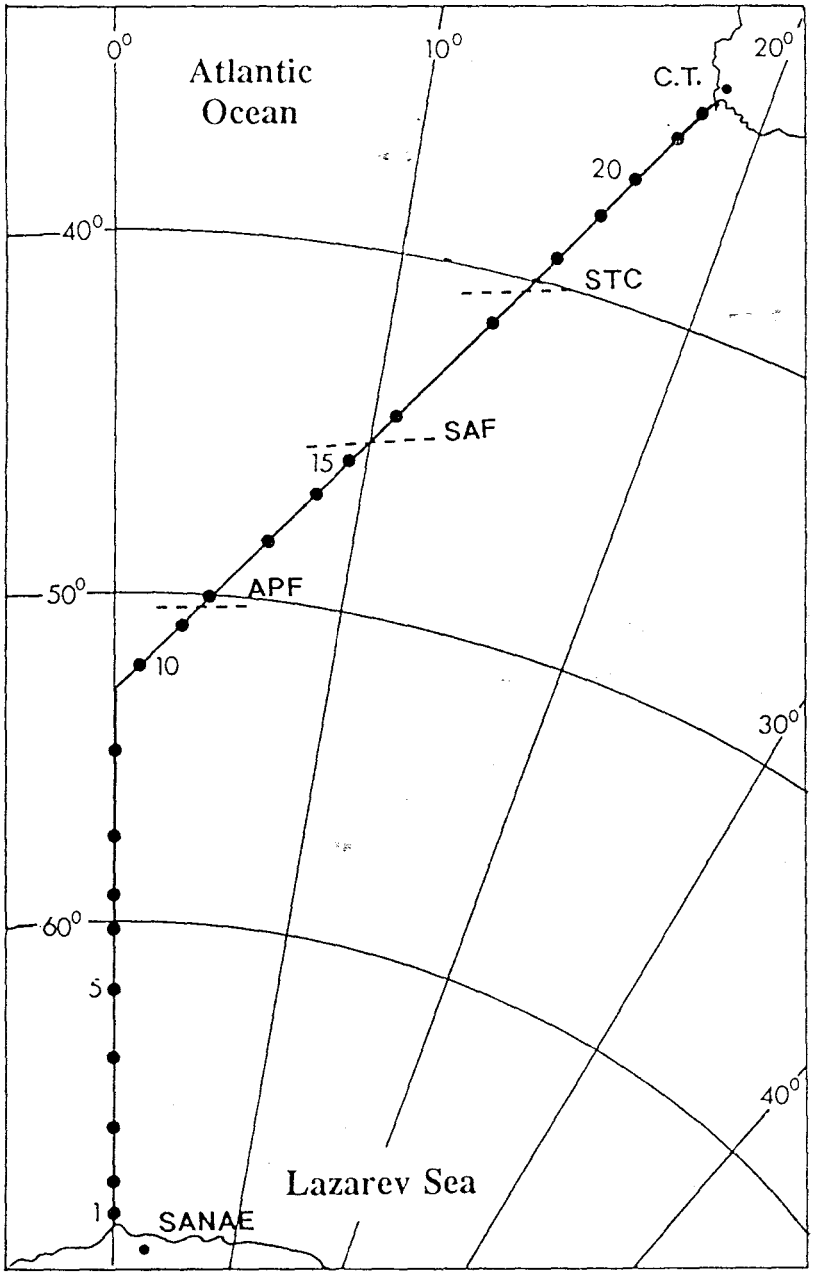

Figure 2.1 Cruise track and position of the stations (every 5th station numbered) occupied during the SAAMES II cruise aboard the *MV. S.A Agulhas* in late austral summer in the Atlantic sector of the Southern Ocean. **C.T.**= Cape Town; **STC** = Subtropical Convergence; **SAF** = Subantarctic Front; **APF** = Antarctic Polar Front.

To assess the dynamics of the algal community during the incubation period, water samples taken from the 1:0 dilution series were fractionated into net- (≥ 20 µm), nano- (2.0 - 20 µm) and pico- (0.2 - 2.0 µm) plankton size fractions at the onset and at the end of the incubation period. Chlorophyll-*a* concentrations were determined fluorometrically (Turner 111 fluorometer) after extraction in 100% methanol (Holm-Hansen & Riemann, 1978).

To estimate the numerical abundance of the protozooplankton community, a 50ml sample of unfiltered seawater was stained with Proflavine (50 µl/ml; 2min), fixed with glutaraldehyde (final conc. 6%) and then filtered (vacuum ≤ 5 cm Hg) through a 2.0 µm Nuclepore filter which had been prestained with Irgalan black (Haas, 1982). Permanent slides were then prepared according to the method of Booth (1987) and frozen at -20°C. Slides were examined within two months of collection at 400 x magnification using a Zeiss fluorescent microscope equipped with a 450-490 excitation filter, a FT 510 chromatic beam splitter and a long pass 528 barrier filter (Haas, 1982). No significant loss in the autofluorescence of the chlorophyll-containing organisms was anticipated (Booth, 1987). Phototrophic organisms were distinguished from heterotrophic organisms by the red autofluorecence of chlorophyll-*a*.

Protozooplankton were separated into the following groups: tintinnids, aloricate ciliates, dinoflagellates and nanoheterotrophs (2 - 20 µm). The number of cells in the 50 ml sample was calculated from the following relation (Waterbury *et al.*, 1986):

$$\frac{\text{No. cells in 100 fields x (total area of filter)}}{\text{(area of 100 fields)}}$$

All data were then multiplied by 20 to express protozooplankton density data as cells l^{-1}.

The apparent growth rate of chlorophyll-a at each dilution is calculated as:

$$\frac{1}{t} \ln \left(\frac{P_t}{P_o} \right)$$

where P_o and P_t are chlorophyll concentrations at the beginning and end of the experiment; *t* is duration of experiment. This is the observed change in chlorophyll in the presence of

grazers. The theoretical growth rate of phytoplankton in the absence of grazers (k) is taken to be the y intercept from the regression analysis between apparent growth rate and dilution (Figure 2.2). The slope of the regression is the instantaneous grazing coefficient (g) of the microzoplankton (Figure 2.2). This regression was calculated by using the computer package, Statgraphics Version 5.0 (Statistical Graphics Corporation).

To normalise chlorophyll values, all data were transformed using the factor log (x + 1) (Legendre & Legendre, 1983). Grazing rates (expressed as %) were transformed using the arcsin transformation (Sokal & Rohlf, 1969). Correlation analysis (Statgraphics, 5.0) was then performed to identify possible relationships between grazing rates and chlorophyll size fraction (Sokal & Rohlf, 1969).

2.3 Results

Chlorophyll distribution

Chlorophyll concentrations at the surface and at the subsurface chlorophyll maximum (SCM) did not differ dramatically (Figure 2.3). Peaks in chlorophyll concentration were identified in the neritic waters of Antarctica, in the marginal ice zone (MIZ), in the vicinity of the Antarctic Polar Front (APF), at the Subtropical Convergence (STC) and in continental shelf waters south of Africa. South of the APF, total chlorophyll concentration in surface waters was generally higher than at the SCM. North of the APF, the situation was reversed, with chlorophyll concentrations at the SCM higher than in surface waters, except in continental shelf waters south of Africa.

Size fractionated chlorophyll

The contribution of the three size fractions to total chlorophyll concentration differed north and south of the APF (Figures 2.4A and 2.4B). The netphytoplankton contribution to total chlorophyll concentration was greatest at stations south of 57^{0}S (station 8), with the exception of station 2 in the subsurface waters and station 5 at the SCM. This pattern extended to 53^{0}S (station 10) in the surface waters (Figure 2.4A). In the surface waters north of 57^{0}S, the nanophytoplankton dominated the chlorophyll concentration. At the SCM, nanophytoplankton dominated chlorophyll concentration between stations 8 and 11, while netphytoplankton

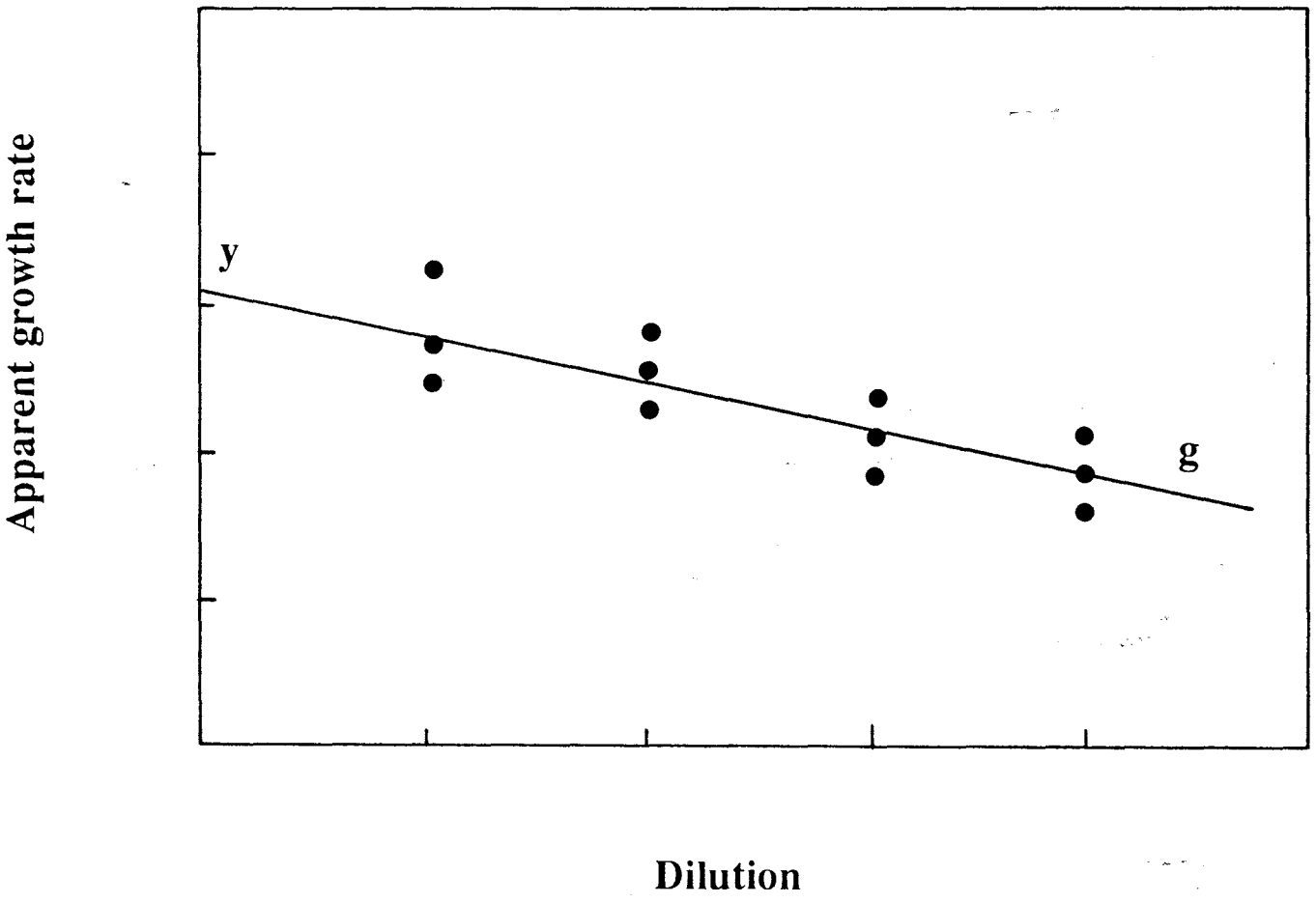

Figure 2.2 Interpretation of an idealised dilution experiment. Slope of the regression is the instantaneous grazing coefficient (g); intercept of the y axis is the instantaneous algal growth coefficient (k).

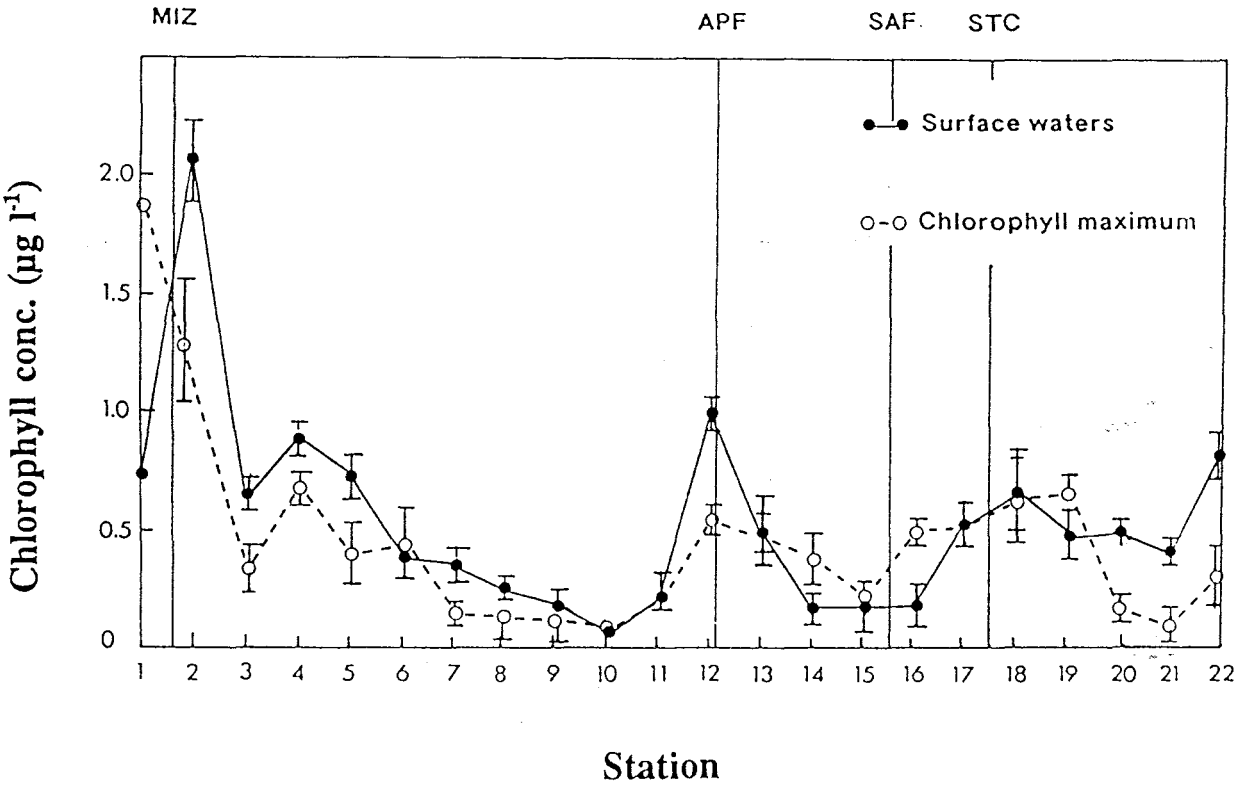

Figure 2.3 Total chlorophyll-*a* distribution in surface waters and at the subsurface maximum (SCM). **MIZ** = marginal ice zone; **APF** = Antarctic Polar Front; **SAF** = Subantarctic Front; **STC** = Subtropical Convergence.

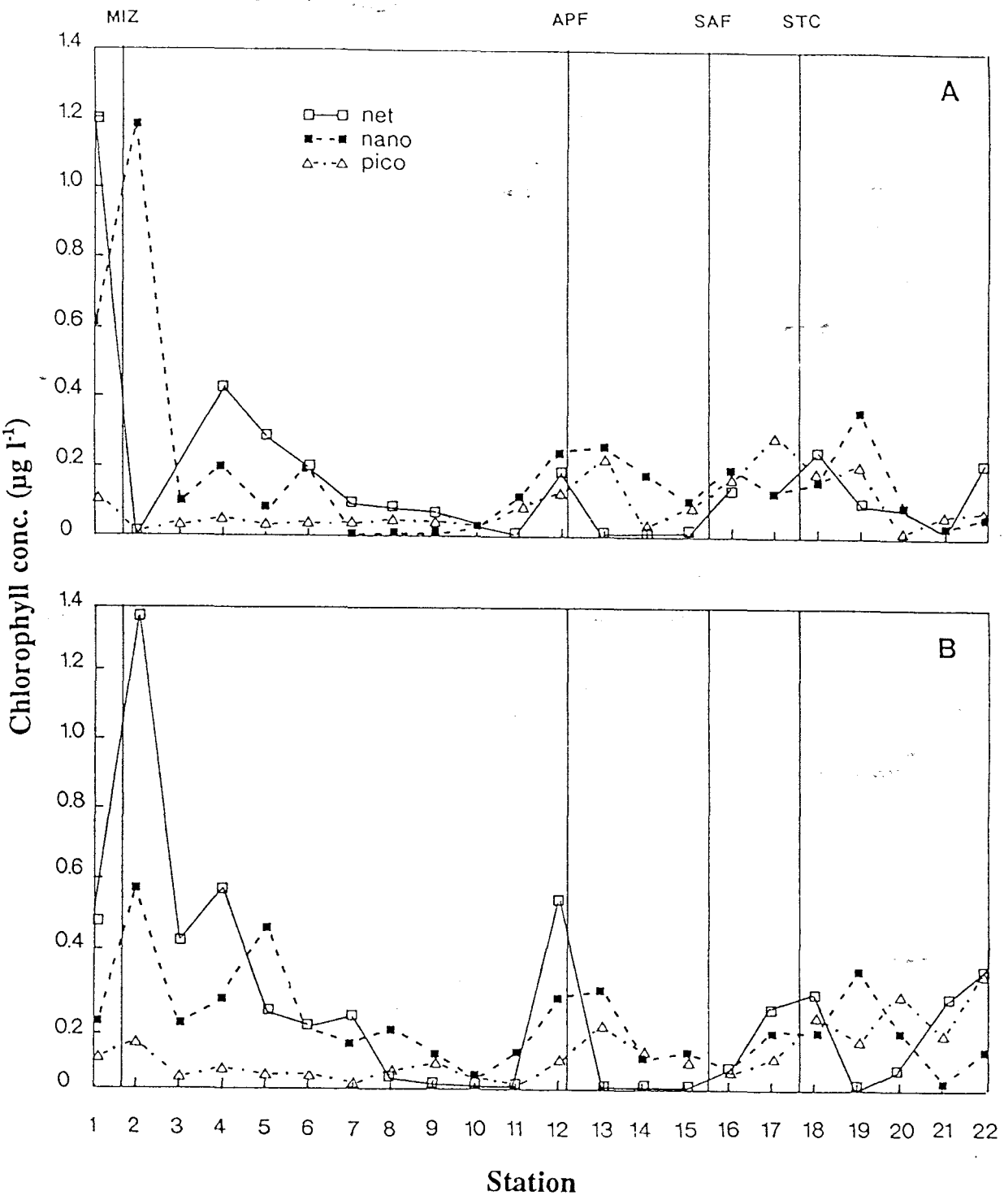

Figure 2.4 Size fractionated chlorophyll-*a* distribution in surface waters (**A**) and at the SCM (**B**). **MIZ** = marginal ice zone; **APF** = Antarctic Polar Front; **SAF** = Subantarctic Front; **STC** = Subtropical Convergence.

dominated in the region of the APF. The picophytoplankton contribution to total chlorophyll concentrations at all stations south of the APF was less than 5%.

North of the APF, the contribution of the three fractions to total chlorophyll concentrations varied considerably, with the smaller size fractions typically dominating. The contribution of netphytoplankton to total chlorophyll concentration north of the APF was greatest in the region of the STC and in continental shelf waters south of Africa. The nanophytoplankton dominated at stations immediately north of the APF and at station 19 at both depths. The contribution of picophytoplankton increased northwards towards Cape Town, dominating at station 17 in the surface waters and station 20 at the SCM.

Population structure

Total protozooplankton abundance co-varied with chlorophyll concentration at both depths (Figures 2.3 and 2.5). Indeed, chlorophyll-*a* concentration accounted for ≈ 40% of the variance associated with microzooplankton abundance in the surface waters ($P < 0.05$). Peaks in protozooplankton abundance at both depths were recorded in the vicinity of the APF and Subantarctic Front (SAF). At the STC only the surface waters exhibited a peak in concentrations. In addition, in surface waters microzooplankton abundance increased dramatically in the region of the MIZ, while at the SCM peaks were identified in the region of 63^0S (station 3) and at station 20, where a dinoflagellate bloom (dominated by *Ceratium* spp. and *Gymnodinium* spp.) was found. At both depths, abundances decreased dramatically in continental shelf waters (stations 21 and 22). Although the contribution of the various components of the microzooplankton assemblage varied between depths, total abundances did not differ significantly ($t = 0.281$; $P < 0.05$).

Nanoheterotrophs dominated numerically at all stations with the exception of station 20 at the SCM where a dinoflagellate bloom was identified (Table 2.1A and 2.1B). Densities ranged between 981 and 2310 ind.l^{-1} in surface waters and between 980 and 2100 ind.l^{-1} at the SCM (Table 2.1A and 2.1B). The > 20 µm fraction was dominated numerically by ciliates (tintinnids and aloricate ciliates). The contribution of the tintinnids to total ciliate densities was less than that of the aloricate ciliates at all stations. Dinoflagellates were the second most abundant component of the > 20 µm fraction.

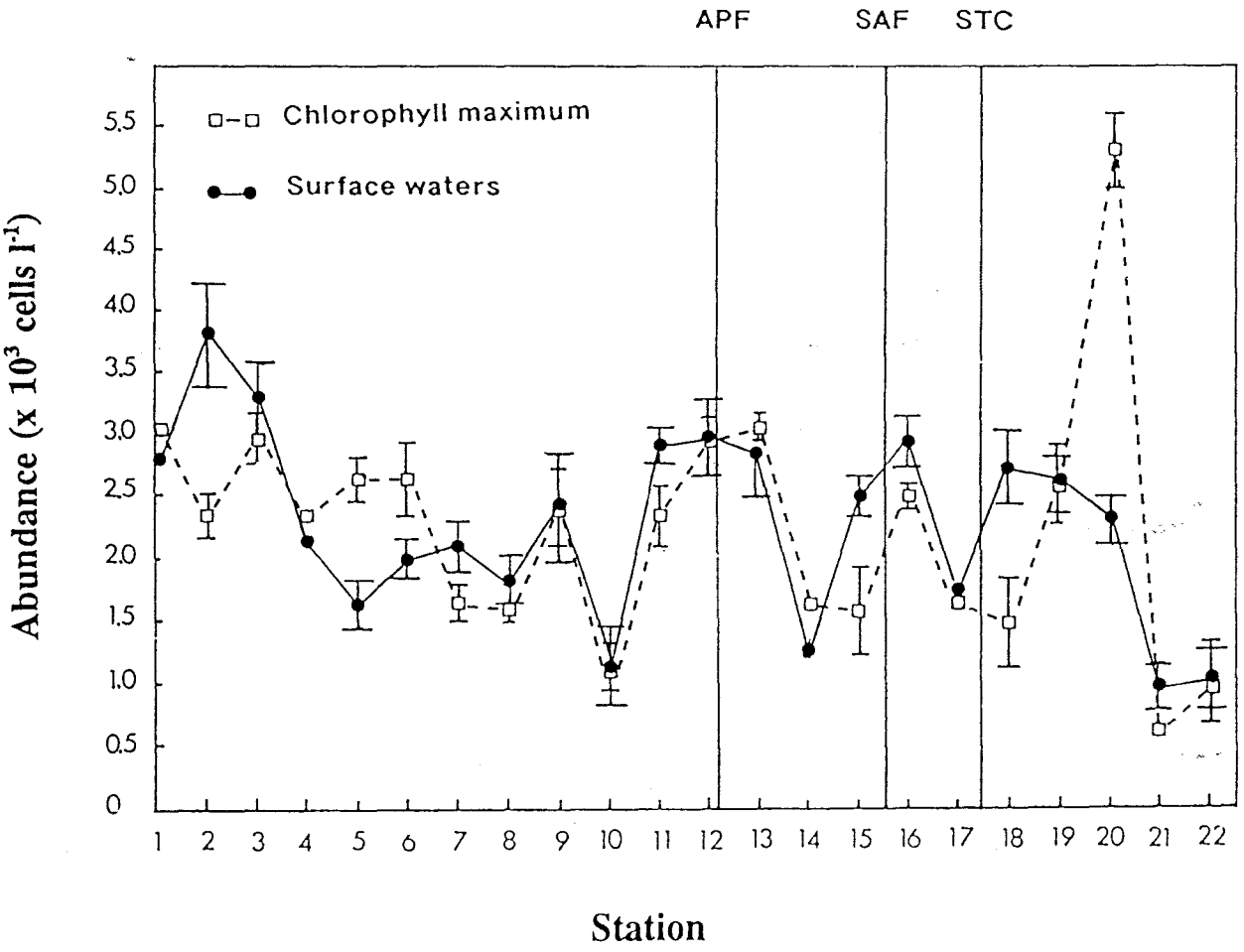

Figure 2.5 Total protozooplankton abundance along the SANAE- Cape Town transect in units of cells. l^{-1}. **MIZ** = marginal ice zone; **APF** = Antarctic Polar Front; **SAF** = Subantarctic Front; **STC** = Subtropical Convergence.

Table 2.1A Composition of the heterotrophic protozooplankton assemblages in the surface waters during the SAAMES II cruise of austral summer (Jan./Feb.) 1993. Results are expressed as ind.l^{-1}.

Stations	Aloricate ciliates	Heterotrophic Tintinnids	Heterotrophic Dinoflagellates	Nano-heterotrophs
1	438	189	377	1782
2	695	202	502	1974
3	440	185	343	2311
4	565	98	468	981
5	387	36	176	1003
6	422	351	194	1033
7	367	88	326	1321
8	290	35	297	1221
9	406	17	453	1487
10	229	20	264	581
11	633	88	713	1437
12	476	88	467	1245
13	405	141	581	1742
14	264	17	282	704
15	572	35	493	1408
16	651	88	651	1566
17	312	101	131	1547
18	554	220	440	1566
19	1336	504	840	978
20	377	112	547	1078
21	73	17	74	843
22	211	35	198	553

Table 2.1B Composition of the heterotrophic protozooplankton assemblages at the chlorophyll maximum during the SAAMES II cruise of austral summer (Jan./Feb.) 1993. Results are expressed as ind.l^{-1}.

Stations	Aloricate ciliates	Heterotrophic Tintinnids	Heterotrophic Dinoflagellates	Nano-heterotrophs
1	397	88	352	2117
2	510	255	528	983
3	763	101	662	1021
4	565	131	652	963
5	422	181	317	1830
6	532	134	467	1468
7	216	131	323	986
8	320	180	243	1034
9	379	88	443	1543
10	282	35	229	968
11	299	0	581	1478
12	261	17	436	1331
13	528	107	528	1830
14	299	17	422	915
15	368	88	296	867
16	387	123	669	1443
17	437	88	121	987
18	422	158	290	563
19	498	285	498	1323
20	123	194	408	968
21	61	14	27	560
22	142	0	211	669

Microzooplankton grazing

Instantaneous growth and grazing coefficients are listed in Tables 2.2A and 2.2B, which show that significant linear correlations ($P < 0.05$) were found between dilution and apparent phytoplankton growth rates.

Pigment specific coefficients (k) were highly variable along the transect. Increased growth rates were recorded in the vicinity of the MIZ, APF, STC and in the continental shelf waters of Africa (Tables 2.2A and 2.2B). At both depths, the lowest growth coefficients were recorded in the neritic waters of Antarctica, while the highest growth rates were recorded in the vicinity of the APF. At the SCM, algal growth coefficients ranged between 0.24 and 1.86 d^{-1}, equivalent to 0.35 and 2.68 chlorophyll doublings d^{-1}. At the SCM, algal growth coefficients were in the same range, between 0.17 and 1.87 d^{-1} or between 0.25 and 2.69 chlorophyll doublings d^{-1}.

Instantaneous grazing coefficients of microzooplankton in the surface waters ranged between 0 and 0.33 d^{-1}. This level of grazing represents a loss of 0 to 28% of the initial standing stock, or between 0 and 46% of the potential production along the transect. At the SCM, instantaneous grazing coefficients ranged from between 0 to 0.58 d^{-1}. This level of instantaneous grazing activity is equivalent to a loss of 0 to 44% of the initial standing stock and 0 to 60% of the potential production along the transect. Analysis of variance and multiple range test indicate that both the initial standing stock and potential production removed were not significantly different at the two depths considered ($F = 0.84$; $F = 0.29$; $P > 0.05$).

Table 2.2A Rate estimates with regression coefficients (r^2) and confidence limits of microzooplankton grazing studies conducted in surface waters during late austral summer (Jan/Feb) 1993. (** = $P < 0.05$; * = $P < 0.001$). Values in brackets = standard error. Doublings $d^{-1} = k/\ln 2$ (Gifford, 1988). MIZ = Marginal Ice Zone; APF = Antarctic Polar Front; STC = Subtropical Convergence.

Station number	Chl-*a* conc.	r^2	Growth coeff. k (d^{-1})	Grazing coeff. g (d^{-1})	%Initial stock removed (d^{-1})	%Potential prod. removed (d^{-1})	Chlorophyll doublings (d^{-1})	
1	1.88	0.47*	0.24 (0.04)	0.04 (0.02)	3.72	18.27	0.35	
2	1.01	0.42**	1.68 (0.25)	0.22 (0.09)	19.82	24.26	2.42	**MIZ**
3	0.33	0.49**	1.31 (0.13)	0.15 (0.05)	15.15	19.10	1.89	
4	0.68	0.60*	0.51 (0.07)	0.20 (0.02)	17.60	44.44	0.73	
5	0.39	0.65*	1.59 (0.16)	0.25 (0.06)	22.50	27.56	2.29	
6	0.44	0.44**	1.24 (0.07)	0.07 (0.02)	6.82	9.26	1.79	
7	0.14	0.32**	1.34 (0.10)	0.18 (0.02)	14.29	20.51	1.93	
8	0.13	0.61**	0.53 (0.12)	0.10 (0.05)	7.69	22.22	0.77	
9	0.13	0.57**	0.87 (0.11)	0.11 (0.01)	7.69	16.67	1.25	
10	0.10	0.82*	0.97 (0.04)	0.22 (0.02)	20.00	31.25	1.39	
11	0.19	0.72*	0.85 (0.04)	0.06 (0.02)	5.26	8.00	1.22	
12	0.54	0.66**	1.86 (0.12)	0.33 (0.04)	27.78	33.44	2.68	**APF**
13	0.48	0.82*	0.96 (0.05)	0.09 (0.02)	8.33	12.98	1.39	
14	0.37	0.74*	0.81 (0.10)	0.19 (0.10)	16.22	34.78	1.17	
15	0.19	0.31**	1.11 (0.15)	0.00 (0.00)	0.00	0.00	1.16	
16	0.49	0.87*	1.11 (0.13)	0.20 (0.07)	22.50	27.00	1.60	
17	0.52	0.72*	0.87 (0.03)	0.08 (0.01)	7.69	12.50	1.25	**STC**
18	0.62	0.65**	0.57 (0.07)	0.13 (0.03)	12.90	29.17	0.83	
19	0.66	0.58**	1.80 (0.11)	0.26 (0.09)	22.72	27.32	2.60	
20	0.16	0.34**	0.87 (0.10)	0.10 (0.02)	12.50	15.77	1.26	
21	0.09	0.43**	0.65 (0.09)	0.05 (0.01)	10.00	11.11	0.93	
22	0.31	0.35**	0.91 (0.10)	0.24 (0.04)	22.58	34.78	1.32	

Table 2.2B Rate estimates with regression coefficients (r^2) and confidence limits of microzooplankton grazing studies conducted at the chlorophyll maximum during late austral summer (Jan/Feb) 1993. (** = $P < 0.05$; * = $P < 0.001$). Values in brackets = standard error. Doublings d^{-1} = k/ln2 (Gifford, 1988). MIZ = Marginal Ice Zone; APF = Antarctic Polar Front; STC = Subtropical Convergence.

Station number	Chl-*a* conc.	r^2	Growth coeff. k (d^{-1})	Grazing coeff. g (d^{-1})	%Initial stock removed (d^{-1})	Potential prod. removed (d^{-1})	Chlorophyll doublings (d^{-1})	
1	0.69	0.40**	0.17 (0.02)	0.02 (0.01)	2.02	15.74	0.25	
2	2.07	0.62*	1.50 (0.12)	0.18 (0.04)	16.47	21.22	2.16	**MIZ**
3	0.64	0.63*	1.49 (0.18)	0.26 (0.06)	23.21	29.82	2.14	
4	0.89	0.62*	0.46 (0.07)	0.11 (0.03)	10.45	28.84	0.67	
5	0.73	0.76*	1.64 (0.13)	0.26 (0.05)	23.02	28.38	2.37	
6	0.39	0.43**	0.49 (0.10)	0.27 (0.08)	23.84	60.97	0.71	
7	0.36	0.31**	1.71 (0.15)	0.58 (0.09)	44.17	53.99	2.47	
8	0.26	0.61**	0.76 (0.04)	0.13 (0.01)	12.31	22.95	1.10	
9	0.19	0.54*	0.70 (0.02)	0.05 (0.01)	4.89	9.32	1.00	
10	0.08	0.84*	1.34 (0.08)	0.18 (0.03)	16.25	22.12	1.93	
11	0.21	0.77*	1.87 (0.30)	0.31 (0.04)	26.67	31.56	2.70	
12	0.99	0.64*	1.44 (0.10)	0.25 (0.04)	22.12	28.98	2.08	**APF**
13	0.49	0.82*	0.81 (0.11)	0.10 (0.01)	10.20	16.88	1.17	
14	0.17	0.48**	0.72 (0.10)	0.17 (0.05)	15.88	30.73	1.04	
15	0.18	0.37**	0.07 (0.01)	0.00 (0.00)	0.00	0.00	0.10	
16	0.18	0.39**	0.71 (0.28)	0.26 (0.10)	22.78	45.16	1.02	
17	0.53	0.38*	0.74 (0.10)	0.10 (0.07)	9.43	18.24	1.06	**STC**
18	0.66	0.80*	0.54 (0.05)	0.14 (0.02)	13.63	31.29	0.78	
19	0.47	0.80*	0.41 (0.03)	0.07 (0.01)	6.38	20.17	0.59	
20	0.50	0.47**	0.06 (0.01)	0.01 (0.00)	11.45	21.88	0.09	
21	0.09	0.75*	0.44 (0.08)	0.11 (0.03)	10.00	29.09	0.63	
22	0.83	0.83*	0.69 (0.02)	0.06 (0.08)	6.09	11.77	0.99	

Dynamics of algal community

The netphytoplankton size fraction increased in concentration at 16 stations in the surface waters and 15 stations at the SCM (Figure 2.6). The mean increase was 7.5% in the surface waters and 8.4% at the SCM. The greatest increase in netphytoplankton concentration at both depth was recorded at station 21. The nanophytoplankton concentration decreased at 14 stations in the surface waters (mean decrease = 9.5%) and the SCM (mean decrease = 10.8%). The picophytoplankton fraction decreased at 17 stations in the surface waters and at 14 stations at the SCM.

Analysis of variance (ANOVA) and multiple range test indicated that at both depths the change in netphytoplankton concentration was significantly different from the percentage change in the nanophytoplankton and picophytoplankton concentrations ($F = 6.03$ in surface waters; $F = 3.35$ at the SCM; $P < 0.05$ in both cases).

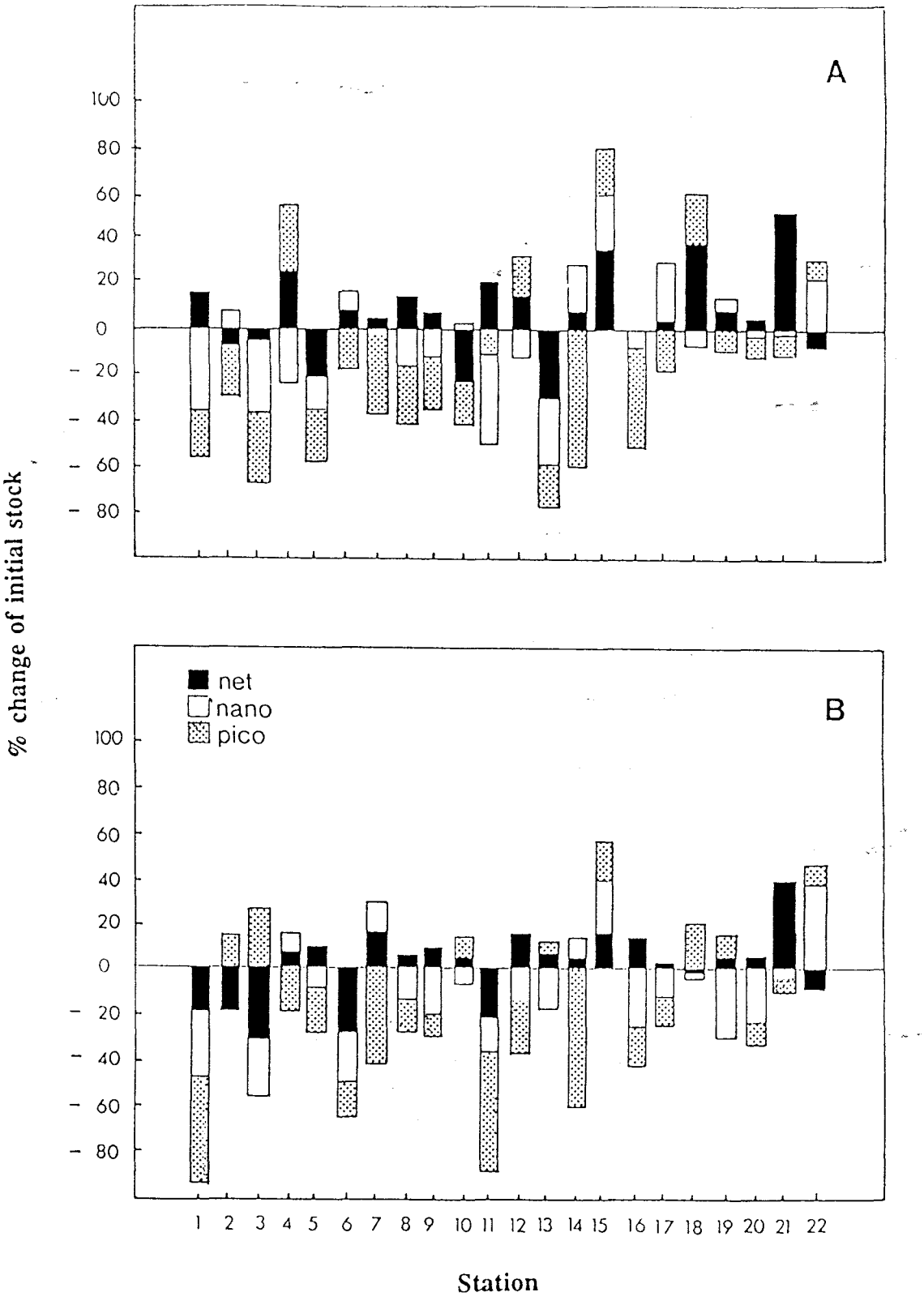

Figure 2.6 Changes in the contribution of the chlorophyll fractions during incubations in surface waters (**A**) and at the SCM (**B**) carried out in Jan.- Feb. 1993. Results are expressed as % increase or decrease of initial concentrations.

2.4 Discussion

The dilution technique is widely employed to estimate microzooplankton herbivory and provides a simultaneous estimation of algal growth and mortality with the minimal manipulation of the natural assemblages (Landry & Hassett, 1982; Paranjape, 1990; Verity & Vernet, 1993). A potential source for the underestimation of microzooplankton grazing during this study may have been the water sampling method employed. Differences in the efficiency of sampling of the different size classes and damage to delicate, soft bodied organisms in water samples collected by submersible pumps have been documented (James, 1991). However, comparative studies of microzooplankton samples collected by pump and sampling bottle found no significant differences in the compositions of the assemblages (Herman *et al.*, 1984; Paranjape, 1991). Indeed, densities of protozooplankton assemblages recorded during this study were in the same range reported from previous studies in the Southern Ocean (Garrison & Buck, 1989; Garrison, 1991). Furthermore, our estimates of microzooplankton grazing on phytoplankton were in the same range reported from other experiments employing the dilution technique (Table 2.3). These considerations suggest that underestimation of microzooplankton impact associated with pump sampling was minimal during this study.

Algal growth coefficients along the transect ranged between 0.06 and 1.87 d^{-1} (0.25 to 2.70 chlorophyll doublings d^{-1}) (Table 2.2A and 2.2B). Highest growth rates were recorded in the marginal ice zone (MIZ), at oceanic fronts and particularly the Antarctic Polar Front (APF) and the Subtropical Convergence (STC); and in the neritic waters off Africa (Table 2.2A and 2.2B). Elevated algal production in these areas of the Southern Ocean are well documented (El-Sayed, 1988; Jacques, 1989). The highest growth rates along the transect were generally recorded at stations dominated by the < 20 µm chlorophyll fraction. These growth rates are consistent with results of microzooplankton grazing studies conducted in regions where the < 20 µm fraction dominated chlorophyll biomass (Gifford, 1988; Gallegos, 1989; Wiese & Scheffel-Moser, 1990; Verity *et al.*, 1993). Interfrontal algal growth rates compare well with results of dilution experiments conducted in open ocean environments (Strom & Welschmeyer, 1991).

Table 2.3 Comparative results of microzooplankton grazing experiments conducted in various oceanic environments employing the dilution technique. Values omitted from the table were not listed in the reference.

Author	Region	Growth coefficient (k) d^{-1}	Grazing coefficient (g) d^{-1}	% Initial standing stock removed d^{-1}	% Potential production removed d^{-1}
Landry & Hassett, 1982	Coastal, Washington	0.46-0.63	0.07-0.29	6-24	-
Paranjape, 1987	Arctic Sea, Pacific	0.00-0.34	0.08-0.17	5-31	13-114
Burkill *et al.*, 1987	Celtic Sea	-0.07-1.04	0.14-1.66	13-65	-
Gifford, 1988	Halifax harbour	0.00-1.44	0.00-1.96	38	0-100
Taylor & Haberstroh, 1988	Bransfield Strait	-0.28-0.49	0.00-0.77	10.2	-
Paranjape, 1990	Grand Bank	0.27-0.708	0.16-0.43	20-30	13-105
Strom & Welschmeyer, 1991	Arctic Sea, Pacific	0.02-0.66	0.1-0.59	40-50	-
Present study	South Atlantic and Southern Ocean	0.24-1.86	0.00-0.33	0-59	0-60

Instantaneous grazing coefficients of the microzooplankton assemblages ranged between 0 and 0.58 d^{-1} (Table 2.2A and 2.2B). This level of grazing is equivalent to a daily loss of 0 - 44% of the initial standing stock or 0 to 60% of potential phytoplankton production. Our results are in the same range reported from similar studies in the northern hemisphere (see Table 2.3). Several criteria are thought to involve food selection, including prey size, motility and surface characteristics (Strom & Welschmeyer, 1991; Hansen *et al.*, 1994; Peters, 1994). Among these, prey size is considered to be the most important. A recent study suggests that the optimum predator: prey ratio volume is 18:1 for ciliates and 8:1 for flagellates (Hansen *et al.*, 1994). On the basis of the species composition of the protozooplankton assemblages recorded along the transect at both depths, grazing impact should be greatest at stations dominated by the < 20 µm chlorophyll fraction. Indeed, grazing impact of microzooplankton was greatest at stations dominated by the < 20 µm chlorophyll fraction (Figure 2.4; Tables 2.2A and 2.2B). The lower grazing rates generally recorded at stations dominated by the larger netphytoplankton can be ascribed to morphological constraints on protozooplankton feeding (Graziano, 1989). It is worth noting that in the surface waters, the lowest grazing impact was recorded at stations dominated by chain-forming species of the genera *Chaetoceros*, *Nitzschia* and *Corethron* (i.e. stations 1, 11, 15, 20, 21). Exceptions presented may reflect the presence of large dinoflagellates, which are able to consume prey up to three times their size (Jacobson & Anderson, 1986; Suttle *et al.*, 1986; Hansen, 1991). Although grazing rates have been demonstrated to be temperature dependent (Peters, 1993), the results of this investigation suggest that grazing impact was independent of temperature during this study (Figures 2.7A and 2.7B).

Station 15 was characterised by a lack of decrease in concentration of phytoplankton at both depths over the duration of the incubation period (Table 2.2A and 2.2B). This occurred despite relatively high cell densities of 3000 $ind.l^{-1}$ (Table 2.1A and 2.1B). The impact of grazing on phytoplankton growth is largely determined by the phase of phytoplankton growth (Banse, 1991). During the exponential phase, the growth rate of the algae exceeds the grazing rate of the microzooplankton (i.e. $k > g$). This results in an increase and accumulation of chlorophyll biomass in the absence of larger herbivores. An indication of the growth status of the local phytoplankton can be derived from the photosynthetic capacity (PC).

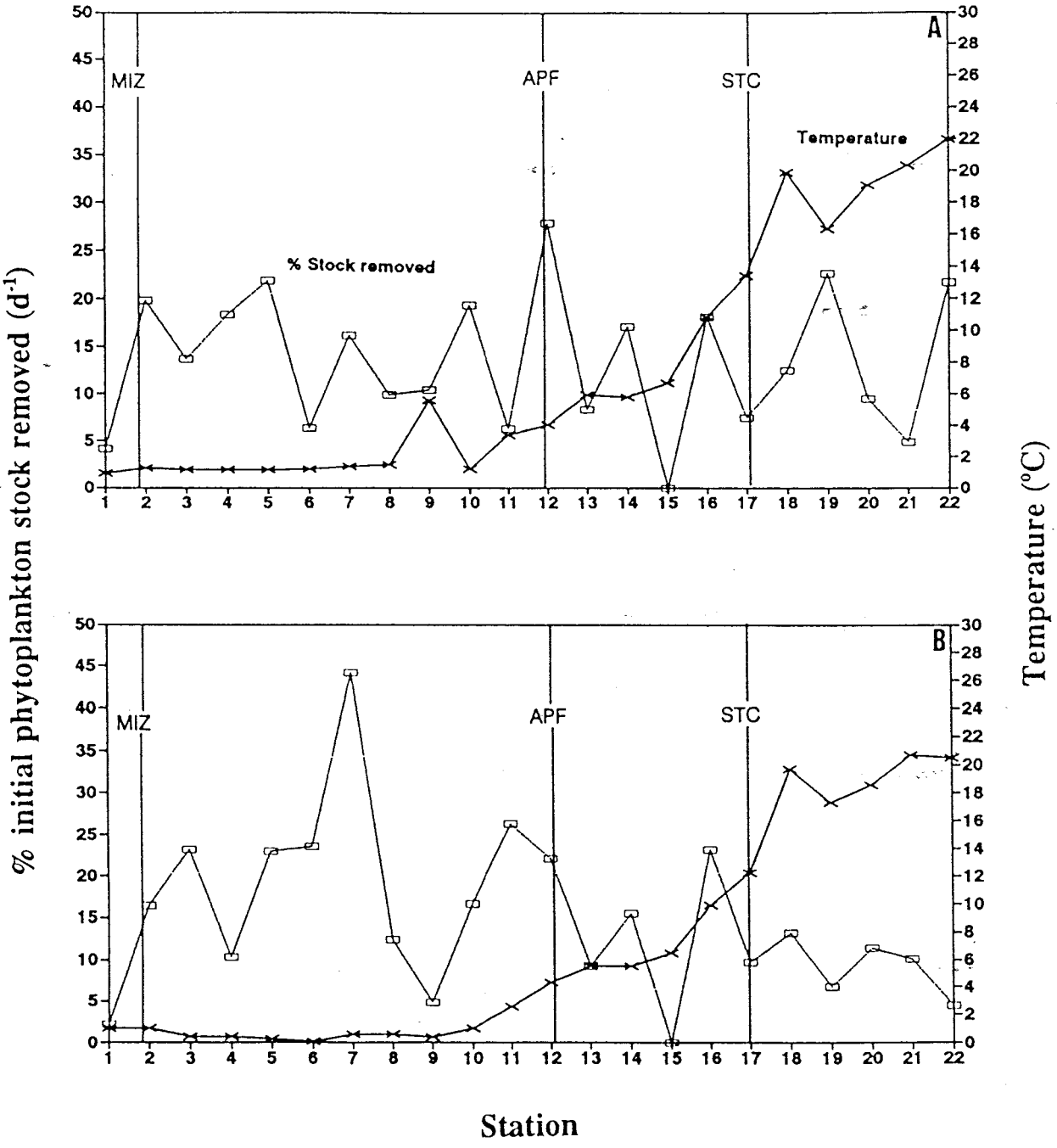

Figure 2.7 Temperature profile and % initial standing stock removed along the SANAE-Cape Town transect in the surface waters (**A**) and chlorophyll maximum (**B**) during the SAAMES II cruise in late austral summer (Jan./Feb.) 1993. **MIZ** = marginal ice zone; **APF** = Antarctic Polar Front; **STC** = Subtropical Convergence.

This provides an index of the physiological status of a phytoplankton community. Results of an unpublished production study (R.K Laubscher, Southern Ocean Group, Rhodes University) show that the PC value of the phytoplankton community at station 15 was among the highest of the entire transect. This suggests that the phytoplankton community at station 15 was in the exponential phase of growth, resulting in an increase in biomass during the grazing experiments.

A preliminary study of algal dynamics in the incubation bottles demonstrated that the nano- and the picophytoplankton contribution to total chlorophyll decreased over the duration of the incubation period (Figure 2.6). Bottling effects on plankton communities are well documented (Venrick *et al.*, 1977; Cullen *et al.*, 1986; Taguchi *et al*, 1993). Changes in the contribution of the phytoplankton fractions to total chlorophyll may result from nutrient limitation and the filtration procedure, including cell breakage and incomplete recovery of cells by the filter (Hilmer & Bate, 1989) or grazing by zooplankton. Changes associated with nutrient limitation and the filtration procedure will generally decrease the contribution of netphytoplankton, in the former case because of physiological limitations for the uptake of nutrients associated with their size (Fogg, 1991) and in the latter because of the fragile frustules. A potential source for the decrease in the < 20 µm fractions may be selective grazing by the microzooplankton assemblages in the incubation bottles. The decrease in the nano- and picophytoplankton concentrations is consistent with the species composition of the protozooplankton assemblages recorded (dominated by nanoheterotrophs and ciliates). Ciliates and nanoheterotrophs generally feed on particles < 20 µm (Rassoulzadegan *et al.*, 1988; Mazumder *et al.*, 1990; Hansen *et al.*, 1994).

Throughout the cruise period, protozooplankton abundance co-varied with phytoplankton abundance (Figures 2.3 and 2.5). Numerous field studies have demonstrated the coupling between microzooplankton abundance and phytoplankton concentration (Silver *et al.*, 1984; Wasik & Mikolajczyk, 1990; Gowing & Garrison, 1991; 1992; Hansen, 1991). In particular, strong correlations between tintinnid growth rates and food availability have been demonstrated (Verity, (1986) cited in Pierce & Turner, 1992). The distribution and abundance of both ciliates and dinoflagellates have been shown to be greatest in the upper 50m of the water column where the chlorophyll concentration is greatest (Silver *et al.*, 1984; Wasik & Mikolajczyk, 1990; Gowing & Garrison, 1991; 1992). Jonsson (1989) suggests that ciliates

may influence their own vertical distribution through direct motility and effects of geotaxis and turbulence. This implies that, as predators, they would be able to orientate themselves towards prey and therefore meet their metabolic demands at lower prey concentrations (Jonsson, 1989). In addition, ciliates would remain in areas rich in prey. Analysis of variance indicated that neither the percentage initial stock nor potential primary production removed by microzooplankton grazing was significantly different between depths. This suggests that the remaining components of the microzooplankton assemblage may also be able to actively determine their position within the water column, aggregating in regions where chlorophyll concentrations are highest. However, this may also represent a local response (increased specific growth rate) of the microzooplankton assemblage to enhanced levels of phytoplankton biomass.

Densities of the various components of the protozooplankton assemblages along the transect varied considerably between stations (Tables 2.1A and 2.1B). Nanoheterotrophs (< 20 μm) dominated at all stations, with ciliates dominating the > 20 μm protozooplankton assemblage. The fixation of the samples with 6% glutaraldehyde solution, however, may have resulted in the underestimation of the ciliate contribution to total protozooplankton counts (James, 1991). According to Garrison (1991), an extreme range of variability within individual studies is a key feature of microzooplankton studies in Antarctic waters. Indeed, mesoscale patchiness is highly developed in the Southern Ocean within both the phytoplankton and the zooplankton (Alder & Boltovskoy, 1991; Perissinotto & McQuaid, 1992). Factors that may influence the distribution and abundances of microzooplankton species include habitat availability (pack ice) and biological interactions. For example, Alder & Boltovskoy (1991) found a high spatial correlation between dinoflagellates and tintinnids in Antarctic waters, which they ascribed to a common food source (silico-flagellates) or the predatory nature of tintinnids, which feed on dinoflagellates (Stoecker *et al.*, 1984).

Microzooplankton grazing along the transect removed on average 14.4% of the initial standing stock (≈ 24.1% of potential production; Tables 2.2A and 2.2B). Grazing experiments, however, were conducted in the absence of potential predators > 200 μm. A preliminary study of gut contents of adult *Euphausia superba* (n = 5) in the region of the APF indicates that protozooplankton (*Ceratium* spp., *Dinophysis* spp., *Amphisolenia* spp and *Protoperidinium* spp) constitute one third of all cells identified in the gut (P.W. Froneman, unpublished data).

This result is consistent with the findings of Hopkins & Torres (1989), which demonstrated that macrozooplankton are major consumers of protozooplankton. This suggests that microzooplankton grazing impact was overestimated and highlights the need for future studies on species interactions among the various size classes of zooplankton.

Microzooplankton grazing has been suggested as a potential reason for the low chlorophyll concentrations recorded in the Southern Ocean (Bjornsen & Kuparinen, 1991). However, the low microzooplankton grazing rates measured during this study suggest that in the Southern Ocean the role of microzooplankton in carbon flux may be minor and that the bulk of the carbon is either lost to sedimentation (von Bodungen *et al.*, 1986; 1988; Fischer *et al.*, 1988) or is channelled into the larger herbivores (meso and macrozooplankton). This result partially supports the model proposed by Huntley *et al.* (1991), which suggests that up to 80% of the net production in the Southern Ocean is channelled directly to the macrozooplankton. However, the relative importance of microzooplankton vs. macrozooplankton may shift seasonally as well as spatially when the size of the phytoplankton in the ecosystem shifts (Pierce & Turner, 1992). Size fractionated grazing studies suggest that in areas where the nano- and picophytoplankton size fractions dominate, the relative importance of microzooplankton would increase along with their grazing impact on total production. The total carbon channelled into the microzooplankton component would thus increase accordingly. Consequently, the role of microzooplankton in carbon flow within the Southern Ocean should differ north and south of the APF as the netphytoplankton fraction dominates in Antarctic waters while smaller fractions (nano-/picophytoplankton) dominate in sub-Antarctic waters (Figure 2.4; Laubscher *et al.*, 1993). The claim of Moloney (1992) that ≈ 60% of the primary production enters the microbial food web is likely to apply only to regions north of the APF, where the contribution of the smaller fractions to total chlorophyll increases.

This has important implications for the biological pump as carbon flux through the micro and mesoplanktonic food webs differ substantially (Michaels & Silver, 1988). Microzooplankton produce relatively small faecal minipellets, which have low sinking rates, while meso/macroplankton produce larger, faster sinking faecal pellets (Fortier *et al.*, 1994). Larger faecal pellets cause a rapid downward flux of organic material from the euphotic zone to the deep sea. This flux is reinforced by diel migrations of most mesoplankton over

hundreds of meters (Longhurst, 1991; Perissinotto & McQuaid, 1992). Through these pathways, production originating in the euphotic zone is consumed and transported below the zone of regenerated production, and the carbon available to the higher trophic levels is reduced.

Microzooplankton minipellets are decomposed together with dissolved and particulate organic matter by heterotrophic bacteria in the zone of regenerated production (Azam *et al.*, 1983; Sherr & Sherr, 1988). Heterotrophic bacteria in turn represent a considerable source of secondary biomass production via the consumption by ciliate and flagellate bacterivores (Anderson & Fenchel, 1984; Albright *et al.*, 1987). Carbon that would otherwise be lost is, therefore, made available again as the microzooplankton in turn are consumed by metazoan grazers (Hewes *et al.*, 1985; Gifford, 1991; Gifford & Dagg, 1988; 1991).

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CHAPTER 3

STRUCTURE AND GRAZING OF THE MICROZOOPLANKTON COMMUNITIES OF THE SUBTROPICAL CONVERGENCE AND A WARM-CORE EDDY IN THE ATLANTIC SECTOR OF THE SOUTHERN OCEAN

Abstract

The comminity structure and grazing dynamics of microzooplankton were investigated at 15 stations during the SAAMES III cruise to the region of the Subtropical Convergence (STC) and across a warm-core eddy in subantarctic waters during austral winter (June/July) 1993. Microzooplankton abundance co-varied with the combined concentration of the nano- and picophytoplankton size fractions. Nano-flagellates dominated numerically at all stations while the > 20 µm fraction was generally dominated by ciliates (oligotrichs and tintinnids). Mixotrophs comprised between 0 and 5% of total chlorophyll concentration. Production in the region showed a weak seasonal trend with the exception of stations in the vicinity of the STC. Instantaneous growth and grazing co-efficients exhibited clear spatial trends, with the highest rates recorded at the edge of the eddy and in the region of the STC. Instantaneous grazing rates at stations at the edge of the eddy and at the STC varied from 0.347 to 0.701 d^{-1}, equivalent to a loss of 30-51% of the initial standing stock and between 56 and 69% of the potential primary production. In the warm core eddy, subantarctic and Agulhas waters, instantaneous grazing rates ranged from 0.281 to 0.433 d^{-1}. This is equivalent to a loss of 24 - 35% of the initial standing stock and between 59 and 83% of the potential primary production. Size selectivity experiments suggest that microzooplankton preferentially graze on the pico- and nanophytoplankton size fractions. The results of this study show that the bulk of photosynthetically fixed carbon is channelled into the microbial loop during austral winter. This implies that the carbon pump is relatively inefficient during winter and that atmospheric CO_2 drawdown via sinking of organic matter into deep water may be very limited in this area.

3.1 Introduction

The Subtropical Convergence (STC) is one of the major frontal systems of the Southern Ocean and constitutes its northern boundary (Deacon, 1982; Lutjeharms & Valentine, 1988). Production in the region of the front shows no seasonal trends, with periods of elevated production alternating with periods of lower production throughout the year (Comiso *et al.*, 1993). According to Dower & Lucas (1993), the STC may represent an important biogenic sink for atmospheric CO_2, and may account for between 0.5 and 0.8% of the total global ocean production.

The interaction of the Agulhas Retroflection Current (ARC) with the northern boundary of the STC in the oceanic region south of Africa results in a high variability in currents and the formation and shedding of warm-core eddies (Lutjeharms & Valentine, 1988; Duncombe Rae, 1991). These eddies subsequently move southwards across the STC, contributing to meridional heat flux into the Southern Ocean and to the transfer of salt between the south Indian Ocean and the south Atlantic (Lutjeharms & Gordon, 1987; Duncombe Rae, 1991). Although the effects of these eddies on the physico-chemical parameters of the region have been the subject of several investigations (Lutjeharms & Gordon, 1987; Lutjeharms & Valentine, 1988; Duncombe Rae, 1991), their effects on the biological processes have largely been neglected in the past.

Studies on the effects of eddies on biological processes elsewhere, and particularly in the Gulf Stream and East Australian Current, have demonstrated that eddies may result in localised areas of increased phytoplankton productivity (Tranter *et al.*, 1980; Angel & Fasham, 1983; Smith & Baker, 1985; Franks *et al.*, 1986) and may be important in the transportation of biological populations between different water masses (Angel & Fasham, 1983). Possible ecological consequences of eddy shedding from the ARC may be the transportation of warm water species across the STC which is regarded as an important biogeographical barrier (Deacon, 1982). Also, indirectly these eddies may result in changes in heat and CO_2 flux which could influence the productivity of the region. A recent study by Dower & Lucas (1993) found enhanced productivity rates at the edge of a warm-core eddy shed from the ARC, in generally low productive subantarctic waters.

Several field studies have demonstrated the importance of microzooplankton in the marine environment (see reviews of Porter *et al.*, 1985; Garrison, 1991; Pierce & Turner, 1992). In the Southern Ocean, a recent study by Froneman & Perissinotto (in press), suggests that the impact of microzooplankton on phytoplankton stock may be determined by the contribution of smaller fractions (nano- and picophytoplankton) to total chlorophyll concentration. Indeed, it is well documented that in coastal environments where the phytoplankton are dominated by pico- and nanoplankton, protozooplankton are often the most significant herbivores (Capriulo & Carpenter, 1980; Burkill *et al.*, 1987). This implies that the impact of microzooplankton on the carbon flux of the Southern Ocean may shift seasonally, since the contribution of the netphytoplankton to total chlorophyll decreases during austral winter, when the nano- and picophytoplankton dominate chlorophyll concentration (Garrison *et al.*, 1993). This may have important implications for the CO_2 flux, as a food web dominated by microzooplankton grazing is characterised by little sedimentation of particulate organic carbon (POC) out of the zone of regeneration where it is recycled by the microbial loop (Michaels & Silver, 1988; Longhurst & Harrison, 1989; Longhurst, 1991). Consequently, the transfer of atmospheric CO_2 into deep waters may be reduced. In order to have a better understanding of the Southern Ocean system as a potential biogenic sink for atmospheric CO_2, it is therefore essential that possible seasonal effects be investigated.

In this paper, the community structure and grazing impact of microzooplankton in the region of the Subtropical Convergence (STC) and in a warm-core eddy shed from the Agulhas Retroflection Current were investigated during austral winter (June/ July) 1993. Grazing rates were determined at 15 stations.

3.2 Materials and methods

Microzooplankton grazing experiments were conducted during the third cruise of the South African Antarctic Marine Ecosystem Study (SAAMES III) aboard the *MV. S.A. Agulhas* in mid austral winter (June/July) 1993 (Figure 3.1). Grazing experiments were carried out using water from the surface layer (5 m), by employing the sequential dilution technique (Landry & Hassett, 1982).

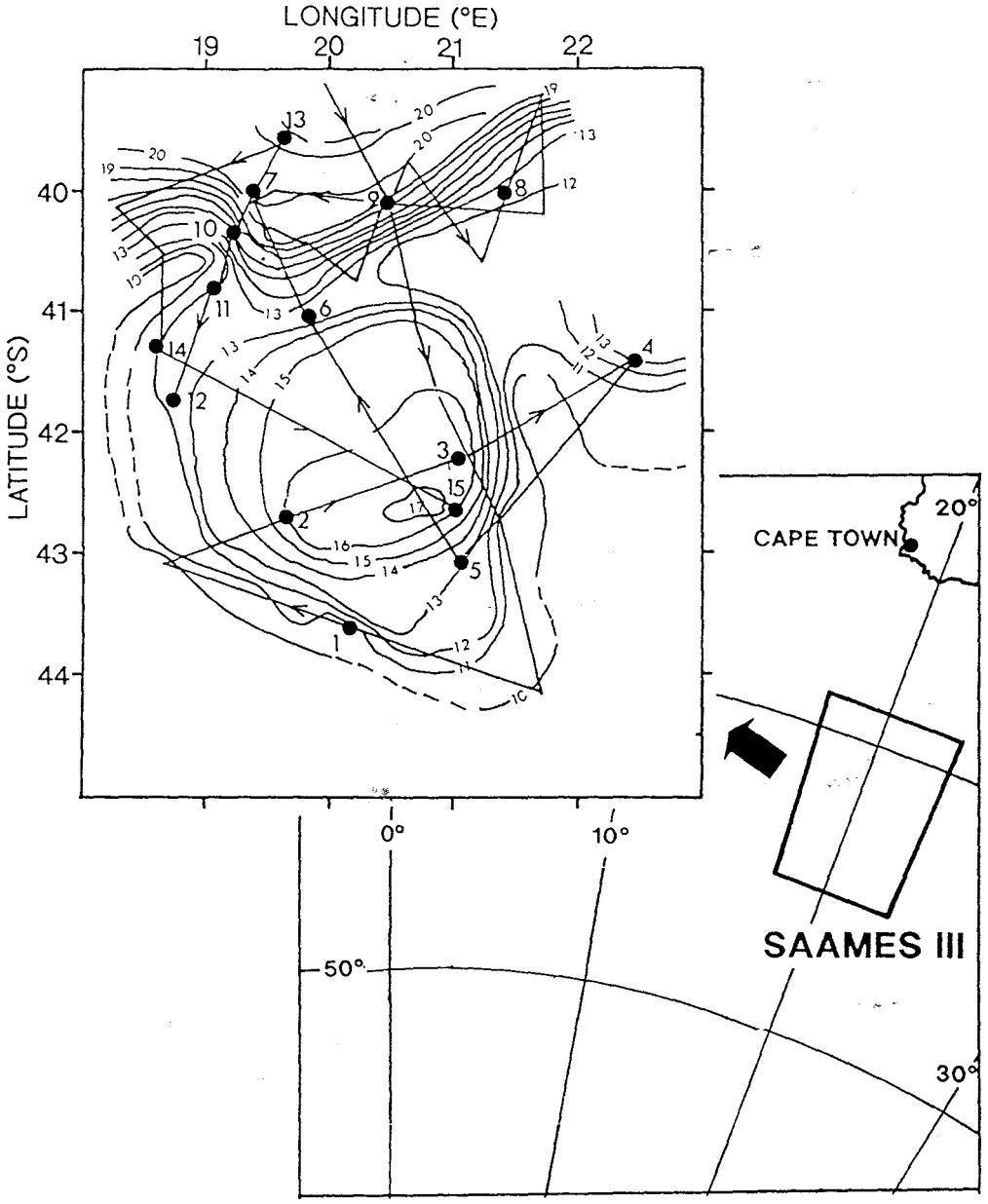

Figure 3.1 Cruise track and position of stations (every station numbered) during the SAAMES III cruise aboard the MV. S.A Agulhas in late austral winter in the Atlantic sector of the Southern Ocean. STC = Subtropical Convergence. Contours are sea surface temperature.

Water samples were obtained using a submersible pump (Flyght-kyokuto model L 40- 25S), operated at a flow rate of ≈ 15 l m^{-1} and supplied to 25 l polyethylene containers through PVC piping. The water was then passed through a 200 µm mesh to separate the microzooplankton fraction. Particle-free water was obtained by passing surface water through a 0.2 µm Milli Q system (Millipore). Dilution series in 2 l acid-washed polyethylene bottles of 1:0 3:1; 1:1; 1:3 filtered to particle-free water were made. Three replicas of each dilution series were prepared. The dilution series were then incubated on deck for 24 hours in perspex incubators cooled with running surface water and screened with shade cloth to simulate light intensity (500 - 1300 µE m^{-2} s^{-1}) at the depth of collection.

Before incubation was begun, water samples (250 ml) for the determination of initial chlorophyll-*a* concentration were taken from each bottle. Bottles were sampled again (250 ml) at the end of the incubation period to determine final chlorophyll-*a* concentration. Chlorophyll-*a* was then fractionated into the net- (≥ 20 µm), nano- (2.0 - 20 µm) and picophytoplankton (0.2 - 2.0 µm) size fractions through multiple serial filtration. Size selectivity studies were also carried out in parallel. Using the same incubation settings, water samples taken from the 1:0 dilution series were fractionated into net (≥ 20 µm), nano (2.0-20 µm) and picophytoplankton (0.2 - 2.0 µm) size fractions at the onset and at the end of the incubation period. Chlorophyll-*a* concentrations were determined fluorometrically (Turner 111 fluorometer), after extraction in 100% methanol (Holm-Hansen & Riemann, 1978).

To identify and enumerate the various components of the microzooplankton community, a 50 ml seawater sample was stained with Proflavine (50 µl/ml; 2 min.), fixed with glutaraldehyde (final conc. 6%) and then filtered (vacuum ≤ 13 cm Hg) through a 2.0 µm Irgalan black prestained Nuclepore filter (Haas, 1982). Permanent slides were then prepared according to the method of Booth (1987) and frozen at -20 ^{0}C. Slides were examined within two months after the cruise using a Zeiss fluorescent microscope equipped with a 450-490 excitation filter, a FT 510 chromatic beam splitter and a long pass 528 barrier filter operated at 400 x magnification (Haas, 1982). No significant loss in the autofluorescence of the chlorophyll containing organisms was anticipated (Booth, 1987). Phototrophic organisms were distinguished from heterotrophic organisms by the red autofluorecence of chlorophyll-*a* (Haas, 1982).

Microzooplankton were grouped into the following protozooplankton groups: tintinnids, aloricate ciliates, dinoflagellates, heterotrophic-nanoflagellates (h-nanoflagellates, < 20 μm) and mixotrophs. Enumerations were converted to cells l^{-1} by employing the equation of Waterbury *et al.* (1986):

$$\frac{\text{No. cells in 100 fields x (total area of filter) x 20}}{\text{(area of 100 fields)}}$$

The apparent growth rate of chlorophyll-a at each dilution is calculated as:

$$\frac{1}{t} \ln \left(\frac{P_t}{P_o} \right)$$

where P_o and P_t are chlorophyll concentrations at the beginning and end of the experiment; t is duration of experiment. This is the observed change in chlorophyll in the presence of grazers. The theoretical growth rate of phytoplankton in the absence of grazers (k) is taken to be the y intercept from the regression analysis between apparent growth rate and dilution (Figure 2.2). The slope of the regression is the instantaneous grazing coefficient (g) of the microzoplankton (Figure 2.2). This regression was calculated by using the computer package, Statgraphics Version 5.0 (Statistical Graphics Corporation).

To normalise the chlorophyll values, all data were transformed using the factor:

$$\log (x + 1) \quad \text{(Legendre \& Legendre, 1983)}$$

Also, grazing data expressed in % were normalised by the arcsin transformation (Sokal & Rolhf, 1969). Partial correlation analysis was then performed on the grazing data to identify possible relationships between grazing rates, temperature and concentrations of chlorophyll size fractions (Sokal & Rolhf, 1969). The computer package Statgraphics Version 5.0 was again used for this analysis.

3.3 Results

Chlorophyll distribution

Chlorophyll concentrations measured along the transect exhibited a clear spatial pattern. Highest concentrations (> 0.5 μg l^{-1}) were recorded at stations at the periphery of the eddy (stations 6 and 12) and in the region of the STC (stations 8-11) (Figure 3.2). An exception was provided by station 1, located at the periphery of the eddy, where the lowest chlorophyll concentration (0.16 μg l^{-1}) along the entire cruise track was recorded. Stations in the warm-core eddy (stations 2, 3, 5 and 15), in subantarctic (stations 4 and 14) and Agulhas waters (stations 7 and 13) were characterised by chlorophyll concentrations < 0.5 μg l^{-1}. The nano- and picophytoplankton size fractions dominated chlorophyll biomass at all stations (Figure 3.2). The picophytoplankton in particular was the dominant component at all but four stations (stations 1, 2, 9 and 10) where the nanophytoplankton dominated chlorophyll concentration. The contribution of the netphytoplankton fraction to total chlorophyll concentration was < 10% at all stations with the exception of station 8 where it contributed ≈ 20% of the total.

Community structure

Microzooplankton abundance generally co-varied with the combined concentrations of the nano- and picophytoplankton (< 20 μm) size-fractions (Figure 3.3). Indeed, ≈ 35% of the variance associated with microzooplankton abundance could be described by the 0.2 - 20 μm size fraction ($P < 0.05$).

H-nanoflagellates (< 20 μm) dominated numerically at all stations (Table 3.1). Densities ranged between 106 and 403 ind.l^{-1}. The > 20 μm microzooplankton fraction was generally dominated by ciliates (oligotrichs and tintinnids), although dinoflagellates dominated at 5 of the 15 grazing stations (Table 3.1). The contribution of tintinnids to total ciliate densities was always less than the contribution of the oligotrichs. The least represented fraction was the plastid-containing microzooplankton or mixotrophs, which comprised < 5% of total cell counts at all stations. Densities of this group never exceeded 14 ind.l^{-1}.

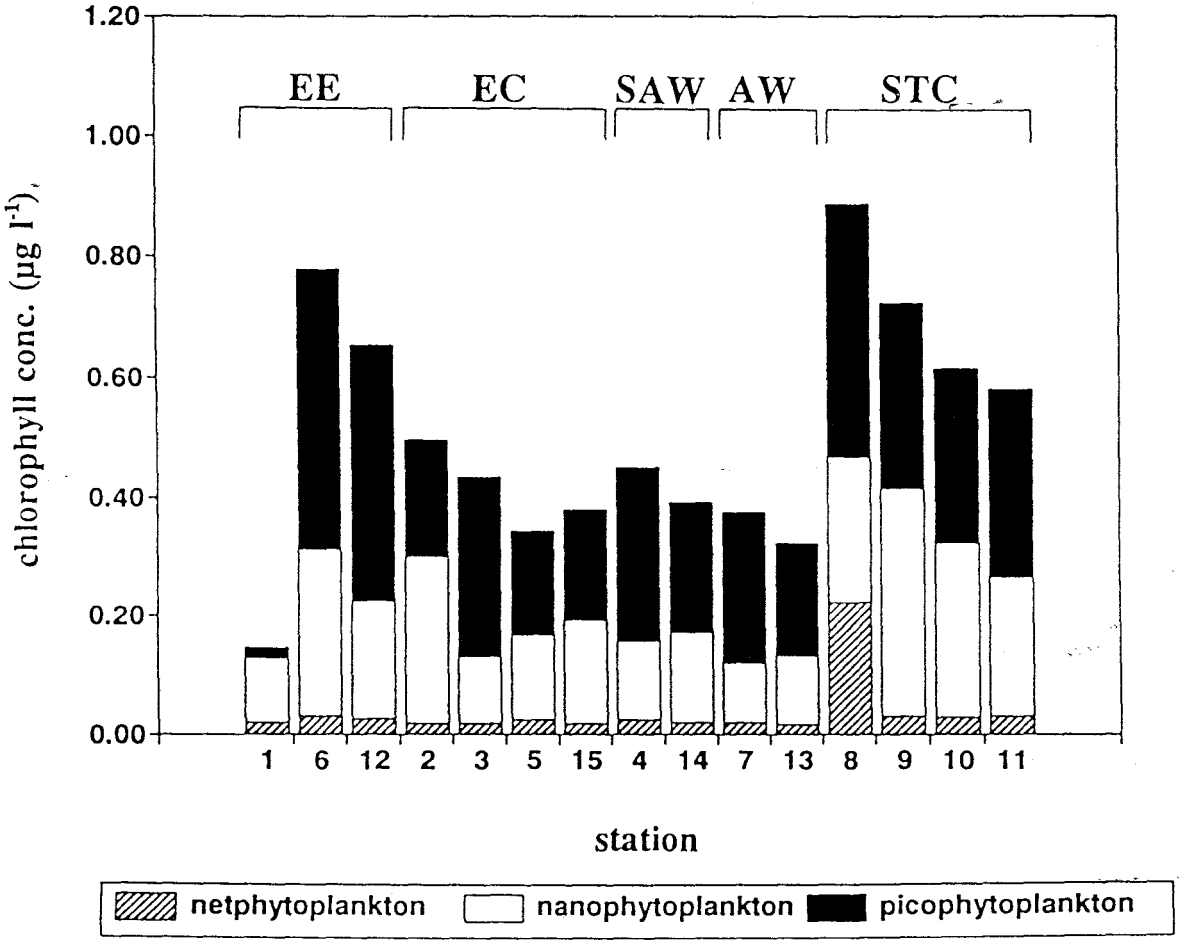

Figure 3.2 Size fractionated chlorophyll-*a* in surface waters along the STC transect during the SAAMES III cruise (June/July, 1993). EE = Eddy edge; EC = Eddy core; STC = Subtropical Convergence; SAW = Subantarctic waters; AW = Agulhas waters.

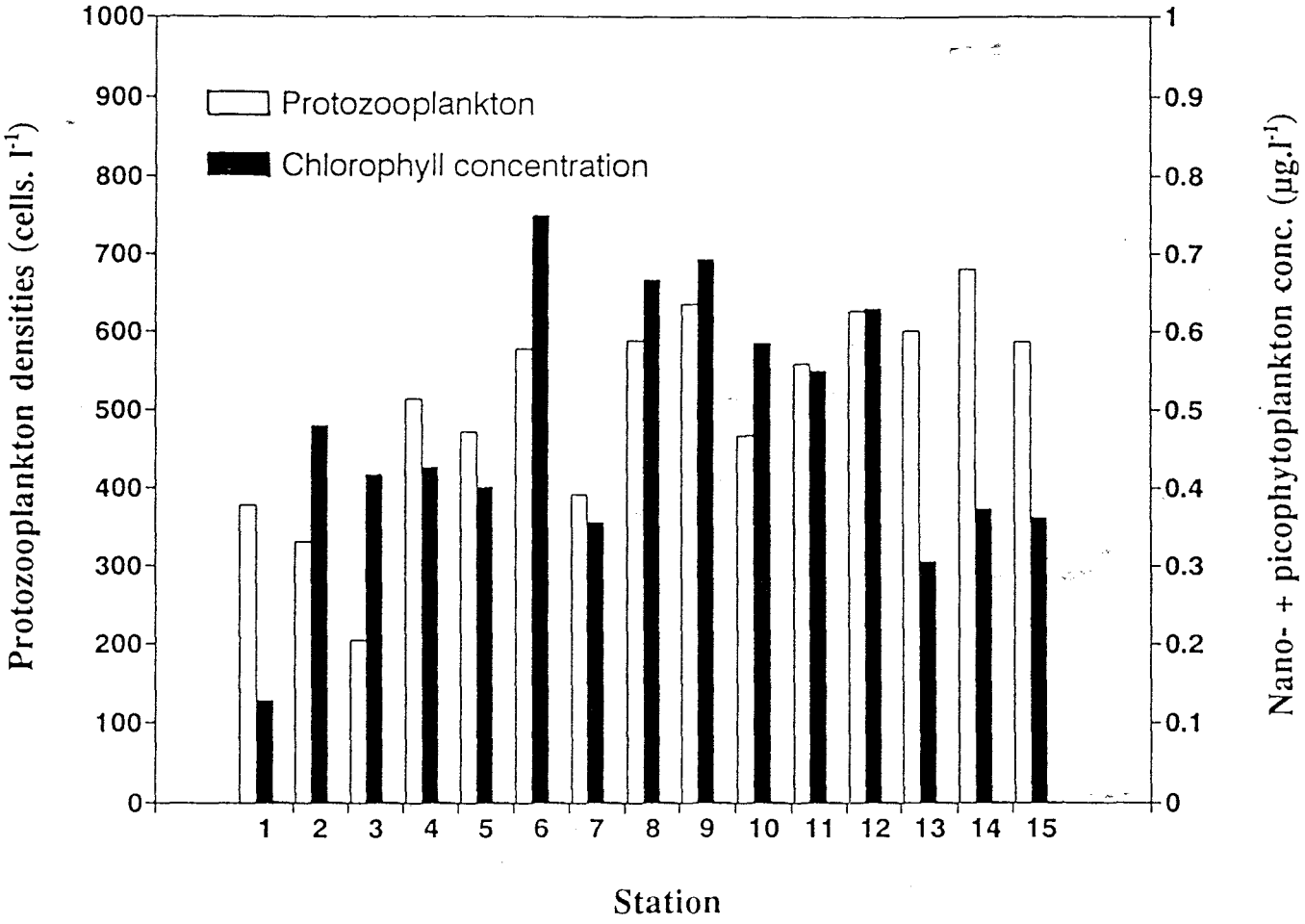

Figure 3.3 Total microzooplankton abundance and combined concentrations of the nano- and picophytoplankton size classes along the SAAMES III transect.

Table 3.1 Abundance of heterotrophic protozooplankton at grazing stations during the SAAMES III cruise (June July) 1993. Results are expressed as ind.l^{-1}.

Station number	Aloricate ciliates	Tintinnids	Dinoflagellates	Nano-heterotrophs	Mixotrophs
1	60	21	64	230	3
2	53	13	71	186	9
3	21	0	32	149	3
4	92	0	96	319	7
5	74	11	82	294	11
6	67	7	57	343	3
7	50	4	124	213	3
8	22	4	27	106	0
9	124	21	117	358	14
10	82	25	50	305	7
11	149	21	128	258	3
12	133	11	112	357	14
13	129	14	124	340	3
14	124	3	146	403	7
15	116	13	142	319	0

Microzooplankton grazing

Instantaneous phytoplankton growth rates, grazing rates and regression coefficients for the grazing experiments at the various oceanographic areas are shown in Table 3.2. During this investigation significant linear regressions ($P < 0.05$) were found between dilution and the apparent phytoplankton growth (Table 3.2). Both growth and grazing coefficients show similar spatial patterns with highest rates recorded at stations at the edge of the eddy and in the vicinity of the STC (Table 3.2). Algal growth coefficients (k) at the edge of the warm-core eddy ranged from 0.55 - 1.32 d^{-1} and between 1.21 and 1.48 d^{-1} at the STC (Table 3.2). These values are equivalent to chlorophyll doubling rates between 0.79 and 1.90 d^{-1} at the edge of the eddy, and between 1.75 and 2.13 d^{-1} at the STC. Within the warm-core eddy algal growth coefficients ranged from 0.51 to 0.71 d^{-1} while within subantarctic waters they were lower between 0.45 - 0.56 d^{-1} (Table 3.2). At stations in the Agulhas waters, the algal growth coefficients ranged from 0.47 to 0.58 d^{-1}. This represents a chlorophyll doubling rate of 0.74 - 0.99 d^{-1} in the core of the eddy, between 0.654 and 0.800 d^{-1} in the subantarctic waters and from 0.71 to 0.84 d^{-1} in the Agulhas waters (Table 3.2).

Grazing coefficients of microzooplankton (g) were highest at stations at the periphery of the warm-core eddy (range: 0.35 - 0.70 d^{-1}) and in the region of the STC (range: 0.53 - 0.72 d^{-1}). This level of instantaneous grazing activity by microzooplankton represents a loss of between 29 and 51% of the initial standing stock at the edge of the warm-core eddy and at the STC, respectively (Table 3.2). The equivalent daily loss of potential production due to grazing in these regions was between 56 and 70% (Table 3.2).

Instantaneous grazing rates of microzooplankton ranged from 0.28 - 0.43 d^{-1} in the warm core eddy, between 0.351 and 0.393 d^{-1} in subantarctic waters and from 0.30 to 0.33 d^{-1} in the Agulhas waters (Table 3.2). This level of grazing is equivalent to a loss of between 25 -33% of the initial standing stock in the three regions (Table 3.2). The potential primary production removed ranged between 62 and 83% of the total (Table 3.2).

Partial Correlation Analysis performed between grazing data and selected variables indicated that the initial standing stock removed was correlated positively to the concentrations of pico- ($t = 3.31$; $P < 0.001$) and nanophytoplankton ($t = 1.87$; $P < 0.05$). Also, the relationship

between growth rate of phytoplankton and grazing impact of microzooplankton was significant ($t = 10.04$; $P < 0.001$). To test whether this relationship was the result of auto correlation, a Box Jenkins test was applied to the data set. A significant correlation was again obtained between microzooplankton grazing and the growth rate of phytoplankton ($r^2 = 0.51$; $P < 0.05$) indicating that the relationship was not artefactual.

Table 3.2 Grazing rate and impact parameters of microzooplankton assemblages of the region of the Subtropical Convergence during the SAAMES III cruise in the Atlantic sector of the Southern Ocean in late austral winter (June/July) 1993. (** = $P < 0.001$; * = $P < 0.05$). Values in parenthsis = standard error. Chlorophyll doublings $d^{-1} = k/\ln 2$ (Gifford, 1988).

oceanographic area	station	chl-*a* conc.	r^2	growth coeff. k (d^{-1})	grazing coeff. g (d^{-1}).	%initial stock removed	%potential prod. removed	chlorophyll doublings (d^{-1})
eddy edge	1	0.160	0.48*	0.55 (0.123)	0.35 (0.046)	29.38	69.83	0.791
	6	0.780	0.53*	1.32 (0.178)	0.70 (0.091)	50.38	68.84	1.900
	12	0.656	0.76**	1.30 (0.073)	0.67 (0.078)	48.93	67.35	1.847
eddy core	2	0.498	0.66**	0.51 (0.101)	0.40 (0.031)	33.33	82.63	0.740
	3	0.436	0.50*	0.71 (0.039)	0.38 (0.071)	31.42	61.83	1.019
	5	0.395	0.55*	0.58 (0.084)	0.28 (0.011)	24.55	55.62	0.838
	15	0.380	0.54*	0.69 (0.102)	0.43 (0.037)	35.26	70.37	0.994
subantarctic waters	4	0.450	0.66*	0.45 (0.098)	0.35 (0.013)	29.56	81.08	0.654
	14	0.393	0.35*	0.56 (0.107)	0.39 (0.037)	32.56	76.36	0.800
Agulhas waters	7	0.376	067**	0.47 (0.081)	0.34 (0.044)	28.45	77.73	0.708
	13	0.321	0.57*	0.58 (0.053)	0.30 (0.065)	25.55	71.54	0.838
Subtropical Convergence	8	0.889	0.89**	1.23 (0.153)	0.60 (0.096)	45.11	63.58	1.779
	9	0.725	0.74**	1.21 (0.108)	0.66 (0.080)	48.41	68.99	1.751
	10	0.614	0.32*	1.32 (0.127)	0.53 (0.073)	41.21	56.25	1.901
	11	0.581	0.39*	1.48 (0.278)	0.72 (0.103)	51.12	66.29	2.129

Size selectivity

Microzooplankton preferentially grazed the smaller size fractions (Figure 3.4). Picophytoplankton was the most intensely grazed fraction, decreasing in concentration during grazing experiments at 11 of the 15 stations. Mean decrease in concentration along the cruise track was 20.8%. Nanophytoplankton was the second most intensely grazed fraction, decreasing in concentration at 7 stations, with a mean decrease of 7.6% along the transect. Analysis of variance (ANOVA) and multiple range tests indicate that the % pico- and nanophytoplankton removed were not significantly different ($F = 0.712$; $P = 0.41$). The least grazed fraction was the netphytoplankton, which during the incubation period increased in concentration at 14 of the 15 stations examined (Figure 3.4).

3.4 Discussion

The role of microzooplankton in determining carbon flux in marine systems has been the subject of extensive investigations (see review of Pierce & Turner, 1992). The results of many studies suggest that the impact of microzooplankton varies greatly over both spatial and temporal scales. Recent studies have shown that the impact of microzooplankton on phytoplankton standing stock is largely determined by the contribution of the smaller chlorophyll fractions to total chlorophyll concentration (Froneman & Perissinotto, in press). Indeed, microzooplankton may remove > 70% of potential primary production in subarctic regions where the < 20 µm fractions dominate chlorophyll concentration (Paranjape, 1987; Verity *et al.*, 1993). During this study, microzooplankton removed > 50% of potential production, suggesting that microzooplankton may represent the major route for the uptake of organic carbon (Table 3.2). This is consistent with results from previous studies conducted in regions where the < 20.0 µm chlorophyll fractions dominate chlorophyll biomass (see results of Gifford, 1988).

The highest algal growth coefficients during the study were recorded in the areas of higher production, at the Subtropical Convergence (STC) and at the edge of the warm -core eddy while the lowest coefficients were associated with the regions of lower production, in the subantarctic and Agulhas waters and in the core of the eddy (Table 3.2). Growth rates in winter were generally lower than summer growth rates suggesting a seasonal influence on

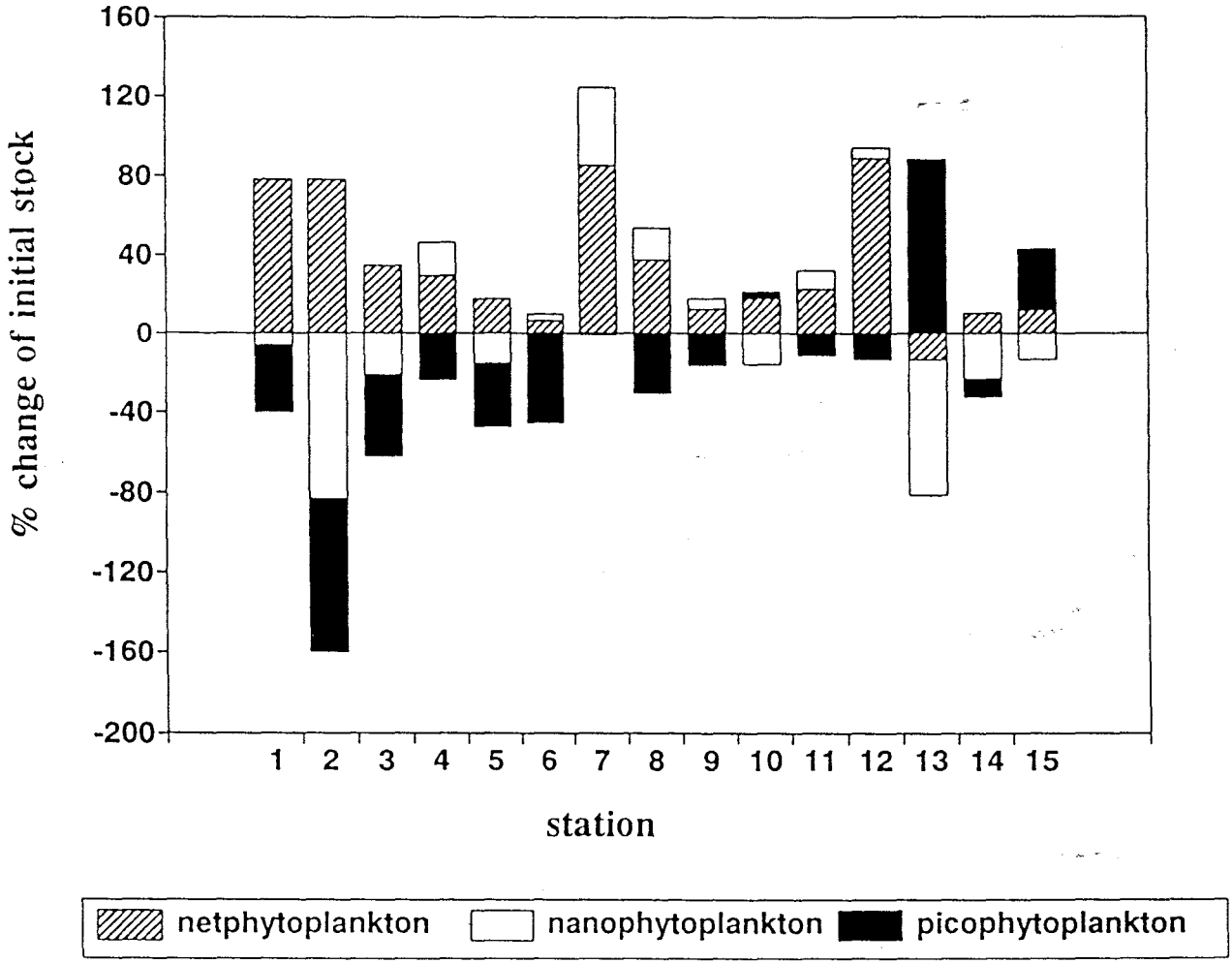

Figure 3.4 Results of size selectivity grazing studies. Results are expressed as percentage increase or decrease of initial concentrations.

production (Froneman & Perissinotto, in press). The result is consistent with size fractionated primary production study (determined by measuring ^{14}C uptake) conducted simultaneously with the microzooplankton grazing studies (Laubscher, unpublished). The exceptions presented were at stations in the vicinity of the STC where winter growth rates were in the same range as summer growth rates (Froneman & Perissinotto, in press). This result is consistent with the findings of Comiso *et al.* (1993) which demonstrated that production at the front shows no seasonal trends. The estimates estimates of algal growth in the low productive areas during this study compare well with a similar study conducted in the Bransfield Strait (Taylor & Haberstroh, 1988). The high algal growth coefficients recorded at the STC and edge of the eddy are among the highest published values in the Southern Ocean. Elevated production in the region of the STC and at the edge of warm-core eddies are well documented and are thought to be the result of a favourable light environment conferred by increased water column stability (El-Sayed, 1988; Dower & Lucas, 1993; Comiso *et al.*, 1993). The spatial differences in production rates and chlorophyll concentrations are reflected in the amount of initial standing stock and potential production removed daily by microzooplankton (Table 3.2).

The initial standing stock removed by microzooplankton grazing was highest at the edge of the warm-core eddy and in the region of the STC (Table 3.2). Several factors have been implicated in determining community grazing rates including temperature, predator abundance, and ingestion rates as determined by the food concentration (Strom & Welschmeyer, 1991; Peters, 1994). During the present study, the relationship between grazing impact and temperature was not significant ($P < 0.05$). However, the grazing impact of microzooplankton was significantly correlated to protozooplankton abundance (Figure 3.5A) and to concentrations of the < 20 µm chlorophyll fraction (Figure 3.5B). These results suggest that the high grazing impact of microzooplankton at the edge of the eddy and in the vicinity of the STC result from the high predator/prey concentrations. Also, the close coupling between phytoplankton growth rates and grazing impact suggest that the phytoplankton/microzooplankton transfer efficiency will be high. Exceptions presented may reflect the wide range of trophic interactions of microzooplankton reported in the literature (Pierce & Turner, 1992; Peters, 1994). For example, non phytoplankton food such as bacteria

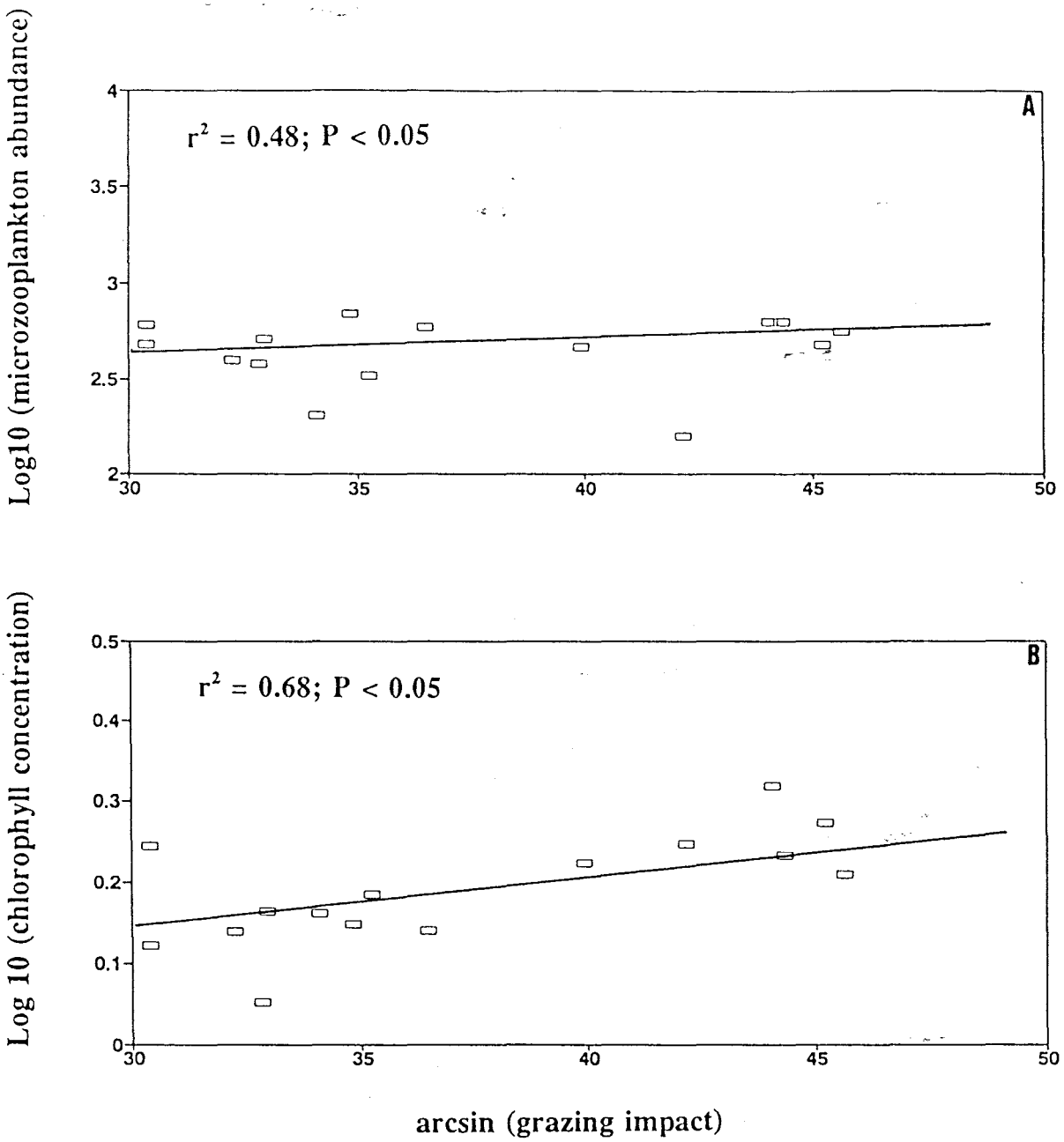

Figure 3.5 Relationship between % initial standing stock removed, protozooplankton abundance (**A**) and < 20 µm chlorophyll fraction (**B**) along the SAAMES III cruise in late austral winter (June/July) 1993.

may represent an important trophic resource. Unfortunately, no information is available on microzooplankton bactivory.

The levels of potential primary production removed showed a weak inverse spatial pattern to the initial standing stock removed (Table 3.2). The lowest levels were recorded in the region of the STC and at the edge of the eddy (Table 3.2). The impact of microzooplankton grazing on potential algal production is largely determined by the growth phase of the algae (Banse, 1991). Under favourable conditions, algal productivity exceeds microzooplankton grazing rates (i.e. $k > g$), resulting in the accumulation of phytoplankton biomass in the absence of larger grazers (Banse, 1991). Phytoplankton production was highest at the edge of the warm -core eddy and in the region of the STC (Table 3.2). The impact of microzooplankton on potential algal production in these regions should be lower when compared to regions of lower production (subantarctic and Agulhas waters and in the warm-core eddy). Indeed, the mean potential primary production removed from the STC and the edge of the eddy was ≈ 65%, compared to ≈ 73% in areas of lower productivity. These results compare favourably with microzooplankton grazing studies conducted in subarctic waters during a bloom, where 80-100% of the potential algal production was grazed when ≈ 80% of the chlorophyll passed through a 10 µm mesh (Verity *et al.*, 1993).

The concentrations of the nano- and picophytoplankton size fractions decreased during the size selectivity grazing study (Figure 3.4). An indication of the growth status of phytoplankton can be derived from the photosynthetic capacity (PC) value. Preliminary results of size fractionated production studies conducted during the cruise, indicate that chlorophyll normalised production rates (PC) were highest in the picophytoplankton and nanophytoplankton size fractions (Laubscher, unpublished). The decreases in concentration of the nano- and picophytoplankton fractions suggest, therefore, that microzooplankton are preferentially grazing on particles < 20 µm. Indeed, during this study, the relationship between grazing impact and < 20 µm chlorophyll fraction was significant ($P < 0.05$). This result is consistent with the community composition of the microzooplankton assemblages which were numerically dominated by nano-heterotrophs throughout the study (Table 3.1). Also, changes in the concentrations of the nano- and picophytoplankton were not significantly different suggesting that microzooplankton are able to feed efficiently on all particles < 20 µm. It is

well documented that nano-heterotrophs and ciliates consume particles < 20 µm (Burkill *et al.*, 1987; Rassoulzadegan *et al.*, 1988; Verity & Vernet, 1993).

Densities of protozooplankton were < 1000 ind. l^{-1} throughout the study (Table 3.1). Despite high food availability, densities were up to 50% lower than during a similar study undertaken in austral summer (Froneman & Perissinotto, in press). Generally, in the region of the STC chlorophyll concentration shows no seasonal trends (Comiso *et al.*, 1993). However, although absolute phytoplankton biomass does not vary seasonally, there is evidence suggesting that the composition of the phytoplankton changes from a community dominated by netphytoplankton in summer, to a community dominated by the nano- and picophytoplankton in winter (Hattori & Fukuchi, 1989; Laubscher *et al.*, 1993). A recent study by Pakhomov *et al.* (1994) found high species richness and biomass in the macrozooplankton community in the region of the STC during winter, despite the absence of the netphytoplankton on which they normally feed. The low microzooplankton densities recorded during this survey may, therefore, be due to predation by larger zooplankton which may switch from herbivory to carnivory in the absence of their preferred food. A similar situation occurs in the Weddell and Scotia Seas where zooplankton feed on microzooplankton during austral winter when nanoautotrophs dominate chlorophyll concentrations (Garrison *et al.*, 1993).

The mixotrophic component was the most poorly represented in the microzooplankton assemblage, exhibiting densities ranging from 0 to 14 ind.l^{-1} (Table 3.1). These low densities may be due to predation or to light limitation associated with seasonality. It is unlikely, therefore, that they contribute significantly to the primary production in the region. Assuming a chlorophyll-*a* content of ≈ 21 - 94 pg. $cell^{-1}$ per mixotrophic ciliate (Garrison *et al.*, 1993), the mixotrophs would have accounted for 0-5% of total chlorophyll-*a* concentration. These estimates compare well with the results of Garrison *et al.* (1993) who found that mixotrophs comprised between 1 - 6% of the total chlorophyll of the ice edge zone of the Weddell and Scotia Seas during austral winter. The low densities of the mixotrophic component suggest that they may not play a significant role in community functioning with respect to carbon flux in winter.

During this study, microzooplankton grazing in the region of the STC removed > 50% of all potential production (Table 3.2). This suggests that the greater part of photosynthetically fixed carbon in the region is channelled into the microzooplankton fraction. As a result, carbon flux into deep water may be reduced in that little sedimentation of organic carbon occurs as the minipellets produced by the microzooplankton remain in suspension. In addition, the faecal pellets are readily decomposed by bacteria in the microbial loop (Michaels & Silver, 1988). Bacteria in turn represent a large reservoir of carbon and nitrogen for bacterivores such as nano-heterotrophic flagellates (Andersen & Fenchel, 1984; Gast, 1985; Albright *et al.*, 1987; McManus & Furhman, 1988; Reid & Karl, 1990; Kirchman *et al.*, 1993). Consequently, particulate organic carbon (POC) flux below the zone of regeneration is reduced as the carbon is recycled within the microbial loop, resulting in the reduction of the biological drawdown of atmospheric CO_2 in the region (Longhurst & Harrison, 1989; Longhurst, 1991). The importance of the STC as a potential biogenic sink for atmospheric CO_2 as proposed by Dower & Lucas (1993) appears, therefore, to vary seasonally. It should also be noted that the carbon: chlorophyll ratio can vary widely, depending on phytoplankton species composition and nutrient conditions, and may affect substantially the POC flux to depth.

While the results presented here apply only to a small portion of the Southern Ocean, there is growing evidence suggesting that in a large variety of oceanic areas the largest portion of photosynthetically fixed carbon during austral winter is also channelled to the microzooplankton. The predominance of nanoautotrophs (the preferred food particle size of microzooplankton) in Antarctic waters during austral winter has been documented in various studies (Garrison *et al.*, 1993; Kivi & Kuosa, 1994). This suggests that heterotrophic protozooplankton may be the most important sink of winter phytoplankton production. In support of this, a recent study conducted in the Weddell and Scotia Seas during winter showed that microzooplankton grazing itself was sufficient to prevent biomass accumulation in the water dominated by nanoautotrophs (Garrison *et al.*, 1993).

Shifts in the structure of the food web can alter the magnitude of particulate fluxes to the interior of the ocean (Roman *et al.*, 1993). In the Southern Ocean, a seasonal shift in the contribution of the various size fractions to total chlorophyll concentration may dramatically alter the biological role of the system. During summer, only $\approx$ 25% of the photosynthetically

fixed carbon is consumed by the microzooplankton (Froneman & Perissinotto, in press). This would suggest that the bulk of the production is processed by meso- and macrozooplankton. Large faecal pellets produced by these grazers, coupled with diel migrations would result in a net downward flux of POC below the zone of regeneration (Longhurst & Harrison, 1989; Longhurst, 1991). Also, changes in sinking of POC are consistent with increases/ decreases in macrozooplankton biomass (Roman *et al.*, 1993). During summer, therefore, the model proposed by Huntley *et al.* (1991), which suggests that up to 80% of net production is channelled into the larger grazers, appears to apply, suggesting that the biological pump is efficient in the drawdown of atmospheric CO_2.

However, during winter, a shift in the size of the phytoplankton results in the bulk of the photosynthetically fixed carbon being channelled into the microzooplanktonic food chain (Garrison *et al.*, 1993). This provides partial support for the proposed model of Moloney (1992) in which it is suggested that up to 60% of the production is processed by the microbial loop. The net result of this reduction in the POC flux leaving the euphotic zone is that atmospheric CO_2 drawdown by the biological pump in the Southern Ocean is reduced during winter. Oceanic features such as warm core eddies may, however, introduce small mesoscale changes in carbon flux, through the enhancement of phytoplankton productivity usually observed at their edges (Dower & Lucas, 1993).

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CHAPTER 4

SEASONAL VARIATIONS IN MICROZOOPLANKTON GRAZING IN THE REGION OF THE SUBTROPICAL CONVERGENCE

Abstract

Microzooplankton grazing and community structure were investigated in the region of the Subtropical Convergence (STC) during three cruises of the South African Antarctic Marine Ecosystem Study (SAAMES) in austral summer (Jan./Feb., 1993; Dec./Jan. 1995) and winter (June/July, 1993). Chlorophyll-*a* concentrations were consistently dominated by the < 20 µm size fraction during all three cruises while the contribution of the microphytoplankton (> 20 µm) to total chlorophyll-*a* concentrations varied considerably between cruises. Microzooplankton communities were numerically dominated by protozoans comprising ciliates (aloricates and tintinnids) and dinoflagellates. Phytoplankton production in the vicinity of the STC showed no seasonal trends. However, marked seasonal differences were observed in the size structure of the phytoplankton. The grazing impact of microzooplankton was highest when the < 20 µm chlorophyll fraction contributed > 95% of the total. Under these conditions, the instantaneous grazing rates ranged between 0.15 and 0.66 d^{-1}. These correspond to daily losses of 14-48% of the initial standing stock and between 45 and 81% of the potential primary production. At stations where microphytoplankton contributed significantly (≈ 20%) to total chlorophyll concentrations, the grazing coefficients were lower, ranging between 0 and 0.26 d^{-1}. This corresponds to a loss of < 25% of the initial standing stock, or between 0 and 32% of the potential production. The results of this study suggest that microzooplankton represent the main grazing sink for production when the < 20 µm chlorophyll size class dominates. Thus, the efficiency of the biological pump in the vicinity of the STC may vary considerably over time, reflecting shifts in phytoplankton production rates and especially the size composition of the phytoplankton community. During periods when small phytoplankton cells dominate, the biological pump may be relatively inefficient in exporting biogenic carbon to depth because of the close coupling between microzooplankton and the microbial loop.

4.1 Introduction

The Subtropical Convergence (STC) is one of the major oceanic fronts of the Southern Ocean and separates subantarctic waters in the south from subtropical waters in the north (Lutjeharms & Valentine, 1984; Lutjeharms *et al.*, 1993). The front is characterised by strong horizontal temperature and salinity gradients and separates water masses with very different physico-chemical properties (Lutjeharms *et al.*, 1993). Consequently, the STC represents a strong biogeographical barrier to the distribution of phytoplankton (Deacon, 1982; Froneman *et al.*, 1995), zooplankton (Pakhomov *et al.*, 1994), cephalopods (Voss, 1985) and birds (Abrams, 1985).

The region of the front typically exhibits chlorophyll biomass enhancements (Laubscher *et al.*, 1993; Weeks & Shillington, 1994; Froneman *et al.*, 1995). Several hypotheses have been proposed for elevated chlorophyll concentrations at the front, including passive transport (Lutjeharms & Walters, 1985; Franks, 1992) and increased *in situ* phytoplankton production rates resulting from increased water column stability (Laubscher *et al.*, 1993). High regional rates of primary production may also be due to the input of dissolved iron from shelf sediments (Comiso *et al.*, 1993; Sullivan *et al.*, 1993; de Baar *et al.*, 1995). Composite satellite data in the region of the front show no seasonal trends in chlorophyll biomass, with periods of elevated chlorophyll concentrations alternating irregularly with periods of lower phytoplankton biomass (Comiso *et al.*, 1993).

On the other hand, recent studies in waters south of Africa suggest that the size composition of the phytoplankton exhibits a marked seasonal trend, with the microphytoplankton (> 20 µm) size class dominating in summer and the nanophytoplankton (< 20 µm) in winter (Weber & El-Sayed, 1987; Lutjeharms *et al.*, 1994). When phytoplankton communities are dominated by the nano- and pico- fractions, microzooplankton are often the most significant herbivores (Burkill *et al.*, 1987). Indeed, a recent study in the vicinity of the STC during winter has shown that microzooplankton represent the main biological sink for primary production when the picophytoplankton dominates total chlorophyll (Lutjeharms *et al.*, 1994). Under these conditions, carbon flux from the surface waters to the deep ocean would be dramatically reduced as nutrients are recycled within the zone of regeneration due to the close

coupling of the microzooplankton and the microbial loop (Sherr & Sherr, 1987; Longhurst, 1991). Thus, biological processes could act antagonistically to the efficient transfer of organic carbon from surface to deep waters (Longhurst, 1991).

In contrast, where microphytoplankton dominate total chlorophyll concentration, macro- and mesozooplankton are expected to be the dominant grazers. Carbon flux from the surface waters to the deep ocean would be high due to the vertical migration patterns of the grazers and their large, fast sinking faecal pellets which transport production originating from the surface waters to depth (Perissinotto & McQuaid, 1992; Fortier *et al.*, 1994). It should be noted, however, that the role of mesozooplankton faecal pellets in the transfer of biogenic carbon to depth is not always important as recent studies have shown that copepods may in fact be responsible for the retention of faecal pellets in the surface waters (Gonzalez & Smetacek, 1994). The sedimentation of large microphytoplankton cells may further contribute to carbon flux (von Bodungen *et al.*, 1986). The biological pump would therefore, be relatively efficient in exporting biogenic carbon to the deep ocean (Longhurst, 1991).

The large area of the STC suggests that this front may be an important biogenic sink for atmospheric carbon dioxide. Indeed, studies in the vicinity of the front have shown that large gradients in the partial pressure of CO_2 in surface waters can be related to enhanced biological activity at the front (Poisson *et al.*, 1993). A recent study conducted in the waters south of Africa, has estimated that the production at this front may be responsible for between 0.5-0.8% of the total global oceanic production (Dower & Lucas, 1993). However, the transfer of biogenic carbon to depth by the biological pump in this region may vary widely because of shifts in the size composition of phytoplankton which mediate changes in the grazing impacts of the various classes of herbivores. The aim of this study is to present seasonal data over a period of two years on the role of microzooplankton grazing in carbon cycling in the region of the STC to the south of Africa.

4.2 Materials and methods

Microzooplankton grazing experiments were conducted during three cruises within the South African Antarctic Marine Ecosystem Study (SAAMES II, III and IV) in late austral summer

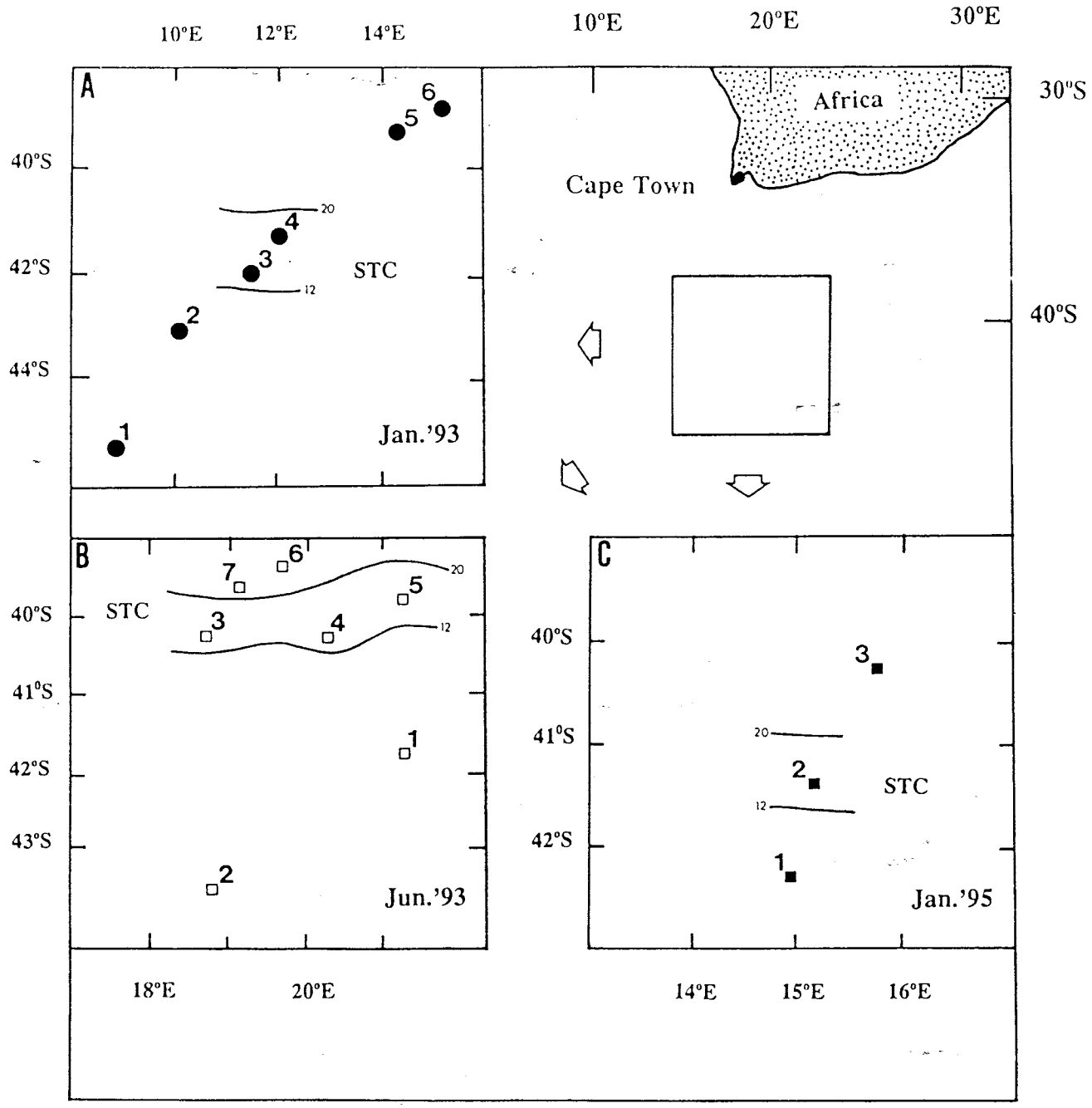

Figure 4.1 Area of investigation and position of microzooplankton grazing stations in the region of the Subtropical Convergence (STC). The isotherms of 12°C and 20°C shown in the insets delimit the average southern and northern boundaries of the STC.

(Jan./Feb., 1993; Dec./Jan., 1995) and winter (June/ July, 1993) (Figure 4.1). All experiments were carried out using water from the surface chlorophyll maximum (5-10 m), by employing the dilution technique (Landry & Hassett, 1982).

Water for the grazing experiments was collected with a submersible pump or Niskin sampling bottles. Previous studies have shown that water samples collected with a submersible pump do not differ significantly from samples collected with sampling bottles (Herman *et al.*, 1984; Paranjape, 1991). For each experiment, 20 l carboys were filled with natural seawater. The water was then gently filtered through a 200 µm mesh to isolate the microzooplankton component. Particle-free water was obtained by passing surface water (obtained with a shipboard metal free pump, Iwaki Magnetic Pump, operated at a flow rate of ≈ 5 l min^{-1}) through a 0.2 µm Milli Q system (Millipore). Dilution series in ratios of 1:0: 3:1; 1:1 and 1:3 of natural to particle-free water were then prepared in 2 l acid-washed polyethylene bottles. Three replicates for each dilution series were prepared. The dilution series were then incubated on deck for 24 h in perspex incubators cooled with running surface waters and screened with shade cloth (neutral spectral irradiance) to simulate light intensity at the depth of collection.

Before the incubations were begun, a 250 ml water sample was taken for initial chlorophyll-a concentration from each bottle. The corresponding bottles were sampled again at the end of the incubation to determine the final pigment concentration. After extraction for 6-12 h in methanol, the fluorescence of the suspension was measured before and after acidification with a few drops of 10% HCl using a Turner 111 fluorometer calibrated with pure chlorophyll-*a* (Holm-Hansen & Riemann, 1978). To determine the size-fractionated pigment concentrations at each of the grazing stations, a single 250 ml water sample was fractionated into the micro- (200 - 20 µm), nano- (2.0 - 20 µm) and picophytoplankton (2.0 - 0.2 µm) fractions. Chlorophyll-*a* and phaeopigment concentrations were then determined fluorometrically as above.

Two techniques were employed to identify and enumerate the various components of the microzooplankton assemblages. During the first two cruises (1993), a 50 ml water sample was stained with Proflavine (Haas, 1982), fixed with glutaraldehyde (final conc. ≈ 6%) and gently

filtered (vacuum < 5cm Hg) through a 2.0 μm Irgalan black-stained Nuclepore filter. Permanent slides were then prepared according to the method of Booth (1987) and frozen at -20°C until the analysis. The slides were examined using a Zeiss epifluorescence microscope equipped with a 450-490 excitation filter, an FT 510 chromatic beam splitter and a long-pass 528 barrier filter (Haas, 1982). All slides were examined within two months of collection. During the third cruise, a 250 ml water sample was passed gently through a 200 μm mesh and fixed with 10% Lugol's solution (Leakey *et al.*, 1994; Stoecker *et al.*, 1994). The water samples were then examined using the Utermöhl settling technique, employing a Nikon TMS inverted microscope operated at 400X magnification. The microzooplankton species were identified from the works of Wood (1954) and Boltovskoy (1981).

Quantitative microphytoplankton samples were collected from a depth of ≈ 5 m using a 20 μm mesh filtration unit (Berman & Kimor, 1983) connected to the shipboard pump (as above). A constant volume of 20 l was filtered for each sample and preserved in 250 ml of 2% hexamine-buffered formalin solution. The species composition of each sample was then determined from a 10 ml subsample (4% of the total, equivalent to 0.8 l of the original seawater filtered) using the Utermöhl settling technique as above. A minimum of 100 fields or 500 cells per sample were counted. For the identification of the microphytoplankton species, the works of Priddle & Fryxell (1985) and Boden & Reid (1989) were used as main references.

The apparent growth rate of chlorophyll-a at the observed dilution was calculated by the exponential model of Landry & Hassett (1982):

$$P_t = P_o\, e^{(k-g)t}$$

where P_t = chlorophyll-a concentration at time t; P_o = initial chlorophyll-a concentration; k and g are the instantaneous algal growth and microzooplankton grazing coefficients, respectively. The coefficients were determined from regression analysis (95% confidence limits) between the apparent growth rate of chlorophyll-*a* and dilution using the computer package Statgraphics, Version 6.0 (Statistical Graphics Corporation, 1992).

Correlation analyses were performed to identify possible relationships between grazing impact, chlorophyll and the measured physico-chemical parameters. Grazing rate data expressed as % were transformed using arcsin transformation (Sokal & Rohlf, 1969) while the remaining data were transformed using the model:

$$\log (x + 1)$$

A Box-Jenkins test was then applied to the data to detect whether the significance of the correlation was spurious. The computer programme Statgraphics was again employed for this analysis.

4.3 Results

Phytoplankton concentrations and size composition

Size fractionated chlorophyll-*a* samples collected during the microzooplankton grazing studies indicated that the < 20 µm phytoplankton fraction dominated the total chlorophyll stock at all stations (Figure 4.2). Indeed, with the exception of the first summer cruise in 1993, where microphytoplankton comprised ≈ 15- 20% of the total chlorophyll concentration (Figure 4.2A), the contribution of this fraction to total chlorophyll was generally < 5%. An exception was station 5 during the winter cruise, where microphytoplankton formed ≈15% of the total chlorophyll-*a* (Figure 4.2B). During the summer cruises, chain-forming diatom species of the genera *Chaetoceros* and *Nitzschia* numerically dominated the microphytoplankton assemblages, while in winter the most abundant species was *Pseudoeunotia doliolus*.

Chlorophyll-*a* concentrations during the summer 1993 cruise were dominated by nanophytoplankton with the exception of station 4 and 6 where the picophytoplankton dominated total chlorophyll (Figure 4.2A). The contribution of the microphytoplankton to total chlorophyll was < 20% at all stations. An exception was station 5, where microphytoplankton contributed 39% of the total chlorophyll. Total chlorophyll-*a* concentrations seemed to be highest in the Agulhas waters while the lowest levels were recorded in the subantarctic waters and at the station located at the southern boundary of the STC. Chlorophyll-*a* concentrations increased substantially at the northern boundary of the front (Figure 4.2A).

In winter, chlorophyll-*a* concentrations were dominated by picophytoplankton (Figure 4.2B). The contribution of microphytoplankton to total chlorophyll was < 10% with the exception of station 5 where it contributed 26% of the total. Total chlorophyll-*a* concentrations were highest at stations located in the vicinity of the STC, ranging between 0.61 and 0.89 μg l^{-1}.

Stations north and south of the front were characterised by similar pigment levels (Figure 4.2B). At stations located south of the front, chlorophyll-*a* concentrations were 0.39 and 0.45 μg l^{-1}, while north of the STC, the chlorophyll concentrations were 0.32 and 0.38 μg l^{-1} at stations north of the front (Figure 4.2B).

During the second summer cruise, in January 1995, the contribution of the various size fractions to total chlorophyll-*a* differed in the samples from north and south of the STC (Figure 4.2C), with nanophytoplankton dominating to the south of the convergence and picophytoplankton to the north. Chlorophyll-*a* concentrations were highest at stations in the Subantarctic waters (0.98 μg l^{-1}) and the lowest in the waters of the Agulhas system (0.27 μg l^{-1}; Figure 4.2C). Intermediate chlorophyll-*a* concentrations (0.60 μg l^{-1}) were recorded inside the STC (Figure 4.2C).

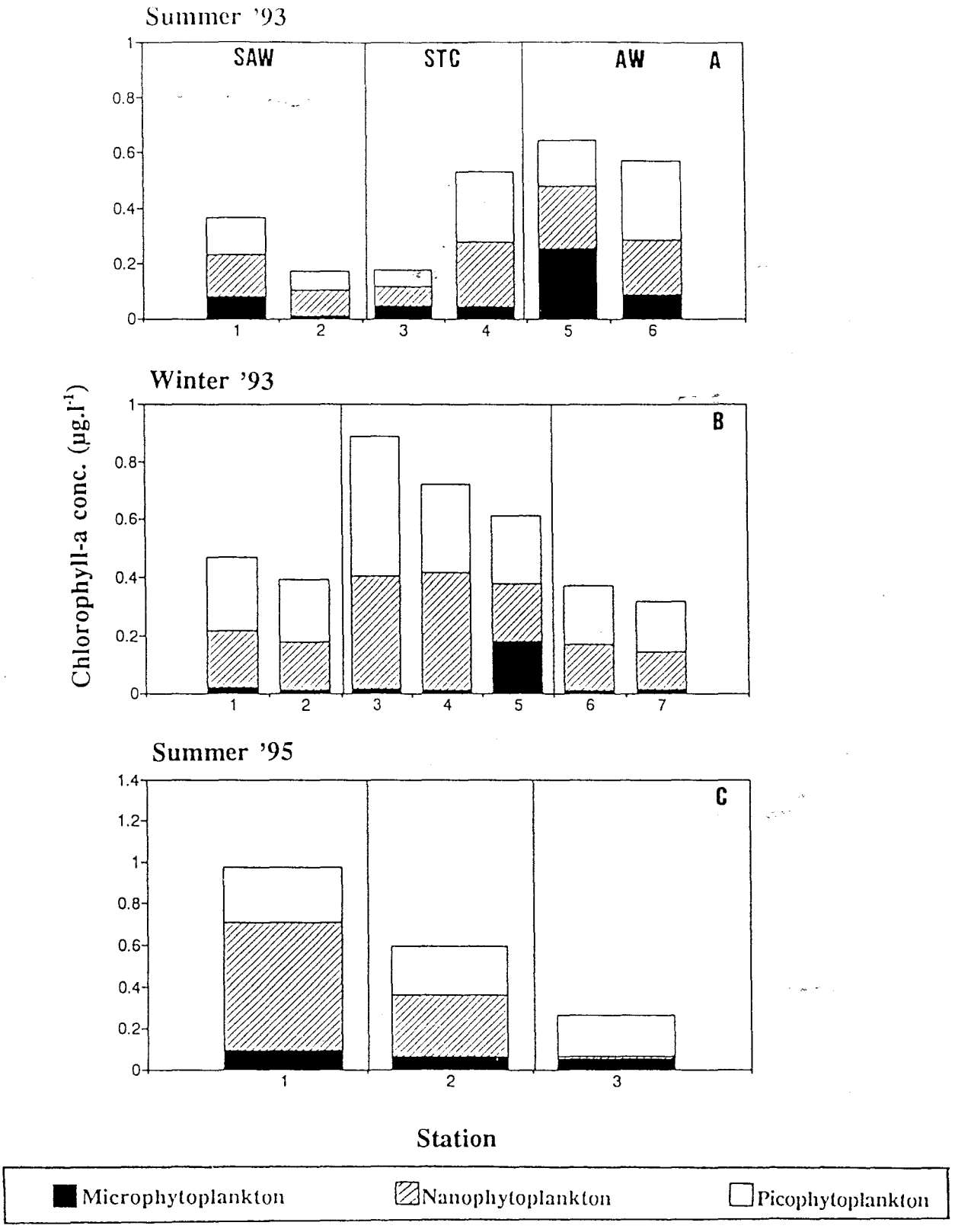

Figure 4.2 Size fractionated chlorophyll-a concentrations during the summer (Jan./Feb.), 1993 (**A**); winter (Jun./Jul.) 1993 (**B**); and summer (Jan.) 1995 (**C**) cruises. SAW = Subantarctic waters; STC = Subtropical Convergence; AW = Agulhas waters.

Microzooplankton community structure

Total microzooplankton abundance during the summer 1993 cruise increased from south to north with the exception of station 4 located at the northern boundary of the STC where densities decreased (Figure 4.3A). Microzooplankton were numerically dominated by protozoans, with ciliates (aloricate and tintinnids) being the dominant group. Densities of ciliates ranged between 280 and 1080 ind.l^{-1}. Dinoflagellates constituted the second most numerous group with densities ranging from 280 to 840 ind.l^{-1}. Well represented species included *Protoperidinium*, *Amphidinium* and *Amphisolenia*. Amongst the larger protozoans, two species of foraminiferans were recorded, *Acanthochiasma* and *Globigerina*. Densities of these were however less than 25 cells. l^{-1}.

During the winter cruise, the dominant components of the microzooplankton assemblages were similar to those observed during the summer 1993 cruise. However, total abundances of microzooplankton during winter were nearly an order of magnitude lower than summer densities (Figure 4.3B). Ciliates (aloricate and tintinnids) were again the dominant group, with densities ranging between 90 and 120 ind.l^{-1}. Dinoflagellate densities ranged between 100 and 150 ind. l^{-1}. Microzooplankton abundance showed no distinct patterns although a marked decrease in total abundance was recorded at station 4, located within the STC (Figure 4.3B).

During the second summer cruise (Jan. 1995), microzooplankton densities were in the same range as those of the summer 1993 (Figure 4.3C). Protozoans, chiefly ciliates and tintinnids, were again the dominant components of the microzooplankton assemblages. Densities of aloricate ciliates ranged between 625 and 1275 ind.l^{-1} and those of tintinnids between 75 and 250 ind. l^{-1}. Amongst the dinoflagellates, species of the genus complex *Protoperidinium* and *Amphidinium* were the most abundant, exhibiting densities in the range of 225-400 ind.l^{-1}. Also, well represented amongst the dinoflagellates were species of the genera *Amphisolenia*. Densities of these two genera exceeded 75 ind.l^{-1} at all stations. Two species of foraminiferans were identified, *Acanthochiasma* sp. and *Globigerina* sp. Densities of these large protozooplankton varied between 25 and 50 ind.l^{-1}.

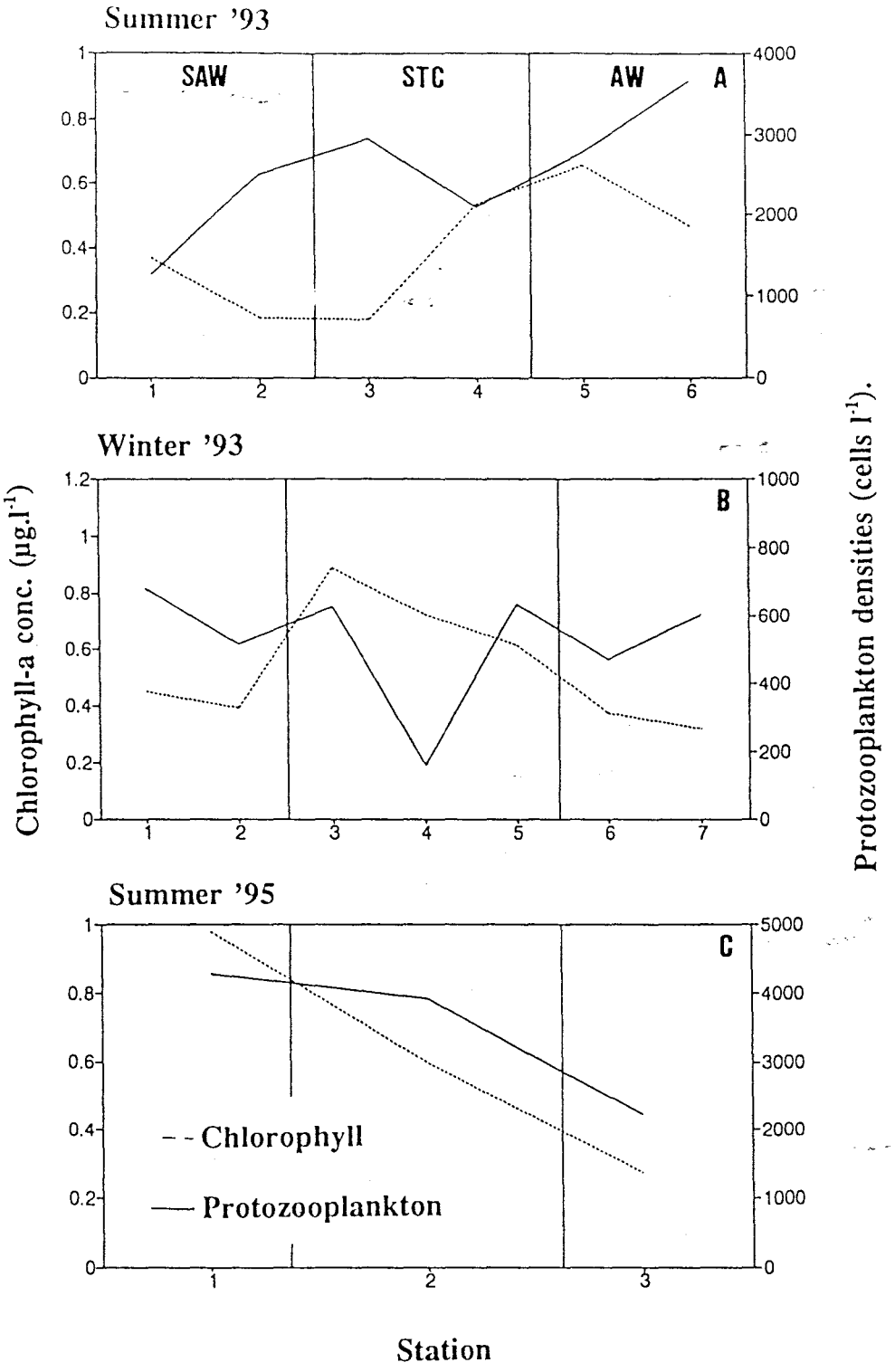

Figure 4.3 Total chlorophyll-*a* and protozooplankton densities during the summer (Jan./Feb.), 1993 (**A**); winter (Jun./Jul.) 1993 (**B**); and summer (Jan.) 1995 (**C**) cruises. SAW = Subantarctic waters; STC = Subtropical Convergence; AW = Agulhas waters.

Grazing experiments

The instantaneous growth and grazing coefficients derived from the microzooplankton grazing experiments conducted during the three cruises are shown in Tables 4.1 to 4.3. In all experiments, the relationship between apparent growth rate and observed dilution factor was significantly linear ($P < 0.05$).

During the first summer cruise (1993), both grazing and growth rate estimates tended to be highest in the region of the STC (Table 4.1). Instantaneous growth coefficients of phytoplankton ranged between 0.07 and 0.81 d^{-1}, equivalent to rates of 0.10 - 1.17 chlorophyll doublings d^{-1}. Instantaneous grazing coefficients of microzooplankton on phytoplankton ranged from 0.00 to 0.26 d^{-1}, corresponding to daily losses of between 0 and 17.1% of the initial standing stock, or between 0.1 and 32.0% of the phytoplankton potential production (Table 4.1).

During the winter cruise, instantaneous growth and grazing coefficients exhibited a clear spatial pattern with the highest rates recorded in the vicinity of the STC (Table 4.2). Indeed, both grazing and growth coefficients in the vicinity of the STC were significantly higher than rates to the north and south of the front ($F = 22.1$; $F = 98.6$; $P < 0.01$). Instantaneous growth coefficients of phytoplankton ranged from 0.45 to 1.32 d^{-1}, equivalent to between 0.66 and 1.90 chlorophyll doublings d^{-1}. Instantaneous grazing coefficients of microzooplankton ranged between 0.34 and 0.66 d^{-1}. This corresponds to a daily loss of between 29.6 and 48.4% of the initial standing stock, or between 56.3 and 81.1% of the daily potential primary production (Table 4.2).

During the second summer cruise (1995), no spatial patterns in either the growth or grazing coefficients were evident (Table 4.3). The highest values were recorded in Agulhas waters north of the STC, while the lowest occurred in the region of the STC (Table 4.3). Phytoplankton growth coefficients ranged between 0.27 and 0.36 d^{-1} (0.38 to 0.52 chlorophyll doublings d^{-1}). Instantaneous grazing coefficients of microzooplankton ranged from 0.150 to 0.25 d^{-1}, which correspond to a loss of between 14.0 and 21.8% of the initial standing stock. The percentage of potential primary production removed by microzooplankton at these stations ranged from 45.4 to 75.3% (Table 4.3).

Pearson correlation analysis indicated that the microzooplankton grazing impact was not significantly correlated to temperature or total chlorophyll concentration. The relationship between grazing and growth coefficients during both the summer and winter cruises (1993) was, however, significant ($r^2 = 0.81$; $r^2 = 0.61$ respectively; $P < 0.05$). Using the Box-Jenkins test, significant correlations were again obtained between growth and grazing coefficients during the summer and winter 1993 cruises ($r^2 = 0.44$; $r^2 = 0.40$ respectively; $P < 0.05$), suggesting that the significance of the relationship was not spurious.

4.4 Discussion

Biogeographic studies in the waters south of Africa have indicated that the Subtropical Convergence (STC) represents a strong barrier to the distribution of warmer water phytoplankton and zooplankton species (Deacon, 1982; Pakhomov *et al.*, 1994; Froneman *et al.*, 1995). During this study, the widely distributed subtropical diatom species *Hemiaulus hauckii,* an indicator of Agulhas waters (Boden *et al.*, 1988; Boden & Reid, 1989), was found north of the STC but was absent from subantarctic waters. A separate study conducted during the winter cruise showed that the STC also represents a strong biogeographical barrier to macrozooplankton distribution (Pakhomov *et al.*, 1994).

The algal growth coefficients were highest in the vicinity of the STC during the summer and winter cruises of 1993 (Tables 4.1 and 4.2). Elevated chlorophyll-*a* concentrations were, however, only recorded in the vicinity of the STC during the winter cruise (Figure 4.2B). Our grazing studies show that during the summer cruise, microzooplankton removed < 10% of the initial standing stock (Table 4.1). These data suggest that the absence of elevated chlorophyll-*a* concentration during the summer (1993) cruise may have resulted from grazing by larger zooplankton. Indeed, a recent study conducted across the STC in the mid Atlantic Ocean has shown a strong negative correlation between mesozooplankton biomass and surface chlorophyll concentrations in the vicinity of the front (Barange *et al.*, in press). The generally lower growth rates of phytoplankton recorded in the waters north of the STC are attributable to nutrient impoverishment of the Agulhas waters (Allanson *et al.*, 1981).

Table 4.1 Grazing rates and impact parameters derived from microzooplankton grazing experiments conducted during the SAAMES II study conducted in late austral summer (Jan./Feb) 1993. Significance levels: * = $P < 0.01$; ** = $P < 0.05$. Values in brackets represent standard errors. **Stations 1 & 2** = Subantarctic waters; **Stations 3 & 4** = Subtropical Convergence; **Stations 5 & 6** = Agulhas waters.

Station	Chl-a conc. ($\mu g\ l^{-1}$)	r^2	Growth coeff. (k) d^{-1}	Grazing coeff. (g) d^{-1}	% Initial stock removed (d^{-1})	% Potential prod. removed (d^{-1})	Chlorophyll doublings (d^{-1})
1	0.368	0.74*	0.81 (0.10)	0.19 (0.01)	17.12	30.69	1.17
2	0.183	0.37**	0.07 (0.01)	0.00 (-)	0.10	0.10	0.10
3	0.177	0.39**	0.71 (0.21)	0.26 (0.09)	6.78	13.25	1.02
4	0.532	0.38**	0.74 (0.11)	0.10 (0.07)	9.77	18.89	1.06
5	0.655	0.80*	0.54 (0.05)	0.14 (0.02)	13.28	31.91	0.78
6	0.465	0.80*	0.41 (0.03)	0.07 (0.01)	6.66	19.83	0.59

Table 4.2 Grazing rate and impact parameters of microzooplankton grazing studies conducted during the SAAMES III study in late austral winter (Jun./Jul.) 1993. Significance levels: * = P < 0.01; ** = P 0.05. **Stations 1-2** = Subantarctic waters; **Stations 3-5** = Subtropical Convergence; **Stations 6-7** = Agulhas waters. Values in brackets represent standard errors.

Station	Chl-a conc. ($\mu g\ l^{-1}$)	r^2	Growth coeff. (k) d^{-1}	Grazing coeff. (g) d^{-1}	% Initial stock removed (d^{-1})	% Potential prod. removed (d^{-1})	Chlorophyll doublings (d-1)
1	0.450	0.66**	0.45 (0.09)	0.35 (0.01)	29.56	81.08	0.65
2	0.393	0.35**	0.56 (0.11)	0.39 (0.04)	32.56	76.36	0.80
3	0.889	0.89*	1.23 (0.15)	0.60 (0.09)	45.11	63.58	1.78
4	0.725	0.74**	1.21 (0.11)	0.66 (0.08)	48.41	68.99	1.75
5	0.614	0.32*	1.32 (0.13)	0.53 (0.07)	41.21	56.26	1.90
6	0.376	0.67*	0.47 (0.08)	0.34 (0.04)	28.45	77.73	0.71
7	0.321	0.57*	0.58 (0.05)	0.30 (0.07)	25.55	71.54	0.84

Table 4.3 Grazing rate and impact parameters of microzooplankton grazing experiments conducted during the SAAMES IV study in late austral summer (Jan.) 1995. Significance levels: * = $P < 0.01$; ** = $P < 0.05$. **Station 1** = Subantarctic waters; **Station 2** = Subtropical Convergence; **Station 3** = Agulhas System waters. Values in brackets represent standard errors.

Station	Chl-a conc. ($\mu g\ l^{-1}$)	r^2	Growth coeff. (k) d^{-1}	Grazing coeff. (g) d^{-1}	% Initial stock removed (d^{-1})	% Potential prod. removed (d^{-1})	Chlorophyll doublings (d^{-1})
1	0.979	0.70*	0.36 (0.01)	0.15 (0.04)	13.97	45.37	0.52
2	0.597	0.61**	0.27 (0.01)	0.16 (0.03)	14.74	62.98	0.38
3	0.272	0.88**	0.34 (-)	0.25 (0.01)	21.76	75.31	0.49

Decreases in microzooplankton densities were recorded at a single station in the vicinity of the STC, during both the summer and winter cruises of 1993 (Figure 4.3A and 4.3B). Zooplankton studies conducted during the winter cruise found high species richness and biomass of macrozooplankton (tunicates and euphausiids) in the region of the front despite the absence of the microphytoplankton on which they normally feed (Pakhomov *et al.*, 1994). Shifts in zooplankton prey in the absence of phytoplankton are well documented (Landry, 1981, cited in Hopkins & Torres, 1989). Thus, the coincidence of high densities of macrozooplankton with low densities of microzooplankton during winter suggests that the macrozooplankton may have been feeding on the microzooplankton due to the scarcity of phytoplankton. Indeed, recent gut analyses conducted in the Atlantic sector of the Southern Ocean indicate that microzooplankton comprise a significant proportion of the diet of some macrozooplankton species (Pakhomov, pers. comm.; pers. observation). While the impact of macrozooplankton on microzooplankton could be reduced in winter due to seasonal adjustments of metabolic rates, recent studies suggest that seasonality is very reduced in the region of the STC (Comiso *et al.*, 1993; Pakhomov *et al.*, 1994). Although summer macrozooplankton communities are similar in composition to winter communities (Pakhomov *et al.*, 1994), the impact of macrozooplankton feeding on microzooplankton is much reduced in summer as microphytoplankton are present in higher concentrations. This suggests that the STC represents an area of increased trophic interaction mediated by phytoplankton size composition and concentration.

During this study, microzooplankton removed between 0 and 48% of the initial phytoplankton standing stock (Tables 4.1 to 4.3). This compares well with similar studies employing the dilution technique in various oceanic environments (Gifford, 1988; Paranjape, 1987; 1990; Verity & Vernet, 1992; Verity *et al.*, 1993). The results also show that their impact on the initial standing stock is highest when the contribution of the microphytoplankton is < 5% of the total stock (Figure 4.2). Most laboratory and field studies of microzooplankton grazing dynamics suggest that microzooplankton preferentially graze on particles < 20 µm (Hansen *et al.*, 1994; Peters, 1994). In particular, a strong trophic link between nano- and picoplankton concentrations and microzooplankton has been observed on many occasions (Jonsson, 1986). Although temperature has been implicated as a key factor controlling grazing rates of protozooplankton (Choi & Peters, 1992), the relationship between grazing impact and

temperature range (between 11 and 22°C) during our studies was not significant. Our data indicate that microzooplankton grazing impact in the region of the STC was determined primarily by the contribution of the < 20 µm chlorophyll fraction to total chlorophyll concentrations. This result is consistent with similar studies conducted both in the Southern Ocean (Lutjeharms *et al.*, 1994) and in the Gulf of Mexico (Fahnenstiel *et al.*, 1995). The dynamic nature of microzooplankton grazing is demonstrated by the high grazing impact on the generally faster growing phytoplankton in the vicinity of the front (Tables 4.1-4.3). The relationship between grazing and growth rates was significant ($P < 0.05$) during the 1993 cruises. This suggests a close coupling between growth rates of phytoplankton and grazing by microzooplankton, and provides further evidence of the important role that microzooplankton play in the carbon cycling in the Southern Ocean.

Microzooplankton grazing impact was lowest during the first summer cruise, in Jan.- Feb. 1993 (Table 4.1) when microzooplankton removed < 15% of the initial standing stock. This is equivalent to < 25% of the potential phytoplankton production (Table 4.1). A feature of this cruise was the high contribution of microphytoplankton (≈ 20%) to total chlorophyll-*a* (Figure 4.2). Indeed, microphytoplankton concentrations during the summer 1993 cruise were significantly higher than those recorded during the other cruises ($F = 3.94$; $P < 0.05$). Analysis of the microphytoplankton species composition at the stations occupied during this cruise showed that chain-forming diatoms of the genera *Chaetoceros* and *Nitzschia* and large diatoms such as *Rhizosolenia* spp. were the most numerous. Although several studies have demonstrated that dinoflagellates are able to consume microphytoplankton (Jacobson & Anderson, 1986; Suttle *et al.*, 1986; Hansen *et al.*, 1994), the available literature on the whole largely suggests that microphytoplankton are not grazed by microzooplankton due to morphological constraints associated with feeding. In particular, the ciliates which were the dominant component of the microzooplankton during this study, show a strong preference for cells < 20 µm (Rassoulzadegan *et al.*, 1988; Hansen *et al.*, 1994; Peters, 1994). This suggests that, under conditions when microphytoplankton dominate chlorophyll-*a* at the STC, sedimentation of phytoplankton cells or grazing by larger metazoan grazers form the primary sinks for photosynthetically fixed carbon. Similarly, in the Atlantic sector of the Southern Ocean, microzooplankton consume < 25% of the potential phytoplankton production when

microphytoplankton dominate chlorophyll-*a* concentrations (Froneman & Perissinotto, in press). These findings fit well with the model of Huntley *et al.* (1991) which estimates that ≈ 80% of the net production enters the macrozooplankton fraction.

The role of the STC as a biological sink for atmospheric CO_2, proposed by Dower & Lucas (1993), may change substantially over time. This variability seems to be mediated by changes in phytoplankton production rates and chlorophyll biomass (Comiso *et al.*, 1993; Sullivan *et al.*, 1993) and shifts in the size composition of the phytoplankton (Figure 4.2). Unfortunately, in the absence of sediment trap collections during the investigation, no conclusive evidence can be presented on the magnitude and seasonal variations of the flux of organic carbon in the area. It may be useful, however, to consider some circumstantial evidence derived from more general studies. Shifts in the composition of the phytoplankton may dramatically affect the flux of particulate organic carbon (POC) from surface waters to the deep ocean. For example, when the < 20 µm chlorophyll fraction dominates total chlorophyll concentration, the bulk of the phytoplankton production would be channelled to the microzooplankton and therefore, the microbial loop. This suggests that relatively little carbon may be exported to the deep ocean because most of the organic matter is recycled within the zone of regeneration (Longhurst, 1991; Fahnenstiel *et al.*, 1995). A recent study conducted in the Gulf of Mexico showed that sedimentation rates of the < 20 um chlorophyll fraction were < 1% of total growth where microzooplankton constituted the major grazers (Fahnenstiel *et al.*, 1995). Also, although macrozooplankton feeding on microzooplankton may represent an important source of carbon flux in winter, the efficiency of the biological pump is reduced due to the increase in trophic steps. In this context, therefore, our results provide some support for the model proposed by Moloney (1992) which suggests that the microbial loop represents the major sink for phytoplankton production in the Southern Ocean. This would appear to be the case at the Subtropical Convergence during winter.

In contrast, during periods when the microphytoplankton dominate chlorophyll-*a* biomass, the macro- and mesozooplankton appear to represent the major trophic route for the uptake of phytoplankton production, thereby resulting in the direct transport of carbon to the deep ocean via vertical migrations (Perissinotto & McQuaid, 1992; Fortier *et al.*, 1994) and the production of large, fast sinking faecal pellets (Roman *et al.*, 1993; Fortier *et al.*, 1994). The

sinking of dead, senescent phytoplankton cells may further contribute carbon flux to the deep ocean (von Bodungen *et al.*, 1986). The biological pump will, therefore, be particularly efficient in the transfer of biogenic carbon to depth (Longhurst, 1991). Our data indicate that while the use of satellite composite data provide invaluable information on total chlorophyll-*a* distribution, these should be used in combination with size fractionated chlorophyll-*a* data to account for the differential partitioning of carbon between size classes of grazers and the biochemical processes operating in the region.

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CHAPTER 5

DYNAMICS OF MICROPLANKTON COMMUNITIES AT THE ICE EDGE ZONE OF THE LAZAREV SEA DURING A SUMMER DROGUE STUDY

Abstract

Microzooplankton grazing and community structure were investigated in austral summer 1995 during a Southern Ocean Drogue and Ocean Flux Study (SODOFS) at the ice edge zone of the Lazarev Sea. Grazing was estimated at the surface chlorophyll maximum (5-10 m) by employing the sequential dilution technique. Chlorophyll-*a* concentrations were dominated by chain-forming microphytoplankton (> 20 µm) of the genera *Chaetoceros* and *Nitzschia.* Microzooplankton were numerically dominated by ciliates (aloricate and *Strombidium* spp.) and dinoflagellates (*Protoperidinium* sp. and *Gymnodinium* sp.). Instantaneous growth rates of nanophytoplankton (< 20 µm) varied between 0.019 and 0.080 d^{-1}, equivalent to between 0.03 and 0.16 chlorophyll doublings d^{-1}. Instantaneous grazing rates of microzooplankton on nanophytoplankton varied from 0.012 to 0.052 d^{-1}. This corresponds to a nanophytoplankton daily loss of between 1.3 and 7.0% (mean = 3.7%) of the initial standing stock, and between 48 and 97% (mean = 70.4%) of the daily potential production. Growth rates of microphytoplankton (> 20 µm) were lower, varying between 0.011 and 0.070 d^{-1}, equivalent to 0.015 - 0.097 chlorophyll doublings d^{-1}. At only three of the 10 stations did grazing by microzooplankton result in a decrease in microphytoplankton concentration. At these stations instantaneous grazing rates of microzooplankton on microphytoplankton ranged between 0 and 0.015 d^{-1}, equivalent to a daily loss of < 1.5% (mean = 1.11%) of initial standing stock and less than 40% (mean = 28.55%) of the potential production. Time series grazing experiments conducted at 6 h intervals did not show any diel patterns of grazing by microzooplankton. The results of this investigation show that microzooplankton grazing at the ice-edge was not sufficient to prevent chlorophyll-*a* accumulation in regions dominated by microphytoplankton. Here, the major biological routes for the uptake of carbon therefore appears to be grazing by metazoans or the sedimentation of phytoplankton cells. Under these conditions, the biological pump will be relatively efficient in the drawdown of atmospheric CO_2.

5.1 Introduction

The fate of photosynthetically fixed carbon in marine environments can dramatically affect the magnitude of particulate flux, and hence the efficiency of the biological pump in the uptake of atmospheric CO_2 (Longhurst, 1991). Although sinking of dead or senescent phytoplankton cells contributes significantly to the magnitude of carbon flux (Schnack, 1985; von Bodungen *et al.*, 1986; Michaels & Silver, 1988), grazing by zooplankton represents the primary biological route for the transfer of organic carbon from the surface waters to the interior of the ocean. The extent of carbon flux through grazers is strongly determined by the community structure of the consumers and the subsequent partitioning of photosynthetically fixed carbon (Michaels & Silver, 1988; Roman *et al.*, 1993). In regions where macro- and mesozooplankton consume the bulk of the phytoplankton production, the organic flux from the surface waters to the deep ocean in the form of large, compact and fast sinking faecal pellets, is generally high (Schnack, 1985; von Bodungen, 1986; Cadee *et al.*, 1992; Gonzalez, 1992a; Fortier *et al.*, 1994). Carbon flux below the zone of regeneration is further enhanced as many of the larger herbivores undertake vertical migrations from the surface waters where they feed to below the zone of regeneration (Fortier *et al.*, 1994). Production originating in the surface waters is, therefore, transported below the zone of regeneration.

In contrast, phytoplankton consumed by microzooplankton contribute less to particulate flux for several reasons: **1**. the close coupling between the microzooplankton and the microbial loop results in the recycling of nutrients in the zone of regeneration (Sherr & Sherr, 1988). **2**. microzooplankton produce small faecal pellets (minipellets) which remain in suspension for long periods (Nothig & von Bodungen, 1989; Elbrachter, 1991; Gonzalez; 1992b); **3**. many protozoans, the dominant component of the microzooplankton (Garrison & Buck, 1989), sequester chloroplasts (Stoecker *et al.*, 1987); **4**. microzooplankton do not undergo vertical migration, thus nutrients contained within the microzooplankton are not transported below the zone of regeneration; **5**. a substantial proportion of the faecal carbon may be retained in the zone of regeneration as a result of coprophagy (Nothig & von Bodungen, 1989); Therefore, there appears to be little material available for direct export to the deep ocean.

A major feature of the Southern Ocean is sea ice, which in winter may extend as far north as 56°S (Sullivan *et al.*, 1993). Associated with the retreating ice during summer are phytoplankton blooms which are thought to result from increased *in situ* production associated with increased water column stability imparted by ice melt (Heywood & Whitaker, 1984; Horner, 1985; Smith & Nelson, 1986; Smith & Sakshaug, 1990). The release of epontic cells during ice melt further contributes to increased chlorophyll concentrations in this region (Smith & Nelson, 1986). The species and size composition and maximum biomass reached by ice-edge blooms are, however, greatly variable (Lancelot *et al.*, 1993; Kang & Fryxell, 1993). Although microphytoplankton generally dominate the ice-edge phytoplankton blooms, moderate nanophytoplankton blooms have been recorded in the Weddell Sea (Lancelot *et al.*, 1993).

Studies in marine environments have shown that when nano- and picophytoplankton dominate phytoplankton communities, microzooplankton are often the most significant herbivores (Garrison *et al.*, 1993; Kivi & Kuosa, 1994; Lutjeharms *et al.*, 1994). In contrast where food webs are dominated by microphytoplankton, metazoans often represent the sink for primary production (Huntley *et al.*, 1989). Thus the pathways of energy flux may vary according to the size composition of phytoplankton at the ice edge zone.

It has been estimated that production at the MIZ contributes ≈ 40% of the annual primary production south of the Antarctic Divergence (Smith & Sakshaug, 1990). The fate of the photosynthetically fixed carbon in this region is therefore of particular importance for the total carbon budget. This study was initiated with the aim of characterising and quantifying the grazing impact of microzooplankton at the ice-edge zone of the Lazarev Sea, and provide data on temporal changes in microzooplankton grazing within the same body of water.

5.2 Materials and methods

Microzooplankton grazing experiments were conducted at 14 stations during the Southern Ocean Drogue and Ocean Flux Study (SODOFS) in austral summer (Dec./Jan.) 1995 (Figure 5.1). The grazing experiments were carried out at the surface chlorophyll maximum (5-10 m) by employing the seawater dilution technique (Landry & Hassett, 1982).

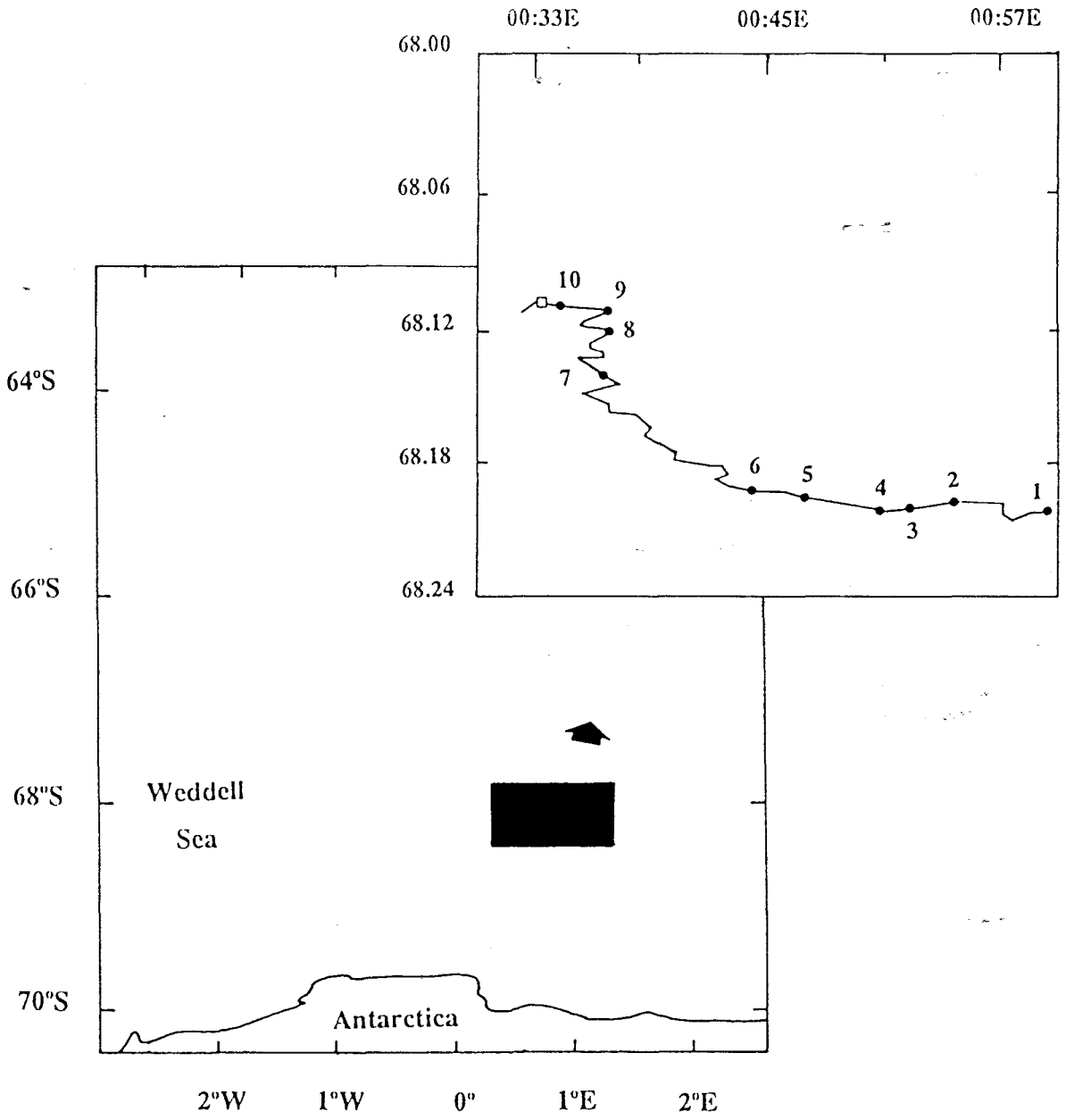

Figure 5.1 Drogue drift track and position of microzooplankton grazing studies conducted during the Southern Ocean Drogue Ocean Flux Study (SODOFS) cruise in austral summer (Dec./Jan.) 1995. ▬ denotes the position of the time series grazing experiment.

Water samples for the grazing experiments were collected with 8 l Niskin bottles. For each experiment, 20 l polyethylene carboys were filled with seawater. The water in the carboys was then gently passed through a 200 µm mesh to isolate the microzooplankton community. Particle free water was obtained by passing surface water (obtained using a shipboard pump Iwaki Magnetic Pump operated at a flow rate of ≈ 5 l min^{-1}) through a 0.2 µm Milli Q (Millipore) filtration system. Dilution series in ratios of 1:0; 3:1; 1:1 and 1:3 in 2 l polyethylene bottles of filtered to unfiltered seawater were then prepared. The dilution series were incubated on deck for 24 h in perspex incubators cooled with running surface water and screened with shade cloth (neutral spectral transmission) to simulate light intensity at the depth of collection. To assess diel patterns of grazing by microzooplankton, duplicate time series grazing experiments were conducted over 24 h with sampling at 6 h intervals, beginning at 06h00. During the entire study, wind speed, surface irradiance and cloud cover were monitored.

Before the incubations were begun, water samples (250ml) were taken for the initial chlorophyll-*a* concentration and microzooplankton abundance. The corresponding bottles were sampled again at the end of the incubation to determine the final chlorophyll-*a* concentrations. Chlorophyll-*a* was fractionated into the nano- (< 20 µm) and microplankton (> 20 µm) size classes. The picophytoplankton size class (0.2 - 2.0 µm) was not sampled during this study as it constituted ≤ 5% of the total stock throughout the period of the investigation. Chlorophyll-*a* concentrations were determined flourometrically (Turner 111 fluorometer) after extraction in 100% methanol for 6-12 h (Holm-Hansen & Riemann, 1978).

To identify and enumerate the various components of the microzooplankton communities at each grazing station, a 250 ml sample of natural seawater was passed gently through a 200 µm mesh and fixed with 10% Lugols' solution (Leakey *et al.*, 1994a; Stoecker *et al.*, 1994). The water samples were then examined using the Utermohl settling technique and employing a Nikon-TMS inverted microscope operated at X400 magnification (Reid, 1983). A minimum of 500 cells or 100 fields were counted for each sample.

The apparent growth rate of chlorophyll-a at each dilution is calculated as:

$$\frac{1}{t} \ln \left(\frac{P_t}{P_o} \right)$$

where P_o and P_t are chlorophyll concentrations at the beginning and end of the experiment; *t* is duration of experiment. This is the observed change in chlorophyll in the presence of grazers. The theoretical growth rate of phytoplankton in the absence of grazers (*k*) is taken to be the y intercept from the regression analysis between apparent growth rate and dilution (see Figure 2.2). The slope of the regression is the instantaneous grazing coefficient (*g*) of the microzoplankton (Figure 2.2). This regression was calculated by using the computer package, Statgraphics Version 5.0 (Statistical Graphics Corporation).

Correlation analysis was performed to identify possible relationships between grazing rate, temperature and chlorophyll. Grazing rate data, expressed as a %, were transformed using the arcsin transformation (Sokal & Rolhf, 1969), while chlorophyll concentration values were transformed using the factor: log (x + 1) (Legendre & Legendre, 1983). The computer package, Statgraphics, Version 5.0, was again used for this analysis.

5.3 Results

Chlorophyll-*a* and phytoplankton

The contribution of the nano- (< 20 μm) and microphytoplankton fractions to total chlorophyll-*a* concentrations varied considerably during the drogue study. However, the contribution of the microphytoplankton, with 54-70% of total chlorophyll, was always greater than that of the nanophytoplankton fraction (Figure 5.2). Microphytoplankton concentrations ranged between 0.215 and 0.581 μg l^{-1} and were dominated by chain-forming *Chaetoceros* spp. and *Nitzschia* spp., and large cells such as *Corethron criophilum* and *Rhizosolenia* spp. (Table 5.1). Concentrations of nanophytoplankton ranged from 0.172 to 0.356 μg l^{-1} and were dominated by unidentified flagellates.

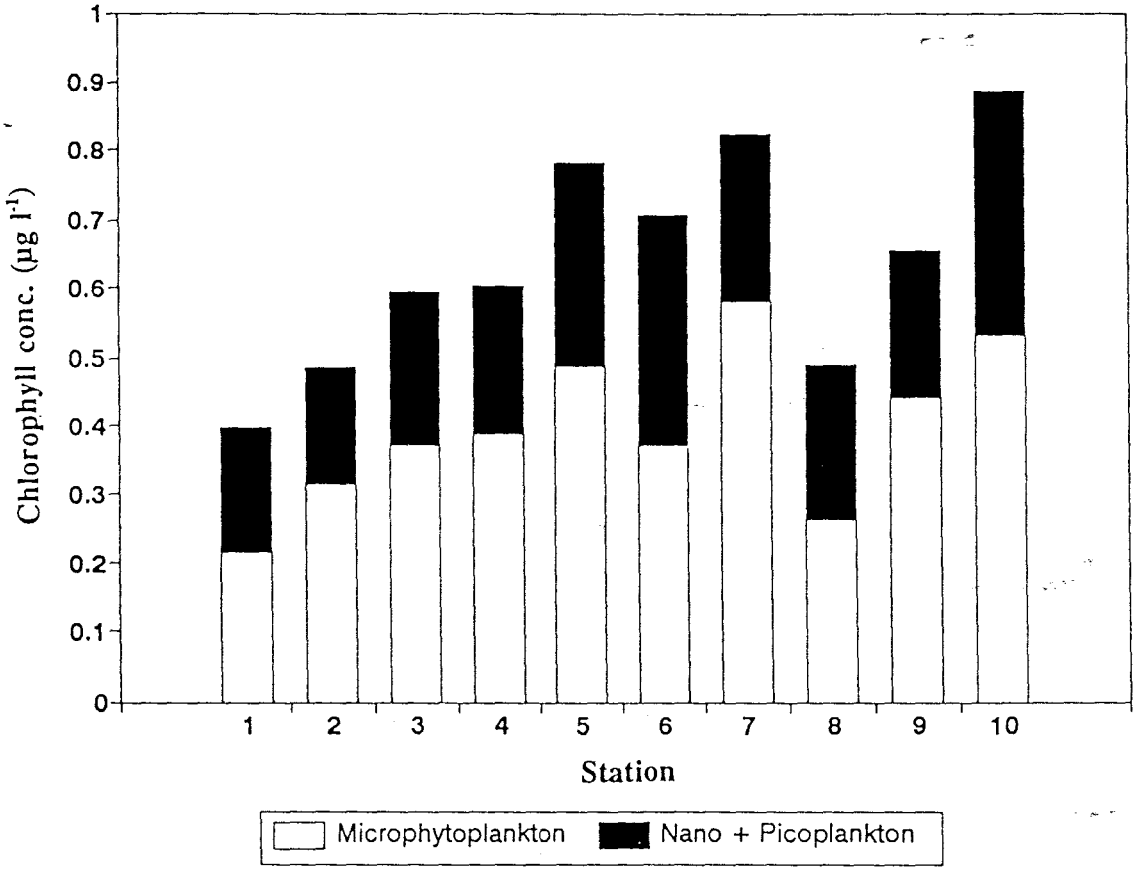

Figure 5.2 Size fractionated surface chlorophyll-*a* concentrations during the SODOFS cruise of late austral summer (Dec./Jan.) 1995.

Table 5.1 Composition of microplankton communities during the Southern Ocean Drogue and Ocean Flux Study (SODOFS) experiment conducted in austral summer (Dec./Jan.) 1994/ 1995. Results expressed are cells l^{-1}.

	Day									
Species	1	2	3	4	5	6	7	8	9	10
Diatoms										
Astermophalus sp.	25	75	-	100	-	200	-	50	-	25
Biddulphia sp.	-	50	50	75	-	200	75	-	25	75
Cylindrotheca closterium	125	225	75	325	150	250	125	50	225	125
Dactyliosolen antarcticus	75	-	25	125	50	-	75	50	50	50
Chaetoceros sp.	375	575	800	575	625	525	475	1225	1050	900
C. atlanticus	175	125	300	125	50	275	225	225	100	175
C. dichaeta	525	1200	925	850	1425	925	875	1350	1550	1600
Concinodiscus sp.	25	-	25	50	25	-	-	-	-	-
Corethron criophilum	250	275	475	450	675	425	650	300	600	575
Eucampia antarctica	-	-	50	25	-	-	25	25	-	50
Navicula sp.	150	125	25	-	175	-	25	75	175	25
Nitzschia sp. (cells)	625	450	525	175	575	225	100	475	400	375
Nitzschia sp. (chains)	425	380	500	375	175	250	225	175	725	125
N. pelagica	75	75	75	125	150	200	175	50	50	75
Pseudonitzschia group	75	175	100	100	25	100	25	-	175	-
Rhizosolenia sp.	150	75	150	175	125	250	175	150	200	125
Proboscia alata	200	175	100	125	225	150	125	150	225	175
R.hebetata var. semispina	50	75	50	100	25	75	50	50	75	25
Thalassiosira sp.	25	100	150	25	75	150	75	50	50	50
Silicoflagellates										
Distephanus speculum	200	550	175	50	100	150	675	525	200	50
Dinoflagellates										
Amphisolenia sp.	125	175	275	225	325	325	350	175	400	350
Amphidinium sp.	25	25	75	150	175	275	125	50	25	100
Ceratium sp.	25	50	25	-	-	50	25	25	25	75
Dinophysis sp.	75	25	-	25	25	50	-	25	25	50
Gymnodinium sp.	275	300	350	250	275	300	275	325	325	325
Gonyaulax sp.	-	-	-	-	25	25	-	-	-	-
Protoperidinium sp.	375	475	750	725	700	650	475	725	375	575
Ciliates										
Strombidium sp.	50	125	200	325	225	175	200	75	75	175
Aloricate ciliates	1600	1475	1075	1400	1225	1325	1525	1625	1575	1725
Tintinnids	25	50	75	50	100	75	25	75	75	100
Foraminiferans										
Acanthochiasma sp.	-	-	-	25	-	-	25	-	-	-
Globigerina sp.	-	-	-	25	25	-	25	-	25	-
Nanoplankton	2225	2450	2475	1950	2325	2650	1975	2000	2475	2600

Microzooplankton community and species composition

During the entire drogue study, the microzooplankton community was numerically dominated by protozoans (Table 5.1), with densities ranging between 2550 and 3400 ind.l^{-1}. The ciliates, comprising aloricate ciliates (Oligotrichs), *Strombidium* spp. and tintinnids dominated numerically the protozooplankton stock, accounting for 48-62% of the total. Aloricate forms constituted the main component of the ciliate group, with densities ranging between 1075 and 1725 ind. l^{-1}. Members of the Strombidiidae (*Strombidium* spp.) were the second most numerous component of this group and their densities ranged from 50 to 325 ind. l^{-1}. Tintinnid densities were always < 100 ind. l^{-1} (Table 5.1).

Among the flagellates, members of the genus *Protoperidinium* were the most abundant species with densities ranging from 375 to 725 ind. l^{-1} (Table 5.1). The second most abundant dinoflagellates during the study were *Gymnodinium* spp. with densities ranging between 250 and 450 ind. l^{-1}. Also, well represented were unidentified species of the genus *Amphidnium* (densities of between 125 and 400 ind. l^{-1}) and *Amphisolenia* spp. (densities range from 25 to 275 ind. l^{-1}). Amongst the dinoflagellates, the least well represented group recorded during the study was the genus *Gonyaulax*. Densities of this group were always < 25 ind. l^{-1}.

Abundances of larger protozooplankton, e.g. foraminiferans, were low (< 25 ind. l^{-1}) throughout the drogue study. Two species of foraminiferans, *Acanthochiasma* sp. and *Globigerina* sp. were recorded (Table 5.1).

Grazing rates

Instantaneous growth and grazing co-efficients with the confidence limits derived from the grazing experiments are shown in Tables 5.2 and 5.3. In all dilution experiments, the relationship between apparent growth rate and dilution was significantly linear ($P < 0.05$ in all cases).

No temporal patterns in growth or grazing were identified in the nanophytoplankton community (Table 5.2A) . Instantaneous growth rates (k) of the nanophytoplankton ranged between 0.02 and 0.08 d^{-1}. This level of growth is equivalent to between 0.03 and 0.12 chlorophyll doublings d^{-1} (Table 5.2A). Instantaneous grazing rates (g) of microzooplankton

on nanophytoplankton ranged from 0.01 to 0.05 d^{-1}. These correspond to daily losses of between 1.3 and 7.0% (mean = 3.8%) of the initial standing stock and 48 - 97% (mean = 70.4%) of the potential primary production of the nanophytoplankton fraction. During these experiments the relationship between algal growth and grazing mortality were always significant (r^2 = 0.58; P < 0.05). Using a Box Jenkins test to determine whether this relationship was due to autocorrelation, a significant correlation was again obtained between microzooplankton grazing and algal growth (r^2 = 0.45; P < 0.05) indicating that this relationship was real.

Instantaneous growth rates in the microphytoplankton fraction were lower and ranged between 0.02 and 0.07 d^{-1} (Table 5.2B). These rates correspond to chlorophyll doubling rates ranging between 0.02 and 0.10 d^{-1}. Microzooplankton grazing resulted in a decrease in the microphytoplankton concentration in only three experiments (Table 5.2B). Here, the instantaneous grazing rates of microzooplankton on microphytoplankton ranged between 0.008 and 0.01 d^{-1}. This level of grazing corresponds to a daily loss of initial standing stock < 1.5% (range 0.9 - 1.5%) or < 40% of the potential production (range 19 - 41%). Pearson and 5th Order Partial correlation analysis between microzooplankton abundance, herbivory and chlorophyll concentration showed no significant relationships during the entire investigation.

During the time series grazing experiments, both the growth co-efficients of the nano- and microphytoplankton fractions and microzooplankton grazing impact on the two fractions were in the same range as that obtained in the previous grazing experiments (Tables 5.3A and 5.3B). Grazing by microzooplankton resulted in a decrease in nanophytoplankton concentration during all experiments (Table 5.3A), while decreases in the microphytoplankton concentration were observed only at three stations (Table 5.3B). No diel patterns in grazing impact of microzooplankton on nano- or microphytoplankton were evident during the time series experiments (Tables 5.3A and 5.3B). Indeed, analysis of variance indicates that the grazing impact of microzooplankton on the nanophytoplankton and microphytoplankton did not differ significantly between different times of the day (F = 0.897; F = 0.352 respectively; P < 0.05 in all cases).

Table 5.2A Estimates of phytoplankton production in the < 20 μm size fraction and grazing by microzooplankton measured with the dilution technique during a Southern Ocean Drogue and Ocean Flux Study (SODOFS). Values in brackets are standard error. Significance levels :* = P< 0.05; ** = P< 0.01.

Station number	Chl-a (μg l^{-1})	r^2	Growth coeff. (k) d^{-1} ($x10^{-2}$)	Grazing coeff. g (d^{-1}) ($x10^{-2}$)	% Initial stock removed (d^{-1})	%Potential prod grazed (d^{-1})	Chlorophyll doublings d^{-1}
1	0.183	40.04*	1.85 (0.005)	-1.24 (0.002)	1.26	67.31	0.027
2	0.172	49.55*	7.96 (0.004)	-4.78 (0.002)	4.71	60.83	0.115
3	0.224	49.56*	7.58 (0.003)	-3.58 (0.001)	3.57	48.30	0.109
4	0.215	43.18*	6.21 (0.005)	-4.68 (0.002)	4.65	76.09	0.082
5	0.296	52.18**	4.52 (0.002)	-2.01 (-)	3.71	44.62	0.065
6	0.338	66.87**	7.16 (0.003)	-4.22 (-)	4.37	59.76	0.103
7	0.245	57.71**	3.36 (0.004)	-3.16 (0.001)	3.26	94.37	0.048
8	0.226	69.31*	4.68 (0.002)	-3.98 (-)	0.42	85.03	0.067
9	0.215	56.31*	4.68 (0.001)	-4.56 (0.002)	4.65	97.09	0.015
10	0.356	51.53*	7.41 (0.008)	-5.16 (0.003)	6.99	70.33	0.107

Table 5.2B Estimates of phytoplankton production in the > 20 µm size fraction and grazing by microzooplankton measured with the dilution technique during a Southern Ocean Drogue and Ocean Flux Study (SODOFS). Values in brackets are standard error. Significance levels: * = P< 0.05; ** = P< 0.01.

Station number	Chl-a (µg l^{-1})	r^2	Growth coeff. k (d^{-1}) ($x10^{-2}$)	Grazing coeff. g (d^{-1}) ($x10^{-2}$)	% Initial stock removed (d^{-1})	%Potential prod.grazed (d^{-1})	Chlorophyll doublings d^{-1}
1	0.215	46.81*	6.14 (0.003)	-1.49 (0.001)	1.49	25.00	0.088
2	0.315	59.73*	2.31 (0.01)	-0.93 (0.004)	0.94	40.76	0.033
3	0.371	48.55**	2.04 (0.004)	3.14 (0.002)	-	-	0.029
4	0.389	47.60*	1.06 (0.002)	0.14 (-)	-	-	0.015
5	0.488	75.36**	1.09 (0.003)	0.55 (-)	-	-	0 016
6	0.371	39.63**	1.67 (0.002)	0.21 (-)	-	-	0.024
7	0.581	53.37*	2.32 (0.002)	0.28 (-)	-	-	0.032
8	0.264	59.43*	6.76 (0.005)	0.38 (-)	-	-	0.097
9	0.441	50.01*	4.73 (0.001)	-0.87 (0.001)	0.91	19.19	0.068
10	0.532	76.23**	1.19 (0.003)	0.67 (0.001)	-	-	0.017

Table 5.3A Diel variations in the < 20 μm phytoplankton and grazing by microzooplankton during a Southern Ocean Drogue and Ocean Flux Study (SODOFS). Values in brackets are standard error. Significance levels: * = P< 0.05; ** = P< 0.01.

Time	Chl-a ($\mu g\ l^{-1}$)	r^2	Growth coeff. (k) d^{-1}	Grazing coeff. (g) d^{-1}	% Initial stock removed (d^{-1})	%Potential prod grazed (d^{-1})	Chlorophyll doublings d^{-1}
06h00							
1	0.387	58.13*	2.98 (0.005)	-2.56 (0.003)	2.53	86.32	0.043
2	0.401	47.69*	3.01 (0.008)	-1.79 (0.004)	1.97	60.41	0.043
12h00							
1	0.356	48.90*	3.41 (0.001)	-3.16 (0.005)	3.12	92.68	0.049
2	0.403	53.19**	2.01 (0.003)	-1.11 (0.004)	1.09	56.22	0.029
18h00							
1	0.304	49.57*	4.31 (0.007)	-2.78 (0.003)	2.73	64.93	0.062
2	0.289	68.33**	3.63 (0.003)	-2.01 (0.009)	1.76	56.60	0.052
0h00							
1	0.424	41.89*	4.56 (0.009)	-3.89 (0.007)	3.82	85.53	0.066
2	0.427	67.97*	4.03 (0.005)	-2.96 (0.003)	2.92	73.86	0.058

Table 5.3B Diel variations in the > 20 µm production and grazing by microzooplankton during a Southern Ocean Drogue and Ocean Flux Study (SODOFS). Values in brackets are standard error. Significance levels: * = P< 0.05; ** = P< 0.01.

Time	Chl-a ($\mu g\ l^{-1}$)	r^2	Growth coeff. k (d^{-1}) ($x10^{-2}$)	Grazing coeff. g (d^{-1}) ($x10^{-2}$)	% Initial stock removed (d^{-1})	%Potential prod grazed (d^{-1})	Chlorophyll doublings d^{-1}
06h00							
1	0.378	59.31**	4.56 (0.004)	-0.03 (-)	0.31	6.25	0.066
2	0.398	65.23*	4.07 (0.001)	-0.01 (-)	0.50	8.31	0.059
12h00							
1	0.477	78.56*	6.75 (0.003)	-0.04 (0.001)	0.42	1.50	0.097
2	0.394	43.67*	1.72 (0.001)	0.40 (0.003)	-	-	0.025
18h00							
1	0.389	43.92*	3.45 (0.001)	0.03 (-)	-	-	0.050
2	0.401	55.32*	3.67 (0.002)	0.05 (-)	-	-	0.053
0h00							
1	0.400	63.21**	1.31 (0.004)	0.10 (-)	-	-	0.019
2	0.406	59.02*	1.23 (0.001)	0.09 (-)	-	-	0.018

5.4 Discussion

In the course of this study the drogue drifted < 25nm in a westerly direction in approximately 12 days (Figure 5.1), suggesting that it had drifted in the Eastwind Drift flowing adjacent to the Antarctic continent (Gow & Tucker, 1990). Oceanographic data show that the entire study was carried out in the same water mass (Rigg, pers. comm.). Conditions during the experiment were characterised by low wind speeds (between 1.4 and 19.8 knots), and high surface light intensities ranging between 564 and 2797 μE. m^{-2}. s^{-1}. The phytoplankton community was dominated by microphytoplankton which comprised 54-70% of total chlorophyll-*a* measured (Figure 5.2).

During the entire study, the microzooplankton assemblages were numerically dominated by protozooplankton with densities ranging between 2550 and 3400 cells l^{-1} (Table 5.1). The densities and species composition of the protozooplankton assemblages were in the same range as previously found with similar studies conducted at the ice-edge zone of the Weddell Sea (Garrison & Buck, 1989; Garrison, 1991). Assuming that total phytoplankton carbon can be calculated using the equation $\mathbf{C_a = 80chl^{0.6}}$ (Hewes *et al.*, 1990), and the carbon content of microzooplankton calculated from **1 um^3 = 0.19 pg C** (Sime-Ngando *et al.*, 1992), microzooplankton carbon contributed ≤ 5% of total carbon in the < 200 μm fraction during this study. This result is similar to the < 12% cited by Buck & Garrison (1989) in a study conducted at the ice-edge zone of the Weddell Sea. Our estimate is, however, conservative since cell shrinkage of up to 44% in samples fixed with Lugols' solution have been documented (Leakey *et al.*, 1994a).

Growth rate estimates of phytoplankton during the drogue study ranged between 0.02 and 0.07 d^{-1}, equivalent to 0.03 - 0.12 chlorophyll doublings d^{-1} (Tables 5.2A and 5.2B). These results compare well with phytoplankton growth rate estimates obtained in various marine environments employing the dilution technique (Table 5.4). These estimates are, however, lower than estimates obtained during one of our previous studies conducted in the same region (Chapter 2). Bacterial growth rate estimates and size fractionated primary production studies were also low during the entire study (Tibbles, pers. comm.; Laubscher, pers. comm) suggesting that the activity of the entire biological system was low during this period.

Although the elevated production of ice algae is well documented (Smith, 1987; Lizotte & Sullivan, 1991), the degree to which ice algae released during ice melt remain active in the water column is unclear (Smith & Sakshaug, 1990). Ecophysiological studies of ice flora have shown that they exhibit photo-inhibition at high light intensities (Smith & Sakshaug, 1990; Lizotte & Sullivan, 1991). For example, the optimum light intensity (I_k) at which maximum production occurs for ice algae has been shown to be between 13 and 25 $\mu E\ m.^{-2}\ s.^{-1}$ (Jacques, 1983; Smith & Sakshaug, 1990). Thus high light intensities in the surface waters would inhibit growth of phytoplankton communities dominated by ice flora (e.g. *Chaetoceros* spp.). Indeed, photo-inhibition is likely to be of significance in water bodies of high solar irradiance and low wind speeds (Kirk, 1994). This, however, does not preclude high production rates of ice flora at reduced light intensities. Also, the presence of *Rhizosolenia* spp. and *Corethron criophilum*, which are regarded as the second stage in the succession of diatom species (Samyshev, 1991), suggests some degree of overlap between the ice associated and open water communities, possibly reflecting the effects of mesoscale hydrography or diatom succession patterns.

Microzooplankton grazing removed < 10% of the initial standing stock of the nanophytoplankton during the grazing experiments (Table 5.2A and 5.3A). Despite the low impact on the initial standing stock, the grazing impact on the potential production was ≈ 70% (Table 5.2A). This reflects the low growth rates of phytoplankton during the study (Tables 5.2A and 5.2B). The relationship between grazing and growth rate of nanophytoplankton was significant ($P < 0.05$), suggesting a close coupling between phytoplankton and microzooplankton. Although the grazing rates reported in this study (0.03 - 0.05 d^{-1}) are among the lowest reported in the literature (Table 5.4), data suggest that microzooplankton grazing alone was sufficient to control the growth of the nanophytoplankton fraction. This result is consistent with similar studies conducted in the Marginal Ice Zone of the Weddell Sea during spring which demonstrated that protozoan grazing rates were higher than primary production in areas dominated by nanoplankton (Lancelot *et al.*, 1993; Garrison *et al.*, 1993; Kivi & Kuosa, 1994). Therefore, protistan grazing at the ice-edge appears only to control phytoplankton in regions dominated by nanoflagellates (Kivi & Kuosa, 1994; Scharek *et al.*, 1994). Similar grazing patterns have also been reported from studies conducted in the northern hemisphere (Paranjape, 1990; Verity & Vernet, 1992). It must be pointed out, however, that

the use of chlorophyll-*a* as an indicator of grazing excludes other potential food sources such as heterotrophic components. The absence of significant correlations between microzooplankton abundance, grazing impact and nanophytoplankton concentrations may be explained by the wide range of trophic responses reported in the literature.

Table 5.4. Microzooplankton grazing and phytoplankton growth rates derived from grazing studies conducted in various oceanic environments employing the dilution technique.

Author	Region	Grazing coefficient (d^{-1})	Growth coefficient (d^{-1})
Burkill *et al.*, 1987.	Coastal	0.14 - 1.04	-0.07 - 1.04
Paranjape, 1987.	Shelf waters	0.08 - 0.17	-0.01 - 0.30
Gifford, 1988.	Shelf waters	0.02 - 1.44	0.24 - 1.92
Verity & Vernet, 1992.	Shelf waters	0.08 - 0.34	-0.13 - 0.21
Burkill *et al.*, 1993.	Oceanic	0.02 - 0.57	-
Verity *et al.*, 1993.	Oceanic	0.21 - 1.09	-0.05 - 0.97

Throughout the SODOFS study, grazing by microzooplankton generally did not result in a decrease in the microphytoplankton concentration (Table 5.2B and 5.3B). Consequently grazing by microzooplankton is not sufficient to control biomass accumulation in communities dominated by microphytoplankton. The inability of protozoans to graze on microphytoplankton reflects morphological constraints associated with feeding (see Hansen *et al.*, 1994; Peters 1994). Meso- and macrozooplankton grazing studies conducted during the same period showed that grazing by the tunicate, *Salpa thompsoni*, was sufficient to control phytoplankton growth during the pre-bloom period (Perissinotto & Pakhomov, in prep). Under blooms conditions, however, sedimentation of phytoplankton cells was probably the main contributor to carbon flux. Recent studies suggest that ice algae released into the water column have a tendency to form aggregates with high sinking rates (Riebesell *et al.*, 1991, cited by Scharek *et al.*, 1994). Indeed, sediment trap studies conducted at the ice edge zone

have shown the important contribution of sinking phytoplankton cells to carbon flux (von Bodungen *et al.*, 1986; 1988; Matsuda *et al.*, 1987; Fisher *et al.*, 1988).

Microzooplankton grazing did not display any diel patterns throughout the period of investigation (Tables 5.3A and 5.3B). Diel feeding patterns by larger metazoan grazers are primary the result of vertical migrations from below the zone of regeneration to the surface waters as a predator avoidance strategy (Gliwicz, 1986; Perissinotto, 1989; Longhurst, 1991). Depth profile studies of the Southern Ocean have demonstrated that protozooplankton biomass is most concentrated in the upper water column, suggesting a close relationship with the sources of primary production (Garrison & Buck, 1989; Pierce & Turner, 1992; Garrison *et al.*, 1993). Microzooplankton, therefore, do not appear to vertically migrate. Recent studies on the feeding dynamics of ciliates showed no clear-cut diurnal grazing patterns (Kivi & Setälä, 1995). On the basis of their results, Kivi & Setälä (1995) suggest that natural ciliate populations are always, within the framework of their temperature limited metabolism, exercising clearance activity at their maximum possible rates. Similar grazing patterns for flagellates have also been documented (Peters, 1994). These data suggest that protozooplankton do not exhibit diel grazing patterns.

The results of the grazing experiments conducted during this study largely indicate that microphytoplankton are not grazed by microzooplankton (Table 5.2B). This result implies that the sedimentation of phytoplankton cells, or grazing by macro- and mesozooplankton at the ice edge represent the primary trophic route for summer production in regions of the ice edge where microphytoplankton dominate. Indeed, a previous study on microzooplankton grazing in the Southern Ocean showed that microzooplankton removed < 25% of summer production when microphytoplankton dominated chlorophyll biomass (Froneman & Perissinotto, in prep). This provides partial support for the model proposed by Huntley *et al.* (1991) which suggests that up to 80% of the net primary production is channelled into macrozooplankton.

Dramatic seasonal differences in the physical conditions in the MIZ and associated changes in phytoplankton abundance and distributional patterns have been observed (Kang & Fryxell, 1993). Recent studies in the Weddell Sea and Atlantic sector of the Southern Ocean have demonstrated a shift in the size composition of phytoplankton from a community dominated

by microphytoplankton in summer, to one dominated by nanophytoplankton in winter (Garrison *et al.*, 1991; 1993; Leakey *et al.*, 1994b). In winter, therefore, grazing by microzooplankton is sufficient to control the growth of phytoplankton (Garrison *et al*, 1993; Lancelot *et al.*, 1994; Lutjeharms *et al.*, 1994) and particulate organic carbon (POC) flux to the deep ocean is reduced due to the close coupling between the microzooplankton and the microbial loop, with consequent recycling of nutrients in the zone of regeneration (Longhurst, 1991). Evidence of this is presented in a number of sediment trap studies conducted in Antarctic waters (Matsuda *et al.*, 1987). These show that minimum POC flux rates (< 10 mg C m^{-2}. d^{-1}) coincide with the winter months, while the highest rates (120 - 135 mg C m^{-2}. d^{-1}) are recorded in the summer, when microphytoplankton dominate the chlorophyll biomass. This result reflects a decrease in phytoplankton standing stock and lower grazing impact of the meso- and macrozooplankton during winter.

A potentially important source of POC flux in winter may, however, result from carnivory by meso- and macrozooplankton eating microzooplankton in the absence of microphytoplankton. In the Southern Ocean, carnivory on microzooplankton by larger metazoan grazers is well documented for the summer period (Hopkins & Torres, 1989; Hopkins *et al.*, 1993; Perissinotto & Pakhomov, in prep). A general shift to zooplankton prey when phytoplankton become scarce has also been shown in a variety of other oceanic areas (Landry, (1981), cited in Hopkins & Torres, 1989). Recent studies conducted in the Weddell Sea in winter have shown that copepods feeding exclusively on phytoplankton can not meet their metabolic costs (Bathmann *et al.*, 1993). Alternative food sources which could potentially meet these energy demands include detritus and protozooplankton. Also, recent studies conducted in coastal waters west of the Antarctic Peninsula have concluded that carnivory is the dominant trophic mode during winter (Huntley & Norhausen, 1995). These results point to importance of protozoans as trophic links, coupling production in the nano- and picoplankton to the higher trophic levels during winter. However, their contribution to the increase in the number of trophic steps would result in a less efficient biological pump.

5.5 References

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CHAPTER 6

CARNIVORY BY SELECTED MACROZOOPLANKTON SPECIES FEEDING ON MICROZOOPLANKTON IN THE ATLANTIC SECTOR OF THE SOUTHERN OCEAN IN AUSTRAL SUMMER

Abstract

Carnivory by the two dominant euphausiid species *Euphausia crystallorophias* and *E. superba* and the salp *Salpa thompsoni* on microzooplankton was investigated using *in vitro* incubations during the fourth South African Antarctic Marine Ecosystem Study (SAAMES IV) cruise in the marginal ice zone of the Lazarev Sea during late austral summer (Dec./Jan.) 1994/95. Chlorophyll-*a* concentrations used in the incubations varied from 0.203 to 1.430 µg l^{-1} and were dominated by chain forming microphytoplankton (> 20 µm) species of the genera *Chaetoceros* and *Nitzschia*. Microzooplankton densities during the study ranged between 1725 and 2735 cells l^{-1} and were entirely dominated by protozoans, comprising aloricate ciliates and dinoflagellates. Ingestion rates calculated from biovolume: carbon estimates for *E. superba* varied between 50 and 301 µg C ind^{-1}. d^{-1} ($\bar{x}$ = 188 $\pm$ 87.98) and those for *E. crystallorophias* between 58 and 247 µg C ind^{-1}. d^{-1} ($\bar{x}$ = 158 $\pm$ 61.23). *S. thompsoni* ingestion rates were the highest recorded, ranging between 98 and 356 µg C ind^{-1}. d^{-1} ($\bar{x}$ = 232 $\pm$ 83.2). Based on the estimated minimum carbon uptake (MCU) values of the three species reported in the literature, carbon derived from the consumption of microzooplankton alone contributes between 107 and 185% of the MCU requirements for juvenile *E. superba*, between 61 and 75% for *E. crystallorophias* and from 166 to 432% for *S. thompsoni*. These results show that even in the presence of high chlorophyll concentrations, microzooplankton represent an important source of carbon for the three dominant Antarctic macrozooplankton species. Microzooplankton are therefore important trophic intermediates between bacterioplankton, small phytoplankton cells and macrozooplankton.

6.1 Introduction

Recent observations have shown that the role of microzooplankton in aquatic food webs is more important than previously thought. Microzooplankton have been shown to consume a significant proportion of daily primary production (Paranjape, 1990; see review of Pierce & Turner, 1992; Froneman & Perissinotto, in press a and b), to be important in regulating bacterial populations (Andersen & Fenchel, 1984; Albright *et al.*, 1987; Bernard & Rassoulzadegan, 1993) and are important agents in nutrient regeneration (Probyn, 1987; Goeyens *et al.*, 1991). In addition to these roles, microzooplankton may be regarded as important trophic intermediates between the bacterioplankton and the larger meso- and macrozooplankton (Gifford & Dagg, 1988; 1991). On the basis of these observations, the classical paradigm of pelagic food webs simply composed of diatoms, copepods and fish has been revised.

Feeding studies of the larger zooplankton in the Southern Ocean have largely used the gut fluorescent technique to estimate daily rations and grazing impact (Perissinotto, 1992). Because the gut florescent technique uses chlorophyll as an index of feeding, the contribution of heterotrophic food items, e.g. micro- and mesozooplankton, to total daily carbon intake is not measured. Consequently, daily rations of these grazers may be substantially underestimated. Indeed, energy budgets for the dominant Antarctic grazer, *Euphausia superba*, show that carbon derived from the grazing of phytoplankton alone can hardly meet the daily metabolic requirements (Drits & Pasternak, 1993; Pakhomov *et al.*, in press; Perissinotto *et al.*, in press). Similarly, studies conducted in the Weddell Sea during winter showed that carbon derived from phytoplankton accounted for only 2-5% of daily carbon requirements of copepods (Bathmann *et al.*, 1993). Alternative sources of carbon included detritus and protozooplankton. These results indicate that heterotrophic carbon may be an important component of the natural diets of grazers which are traditionally considered herbivorous.

Gut content analyses of the dominant grazers in the vicinity of the marginal ice zone (MIZ) have shown that protozoans comprise a significant proportion of the total number of items identified (Hopkins & Torres, 1989; Hopkins *et al.*, 1993). Indeed, a recent study has shown that protozoans constitute $\approx$ 25% of the total identifiable items in the gut of the two dominant

Antarctic euphausiids, *Euphausia crystallorophias* (Pakhomov *et al.*, in press) and *E. superba* (Perissinotto *et al.*, in press) in the Atlantic sector of the Southern Ocean. These estimates are, however, likely to be underestimated due to the fragility of microzooplankton components. These studies do also fail to provide any quantitative data on the grazing impact of the two euphausiids on microzooplankton populations or the contribution of these organisms to their daily energy intake.

The aim of this study was, therefore, to present the first quantitative grazing rate data of the three dominant macrozooplankton species on microzooplankton in the vicinity of marginal ice zone of the Lazarev Sea during austral summer 1995.

6.2 Materials and methods

Carnivory experiments using selected macrozooplankton feeding on microzooplankton were conducted during the fourth South African Antarctic Marine Ecosystem Study (SAAMES IV) cruise in the marginal ice zone of the Lazarev Sea during summer (Dec./ Jan.) 1994 -1995 (Figure 6.1). The consumption of microzooplankton was estimated employing the techniques of Gifford & Dagg (1988; 1991). The grazing impact of the three most abundant species of the local macrozooplankton community were investigated: *Euphausia crystallorophias*, juvenile *E. superba* and the tunicate, *Salpa thompsoni*.

Macrozooplankton collected with net tows (500 µm Bongo nets) were acclimated in natural seawater for 24 h in 20 l polyethylene carboys under ambient conditions. Prior to the start of the carnivory experiments, 6 replicate samples were prepared in 20 l polyethylene containers filled with natural seawater and allowed to stand for 2 h. According to Gifford (1993), this time period is sufficient to allow for the stabilization of the plankton assemblage in the containers. For each experiment, 2 replicate samples containing only natural seawater were used as controls. In the experimental treatments, 4 replicate samples each containing one individual macrozooplankton were used. The controls and treatments were then incubated on deck under ambient conditions for 24 h. Each container was gently stirred with a plastic spatula at 6 h intervals to prevent the settlement of plankton.

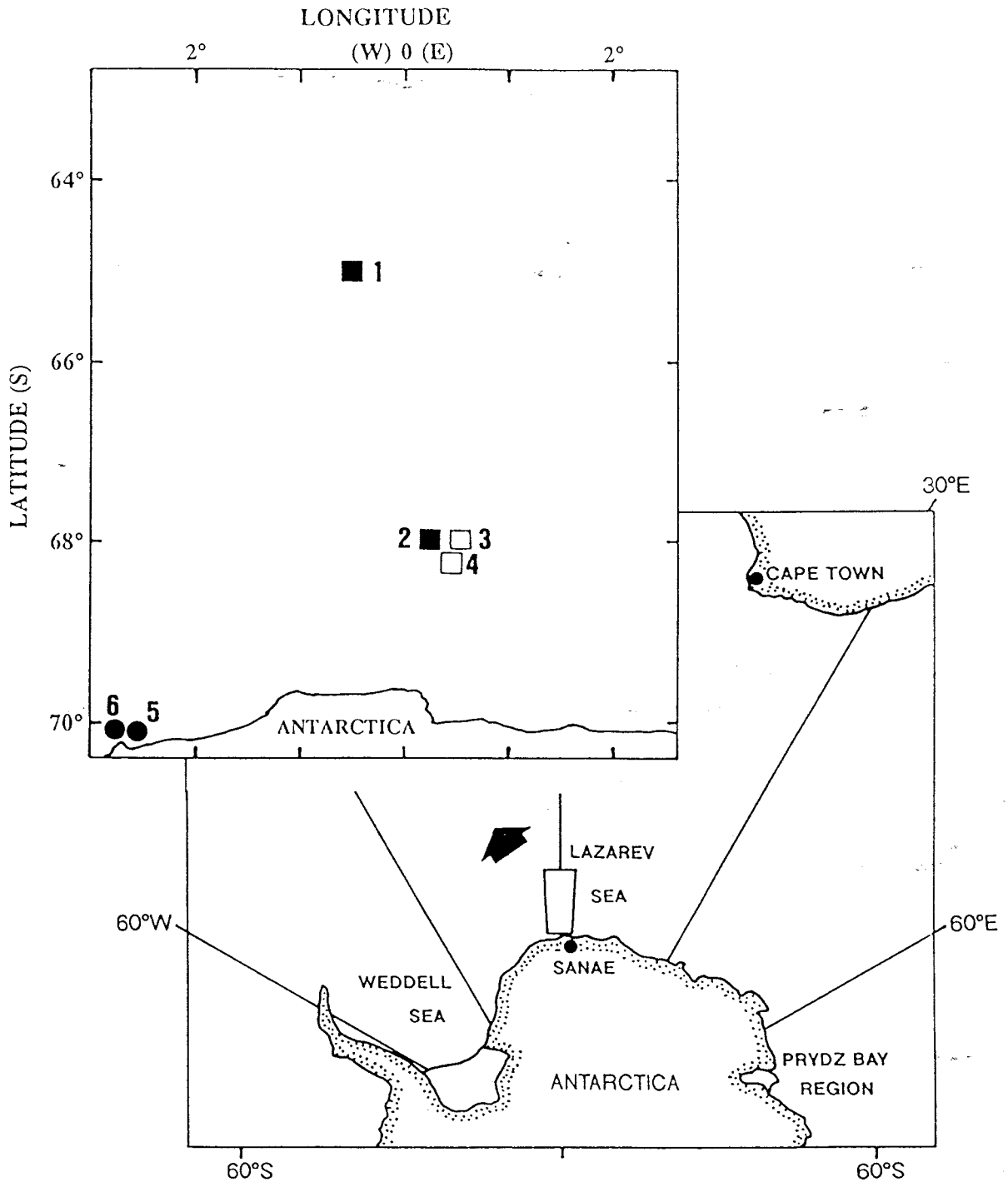

Figure 6.1 Location of the study area with an inset illustrating the position of the stations where the carnivory experiments were conducted during the SAAMES IV cruise to the region of the ice-edge zone of the Lazarev Sea in austral summer (Dec./Jan.) 1994/95. Stations 1 and 2 = *Euphausia superba*; 3 and 4 = *Salpa thompsoni*; 5 and 6 = *E. crystallorophias*.

At the beginning of the experiment, two 250 ml water samples were taken from each container for the determination of initial chlorophyll-*a* concentration and microzooplankton species composition and abundance. This procedure was repeated at the end of the experiments to estimate the final chlorophyll-*a* concentration and microzooplankton densities. Chlorophyll-*a* concentrations were determined fluorometrically (Turner 111 fluorometer) after extraction in 100% methanol for 6 h (Holm-Hansen & Riemann, 1978). Total phytoplankton carbon was estimated employing the equation of Hewes *et al.* (1990):

$$C_a = 80\ chl^{0.6}$$

Water samples for the determination of microzooplankton species composition and abundances were fixed with 10% Lugols solution (Leakey *et al.*, 1994). Microzooplankton species composition and densities were then estimated using the Utermöhl settling technique after sedimentation in a 10 ml settling chamber (Reid, 1983). From each sample, three subsamples of 10ml, representing 30% of the total were counted. A Nikon TMS inverted microscope operated at 400X magnification was used for this analysis. A minimum of 100 fields or 500 cells were counted for each sample. The total carbon of the microzooplankton fraction was estimated by calculating the mean biovolume of 50 ciliates and 50 dinoflagellates (Boltovoskoy *et al.*, 1989). The carbon biomass of the microzooplankton was then estimated assuming that 1 μm^3 = 0.19 pg C (Putt & Stoecker, 1989; Sime-Ngando *et al.*, 1992).

In all experimental treatments macrozooplankton organisms were preserved in buffered formalin at the end of the incubation period. The dry weight of specimens from each grazing study was determined by oven drying at 60°C for 36 h.

The grazing impact of macrozooplankton on microzooplankton was estimated by employing the following equations:

a) growth coefficient (k) of the microzooplankton:

$$C_1 = C_0\ e^{(k(t1-t0))}$$

where C_0 and C_1 are the prey concentrations in the control at time zero (*t0*) and at the end of the experiment (*t1*).

b) grazing coefficient (*g*) of macrozooplankton:

$$C_1 = C_0 (e^{(k-g)(t1-t0)}-1)$$

where C_1 is prey concentration in the experimental treatment at the end of the incubation.

c) the mean prey concentration <C> was derived from:

$$<C> = C_0 (e^{(k-g)(t1-t0)}-1)/(t1-t0)(k-g).$$

d) the clearance rate (F) was calculated using the following equation:

$$F = Vg/N$$

where V is the volume of the incubation chamber; *g* is the grazing coefficient of the macrozooplankton; and N the number of macrozooplankton per experimental container treatment.

Finally, the ingestion rate of macrozooplankton was estimated from:

$$I = F. <C>$$

6.3 Results

Microplankton community structure

The initial conditions at each grazing station are summarised in Table 6.1. Chlorophyll-*a* concentrations during the period investigated ranged between 0.203 and 1.430 µg l^{-1} and were dominated by microphytoplankton. The contribution of the < 20 µm chlorophyll fraction (nano- and picophytoplankton) to total chlorophyll was < 20% at all stations. An exception

occurred in the first *Euphausia crystallorophias* experiment (station 5), where the < 20 µm chlorophyll fraction dominated the total. Among the microphytoplankton, chain-forming diatom species of the genera *Chaetoceros* and *Nitzschia*, as well as large cells such *Corethron criophilum* and *Rhizosolenia* dominated numerically (Table 6.2). In particular, the single most abundant species was *Chaetoceros dichaeta* which contributed > 20% of the total cell counts at all the grazing stations (Table 6.2).

Table 6.1 Initial experimental conditions of the macrozooplankton carnivory experiments conducted during the SAAMES IV cruise in the marginal ice zone of the Lazarev Sea during late austral summer (Dec. /Jan.) 1994/95.

Station	Temperature (°C)	Macrozooplankton species	Total Chl-a conc. (µg l^{-1})	Microzooplankton densities (cells l^{-1})	Autotrophic carbon (µg C l^{-1})	Heterotrophic carbon (µg C l^{-1})
1	-1.15	*Euphausia superba*	0.203	1725	30.73	21.36
2	-1.19	*E. superba*	1.214	2225	89.97	27.55
3	0.40	*Salpa thompsoni*	1.390	2650	94.48	32.81
4	-0.30	*S. thompsoni*	1.430	2735	99.15	33.86
5	-1.27	*Euphausia crystallorophias*	0.204	2765	30.82	34.23
6	-0.86	*E. crystallorophias*	0.406	1800	46.58	22.28

The microzooplankton fraction was entirely dominated by protozoans with densities ranging between 1725 and 2765 cells l^{-1} (Table 6.1). The biomass of the microzooplankton ranged from 21 to 34 µg l^{-1} contributing between 23 and 52% of the total (microphytoplankton and microzooplankton) available carbon during the investigation.

Among the protozoans, dinoflagellates were the most numerous, with densities ranging between 828 and 1340 cells l^{-1}. *Protoperidinium, Gymnodinium* and *Amphisolenia* were identified as the most abundant components of this group. Also recorded were representatives of the genera *Dinophysis, Procentrum* and *Ceratium*. Densities of these taxa were, however, always < 50 cells. l^{-1}. Aloricate forms constituted the main component of the ciliates, with densities ranging between 774 and 1230 cells l^{-1}. The contribution of tintinnids was < 5% of the total at all stations. Abundances of the larger protozoans e.g. acantharians and foraminiferans were always < 5 cells l^{-1}.

Table 6.2 Microphytoplankton species composition and abundance during the SAAMES IV cruise in the region of the Marginal Ice zone of the Lazarev Sea in austral summer (Dec./Jan.) 1994/95. Only species contributing > 5% of total cell counts at each station are listed. Stations 1 and 2 = *Euphausia superba*; 3 and 4 = *Salpa thompsoni*; 5 and 6 = *E. crystallorophias*.

Station	Species composition and % contribution to total cell counts
1	*Chaetoceros* sp. (23%); *C. dichaeta* (30%); *Nitzschia* sp. (12%); *Corethron criophilum* (10%); *Distephanus speculum* (5%); *Rhizosolenia bergonii* (5%); *Navicula* spp. (5%).
2	*Chaetoceros* sp. (19%); *C. dichaeta* (24%); *Nitzschia* sp. (13%); *Corethron criophilum* (8%); *Rhizosolenia bergonii* (5%); *Navicula* sp. (5%).
3	*Chaetoceros* sp. (27%); *C. dichaeta* (22%); *Nitzschia* sp. (13%); *Corethron criophilum* (8%);*Rhizosolenia bergonii* (5%); *Navicula* sp. (5%).
4	*Chaetoceros* sp. (27%); *C. dichaeta* (34%); *Nitzschia* sp. (8%); *N. pelagica* (5%); *Corethron criophilum* (8%); *Rhizosolenia bergonii* (8%); *Navicula* sp. (5%).
5	*Chaetoceros* sp. (27%); *C. dichaeta* (31%); *Nitzschia* sp. (8%); *Rhizosolenia bergonii* (8%); *Navicula* sp. (5%).
6	*Chaetoceros* sp. (38%); *C. dichaeta* (27%); *Nitzschia* sp. (9%); *Corethron criophilum* (9%); *Rhizosolenia begonii* (5%); *Navicula* sp. (5%).

Feeding experiments

The rate estimates for all carnivory experiments conducted are shown in Figures 6.2 and 6.3. Although the clearance and ingestion rates varied considerably between experimental treatments, a *t*-test showed that the grazing impact of macrozooplankton on microzooplankton were not significantly different between experiments ($P > 0.05$). During the first carnivory experiments with *Euphausia superba* juveniles, clearance rates ranged between 229 and 400 ml ind.$^{-1}$ h^{-1} ($\bar{x}$ = 271 ml ind.$^{-1}$ h^{-1}) (Figure 6.2A). This level corresponds to individual ingestion rates ranging from 165 to 1016 cells ind.$^{-1}$ h^{-1}, equivalent to a daily intake of between 50 and 301 µg C ind^{-1} d^{-1} ($\bar{x}$ = 226 µg C ind^{-1} d^{-1})(Figure 6.3A). During the second *E. superba* experiment, the clearance rates were higher, ranging between 121 and 162 ml ind^{-1}. h^{-1} ($\bar{x}$ = 363 ml ind.$^{-1}$ h^{-1}) (Figure 6.2B), equivalent to an ingestion rate of between 405 and 547 cells ind.$^{-1}$ h^{-1}. This implies a daily consumption of between 120 and 162 µg C ind^{-1} d^{-1} ($\bar{x}$ = 149 µg C ind^{-1} d^{-1}) (Figure 6.3B).

Clearance rates of *Salpa thompsoni* during the early summer experiment ranged from 210 to 218 ml ind.$^{-1}$ h^{-1} (Figure 6.2C), equivalent to an ingestion rate of between 510 and 874 cells ind.$^{-1}$ h^{-1}. This level translates into a daily ingestion rate of between 152 and 259 µg C ind.$^{-1}$ d^{-1} ($\bar{x}$ = 173 µg C ind.$^{-1}$ d^{-1}) (Figure 6.3C). The estimated grazing parameters during the second experiment were higher. On this occassion, the clearance rates of salps ranged between 225 and 281 ml ind.$^{-1}$ h^{-1} (Figure 6.2D), or between 328 and 1035 cells ind.$^{-1}$ h^{-1}. Daily carbon ingestion rates from the consumption of microzooplankton ranged between 98 and 356 µg C ind.$^{-1}$ d^{-1} ($\bar{x}$ = 255 µg C ind^{-1} d^{-1}) (Figure 6.3D).

Clearance rates for adult *E. crystallorophias* ranged between 119 and 246 ml ind.$^{-1}$ h^{-1} and between 76 and 150 ml ind.$^{-1}$ h^{-1} during the first and second carnivory experiments, respectively (Figures 6.2E and 6.2F). These rates correspond to ingestion rates varying between 216 and 833 cells ind.$^{-1}$ h^{-1} and between 105 and 805 cells ind.$^{-1}$ h^{-1}, respectively. Daily estimates of carbon derived from the consumption of microzooplankton, ranged between 124 and 247 µg C ind^{-1} d^{-1} ($\bar{x}$ = 173 µg C ind.$^{-1}$ d^{-1}) during the first experiment, and between 59 and 239 µg C ind.$^{-1}$ d^{-1} ($\bar{x}$ = 143 µg C ind^{-1} d^{-1}) during the second experiment.

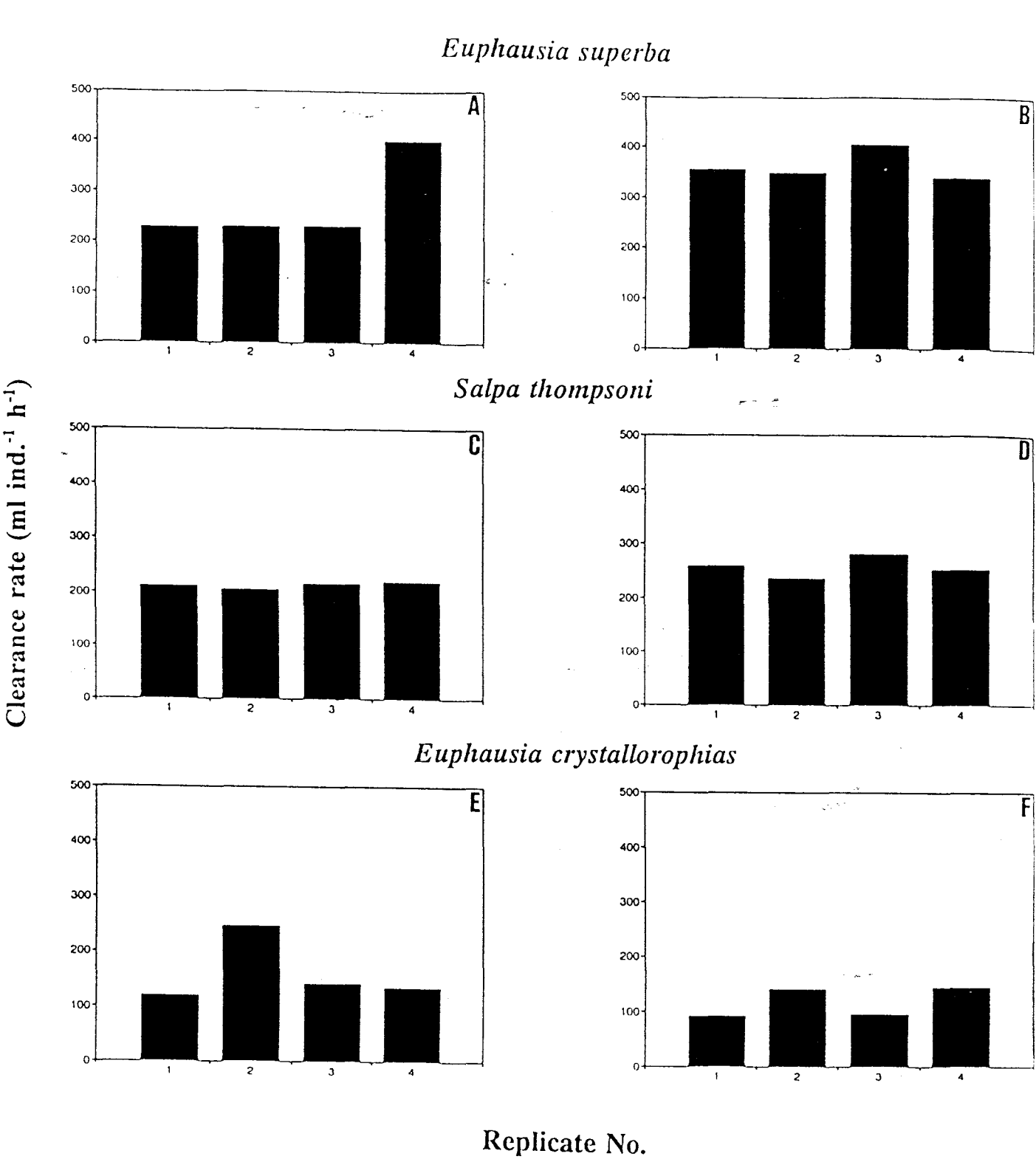

Figure 6.2 Clearance rates of three macrozooplankton species feeding on microzooplankton during the SAAMES IV in austral summer (Dec./Jan.) 1994/95.

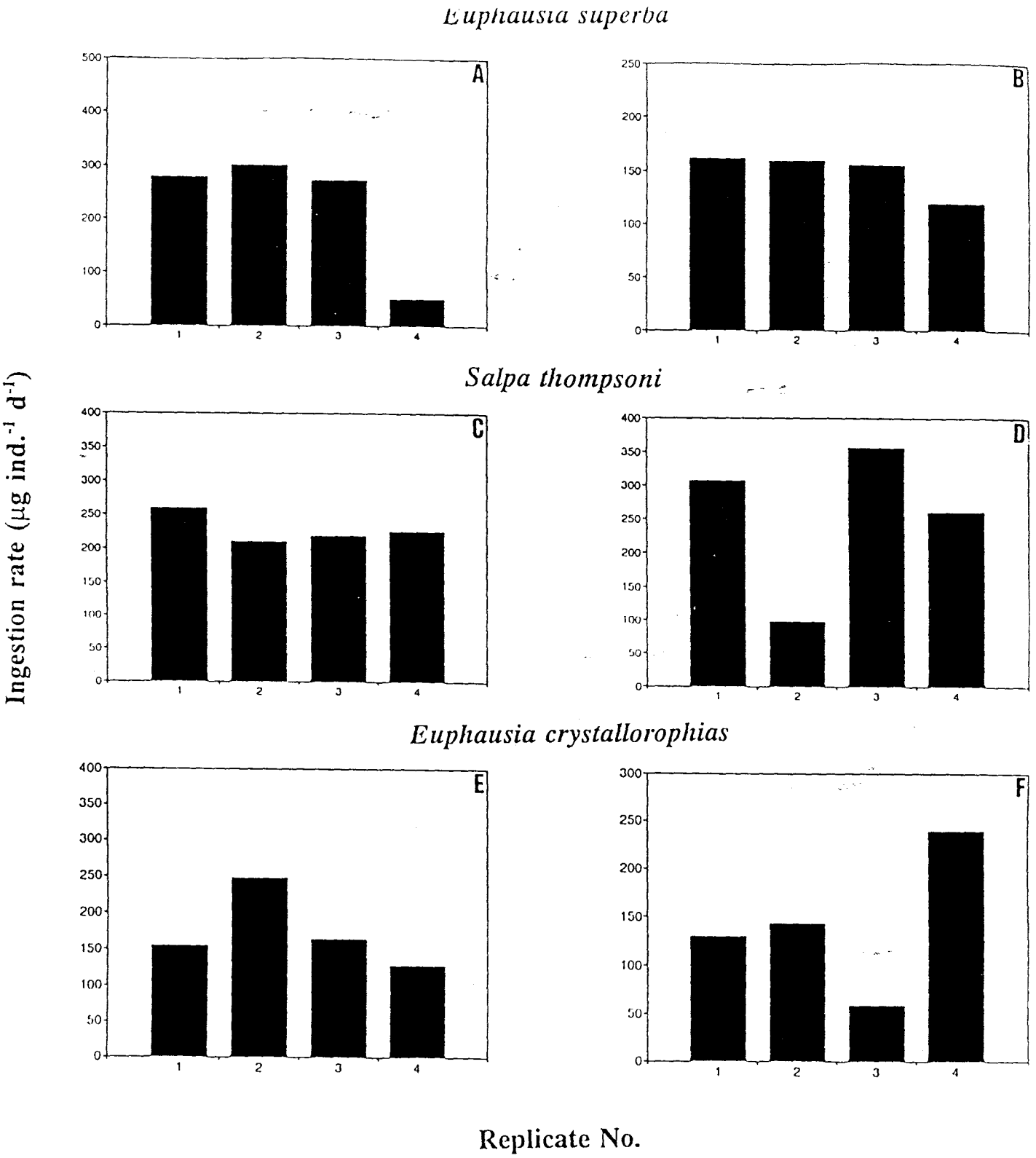

Figure 6.3 Daily carbon ingestion rates derived from the consumption of microzooplankton by three macrozooplankton species. Experiments were conducted during the SAAMES IV cruise to the region of the ice-edge zone of the Lazarev Sea during austral summer (Dec./Jan.) 1994/95.

6.4 Discussion

The substantial consumption of microzooplankton by copepods in the northern hemisphere (Tisleus, 1989; Gifford & Dagg, 1988; 1991; Joeng, 1994) and in the Southern Ocean (Atkinson, 1994) is well documented. Feeding by larger macrozooplankton on microzooplankton is, however, poorly understood (Hopkins & Torres, 1989; Hopkins *et al.*, 1993). The results of our study show that microzooplankton represent an important source of carbon for the three dominant macrozooplankton species of the higher Antarctic throughout the austral summer. Antarctic microzooplankton can, therefore, be regarded as important trophic intermediates between bacterioplankton, small phytoplankton cells and the larger meso- and macrozooplankton.

During the investigation period microplankton assemblages were dominated by chain forming diatoms and protozoans (Table 6.2). The dominance of typical ice associated microphytoplankton species such as *Nitzschia* and *Chaetoceros* (Heywood & Whitaker, 1984; Horner, 1985) suggests that the microphytoplankton community encountered during this study may have originated from ice melt. The numerical dominance of flagellates and ciliates in the microzooplankton community is consistent with the results of previous studies in the marginal ice zone (MIZ) (see review of Garrison, 1991). Our estimates of microzooplankton biomass ranged between 24 and 34 µg C l^{-1} (Table 6.1), and are among the highest values recorded for the Southern Ocean (see review of Garrison, 1991). These elevated microzooplankton abundances are probably the result of increased phytoplankton biomass typically associated with the MIZ during summer (see review of Garrison, 1991).

During our grazing studies, the clearance rates of *Euphausia superba* varied between 121 and 401 ml $ind.^{-1}$ h^{-1} (Figures 6.2A and 6.2B). These estimates are in the same range as those obtained by Price *et al.* (1988). According to Price *et al.* (1988), clearance rates of *E.superba* are strongly affected by the size of the container in which the organisms are incubated and the concentration of prey. Reductions in the filtration rates of *E. superba* feeding on *Chaetoceros* spp. have been documented (Schnack, 1985). During our investigation,

Chaetoceros spp. were identified as being the dominant component of the microphytoplankton assemblages (Table 6.2). However, since *E. superba* is able to feed selectively (Schnack, 1985), the filtration rates on microzooplankton can be considered to be realistic.

The ingestion of carbon derived from microzooplankton by *E. superba* during our study ranged between 50 and 301 µg C ind.$^{-1}$ d^{-1} (Figures 6.3A and 6.3B). According to Holm-Hansen and Huntley (1984), the minimum carbon uptake (**MCU**) of *E. superba* can be calculated from the equation: **MCU (µg C ind.$^{-1}$ h^{-1}) = 0.452 W$^{0.975}$**, where W is the dry weight of an individual krill. Thus, with krill of average size 23 mm (mean dry weight 13.6 mg), the **MCU** for krill during the study was estimated at 0.138 mg C ind^{-1} d^{-1}. The ingestion data obtained in this study show that the amount of carbon derived from the consumption of microzooplankton may comprise between 107 and 185% of the **MCU**. The energy derived from the consumption of microzooplankton is, therefore, sufficient to cover the minimum daily carbon requirements.

Energy budgets for *E. superba* based on its feeding rates on phytoplankton only have concluded that ingestion rates are inadequate to meet daily metabolic requirements (Drits & Pasternak, 1993; Pakhomov *et al.*, in press). *E. superba* requires ≈ 5% of its total body carbon per day for maintenance, activity, growth and reproduction (Clarke & Morris, 1983). In our experiments, the carbon required to meet its daily metabolic requirements would have been 0.270 mg C d^{-1}. The ingestion rates (x = 0.214 mg C d^{-1}) derived from our grazing experiments show that carbon derived exclusively from the consumption of microzooplankton accounts for 79% of the total daily carbon requirements of krill. This is, however, likely to be an overestimation since it is well documented that *E. superba* generally do not ingest diatoms of the genera *Chaetoceros* and *Rhizosolenia* (Maciejewska & Opalinski, 1993; Perissinotto *et al.*, in press). These were the dominant microphytoplankton species during our study. Thus, *E. superba* may have fed selectively on the microzooplankton in the presence of these diatom species. Also a substantial consumption of heterotrophic carbon by macrozooplankton may account for the discrepancy between ingestion and egestion rates recorded in the Antarctic krill (Drits & Pasternak, 1993; Pakhomov *et al.*, in press). Clearly,

the extent of carnivory of macrozooplankton has important implications for the use of the gut fluorescent method for the estimation of daily rations.

The clearance rates of *Salpa thompsoni* (1-2cm total length) ranged between 210 and 285 ml. ind. h^{-1} (Figures 6.2C and 6.2D). These rates are in the same range as those of Reinke (1987) and Huntley *et al.* (1989). However, the rates are generally lower than tunicates of similar size cited in the literature (see Huntley *et al.*, 1989). Reductions in salp clearance rates may result from bottling effects or the inability of salps to regulate their feeding rates. A recent study showed that the feeding efficiency of salps decreased at chlorophyll concentrations > 1 µg l^{-1} (Perissinotto & Pakhomov, submitted). It is worth noting that a similar study conducted in the Southern Ocean measured filtration rates for *S. thompsoni*, of between 410 and 600 ml $ind.^{-1}$ h^{-1} in regions where the chlorophyll concentrations were < 0.6 µg l^{-1} (Drits & Semenova, 1989). Using an *in situ* technique, Perissinotto & Pakhomov (submitted) obtained rates averaging 430 ml. h^{-1} for salps in the size range 1-5 cm length.

During grazing experiments, the ingestion rates of *S. thompsoni* ranged from 98 to 255 µg C. $ind^{-1}.d^{-1}$ (Figures 6.3C and 6.3D). The **MCU** of this salp species can be estimated by assuming that individuals require ≈ 2.57% (mean value) of dry body weigh carbon per day (Ikeda & Bruce, 1986). Salps of 1 cm length with a dry body weight of 2.3 mg would require ≈ 59 µg C d^{-1}. The carbon derived from the consumption of microzooplankton thus contributes between 166 and 432% of the daily **MCU**. Although these values are far in excess of minimum carbon requirements, the **MCU** value does not take into account the high energy demands of salps associated with their rapid growth and reproduction rates (Fortier *et al.*, 1994). Also, faecal pellets produced by salps have a high (up to 37%) carbon content (Fortier *et al.*, 1994). A similar study conducted using the gut fluorescent technique measured consumption rates > 3.6 mg C $ind.^{-1}$ d^{-1}, which is more than 400 times the **MCU** value for salps (Perissinotto & Pakhomov, submitted).

The estimated total daily carbon requirements for salps range between 17 and 25% of body carbon (Huntley *et al.*, 1989; Drits & Pasternak, 1993). Assuming a mean value of 21%, the

daily carbon requirements of *S. thompsoni* during the experiments would have been ≈ 0.483 mg C d^{-1}. Thus, with the daily ingestion rates ranging from 98 to 255 µg C d^{-1}, carbon derived from the consumption of microzooplankton could contribute between 20 and 53% of the total daily ration. It is well established that animal prey are assimilated more efficiently than plant prey (Gifford & Dagg, 1991). Thus, by consuming microzooplankton rather than algal cells, salps may gain energetic advantages.

The amount of carbon derived from the consumption of microzooplankton in *E. crystallorophias* ranged between 142 and 173 µg C. $ind.^{-1}$ d^{-1} (Figures 6.3E and 6.3F). Generally, the Antarctic neritic krill require ≈ 1.72% (mean value) of its carbon body weight to meet its minimum energy demands (Ikeda & Bruce, 1986). Assuming a carbon content of 45% dry weight (Ikeda & Bruce, 1986), the **MCU** for adult *E. crystallorophias*, ≈ 30 mm long (dry weight 30 mg) used in our experiments, was equal to 0.232 mg C d^{-1}. Carbon derived from the consumption of microzooplankton by the Antarctic neritic krill would therefore contribute between 61 and 75% of the daily **MCU**.

On the assumption that adult *E. crystallorophias* also require ≈ 5% of their total body carbon daily, the minimum amount of carbon required to meet all the metabolic costs associated with growth and reproduction can be estimated as described above. Thus, the carbon requirements of an adult *E. crystallorophias* of 30 mm length (dry weight 30 mg) is estimated at ≈ 0.6 mg C d^{-1}. The mean daily C derived from the ingestion of microzooplankton during our study was 0.152 mg C $ind.^{-1}$ d^{-1} which accounts for only 25% of the total daily carbon requirements of *E. crystallorophias* adults. This suggests that consumption of phytoplankton food must have represented the main carbon source for *E. crystallorophias* during the study period or that they were losing weight.

Recent observations suggest that small nano- and picophytoplankton cells dominate the total chlorophyll biomass of the Southern Ocean in winter (Garrison *et al.*, 1993; Kivi & Kuosa, 1994). This suggests that the grazing impact of macrozooplankton on microzooplankton may vary seasonally. Shifts in the diet of many macrozooplankton groups have been recorded in

the absence of microphytoplankton (Landry, 1981). Thus, carnivory by macrozooplankton can be expected to increase dramatically in the absence of the microphytoplankton size fraction. Indeed, a recent study conducted in the Weddell Sea during winter has shown that phytoplankton may accounted for only 2-5% of daily carbon requirements of copepods (Bathmann *et al.*, 1993). Detritus and protozooplankton have been suggested as the major contributors to daily carbon requirements. Also, a recent study conducted in the region of the Antarctic Peninsula in mid-winter has concluded that carnivory by zooplankton is the dominant trophic mode of the pelagic community during this season (Huntley & Nordhausen, 1995). Winter is also the period when the microzooplankton appear to represent the main sink for phytoplankton production (Garrison *et al.*, 1993; Lutjeharms *et al.*, 1994). The consumption of microzooplankton by meso- and macrozooplankton may then represent an important source of carbon flux and provide a mechanism capable of increasing the efficiency of the biological pump.

The results of this investigation indicate that Antarctic microzooplankton represent a significant source of carbon for macrozooplankton and can thus be regarded as important trophic intermediates between bacterioplankton, small phytoplankton cells and the larger metazoan grazers. Future studies should focus on the seasonal variations in macrozooplankton feeding in order to obtain a more accurate estimate of the fate of microzooplankton and their role in carbon flux.

6.5 References

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CHAPTER 7

THE ROLE OF MICROZOOPLANKTON IN THE DIET AND DAILY RATION OF SELECTED ANTARCTIC COPEPODS DURING AUSTRAL SUMMER.

Abstract

Predation of the dominant Antarctic copepod species *Calanus propinquus, Calanoides acutus, Rhincalanus gigas* and *Metridia gerlachei* on microzooplankton was estimated using *in vitro* incubations during the fourth South African Antarctic Marine Ecosystem study (SAAMES IV) cruise to the region of the ice edge in the Lazarev Sea during austral summer (Dec./Jan.) 1994-'95. Initial chlorophyll-a concentrations used in the incubations ranged between 0.19 and 0.93 µg l^{-1} and were dominated by ice associated chain-forming microphytoplankton species of the genera *Chaetoceros* and *Nitzschia* and large diatoms such as *Corethron criophilum* and *Rhizosolenia indica.* The microzooplankton were entirely dominated by protozoans comprising dinoflagellates (*Amphisolenia, Goniaulax, Protoperidinium* sp.) and naked ciliates. Densities of protozoans ranged between 1375 and 2125 cells l^{-1}. Carbon derived from the consumption of microzooplankton was highest for *Metridia gerlachei* ($\bar{x} \approx 9$ µg C ind.$^{-1}$ d^{-1}) and lowest for *Rhincalanus gigas* ($\bar{x} \approx 2$ µg C ind.$^{-1}$ d^{-1}). The mean carbon ingested by *Calanoides acutus* and *Calanus propinquus* was ≈ 6 and 5 µg C ind.$^{-1}$ d^{-1} respectively. Daily rations for *R.gigas* were only 2% body carbon. This low value contrasts with those obtained for the smaller copepods, *Metridia gerlachei, Calanoides acutus* and *Calanus propinquus* where daily rations were 9.1, 4.9 and 5.5% body C, respectively. Based on the minimum carbon uptake (MCU) of the four species examined, carbon derived from microzooplankton contributed > 120% of the MCU. The ingestion rates of the copepod species feeding on microzooplankton were highest for *Metridia gerlachei, Calanoides acutus* and *Calanus propinquus,* suggesting that the smaller copepod species used in this investigation feed more efficiently on microzooplankton than *Rhincalanus gigas.* Copepods are important consumers of microzooplankton and may, therefore, play a major role in reducing the grazing impact of microzooplankton on the local phytoplankton stock.

7.1 Introduction

The role of microzooplankton as trophic intermediates between bacterioplankton, small phytoplankton cells and larger zooplankton is well documented for the northern hemisphere (Stoecker & Capuzzo, 1990; Jonsson & Tiselius, 1992; Pierce & Turner, 1992). In particular, extensive investigations have been carried out on the role of microzooplankton in the natural diets of calanoid copepods (Gifford & Dagg, 1988; 1991; Jonsson & Tiselius; 1992; Pierce & Turner, 1992; Fessenden & Cowles, 1994; Jeong, 1994). The results of these studies have shown that microzooplankton are quantitatively important in meeting the daily dietary requirements of copepods. For example, the copepod *Acartia tonsa* may obtain up to 58% of its total carbon requirements from the ingestion of protozoan prey (Gifford & Dagg, 1989; 1991).

Microzooplankton are a ubiquitous component of the plankton assemblages in the Southern Ocean (Garrison, 1991; Garrison *et al.*, 1993) and are now recognised as major consumers of phytoplankton production (Froneman & Perissinotto, in press). The close coupling between the microbial loop and microzooplankton suggests that little carbon is available for export to depth in regions where microzooplankton are the major grazers (Longhurst, 1991). Grazing by zooplankton on microzooplankton may, however, represent an important source of carbon that can potentially be transferred from the microbial loop to the long-living pool.

Copepods and euphausiids are the two most important components of the zooplankton standing stock of the Southern Ocean (Schnack-Schiel & Mujica, 1994). Since copepods often dominate zooplankton biomass and have a higher production: biomass ratio than larger zooplankton, they account for the bulk of the zooplankton production in the high Antarctic (Boysen- Ennen *et al.*, 1991 cited in Schnack-Schiel & Mujica, 1994; Voronina *et al.*, 1994). Thus, copepods may make a proportionately higher contribution to carbon cycling in the high Antarctic than the larger macrozooplankton, especially in regions where the Antarctic krill, *Euphausia superba*, is scarce.

The role of copepods in carbon cycling in the Southern Ocean is well documented (Conover & Huntley, 1992). The grazing impact of copepods on phytoplankton has been estimated

using *in vitro* (Schnack, 1983; Schnack *et al.*, 1985) and *in situ* (Atkinson, 1992; Lopez & Huntley, 1995) techniques. The contribution of microzooplankton to the natural diets of copepods has, however, not been investigated adequately. Preliminary studies based on gut content analysis, have provided some evidence of the importance of microzooplankton in the natural diets of all the dominant copepod species in the Southern Ocean (Hopkins & Torres, 1989; Hopkins *et al.*, 1993). These results do not, however, provide estimates of daily ration or ingestion rates. Also, due to the fragility of these microzooplankton, it is widely accepted that they are grossly underestimated in studies of gut content analysis (Tanoue & Hara, 1986).

A recent *in vitro* grazing study, conducted by Atkinson (1994), has shown that the consumption of dinoflagellates, ciliates and cryptomonads by the dominant copepods in the shelf region of South Georgia contributes a median of 43% of their total carbon uptake. Furthermore, this study has suggested that larger copepods consume microzooplankton at rates equivalent to those observed using diatoms of similar size (Atkinson, 1994; Atkinson & Shreeve, in press). Small copepods, however, feed selectively on motile taxa such as protozoans (Atkinson, 1994; 1995). This may have important implications for the efficiency of the biological pump in the high Antarctic.

The main aim of this study was to examine the role of microzooplankton in the diet and daily rations of the four dominant Antarctic copepod species in the ice-edge zone of the Lazarev Sea during austral summer.

7.2 Materials and methods

Carnivory experiments with the dominant copepods species were conducted during the fourth South African Antarctic Marine Ecosystem Study (SAAMES IV) cruise in the region of the ice edge zone of the Lazarev Sea during austral summer (Dec./Jan.) 1994-'95 (Figure 7.1). The consumption of microzooplankton by the four dominant copepod species *Calanus propinquus, Calanoides acutus, Rhincalanus gigas* and *Metridia gerlachei* were estimated by employing the methods of Gifford & Dagg (1988; 1991) and Gifford (1993).

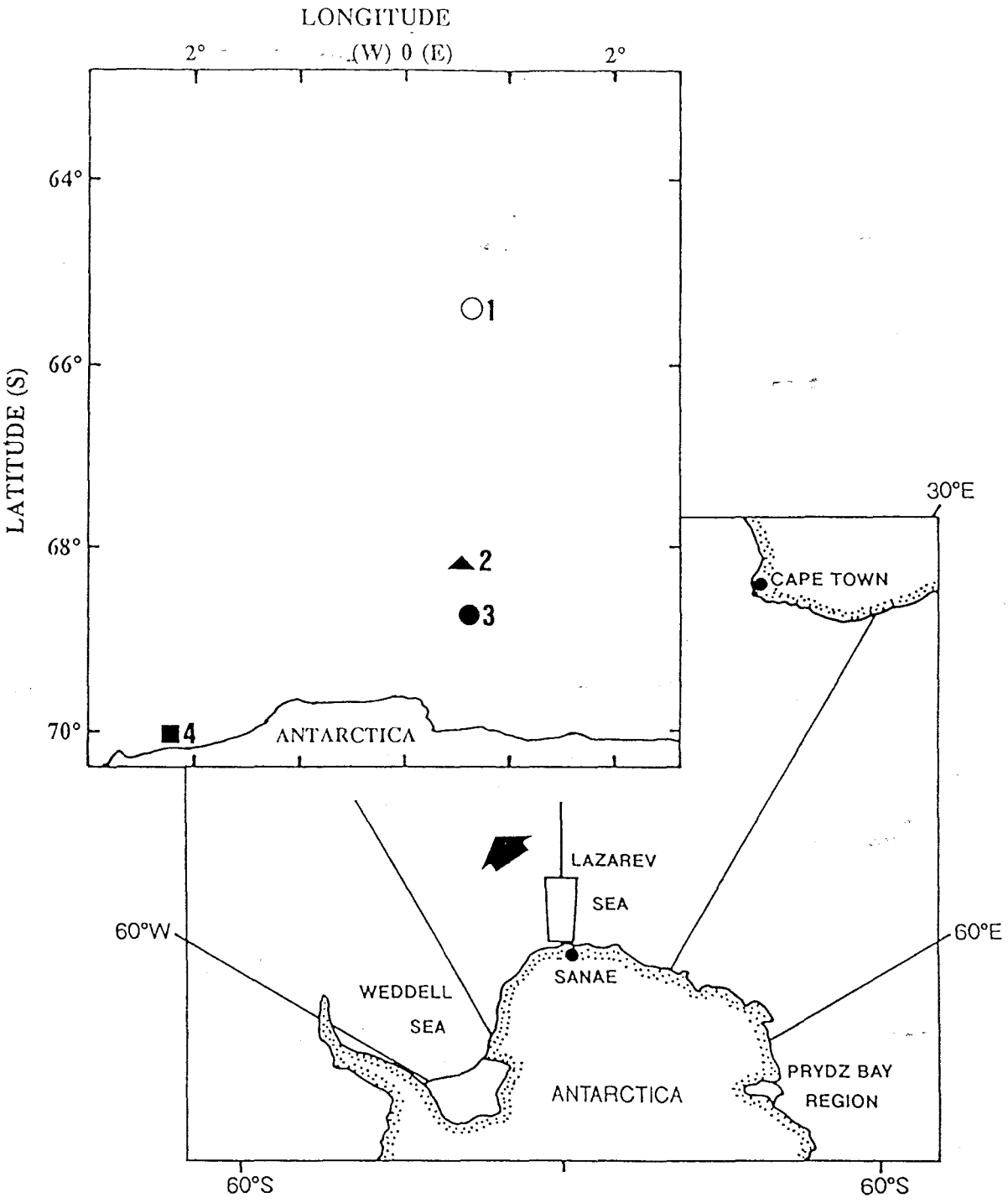

Figure 7.1 Location of the study area with inset showing the position of the stations where carnivory experiments were conducted during the SAAMES IV cruise to the ice-edge zone of the Lazarev Sea in austral summer (Dec./Jan.) 1995. Station 1 = *Calanoides acutus*; 2 = *Rhincalanus gigas*; 3 = *Metridia gerlachei*; 4 = *Calanus propinquus*.

Copepods were collected during net tows (500 µm Bongo net) and acclimated for 24 h in 20 l polyethylene carboys containing natural sea water under ambient conditions on deck. Prior to the onset of the feeding experiments, 6 replicate samples were prepared in 2 l polyethylene bottles filled with natural sea water and allowed to stand for 2 h. According to Gifford (1993), this time period is sufficient for the stabilisation of the plankton assemblages in the containers.

For each experiment, 2 replicate samples containing only natural seawater were used as controls. For the experimental treatments, 4-5 replicate samples each containing 4-5 copepods were used. To prevent the settlement of plankton in the containers, the water in each bottle was gently stirred at 4 hour intervals. At the beginning of the experiments, a 250 ml water sample was taken for chlorophyll-*a* analysis. Another 100 ml water sample was taken for the determination of microzooplankton species composition and abundance. This procedure was repeated at the end of each experiment to estimate the final concentrations of chlorophyll-*a* and microzooplankton densities and composition. Chlorophyll-*a* and phaeopigments concentrations were determined fluorometrically after extraction in 100% methanol for 12 h (Holm-Hansen & Riemann, 1978).

The microzooplankton samples were fixed with 10% Lugols solution (Leakey *et al.*, 1994). For each sample, three 10 ml subsamples, representing 30% of the total sample, were counted. The microzooplankton species composition and densities in each container were then estimated using the Utermöhl settling technique, after sedimentation in a 10 ml settling chamber. A Nikkon TMS inverted microscope operated at X400 magnification was used for this analysis. A minimum of 100 fields or 500 cells were counted for each sample. The total carbon in the microzooplankton fraction was then estimated by calculating the mean biovolume of 50 ciliates and 50 dinoflagellates for each experiment (Boltovskoy *et al.*, 1989). The carbon biomass was estimated by assuming that 1 μm^3 = 0.19 pg C (Sime-Ngando *et al.*, 1992). Copepods used in the experiments were preserved in buffered formalin at the end of the incubation period. The dry weight of each specimen was then determined by oven-drying at 60°C for 36h.

The grazing impact of macrozooplankton on microzooplankton was estimated using the following equations:

a) growth coefficient (k) of the microzooplankton:

$$C_1 = C_0\, e^{(k(t1-t0))}$$

where C_0 and C_1 are the prey concentrations in the control at time zero (t_o) and at the end of the experiment (t_1).

b) grazing coefficient (g) of mesozooplankton:

$$C_1 = C_0\, (e^{(k-g)(t1-t0)}-1)$$

c) the mean prey concentration <C> was derived from:

$$<C> = C_0\, (e^{(k-g)(t1-t0)}-1)/(t_1-t_o)(k-g).$$

d) the clearance rate (F) was calculated using the following equation:

$$F = Vg/N$$

where V is the volume of the incubation chamber; g is the grazing coefficient of the copepods; and N the number of mesozooplankton per experimental container.

Finally, the ingestion rate of mesozooplankton was derived from:

$$I = F <C>$$

7.3 Results

Microplankton community

A summary of the initial experimental conditions for the copepods grazing experiments is presented in Table 7.1. During the incubations, mean chlorophyll-*a* concentrations ranged between 0.187 and 0.931 µg l^{-1}. Size fractionated studies indicated that microphytoplankton dominated total chlorophyll, contributing between 54 and 70% of the total. Amongst the microphytoplankton, chain-forming species of the genera *Chaetoceros* and *Nitzschia* numerically dominated the cell counts. Also well represented were large diatoms such as *Corethron criophilum*, *Rhizosolenia indica* and the silico-flagellate, *Distephanus speculum*. The single most abundant diatom species during the investigation was *Chaetoceros dichaeta* which comprised up to 40% of all cells counted. The concentration of the < 20 µm chlorophyll fraction was always < 0.08 µg l^{-1} and was dominated by unidentified nanoflagellates.

Table 7.1 Summary of the experimental conditions of the copepod feeding experiments in the ice-edge region of the Lazarev Sea during austral summer (Dec./Jan.) 1994/95.

Species	Initial chl-*a* (µg l^{-1})	Protozooplankton (cells l^{-1})	mean prey volume (x 10^3 µm^3)
Rhincalanus gigas	0.907 (± 0.103)	1375 (± 250)	3.71
Metridia gerlachei	0.931 (± 0.080)	1750 (± 125)	3.81
Calanoides acutus	0.187 (± 0.030)	1875 (± 375)	2.71
Calanus propinquus	0.215 (± 0.035)	2125 (± 250)	3.97

The microzooplankton fraction was entirely dominated by protozoans, with densities ranging from 1375 to 2125 cells l^{-1} (Table 7.1). Among these, aloricate forms numerically dominated with densities ranging from 750 to 1375 cells l^{-1}. Tintinnid abundances were always < 100 cells l^{-1}. Dinoflagellates were the second most abundant group with densities ranging between

625 and 750 cells l^{-1}. *Protoperidinium, Amphisolenia* and *Gonyaulax* species were identified as the main components of this group. Abundances of the larger protozoans such as acantharians and foraminiferans were always < 25 cells l^{-1} throughout the study.

Feeding experiments

The rate estimates for all carnivory experiments conducted are shown in Table 7.2. Although the ingestion rates varied between treatments, *t*-tests showed that they were not significantly different ($P > 0.05$).

The clearance rates of *Rhincalanus gigas* during the incubations ranged between 4.1 and 24.3 ml ind.$^{-1}$ h^{-1}, corresponding to ingestion rates of between 54 and 170 microzooplankton cells d^{-1} (Table 7.2). This is equivalent to a carbon-specific ingestion rate of between 3.8 and 11.9 µg C ind.$^{-1}$ d^{-1} ($\bar{x}$ = 8.5 ± 3.1 µg C ind.$^{-1}$ d^{-1}) which corresponds to a daily carbon ration of between 0.8 and 2.8% body carbon ($\bar{x}$ = 2.0% ± 0.7) (Table 7.2). The feeding rates of *Metridia gerlachei* on microzooplankton were amongst the highest recorded during the investigation. Indeed, analysis of variance indicates that the daily ration of *M. gerlachei* was significantly higher that those of the three other copepod species ($F = 12.4$; $P < 0.05$). The clearance rates of *M. gerlachei* ranged from 4.3 to 8.9 ml ind.$^{-1}$ h^{-1}, equivalent to a daily ingestion rate of between 125 and 270 microzooplankton cells ind.$^{-1}$ (Table 7.2). The daily carbon specific ingestion rate ranged betwen 9.0 and 14.8 µg C. ind.$^{-1}$ ($\bar{x}$ = 13.6 ± 4.4 µg C. ind.$^{-1}$ d^{-1}), corresponding to a daily ration of between 5.7 and 13.6% body carbon ($\bar{x}$ = 9.1% ± 3.4; Table 7.2).

The clearance rates of *Calanoides acutus* varied between 4.3 and 8.8 ml ind^{-1}. h^{-1} which corresponds to an ingestion rate of between 110 and 170 microzooplankton cells ind.$^{-1}$ d^{-1} (Table 7.2). This is equivaleny to a carbon-specific ingestion rate of between 4.5 and 7.0 µg C d^{-1} ($\bar{x}$ = 6.2 ± 1.4 µg C d^{-1}), or between 3.9 and 5.7% body carbon d^{-1} ($\bar{x}$ = 4.9% ± 0.9)(Table 7.2). The clearance rates of *Calanus propinquus* during the incubations ranged from 5.6 to 10.1 ml ind.$^{-1}$ h^{-1} which corresponds to an ingestion rate of between 131 and 156 microzooplankton cells ind.$^{-1}$ d^{-1}. These ingestion rates are equivalent to a carbon specific ingestion rate of 9.8 to 11.7 µg C ind.$^{-1}$ d^{-1} ($\bar{x}$ = 10.1 ± 1.3 µg C ind.$^{-1}$ d^{-1}) which correspond to a daily ration of 4.2 to 6.4% body carbon (Table 7.2).

Table 7.2 Summary of grazing results of microzooplankton feeding studies of selected copepod species conducted at the ice-edge zone of the Lazarev Sea during austral summer (Dec./Jan.) 1994/95. Values are means for the 4-5 individuals used in each experiment. F = Clearance rate; I = Ingestion rate; C = carbon specific ingestion rate.

Species	Dry wt. (µg)	C d.wt. (µg)	F (ml ind.$^{-1}$ h^{-1})	I (cells ind.$^{-1}$ d^{-1})	C (µg ind.$^{-1}$ d^{-1})	Ration % body C
Rhincalanus gigas	1097	480	24.3	149	10.5	2.2
	847	371	14.0	116	8.2	2.2
	975	427	10.6	170	11.9	2.8
	901	395	8.0	117	8.2	2.1
	1112	487	4.1	54	3.8	0.8
Metridia gerlachei	347	157	8.5	125	9.0	5.7
	316	143	4.3	270	19.5	13.6
	345	156	8.9	205	14.9	9.5
	338	153	5.1	157	11.4	7.4
Calanoides acutus	295	136	6.8	156	8.1	5.9
	259	113	7.8	143	4.5	3.9
	272	126	8.8	170	5.8	4.6
	281	130	4.3	110	5.7	4.4
	265	122	6.9	135	7.0	5.7
Calanus propinquus	447	195	8.1	131	9.8	5.0
	390	170	7.3	140	10.5	6.2
	429	187	6.9	137	10.3	5.5
	422	184	10.1	156	11.7	6.4
	451	197	5.6	110	8.2	4.2

The ingestion rates of the copepods feeding on microzooplankton, expressed as daily ration plotted against mean body dry mass (see Atkinson, 1994), were highest for the smaller copepods, *C. propinquus*, *C. acutus* and *M. gerlachei* which were represented by the IV-V copepodite stages during the period of the investigation (Figure 7.2).

7.4 Discussion

Microzooplankton are recognised as major consumers of phytoplankton production in the Southern Ocean (Garrison *et al.*, 1993; Froneman & Perissinotto, in press). During this study, all four species of copepods consumed large amounts of microzooplankton in the presence of substantial chlorophyll concentrations (Table 7.2). These results are consistent with those obtained in similar studies conducted in different regions of the Southern Ocean (Conover & Huntley, 1991; Atkinson, 1994; 1995) and provide further support to the growing consensus that microzooplankton represent an important trophic intermediate between bacterioplankton, small phytoplankton cells and metazoan grazers. Predation by copepods, as a consequence, reduces the high grazing impact that microzooplankton generally exert on the phytoplankton stock.

During this investigation, the average daily ration of carbon derived from the consumption of microzooplankton was highest for *Metridia gerlachei* (≈ 9% body C) and *Calanus propinquus* (≈ 6% body C.) (Table 7.2). Indeed, during this study, the mass specific ingestion rates of copepods feeding on microzooplankton were highest for these two species (Figure 7.2). These results are consistent with those obtained in similar studies of copepod grazing in the Southern Ocean (see review of Conover & Huntley, 1991). According to Atkinson (1995), both *M. gerlachei* and *C. propinquus* show a strong preference for motile taxa, suggesting that they may feed selectively on microzooplankton. Recent studies have also shown that maximum clearance rates of *M. gerlachei* and *C. propinquus* appear to be on particles < 200 µm in length (Atkinson, 1994). This is below the average size of the large chain-forming diatom species (*Chaetoceros dichaeta*) recorded during our investigation. By selectively consuming microzooplankton, copepods may gain two major advantages.

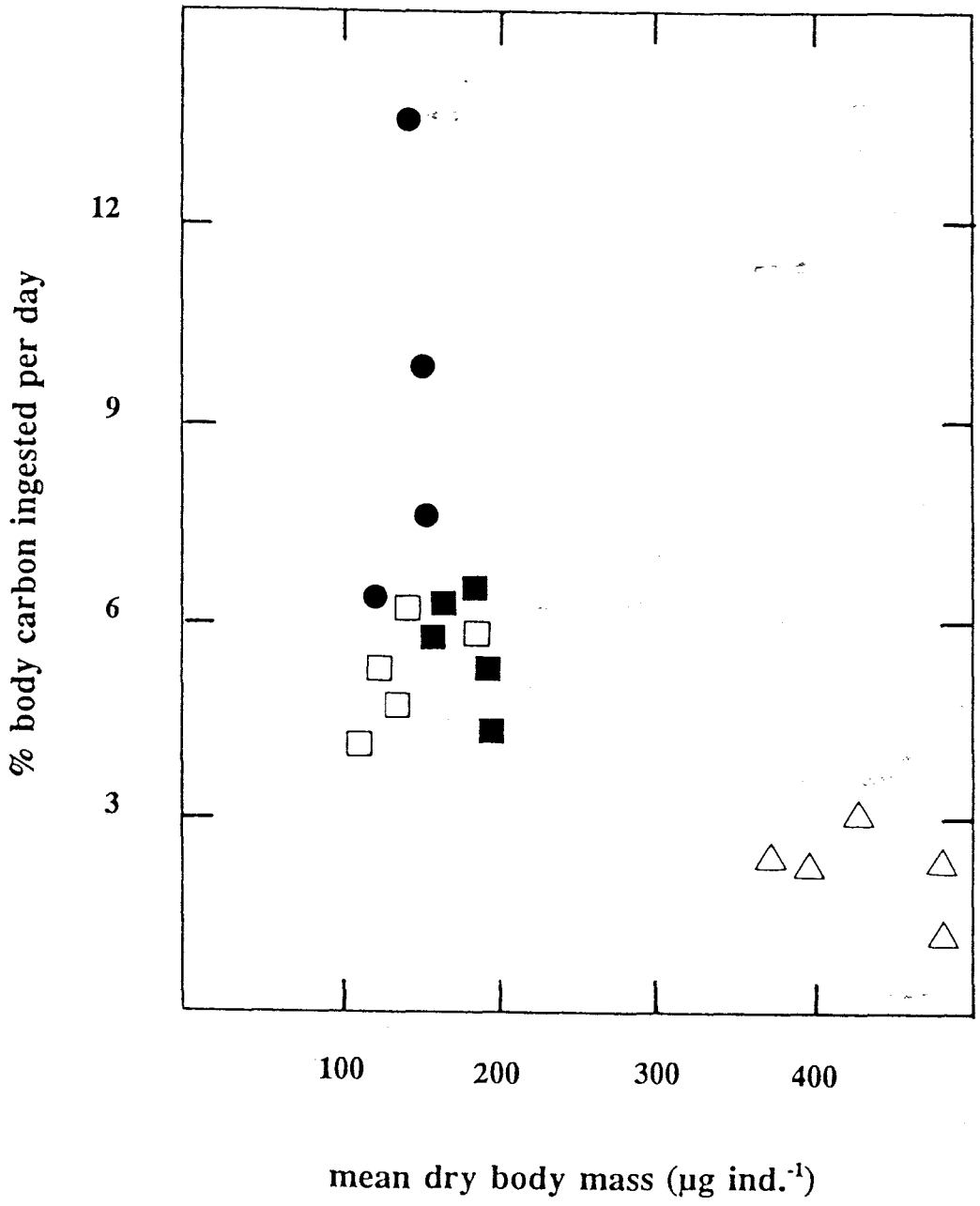

Figure 7.2 Ingestion rates (expressed as daily ration) in relation to body mass. Each data point is a mean value for one experiment involving 4-5 copepods. ● = *Metridia gerlachei*; △ = *Rhincalanus gigas*; □ = *Calanus propinquus*; ○ = *Calanoides acutus*.

Firstly, protozoans represent a more nutritious food source than diatoms (Stoecker & Capuzzo, 1990) and by feeding on smaller prey, copepods may substantially reduce their competition with larger zooplankton species. The implication is that copepods may be the most important grazers of protozoans in the Southern Ocean.

The daily rations of *Rhincalanus gigas* were the lowest recorded during this investigation (Table 7.2). Indeed, analysis of variance indicated that daily rations for this species were significantly different from the other three copepods ($F = 12.83$; $P < 0.001$). There is some evidence in the literature showing that *R. gigas* exhibits low feeding and respiration rates and has low growth efficiencies when incubated *in vitro* (Conover & Huntley, 1991; Atkinson *et al.*, 1992; Drits & Pasternak, 1995). The low daily rations obtained during this study probably reflects the low *in vitro* feeding rates of *R.gigas* reported in the literature (Conover & Huntley, 1991; Atkinson *et al.*, 1992; Drits & Pasternak, 1993).

Carbon derived from the consumption of microzooplankton by the four copepod species ranged between 0.8 and 19.5 µg C ind.$^{-1}$ d^{-1} (Table 7.2). The minimum carbon uptake (**MCU**) of the copepods can be estimated from the daily respiration rates available in the literature (Schnack 1985), assuming that **1 ml O_2 = 4.86 Cal and 10 Cal = 1 mg C** (Vinogradov & Shushkina, 1987). The **MCU** values for the four species are shown in Table 7.3. From these data, it is evident that carbon derived from the consumption of microzooplankton contributes > 120% of the **MCU** for all species. These results indicate that all four species can meet their basic metabolic requirements by feeding on microzooplankton alone.

Table 7.3 Daily carbon ingestion rates, expressed as % minimum carbon uptake (MCU), of selected copepods used in feeding experiments conducted within the ice-edge zone of the Lazarev Sea during austral summer (Dec./Jan.) 1994/95.

Species	MCU (% body C dry wt. d^{-1})	% MCU d^{-1}
Rhincalanus gigas	1.65	122
Metridia gerlachei	1.14	721
Calanoides acutus	3.95	155
Calanus propinquus	2.96	185

The contribution of microzooplankton to the total carbon ration of the four copepod species used in this investigation was estimated from the average daily rations reported in Conover & Huntley (1991). The daily carbon rations obtained from the consumption of microzooplankton for *Calanoides acutus* and *Rhincalanus gigas* were ≈ 17%, while for *Calanus propinquus* and *Metridia gerlachei* the rations were 19 and 24%, respectively. From these results, it is clear that the low daily rations of *R. gigas* reflect the normally low metabolic characteristics of this species (Conover & Huntley, 1991). It is worth noting that our estimates of daily rations calculated by employing the average daily rations of Conover & Huntley (1991) compare well with results obtained in a recent study conducted by Atkinson (1994). In his study protozoans contributed on average 43% of the total daily carbon intake.

It is obvious that during the summer 1994/'95 microzooplankton represented an important source of carbon for all four species of copepods studied (Table 7.2). It should be noted, however, that the estimates do not provide accurate data on the contribution of heterotrophic carbon to total daily intake since some of the taxa represented in the protozoan assemblages were autotrophic. Daily rations derived from the consumption of protozoans have been probably severly underestimated since the contribution of the < 20 µm (nanoplankton) component was not considered during this investigation. Furthermore, the estimates of carbon ingestion were derived from the biovolume of microzooplankton cells fixed with Lugols solution. Recent studies have shown that cell shrinkage in samples fixed with Lugol's solution may be as high as 44% (Leakey *et al.*, 1994). Thus, the estimates of daily rations of copepods feeding on microzooplankton can be considered as conservative values.

Seasonal grazing rates of copepods feeding on microzooplankton are poorly documented (see review of Conover & Huntley, 1991). The results of this study show that microzooplankton represent an important carbon source for copepods. Recent studies conducted during winter in the Weddell Sea have shown that energy derived from the consumption of phytoplankton alone cannot meet the daily metabolic requirements of copepods (Bathmann *et al.*, 1993; Drits *et al.*, 1994). Alternative sources of food have been suggested to meet copepods energetic demands, including detritus and protozoans (Bathmann *et al.*, 1993). A carnivorous feeding mode in copepods has been suggested as a possible mechanism allowing them to remain active during winter when chlorophyll concentrations are low (Atkinson, 1994). Indeed,

another recent study conducted in the vicinity of the Antarctic Peninsula has shown that carnivory by zooplankton represents the dominant trophic mode during winter (Huntley & Nordhausen, 1995). Alternatively, copepods may utilise their lipid and protein supplies to overwinter (Hagen *et al.*, 1993). Microzooplankton appear to represent an important carbon source for copepods in both summer and winter. The ability of copepods to consume both autotrophic and heterotrophic prey is mirrored by that of eupausiids (chapter 6) and may be a necessary adaption to the Antarctic seasonality and patchiness of food distribution (Atkinson, 1994). During winter, in the absence of microphytoplankton (Garrison *et al.*, 1993), the predation impact of copepods on microzooplankton can be expected to increase. During this time, copepod consumption of microzooplankton may promote trophic cascading down to bacteria. For example, the removal of microzooplankton may allow abundances of small phytoplankton cells and bacteria to increase, thus promoting a shift in the size spectrum of the plankton community. These shifts may have important consequences for the biological pump. Copepods can therefore be regarded as the most important consumers of the microbial community and as such may reduce dramatically the high impact of microzooplankton grazing during winter (Garrison *et al.*, 1993; Froneman & Perissinotto, in press).

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CHAPTER 8

FINAL DISCUSSION

Models of the Antarctic food web have traditionally considered large zooplankton species such as krill and copepods to be the most important grazers of phytoplankton in the Southern Ocean (Clarke, 1985; Hempel, 1985). Recently it has been realised that the microbial loop, characterised by microzooplankton and bacterioplankton, represents an important component of pelagic systems in general (Goldman, 1988; Laurion *et al.*, 1995). The importance of the microbial loop in carbon cycling in the Southern Ocean is, however, not clear (Garrison, 1991; Marchant & Murphy, 1994). The results of this study show that microzooplankton represent a key component of the pelagic system in the Southern Ocean, both as grazers of phytoplankton and as trophic intermediates between bacterioplankton, small phytoplankton cells and larger zooplankton.

The Southern Ocean is not as ecologically uniform as implied by the general term (Deacon, 1982; Froneman *et al.*, 1995a; 1995b). South of the Antarctic Polar Front (APF), three oceanic zones are generally identified (Hempel, 1985; Moloney & Ryan, 1995). These are the permanently open ocean zone, the seasonal ice zone and the coastal shelf zone. During spring, the phytoplankton assemblages of the latter two zones are dominated by larger microphytoplankton cells (Hempel, 1985; Moloney & Ryan, 1995). The high contribution of microphytoplankton to total chlorophyll in these regions is coupled to the seasonal ice melt and the subsequent release of epontic cells into the water column (Horner, 1985). In the permanently open ocean zone and the region north of the APF, small nano- and picophytoplankton cells dominate total chlorophyll most of the time (Weber & El-Sayed, 1988; Jacques, 1989; Laubscher *et al.*, 1993; Froneman *et al.*, 1995a; 1995b). The results of the microzooplankton grazing studies reported here suggest that microzooplankton represent the most important grazers in regions where the < 20 µm chlorophyll fraction dominate (Chapters 4 and 5). Indeed, during this investigation the grazing impact of

microzooplankton was significantly correlated to the contribution of the < 20 μm fraction to total chlorophyll. In areas dominated by microphytoplankton, microzooplankton grazing appears to control the concentrations of nano- and picophytoplankton (Chapter 5). These results point to a spatial variation in the grazing impact of microzooplankton mediated by changes in the size composition of phytoplankton. Evidence of this is provided by the results of the first summer investigation, when the mean grazing impact of microzooplankton in the permanently open waters was higher than the grazing impact observed in the MIZ (Chapter 2). It should be noted, however, that nanophytoplankton blooms may be associated with the retreating sea ice (Lancelot *et al.*, 1993). In these areas, microzooplankton can be reagrded as the most important grazers of phytoplankton (Lancelot *et al.*, 1993).

Temporal variations in the role of microzooplankton in carbon cycling in the Southern Ocean may be regulated by seasonal shifts in the size composition of phytoplankton, which are well documented (Garrison *et al.*, 1993; Lutjeharms *et al.*, 1994; Froneman & Perissinotto, in press b). Large microphytoplankton cells dominate the phytoplankton assemblages at oceanic fronts (Laubscher *et al.*, 1993; Froneman *et al.*, 1995a; 1995b) and in the waters south of the Antarctic Polar Front (APF) in early spring (Froneman *et al.*, 1995a; 1995b; Savidge *et al.*, 1995). During late austral summer and winter, however, small nano- and picophytoplankton cells dominate the total phytoplankton (Weber & El-Sayed, 1987; Hewes *et al.*, 1985; Garrison *et al.*, 1993; Kivi & Kuosa, 1994; Leakey *et al.*, 1994). The results of this investigation (Chapter 3) and that of Garrison *et al.* (1993) suggest that in regions where the < 20 μm chlorophyll fractions dominate, microzooplankton represent the most important grazers. These data suggest that, with the exception of the spring diatom blooms associated with the retreating ice, and blooms found at oceanic fronts, the microbial loop represents the major sink for phytoplankton production. These results are in agreement with the proposal of Azam *et al.* (1991) that the microbial loop in Antarctic waters is especially important during winter.

In chapters 6 and 7 it was shown that all the main components of the macro- and mesozooplankton assemblages in the Southern Ocean consume microzooplankton. In particular,

copepods, which can dominate zooplankton biomass in regions where krill are scarce (Voronina *et al.*, 1995), were observed to feed selectively on microzooplankton. Copepods may, therefore, reduce the high grazing impact of microzooplankton on phytoplankton. Although the results of this investigation show that copepods are important grazers of microzooplankton, they contribute little to vertical carbon flux since many produce small buoyant faecal strings and often consume their own faecal pellets (Fortier *et al.*, 1994). Larger zooplankton, e.g. euphausiids and salps, feeding on microzooplankton are more efficient in the transfer of carbon from the microbial loop to depth and thus represent an important leak in the microbial loop (Fortier *et al.*, 1994). It should be noted, however, that long food chains are less efficient in the transfer of carbon to depth than short food chains (Pomeroy & Wiebe, 1988; Moloney & Ryan, 1995). Consequently, the transfer of organic carbon from the surface waters to depth via the microbial loop, is considered inefficient. A further possible effect of copepod grazing on microzooplankton may be trophic cascading (Wickham, 1995). Microzooplankton are the dominant bacterivores in the pelagic system. By selectively grazing microzooplankton, copepods may promote shifts in the size composition of the plankton communities. This may have important implications for the subsequent energy transfer and the biological pump.

The seasonal shift in the size composition of the phytoplankton assemblages may promote increased carnivory by zooplankton on microzooplankton. Recent studies have shown that metazoan grazers may switch from herbivory to carnivory during winter when microphytoplankton cells become scarce (Huntley & Nordhausen, 1995). Since copepods and euphausiids are unable to feed efficiently on cells of < 5 μm (Conover & Huntley, 1991; Fortier *et al.*, 1994), microzooplankton appear to represent the most important food for zooplankton during winter when small cells dominate (Kivi & Kuosa, 1994; Bathmann *et al.*, 1993). Indeed, recent studies conducted in the vicinity of the Antarctic Peninsula concluded that carnivory by zooplankton represents the dominant trophic mode throughout the austral autumn and winter (Huntley & Nordhausen, 1995).

The results of this investigation suggest a strong temporal/seasonal shift in the components and dynamics of the Antarctic ecosystem. This shift appears to reflect changes in the size composition in phytoplankton which facilitates the subsequent partitioning of phytogenic carbon between the various size classes of zooplankton. Sea ice appears to be of particular importance in structuring the Antarctic food web and enhancing ecological variability (Eicken, 1992). A short food web dominated by larger zooplankton appears to exist only during the seasonal retreat of the pack ice, in the waters surrounding oceanic islands and in the vicinity of the oceanic fronts where large microphytoplankton dominate total chlorophyll (El-Sayed, 1988; Jacques, 1989). The key role of the Antarctic krill, *Euphausia superba*, in the grazing dynamics and carbon flux of the Southern Ocean appears to be restricted to early summer, in the region south of the APF and in areas generally dominated by elevated microphytoplankton concentrations such as the waters surrounding oceanic islands and at the major oceanic fronts (El-Sayed, 1988; Jacques, 1989; Laubscher *et al.*, 1993; Froneman *et al.*, 1995a; 1995b).

In the permanently open waters, during early austral summer and winter, the microbial loop represents the main sink for phytoplankton production (Garrison *et al.*, 1993; Froneman & Perissinotto, in press a; b). Predation by larger zooplankton on microzooplankton appears to represent an important trophic pathway in these areas. This is probably related to the inability of large zooplankton to feed efficiently on small phytoplankton cells (Fortier *et al.*, 1994). Indeed, predation on protozoa is expected to be particularly important in marine environments dominated by cells < 5 µm in size (Stoecker & Capuzzo, 1990). Grazing by krill and copepods on ice algae during winter may result in carbon being transported to depth. However, recent studies conducted under pack ice have shown that POC flux in winter (≈ 10 mg C m^{-2}. d^{-1}) is more than an order of magnitude lower than during summer (Matsuda *et al.*, 1987). These data suggest that grazing by larger zooplankton feeding on ice-bound algae during winter contributes little to the transfer of carbon to depth. Thus, in permanently open waters and during winter, long food chains predominate. Under these conditions, the biological pump will be relatively inefficient in the transfer of carbon to depth.

A potentially important source of carbon flux in regions dominated by small phytoplankton cells may be represented by the tunicate *Salpa thompsoni* (Perissinotto & Pakhomov, submitted). Salps are able to retain all particles > 2 μm with 100% efficiency and can therefore be regarded as important consumers of nanophytoplankton (Fortier *et al.*, 1994). During the last decade, salps have shown a consistent increase in biomass in the Prydz Bay region of the Southern Ocean, becoming at times the major contributor to the pelagic stock (Perissinotto & Pakhomov, submitted). Salps are efficient agents in the transfer of carbon to depth (Fortier *et al.*, 1994). In regions where the microbial loop dominates, salps may represent a mechanism capable of enhancing the local efficiency of the biological pump.

An indication of the spatio-temporal variability of the Antarctic food web can be derived from the ratio between new and regenerated production, termed the *f*-ratio (Goldman, 1988). The biological processes of regenerated and new production are distinctly different. The new production system is characterised by nitrate-based production dominated by large diatoms and large herbivorous zooplankton and high flux rates (Goldman, 1988; Goeyens *et al.*, 1991). In contrast, in regeneration-based systems, flux rates are dramatically reduced as the microbial loop represents the sink for phytoplankton production (Goldman, 1988). A study conducted in the Scotia-Weddell Confluence during summer (Nov.- Dec.) showed that the pelagic system evolved from new production system to regenerated production (Goeyens *et al.*, 1991). More recently, high *f*-ratios were found in the Atlantic sector of the Southern Ocean during summer, associated with the marginal ice zone and oceanic fronts suggesting that these areas are characterised by high flux rates (Mantel *et al.*, 1995). These areas can therefore be regarded as regions of export, characterised by an efficient biological pump (Longhurst & Harrison 1989; Longhurst, 1991). In contrast, low *f*-ratios were recorded in the permanent open waters, suggesting that much of the production in these regions is being recycled. During winter, the *f*- ratios were < 0.1 at all stations, suggesting a system dominated by the microbial loop (Mantel *et al.*, 1995). Also, another recent study conducted in the Pacific sector of the Southern Ocean has shown that the region south of 55°S (i.e. the marginal ice zone) represents a small sink for CO_2 while the open waters

north of this region represent a weak source of atmospheric CO_2 (Tilbrook *et al.*, 1995). These data indicate strong spatio-temporal variations in the Antarctic food web and support the model for the Antarctic marine food web proposed in this discussion.

This investigation represents the first long term study of the role of microzooplankton in carbon cycling in the Southern Ocean and has highlighted three aspects of microzooplankton ecology which require further investigation.

1. While it is generally well documented that metazoans contribute significantly to carbon flux (Fortier *et al.*, 1994), little is known about the fate of protozoan faecal pellets. Possible fates for faecal pellets include sedimentation (Nothig & von Bodungen, 1989), ingestion by coprophagy and disintegration in surface waters. Sediment trap studies conducted in high Antarctic waters have shown that dinoflagellate faecal pellets may form a significant proportion of sediment particles (Nothig & von Bodungen, 1989; Buck *et al.*, 1990; Gonzalez, 1992). For example, a study conducted at the ice edge found that 36% of all faecal material collected in the traps had originated from protozoans (Nothig & von Bodungen, 1989). Protozoan faecal pellets may thus be important because of the role they play in the redistribution, recycling and remineralization of organic carbon. Their contribution to total carbon flux, particularly during winter when microzooplankton represent the dominant grazers, requires urgent attention.

2. A preliminary investigation carried out during the winter 1993 cruise (Chapter 3) showed that mixotrophs contributed $\approx$ 5% of total chlorophyll concentrations at all grazing stations occupied. However, recent studies have shown that up to 80% of all ciliates, the dominant component of the microzooplankton assemblages during this investigation, contained functional plastids (Stoecker *et al.*, 1988). Since mixotrophs have a higher production to ingestion ratio than heterotrophic microzooplankton, they are more efficient in producing animal biomass (Stoecker, 1991). Consequently, mixotrophs can play an important role in nutrient recycling and energy dynamics of plankton assemblages on the

Southern Ocean. Also, carbon fixed by mixotrophic ciliates may be more directly utilised by larger zooplankton, particularly in areas where small picophytoplankton dominate.

3. During this investigation, the grazing impact of microzooplankton was shown largely to be restricted to the < 20μm chlorophyll fraction. While nanoheterotrophic flagellates generally feed on small phytoplankton cells, a most recent study has shown that some nanoheterotrophic flagellates are able to enter and feed on large microphytoplankton cells (S. Kuhn pers. comm.). This parasitoid behaviour of the nanoflagellates may occur in up to 90% of all phytoplankton cells (S. Kuhn pers. comm.). As these organisms do not preserve well, they are often overlooked unless living material is examined. Consequently, this behaviour has not been documented in the Southern Ocean as yet. While this parasitoidism may result in heavy losses of phytoplankton biomass, the ability of nanoflagellates to feed on other components of the microplankton assemblages such as dinoflagellates has not been investigated. Parasitoid nanoflagellates may therefore, form an important part of the heterotrophic protozoan community and may represent an important link between small protozoans and larger microphytoplankton cells in the marine pelagic food web of the Southern Ocean.

Future models of the Antarctic food web should focus on spatial and temporal components. In particular, summer/winter differences in the dominant components of the food webs should be given more attention as these may dramatically affect the estimates of carbon flux to depth. This may have important implications for our understanding of the efficiency of the biological pump in the Southern Ocean.

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