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THE RELATIVE ROLE OF PROTOZOANS IN
THE FLUX OF PHYTOPLANKTON
NITROGEN THROUGH PELAGIC
FOOD WEBS

SUSAN GAIL MATTHEWS

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THE RELATIVE ROLE OF PROTOZOANS IN THE FLUX OF
PHYTOPLANKTON NITROGEN THROUGH
PELAGIC FOOD WEBS

by

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ABSTRACT

Experiments were carried out during a cruise in the southern Benguela upwelling region in April 1989 to budget the nitrogen flux through the different microplanktonic compartments leading to copepods. Uptake of nitrate and ammonium by three different size classes of phytoplankton (net-, nano-, and picoplankton) was measured using ^{15}N isotope techniques. Microzooplankton grazing on autotrophic picoplankton and nanoplankton was quantified by predator:prey dilution experiments. Between 7 and 52 copepods in species assemblages representative of the natural communities were incubated in 1 l samples of ambient seawater to examine grazing rates on chlorotic and non-chlorotic microplankton. Copepod and microzooplankton excretion rates were also measured using ^{15}N isotope techniques. Two experiments were performed at night and two during the day.

Nitrogen uptake and regeneration studies revealed that phytoplankton of all size classes showed a consistent preference for ammonium, although nitrate was quantitatively more important for netplankton. Microzooplankton excretion fulfilled most of the phytoplankton ammonium demand, while copepod excretion was only detectable at night.

Competition between microzooplankton and mesozooplankton for phytoplankton prey was minimal, in that the former appeared to graze mainly $<2\ \mu\text{m}$ phytoplankton. Nevertheless, microzooplankton grazers had a significant impact on phytoplankton standing stocks. Microzooplankton grazing rates represented about 5% of phytoplankton biomass under diatom bloom conditions and an average of 46% under post-bloom conditions. On the other hand, copepods removed 18% of phytoplankton biomass under bloom conditions and only 1% under post-bloom conditions.

Copepods appeared to demonstrate a preference for protozoan prey over phytoplankton, in that the percentage of carbon ingested as protozoans exceeded the percentage of carbon available as protozoans. Quantitatively, protozoans made up a highly variable component of the copepod diet. For example, at station 12, where the plankton assemblage was dominated by oligotrichous ciliates, 80% of the ingested nitrogen ration consisted of protozoa. However, the total ingested ration at this station was only 0.6% of that at station 2, with a bloom assemblage, and it is unlikely that such a diet could support a large production potential.

On average only 3% of the nitrogen ingested by protozoa was subsequently transferred to copepods. Microbial pathways thus appear to have a minor rôle in the transfer of nitrogen to higher trophic levels, their function being mainly the regeneration of nitrogen for primary producers.

GENERAL INTRODUCTION

The "microbial loop" hypothesis of Azam et al. (1983) revived interest in the rôle of microzooplankton grazing in marine food webs. It had previously been noted that 10-50% of carbon fixed by primary producers is released as dissolved organic carbon (DOC) (Larsson & Hagström 1982). Bacteria, with their large surface to volume ratios, are capable of rapid assimilation of DOC and other nutrients. The fact that bacterial concentrations in the ocean are nevertheless remarkably constant has been attributed to protozoan grazing, since bacteria are too small to be effectively ingested by most macrozooplankton. A number of authors have shown that heterotrophic flagellates in the size range 3-10 μm are efficient bacterivores (Fenchel 1982, Sieburth & Davis 1982, Goldman & Caron 1985, Porter et al. 1985). Azam et al. (1983) proposed that flagellates were in turn preyed upon by larger microzooplankton, ensuring that the energy released as DOC is returned to the main food chain via this "microbial loop". At each of these trophic levels, much of the carbon originally fixed by bacteria will be lost to the system as respired CO_2 . Thus the amount of carbon available to metazoans may be only a fraction of the initial bacterial production.

Considerable debate has surrounded the rôle of ciliates as bacterivores. Ciliate bacterivory bypasses at least one step in the microbial loop, making the loop more efficient in returning energy to higher order consumers (Sherr & Sherr 1987). Fenchel (1980b) reported that the mechanical properties of cilia result in low clearance rates for ciliates which depend on bacteria-sized particles. This means that these ciliates would require bacterial concentrations of 10^7 - 10^8 bacteria ml^{-1} ; such concentrations are generally not found in oceanic waters, where concentrations of 10^5 - 10^6 bacteria ml^{-1} are more typical. It was therefore proposed that ciliates could only consume bacteria which were either attached to other particles or aggregated together to form larger particles.

However, Fenchel and many others who examined ciliate bacterivory used bacteria-sized latex microspheres to measure clearance rates (Fenchel 1980b, Børnsheim 1984, Jonsson 1986, McManus & Fuhrman 1986). Recently it has been found that ciliates may discriminate against these particles (Pace & Bailiff 1987) and Sherr & Sherr (1987) recorded ciliate bacterivory estimates 10-100x higher using their FLB (fluorescently labelled bacteria) technique than earlier estimates using microspheres. In addition, most of the earlier ciliate bacterivory studies have used tintinnids or large (30-50 μm) aloricate ciliates. More recently, however, small aloricate ciliates with equivalent spherical diameters $<20 \mu\text{m}$, previously overlooked as a result of inferior microscopy techniques, have been found to be a major component of the heterotrophic nanoplankton in diverse marine systems (Gast 1985, Sherr & Sherr 1987, Rassoulzadegan et al. 1988, Sherr et al. 1987, 1989b).

Sherr et al. (1989a) reported that small ciliates were responsible for the largest fraction (61%) of protozoan grazing in a tidal creek. In the more meso- to oligotrophic environment of the Mediterranean, Sherr et al. (1989b) found that small spirotrichs could grow on an exclusive diet of bacteria at a concentration of 10^6 bacteria ml^{-1} , while larger ciliates obtained $<10\%$ of their food ration as bacteria. In addition, Rassoulzadegan et al. (1988) found that ciliates could remove 1-38% of the bacterioplankton production in the N-W Mediterranean, with ciliates smaller than $30 \mu\text{m}$ taking 72% of their food ration as picoplankton-sized food. Thus it seems possible that ciliates can grow in natural concentrations of free bacteria in the ocean. Indeed, Albright et al. (1987) found that ciliates showed a marked preference for free bacteria over aggregated or attached bacteria, and that all ciliates were capable of ingesting free bacteria at concentrations of $6-12 \times 10^6$ bacteria ml^{-1} . Other authors

have also reported ciliate growth at bacterial concentrations of 10^5 - 10^6 ml⁻¹ (Gast 1985, Rivier et al. 1986).

Although there now seems little doubt that ciliates are capable of ingesting bacteria, the quantitative importance of ciliate bacterivory in the ocean is still debatable. However, Lessard & Swift (1985) conclude that although heterotrophic nanoflagellates are more abundant than either dinoflagellates or ciliates, the higher clearance rates on bacterial prey by the latter in their study means that the population clearance rates on all three groups are similar.

The debate concerning the relative importance of the microbial loop as a source of food for higher order consumers or as a sink for fixed carbon via respiratory losses has been termed the "link or sink" controversy (Ducklow et al. 1986, Sherr et al. 1987). Ducklow et al. (1986) supported the argument that bacterioplankton are a sink in planktonic food webs, since they found that only 2% of carbon-14 tracer initially fixed by bacterioplankton was subsequently detected in size classes larger than 1 μ m.

However, the preoccupation with the rôle of bacteria in the microbial loop has resulted in the neglect of other important issues. Photoautotrophs < 5 μ m in size, including cyanobacteria and algal nanoflagellates, are often the most abundant and active producers in diverse marine environments (Bienfang & Takahashi 1983, Joint & Pomroy 1983, Li et al. 1983, Platt et al. 1983). Furthermore, ciliates are generally regarded as being primarily herbivorous (Beers & Stewart 1970, 1971, Beers et al. 1975, 1980, Heinbokel 1978a, 1978b, Smetacek 1981, Stoecker et al. 1981). Cyanobacteria were largely overlooked by Azam et al. (1983) in their microbial loop hypothesis, yet since they are generally larger than heterotrophic bacteria, cyanobacteria are likely to be cleared more effectively by ciliates (Fenchel 1980b).

There have been a number of reports of ciliates ingesting cyanobacteria (Sherr et al. 1986a, Rassoulzadegan et al. 1988). Herbivorous mesozooplankton, such as copepods, are unable to ingest particles $<5 \mu\text{m}$ efficiently (Nival & Nival 1976). Thus ciliates may be important in the repackaging of these small particles into a form exploitable by mesozooplankton (Sherr & Sherr 1988). Laboratory and field feeding experiments have confirmed that copepods do indeed ingest ciliates (Berk et al. 1977, Stoecker & Egloff 1987, Gifford & Dagg 1988, Tiselius 1989).

The aim of the present study was to budget the flux of nitrogen through microzooplankton and mesozooplankton pathways. Uptake of nitrate and ammonium by three size classes of phytoplankton (net-, nano-, and picoplankton) was measured, as well as ammonium excretion by microzooplankton and copepods (Chapter 1). Microzooplankton grazing on phytoplankton (Chapter 2), and copepod grazing on phytoplankton and protozoans (Chapter 3), provided insight into the relative importance of microbial loop and classical type food chains under different environmental conditions (Chapter 4).

CHAPTER 1

NITROGEN UPTAKE AND REGENERATION

INTRODUCTION

Natural assemblages of phytoplankton have been reported to prefer regenerated nitrogen in the form of ammonium and urea (McCarthy et al. 1977), to the extent that ammonium concentrations $> 1 \mu\text{M}$ can suppress nitrate uptake (Eppley et al. 1969, MacIsaac & Dugdale 1969, Paasche & Kristiansen 1982). This preference for reduced nitrogen appears to hold even under conditions of high ambient nitrate concentrations typical of upwelling ecosystems.

The trend of ammonium preference appears to be size-based, with netplankton blooms often developing in response to a large input of nitrate into the euphotic zone through upwelling (Malone 1980), while the smaller size classes utilize mainly regenerated nitrogen as ammonium (Glibert et al. 1982b, Probyn 1985). Thus the relative availability of oxidised or reduced nitrogen may affect the size structure of the phytoplankton community, which in turn influences the structure of the secondary food chain.

If a large portion of the phytoplankton community is to rely on regenerated nitrogen to fulfill its nitrogen demand, one would expect that regeneration should equal or exceed nitrogen uptake. Early regeneration studies focussed on macrozooplankton (Harris 1959). However, it has since become clear that generally not more than 30% of phytoplankton nitrogen demand is supplied by macrozooplankton (see reviews by Harrison 1980, Williams 1981, Bidigare 1983). Instead, development of the ^{15}N tracer technique allowed quantification of the rôle of microzooplankton ($< 200 \mu\text{m}$) as remineralizers. A number of authors have shown that microzooplankton supply the

major share of uptake requirements (Harrison 1978, Caperon et al. 1979, Glibert 1982, Probyn & Lucas 1987).

The purpose of this chapter was to examine the contribution of new nitrogen as nitrate and regenerated nitrogen as ammonium to the net-, nano- and picoplankton community in the euphotic zone in the Southern Benguela, and to establish the relative rôles of macro- and microzooplankton in fulfilling this nitrogen demand.

METHODS

Sampling

Ammonium regeneration by microzooplankton and copepods and uptake of nitrate and ammonium by phytoplankton were measured at four stations in Table Bay in April 1989 aboard the research vessel "Benguela". Water was collected from a depth of 2 m using a Rosette sampler and prescreened through a 300 μm mesh to remove larger zooplankton. A representative assemblage of copepod species was collected with a drifting 300 μm plankton net for the copepod excretion experiments.

Analytical

Particulate matter for each size fraction was concentrated onto glass fibre filters (Whatman GF/F filters were used throughout) and frozen for later analyses. Chlorophyll *a*, corrected for phaeopigments, was measured fluorometrically in 90% acetone extracts (Holm-Hansen et al. 1965), and particulate organic carbon and nitrogen was measured on a Heraeus CHN analyser. Water samples for ambient nitrate determinations were stored frozen and analyzed some weeks later using standard autoanalyzer methods (Technicon II). Water samples for ammonium determinations

were also stored frozen and analyzed back in the laboratory according to the manual procedure of Koroleff (1983), but scaled down to 5 ml samples.

Nitrogen flux experiments

Copepod and microzooplankton excretion, as well as phytoplankton nitrogen uptake, were measured using ^{15}N isotope dilution techniques. A representative assemblage of copepods was incubated in one litre of $^{15}\text{NH}_4\text{Cl}$ spiked water. Microplankton remineralization and uptake were measured by adding either $0.2 \mu\text{mol Na}^{15}\text{NO}_3$ (99.6 at. %) or $0.1 \mu\text{mol }^{15}\text{NH}_4\text{Cl}$ (99.7 at. %) to 6 l of prescreened water. Enrichment of ambient levels with ^{15}N ranged between 4 and 8 atom % for nitrate, and 7 and 15% for ammonium. One liter of each 6 l sample was filtered under vacuum and a portion of the filtrate used for ammonium analyses. Exactly 900 ml of the filtrate was spiked with $10 \mu\text{mol NH}_4\text{Cl}$ to satisfy the mass requirements for atomic spectrometry and stored frozen. The remaining sample was immediately decanted into a 5 l bottle and incubated in deck boxes cooled by flowing surface seawater and exposed to 75% incident light levels. Daytime incubations (stations 6 & X) were of 4-5½ hours duration, while night-time incubations (stations 2 & 12) lasted 10-12 hours.

At the end of the incubation periods, the samples were fractionated into <2, <20 and <300 μm size classes. Exactly 2 l of each sample were gently filtered through a 2 μm Nuclepore membrane before being refiltered onto a precombusted glass fibre filter. Another 2 l of the sample were carefully poured through a 20 μm plankton screen before being filtered onto a GF/F filter, while the remaining litre was filtered directly onto a GF/F filter. The filters were frozen after rinsing with 50 ml 0.2 μm filtered seawater. A portion of the filtrate from the <300 μm fraction was used for ammonium analyses and 900 ml for determination of the aqueous ammonium enrichment was stored frozen as before.

Back in the laboratory, the samples were thawed and sufficient MgO was added to each to raise the pH above 9. A 25 mm glass fibre filter wetted with 0.05 ml 6N H₂SO₄ was then suspended above each sample and the bottles tightly capped. The bottles were left at room temperature for 3 weeks, during which time much of the aqueous ammonium was recovered on the filter. All filters were analyzed for ¹⁵N content by emission spectrometry after a Kjeldahl - Rittenberg oxidation procedure (Fiedler & Proksch 1975). Particulate organic nitrogen (PN) concentrations were also obtained during this procedure.

Nitrate uptake rates (u) were calculated using the following equation (Dugdale & Goering 1967):

$$u = \frac{PE \times PN}{R_o \times T}$$

where PE = percent ¹⁵N enrichment of the particulate fraction in excess of the natural abundance; PN = particulate nitrogen concentration (μmol l⁻¹); T = duration of the incubation in hours and R_o = the calculated aqueous ¹⁵N enrichment at the beginning of the incubation.

Ammonium uptake rates were calculated using a similar equation, in which correction is made for isotope dilution (Glibert et al. 1982c):

$$u = \frac{PE \times PN}{R \times T}$$

where R = exponential average ¹⁵N enrichment: $R = R_o/kt (1 - e^{-kt})$ and

$$k = \frac{-\ln R_t/R_o}{T}$$

R_o and R_t are the measured aqueous enrichments at the beginning and end of an incubation.

Ammonium regeneration rates were calculated from a modified form of the Blackburn (1979) and Caperon et al. (1979) model:

$$r = \frac{\ln(R_t/R_o) \cdot (S_o - S_t)}{\ln(S_t/S_o) \cdot T}$$

where S_o and S_t = aqueous ammonium concentrations at the start and finish of an experiment. In cases where ammonium concentrations remained unchanged over the time course of an experiment, regeneration rates were calculated from the equation of Laws (1984):

$$r = \frac{\ln(R_o/R_t)S_o}{T}$$

RESULTS

Ambient nutrient concentrations and chlorophyll and particulate nitrogen (PN) concentrations for the different size fractions are shown in Table 1. Station 2 phytoplankton comprised a mixed assemblage of large diatoms (*Amphora* sp.) and dinoflagellates (*Ceratium furca*, *Prorocentrum micans*), as well as smaller pennate (*Nitzschia* spp., *Thalassionema* spp.) and chain-forming diatoms (*Skeletonema costatum*, *Thalassiosira* spp.). A large phytoplankton community such as this may be part of a bloom which has already used up much of the ambient nitrate concentration. This would explain the low nitrate concentration at this station, compared to the higher concentrations at the other stations (stations 6, 12 & X). The phytoplankton community

Table 1. Concentrations of N nutrients, chlorophyll a and particulate nitrogen (PN) in net- (300-20 μm), nano- (20-2 μm) and picoplankton (<2 μm) communities.

Stations	N conc. ($\mu\text{mol N l}^{-1}$)		Chl a conc. ($\mu\text{g l}^{-1}$)			PN conc. ($\mu\text{mol N l}^{-1}$)		
	NH_4^+	NO_3^-	net	nano	pico	net	nano	pico
2	0.27	4.89	5.83	5.20	0.60	5.81	4.27	2.23
6	0.70	8.39	0.18	1.78	1.38	1.24	0.97	1.86
12	0.33	8.21	0.53	1.51	0.61	0.31	3.20	2.53
X	0.74	8.83	0.45	1.99	0.80	1.19	0.70	3.41

at these other stations was dominated by autotrophic flagellates of 3-8 μm length. The nanoplankton community at all stations comprised a relatively consistent 54% (range 45-61%) of the total chlorophyll concentration, with much of the interstation variability occurring in net- and picoplankton chlorophyll.

However, of the total particulate nitrogen concentrations, the picoplankton fraction was on average the largest at the four stations. This implies that there may have been large concentrations of heterotrophic bacteria and picoflagellates, as well as small detrital particles, in the upper waters. There was a good correlation ($p \leq 0.001$) between particulate nitrogen and chlorophyll *a* concentrations for the different size classes (Figure 1). The intercept of this plot reveals that $2 \mu\text{mol N l}^{-1}$ of the particulate nitrogen concentration is attributable to heterotrophs or detritus.

Nitrogen uptake

Total nitrogen uptake for the three size fractions at each station is illustrated in Figure 2a. Apart from station 2, where the picoplankton community contributed only 5% of the total chlorophyll concentration, a large percentage (44-56%) of the total nitrogen uptake was attributable to picoplankton activity. It is also evident that there was a fall-off in activity at night (stations 2 & 12). Uptake rates normalized to chlorophyll (Figure 2b) emphasize this trend and confirm that the fall-off is not a result of low phytoplankton biomass at the night-time stations. Netplankton experienced the most marked reduction in activity, while picoplankton was responsible for an average of 63% of total nitrogen uptake at the two night-time stations. This indicates active protein synthesis by picoplankton, and to a lesser degree, by nanoplankton at night, as reported by Cuhel et al. (1984).

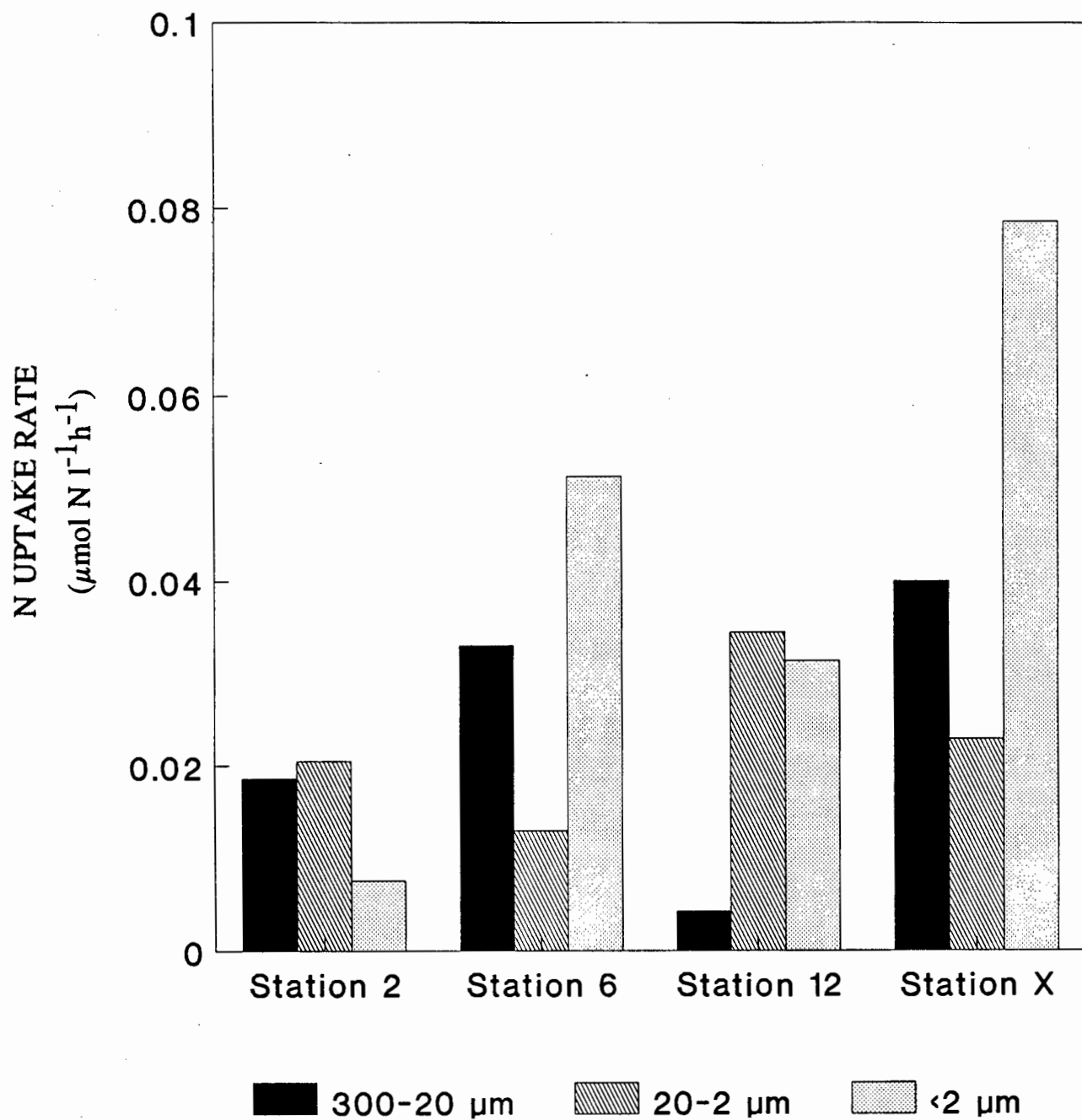


Fig. 2a. Nitrogen uptake rates by the net- (300-20 μm), nano- (20-2 μm) and picoplankton (<2 μm) communities

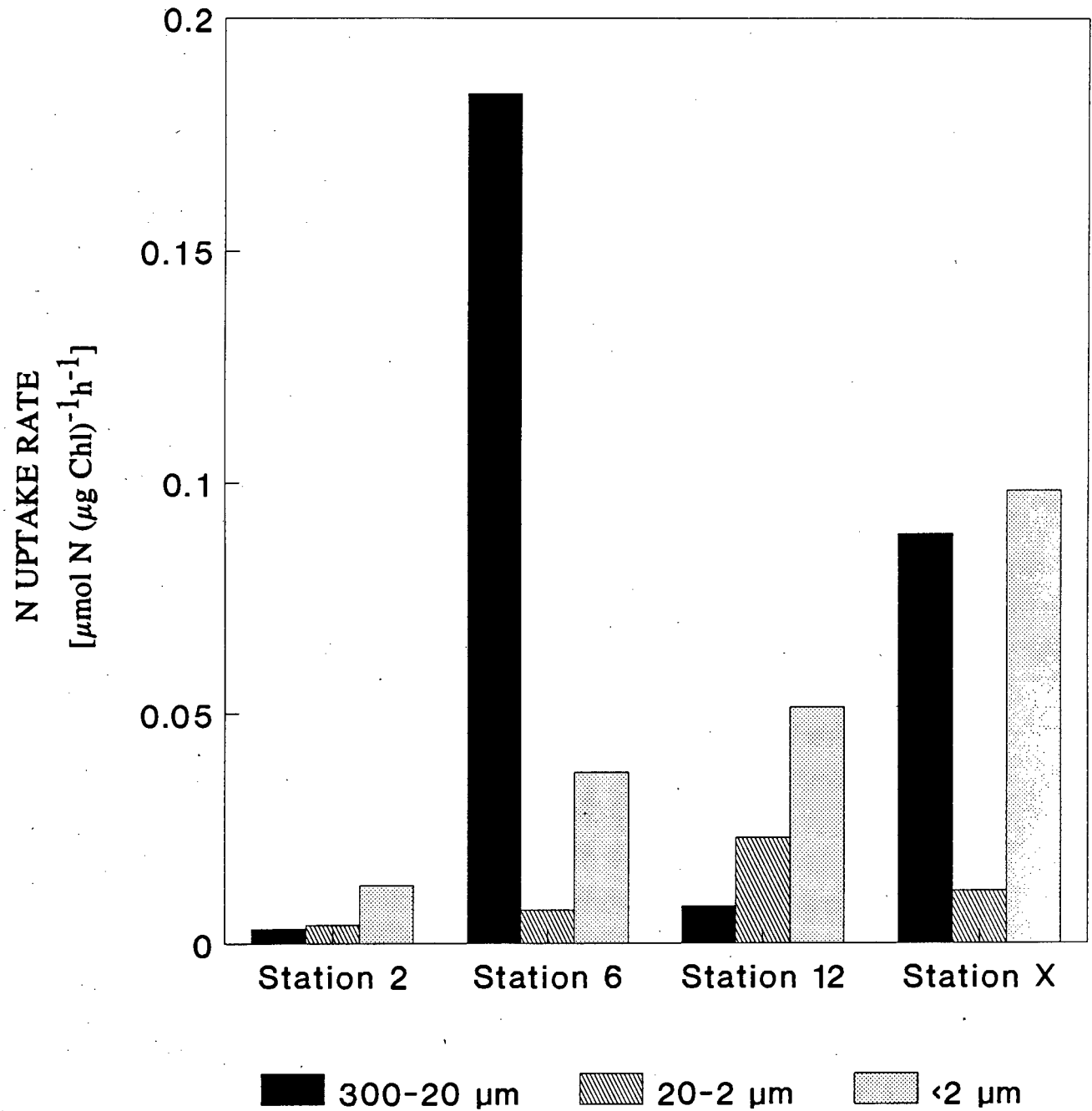


Fig. 2b. Nitrogen uptake rates normalized to chlorophyll concentration, by the net- (300-20 μm), nano- (20-2 μm) and picoplankton (<2 μm) communities

Nitrate and ammonium uptake by the different size fractions is shown in Figure 3. Once these rates are normalized to chlorophyll (Figure 4), it becomes clear that ammonium is relatively more important per unit biomass for the smaller size fractions. This trend is also reflected in the relative preference indices (McCarthy et al. 1977), shown in Figure 5. The picoplankton and nanoplankton communities were considered together, as a relationship between size class and RPI was not consistent for these two size classes. At all stations nitrate was discriminated against by all size fractions, as indicated by the RPIs of less than unity. The $<20 \mu\text{m}$ community showed a stronger preference for ammonium than did the netplankton community.

However, relative preference indices can be misleading in that although they may reveal a preference for ammonium, in fact nitrate, with the higher concentrations characteristic of upwelling areas, may be the major nitrogen source utilized. The f-ratio ($f = \text{nitrate uptake} / \text{total nitrogen uptake}$; Eppley & Peterson 1979) for the netplankton community at all stations ranged from 48 to 100% ($\bar{x} = 69\%$), while that of the $<20 \mu\text{m}$ community ranged from 25 to 49% ($\bar{x} = 37\%$). This indicates that nitrate is quantitatively the more important nutrient for the netplankton community, despite the preference shown for ammonium.

Ammonium regeneration

Ammonium excretion by microzooplankton and copepods, as well as the percentage of ammonium uptake by the phytoplankton community supported by ammonium excretion, is shown in Table 2. Microzooplankton excretion was extremely variable for the four stations, but always made up the majority of the total ammonium excretion.

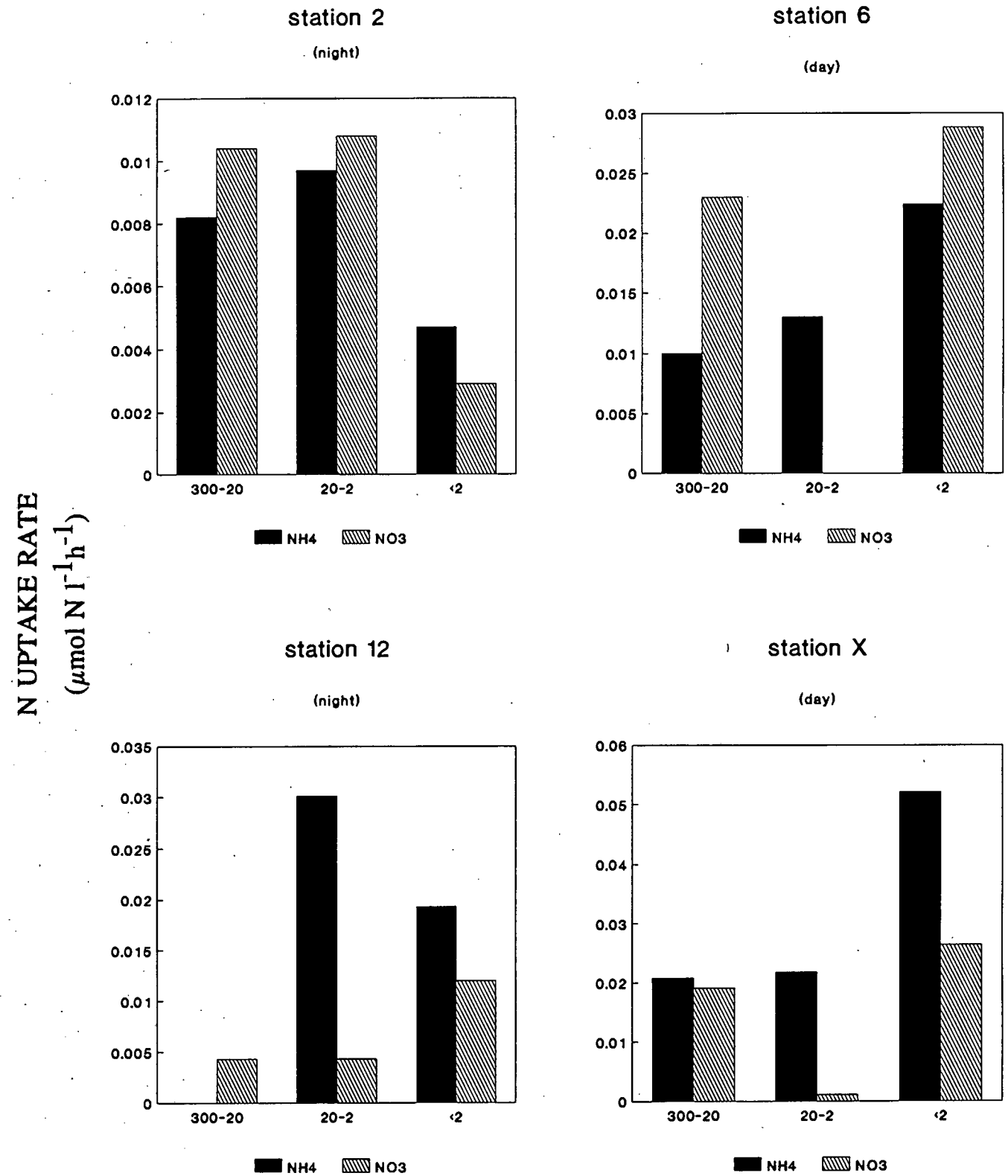


Fig. 3. Ammonium (NH_4^+) and nitrate (NO_3^-) uptake rates at the four stations by the net- (300-20 μm), nano- (20-2 μm) and picoplankton (<2 μm) communities

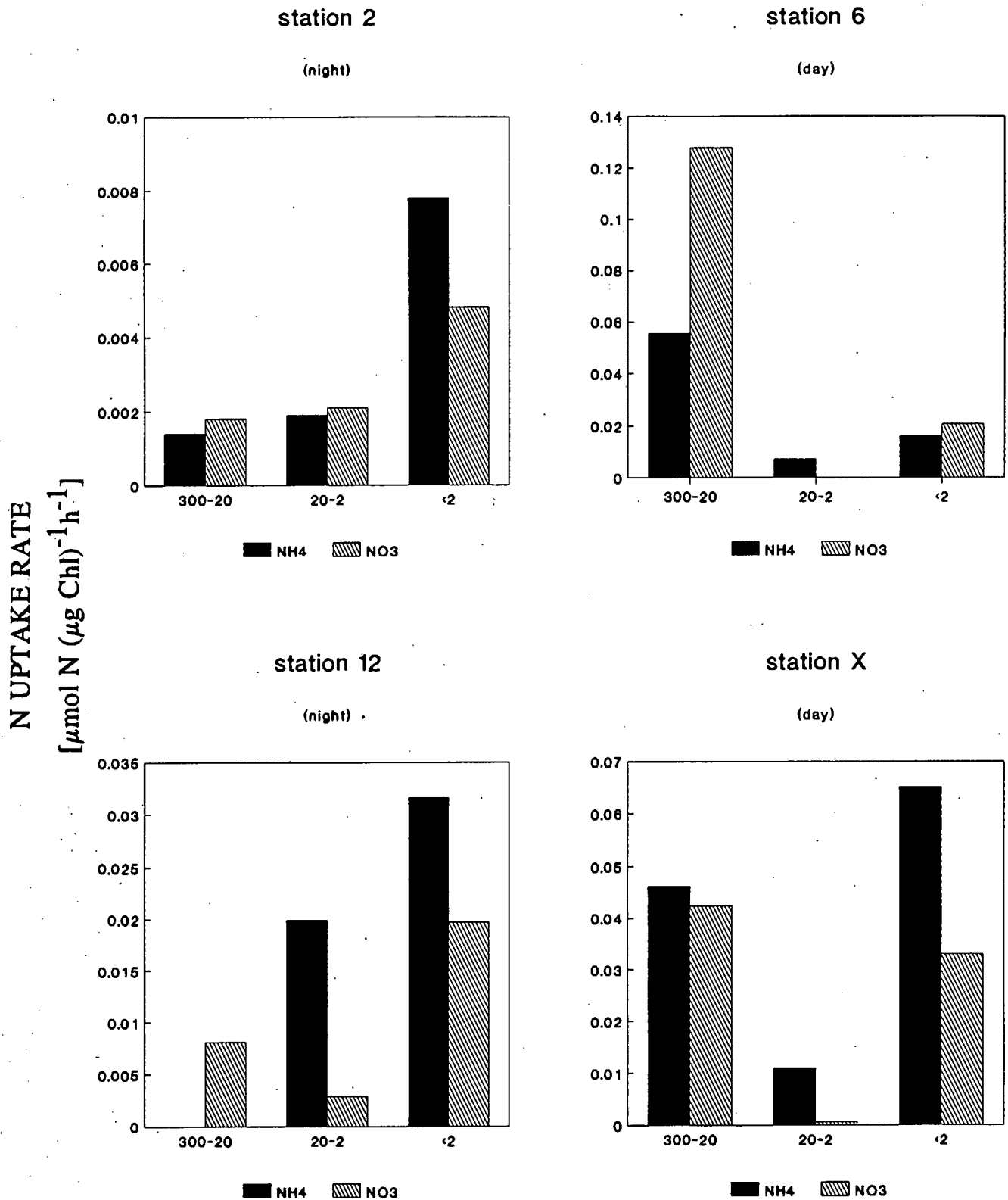


Fig. 4. Ammonium (NH_4^+) and nitrate (NO_3^-) uptake rates normalized to chlorophyll concentration, by the net- (300-20 μm), nano- (20-2 μm) and picoplankton (<2 μm) communities

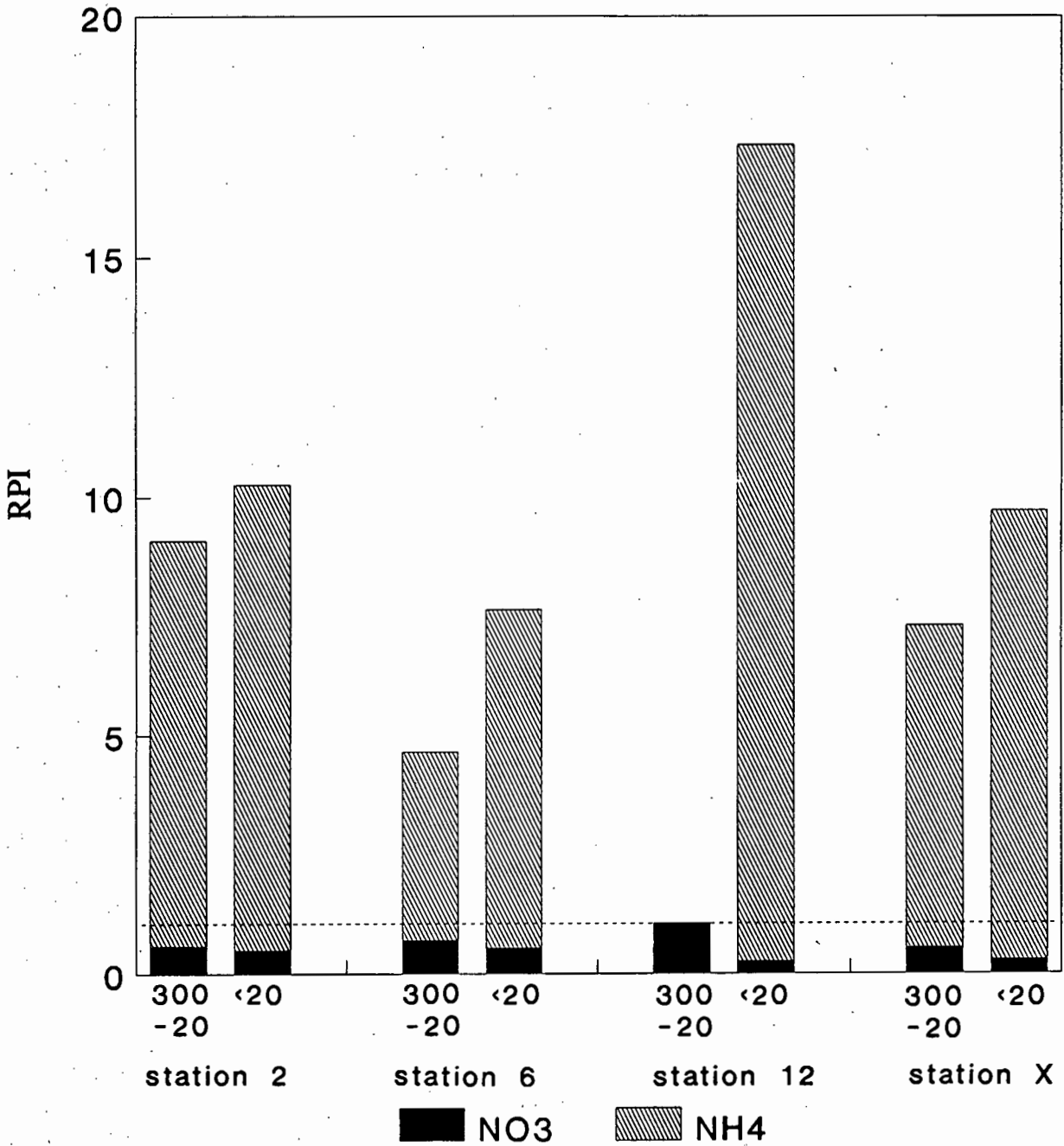


Fig. 5. Relative preference indices (RPIs) for ammonium (NH_4^+) and nitrate (NO_3^-) for the 300-20 μm and <20 μm size classes.

Table 2. Rates of NH_4^+ uptake ($\mu\text{mol N l}^{-1} \text{h}^{-1}$) by phytoplankton ($<300 \mu\text{m}$) and NH_4^+ excretion ($\mu\text{mol N l}^{-1} \text{h}^{-1}$) by microzooplankton and copepods.

	Stations			
	2	6	12	X
Microzooplankton excretion	0.0054	0.0590	0.0220	0.2510
Copepod excretion	0.0017	-	0.0030	-
Total NH_4^+ excretion	0.0071	0.0590	0.0590	0.2510
Total NH_4^+ uptake	0.0226	0.0454	0.0454	0.0946
Excretion/uptake (%)	31	130	55	264

Copepod excretion was undetectable at the day-time stations, 6 & X. This is probably a reflection of the diel vertical migration of the larger copepod species; our copepod counts revealed that the day-time stations were dominated by small species such as *Paracalanus parvus*, *Ctenocalanus vanus* and *Clausocalanus arcuicornis*, while the night-time stations, 2 & 12, also had large communities of *Centropages brachiatus*, *Calanoides carinatus* and *Calanus australis* (see Chapter 3, Table 1). The highest copepod excretion measured at station 2 can be attributed to the larger food supply at this station (see Table 1).

At the night-time stations, ammonium excretion by the microzooplankton and copepod communities could support only 31 to 55% of the phytoplankton ammonium uptake, while supply exceeded demand at the day-time stations (130 & 264%).

DISCUSSION

Nitrogen uptake

The proportion of chlorophyll *a* recovered in the surface water picoplankton fraction (5-41%) is lower than the 25-90% depth integrated estimate of Li et al. (1983) for the eastern tropical Pacific Ocean, but in good agreement with Probyn's (1985) estimate of 2-50% for the Southern Benguela upwelling region. The higher picoplankton chlorophyll *a* estimates in the tropics indicates the greater importance of picoplankton as primary producers in tropical rather than temperate waters.

However, despite the small size of the picoplankton community, it accounted for 16-56% ($\bar{x}=42\%$) of the total nitrogen uptake of the intact communities and was

responsible for the majority of nitrogen uptake during the day. The importance of the smallest size class was also recognized by Nalewajko & Garside (1983), who found that the 0.2-3 μm size class was more active in photosynthesis, as well as nitrogen and phosphate uptake, than larger size classes. Harrison & Wood (1988) reported that picoplankton nitrogen uptake in coastal and oceanic waters averaged >30% of the combined nitrate and ammonium uptake by the intact communities. Once the uptake rates in the present study were normalized to chlorophyll, it became evident that the picoplankton community was especially efficient at night, when assimilation of nitrogen by the larger size classes was substantially reduced.

There was good evidence for nitrogen resource partitioning by the net- and nanoplankton (<20 μm) communities. The RPIs (McCarthy et al. 1977) indicated that all size classes showed a preference of ammonium over nitrate, with the nano- and picoplankton (<20 μm) showing a stronger preference than the netplankton. This trend has been observed in a number of studies (Glibert et al. 1982a, McCarthy et al. 1982, Paasche & Kristiansen 1982, Carpenter & Dunham 1985, Probyn 1985, Probyn & Painting 1985, Owens et al. 1986). However, some authors have reported that nitrate may be preferred under conditions of low (<1 μM) ammonium concentrations (Eppley et al. 1969, MacIsaac & Dugdale 1969, Furnas 1983b). In this study the ambient ammonium concentration was <1 μM at all four stations, but nitrate was never preferred. Similarly, Probyn (1985) found that nitrate was not taken up preferentially even at ammonium concentrations approaching 0.1 μM .

Despite the preference shown for ammonium, nitrate was quantitatively more important for the netplankton community. This supports Malone's (1980) hypothesis that netplankton productivity is regulated primarily by nitrate, whereas nanoplankton productivity is controlled by regenerated nitrogen. This hypothesis was not based on

any direct physiological evidence, but rather on the observation that netplankton blooms often develop in response to a large input of nitrate into the euphotic zone through upwelling. However, size fractionated nitrogen uptake studies by Glibert et al. (1982b) and Probyn (1985) have yielded direct evidence to support the hypothesis. The mean ratio of "new"/total production of 69% for the netplankton community in this study is in close agreement with the depth-integrated value of 67% for the Middle Atlantic Bight (Harrison et al. 1983) and the shelf value of 71% in Probyn's (1985) study.

Picoplankton have been reported to utilize reduced nitrogen more efficiently (per unit phytoplankton biomass) than larger phytoplankton species (Bienfang & Takahashi 1983, Le Bouteiller 1986, Probyn & Lucas 1987). In this study, such a trend was only evident at the night-time stations, when netplankton were relatively inactive. The light-dependence of ammonium and nitrate uptake is well established (MacIsaac & Dugdale 1972, Packard 1973). Kuenzler et al. (1979) reasoned that ammonium uptake, which is relatively light-independent, would proceed at near maximal rates at night, while nitrate uptake would slow down and photosynthesis cease. Nalewajko & Garside (1983) extended the hypothesis to include cell-size-dependent differences in the way algae in the same community perceive their light and nutrient environment. However, if picoplankton exhibit a strong preference for ammonium uptake, which is relatively light-independent, it follows that this community would be more efficient at night than larger size classes.

It should be noted that the f-ratios in the present study may be overestimates, since a large portion of the nitrogen utilized may be in the form of urea. Urea is usually taken up with intermediate preference, ie. ammonium > urea > nitrate. (McCarthy et al. 1977, Probyn & Painting 1985), but some authors have found urea to be the most

important nutrient assimilated (Kaufman et al. 1983, Harrison et al. 1985). However, it is unlikely that the inclusion of urea would markedly affect the size related trend of dependence on regenerated nitrogen in the present study.

Ammonium regeneration

This study supports earlier conclusions that macrozooplankton play a relatively minor rôle as ammonium remineralizers. In the two stations where ammonium excretion by copepods was detectable (at the night-time stations 2 & 12), only 7% of the total ammonium uptake, or 4% of the total nitrogen uptake by the intact communities, could be attributed to copepods. Similarly, Smith (1978) found that ammonium excretion by copepods in a shallow well-mixed estuary supplied only 8% of phytoplankton ammonium demand, while Vargo (1979) reported that ammonium excretion by mixed zooplankton populations in Narragansett Bay accounted for only 4.4% of the nitrogen required for gross annual production. However, Båmstedt (1985) measured ammonium excretion by 19 species of macrozooplankton and estimated that they could fulfill as much as 50% of the nitrogen demand. Smith & Whitley (1977) found that 25% of total nitrogen uptake could be supplied by zooplankton ($>102 \mu\text{m}$) ammonium excretion in the N-W African upwelling region.

Many of these studies do not include measurements of urea excretion. Jawed (1969) and Corner et al. (1976) found that generally $<10\%$ of nitrogen excreted by macrozooplankton is in the form of urea. However, Eppley et al. (1973) and Båmstedt (1985) have reported urea excretion of 50 and 40% of total nitrogen excreted, respectively. Therefore, the present study, and others which exclude urea excretion, may underestimate the proportion of regenerated nitrogen supplied by macrozooplankton. Nevertheless, the total contribution by macrozooplankton to phytoplankton nitrogen demand is unlikely to change significantly. For example,

Harrison et al. (1985) found that the four dominant macrozooplankton species in the polar waters of Baffin Bay supplied only ~3% of the urea-N but ~40% of the ammonium-N requirements of the primary producers.

Microzooplankton ammonium excretion rates recorded in this study were within the range found by other authors in a variety of marine environments (see Table 3, Selmer 1988). Microzooplankton supplied 24-264% of the total ammonium demand and 12-178% of the combined nitrate and ammonium uptake by the phytoplankton community. There is conflicting experimental evidence regarding the time scales over which ammonium fluxes through the microplankton community are coupled. Short-term measurements of ammonium regeneration during the day have generally shown excretion to equal or exceed ammonium uptake (Harrison 1978, Owens et al. 1986, Probyn 1987), in agreement with the present study. Other studies have shown that ammonium assimilation during daylight hours exceeded microzooplankton regeneration (Paasche & Kristiansen 1982, Harrison et al. 1983, La Roche 1983). However, Caperon et al. (1979) and Glibert (1982) have demonstrated that microplankton regeneration and uptake were only in balance when integrated over 24 hours, since remineralization rates at night often exceed uptake rates, while the opposite was true during daylight hours.

It is difficult to explain why deviations from this general pattern occur, such as in the present study. Perhaps detailed analyses of species assemblages and environmental conditions in parallel with nitrogen flux measurements will provide some ground truths regarding diurnal variation in the assimilation/regeneration rates. For example, Longhurst & Harrison (1988) reported that dissolved nitrogen may be lost from the euphotic zone through the diel vertical migration of interzonal zooplankton and nekton

that feed in the euphotic zone at night and excrete nitrogenous compounds at depth by day.

Although no size fractionated regeneration experiments were performed in this study, other authors have reported that the smallest size fractions are generally the most active remineralizers. Glibert (1982) and Probyn (1987) found that the $<10 \mu\text{m}$ and $<15 \mu\text{m}$ size classes contributed the largest fraction of regenerated nitrogen, respectively. This would include the phagotrophic flagellates, which usually dominate the heterotrophic nanoplankton (Sieburth et al. 1978, Sorokin 1979, Sherr et al. 1984). Alternatively, Paasche & Kristiansen (1982) reported that most of the ammonium in the Oslofjord, Norway, in summer, appeared to be produced by juvenile copepods, rotifers, tintinnids and heterotrophic dinoflagellates in the 45-200 μm size fraction.

The rôle of bacteria in nitrogen remineralization is a contentious issue. Bacteria were traditionally seen as the most active remineralizers. However, Azam et al. (1983) proposed that their importance in the food chain is to repackage dissolved organic matter (DOM) into particles suitable for ingestion by flagellates, which are in turn eaten by larger microzooplankton. It is this "microbial loop" which is responsible for nutrient recycling, rather than bacteria themselves.

However, under certain conditions bacteria may be important in nitrogen remineralization. For example, Harrison (1978) reported that 40% of ammonium remineralization in Californian coastal waters could be attributed to particles $<1 \mu\text{m}$, although only 10% of the total particulate matter was in this size class. In a later study on the Atlantic continental shelf, Harrison et al. (1983) found that on average 74% of the microplankton ammonium regeneration was by particles $<1 \mu\text{m}$. Furthermore, Glibert (1982) noted that during the decline of a phytoplankton bloom, much of the

ammonium remineralization was due to the $< 1 \mu\text{m}$ size class, while Probyn (1987) found that the importance of bacteria as remineralizers increased with depth in the euphotic zone.

In conclusion, this study supports Malone's (1980) hypothesis that netplankton productivity is supported by nitrate, while nanoplankton productivity depends on the supply of regenerated nitrogen. Microzooplankton remineralization accounts for the majority of regenerated nitrogen. Since upwelling in the southern Benguela is driven by south-easter winds which only occur in summer, the relative rôles of new and regenerated nitrogen are likely to differ seasonally. In the winter months, phytoplankton production may be supported largely by regenerated nitrogen, implying that the size structure of the phytoplankton community may comprise mainly cells in the nano- and picoplankton size range. This is likely to have a significant effect on the efficiency of the pelagic food chain leading to commercially important fish, such as anchovy.

CHAPTER 2

MICROZOOPLANKTON GRAZING

been favourite study animals. The results from such experiments are then extrapolated to known field concentrations of potential predator and prey organisms. Using this approach Capriulo & Carpenter (1983) calculated that approximately 27% of the annual primary production in Long Island Sound was grazed by tintinnids.

In the natural environment tintinnids are usually outnumbered by the more delicate aloricate ciliates, primarily oligotrichs (Beers & Stewart 1970, Beers et al. 1975, 1980, Smetacek 1981). These animals have been somewhat neglected in laboratory studies, although there is growing evidence of their importance in diverse marine ecosystems. Indeed, Rassoulzadegan & Etienne (1981) found that in their Mediterranean study area tintinnids, which made up only 12.4% of the total oligotrichous ciliate biomass, consumed only about 5% of daily primary production, while the large population of aloricate ciliates was estimated to graze an additional 54% of daily production.

Information on ingestion and feeding rates of natural microzooplankton populations feeding on naturally occurring phytoplankton assemblages is limited by the difficulties of conducting field studies on small animals with similar sized prey. Capriulo & Carpenter (1980) attempted to separate predators from prey in their size fractionation technique, in which anything that passed a 35 μm mesh was considered to be prey, while predators were assumed to be of 35-202 μm in size. However, many ciliates are smaller than 35 μm and these do, in fact, frequently dominate the biomass of the microplankton fraction (Beers et al. 1980). Verity (1986) avoided this problem by using <10 μm and <202 μm fractions as prey and predator communities, respectively. However, in addition to altering the plankton assemblage, so that growth of prey organisms may not be equivalent in the different fractions, the fractionation technique is particularly destructive to the delicate aloricate ciliates (Gifford 1985).

In this chapter, the seawater dilution technique of Landry & Hasset (1982) was employed to measure grazing in natural assemblages of microzooplankton, primarily ciliates, on different size classes of phytoplankton. The advantage of this technique is that it involves minimal manipulation of the community, thus avoiding damage to the aloricate ciliates. The method has also been used successfully in tropical coastal waters by Landry et al. (1984), temperate coastal and oceanic environments by Campbell & Carpenter (1986), Burkhill et al. (1987), Gifford (1988) and Gallegos (1989), and in the eastern Canadian Arctic by Paranjape (1987). The method is discussed in detail below.

METHODS

The Dilution Method

The method is based upon three assumptions: firstly, that phytoplankton growth rates are not density-dependent; secondly, that consumers are not food-satiated at natural prey densities, with ingestion being a linear function of consumer density; and thirdly, that phytoplankton growth can be described by the equation:

$$1/t (P_t/P_0) = k-g$$

where P_0 and P_t are phytoplankton densities at the beginning and end of the experiment; k and g are instantaneous coefficients of population growth and grazing mortality respectively, and t is time.

The first assumption implies that k will be constant if concentrations of nutrients and other growth factors remain constant and non-limiting. The second assumption implies

that g varies directly with the density of consumers, but is not affected by changes in phytoplankton concentration ie. that no feeding thresholds occur at dilute food levels and that feeding is unsaturated at high food levels.

Rates of phytoplankton growth (k) and grazing mortality (g) are calculated from changes in phytoplankton density following incubations of different dilutions of seawater containing the natural microzooplankton assemblage. The observed rate of change of prey density is linearly related to the dilution factor (fraction of unfiltered seawater), since the grazers are diluted with their food. The negative slope of this relationship is the grazing coefficient g , while the y-axis intercept is the phytoplankton growth rate k .

Experimental Design

Experimental seawater was collected from a depth of 2 m with a Rosette sampler and prescreened through a 300 μm mesh to remove larger zooplankton. Half of this water was filtered through Whatman GF/F filters to provide the "particle free" water with which to make up the dilutions. Duplicates of each of the dilutions 100%, 60%, 40%, 20% and 0% were prepared in 300 ml diffusion chambers, which were equipped with 0.1 μm Nuclepore filters, allowing for gaseous and nutrient exchange. The chambers were kept in deck boxes cooled with flowing surface seawater and exposed to 75% incident light levels. Day-time incubations lasted 5½-7 hours, while night-time incubations were of 10½-13½ hours duration.

Once the incubation chambers had been filled with the dilution mixtures, what remained of each mixture was size fractionated into <300, <20 and <2 μm size classes. Duplicate 50 ml subsamples from each fraction were filtered onto 25 mm Whatman GF/F filters and frozen for later chlorophyll analyses. This was repeated at

the end of the incubations for each dilution. In addition, 250 ml of the undiluted experimental water was preserved with 10% Lugol's iodine for enumeration of microzooplankton grazers (mainly ciliates) and their identification by scanning electron microscopy.

Back in the laboratory, Lugol's-preserved microzooplankton were destained with thiosulphate before being stained with Rose Bengal and filtered onto 3 μm Millipore filters. These were then mounted with hydroxypropyl methacrylate to make permanent slides (Crumpton 1987) and the microzooplankton enumerated under a Nikon Alphaphot YS light microscope. Volumes of individual cells were calculated from linear dimensions and a volume:carbon ratio of 0.19 $\text{pg C } \mu\text{m}^{-3}$ was used (Putt & Stoecker 1989). Only protozoans larger than 9 x 12 μm were counted. Nanoflagellates were ignored since many of the smaller flagellates are autotrophic while heterotrophic forms are largely bacterivorous (Fenchel 1982, Sieburth & Davis 1982, Goldman & Caron 1985, Porter et al. 1985). The present study was concerned primarily with the grazing of phototrophs.

Chlorophyll *a* in the different size classes, corrected for phaeopigments, was measured fluorometrically in 90% acetone extracts (Holm-Hansen et al. 1965). Phytoplankton carbon was then estimated from a carbon:chlorophyll ratio of 30 for picoplankton (Joint & Pomroy 1986) and 50 for nano- and netplankton (Ryther et al. 1971, Pitcher 1988).

Clearance and ingestion rates of microzooplankton on the identified prey size categories were then calculated using Frost's (1972) equations, formulated to describe copepod feeding, but with the modification of using initial cell concentration instead of mean concentration, as suggested by Marin et al. (1986).

Samples were prepared for electron microscopy by filtering onto Millipore filters, which were then serially dehydrated to pure alcohol, critical-point dried from carbon dioxide and sputter-coated with gold palladium.

RESULTS

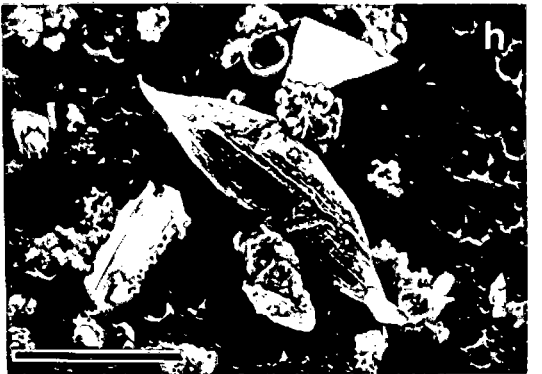
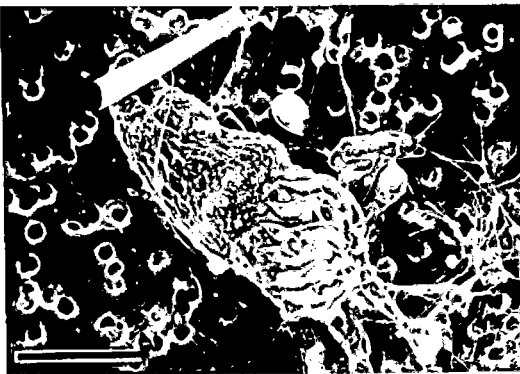
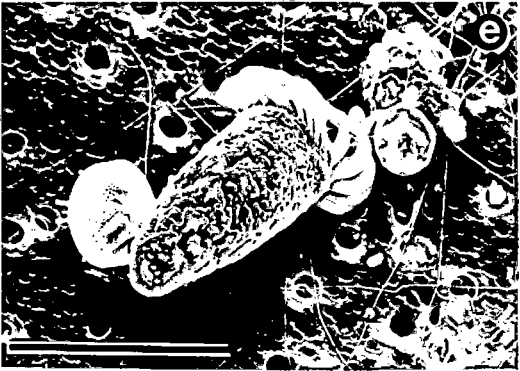
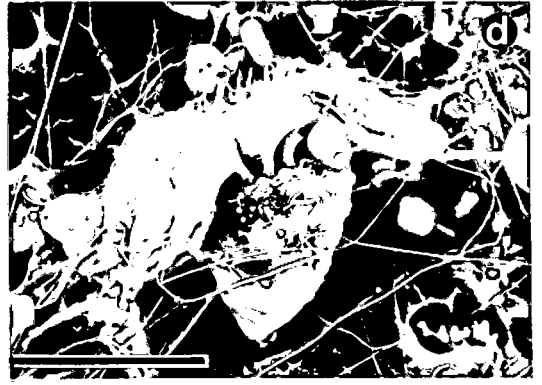
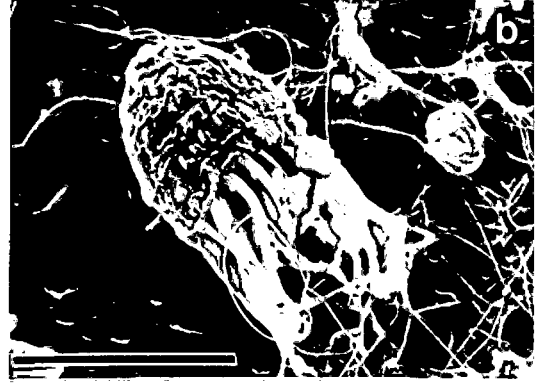
Microplankton composition

Concentrations of microzooplankton ($< 300 \mu\text{m}$) ranged from 7 800 individuals l^{-1} at station 6 to 21 000 individuals l^{-1} at Station 12 (Table 1). The corresponding concentrations expressed in terms of carbon are a reflection of the different species composition at each station. At stations 6, 12 and X the microzooplankton assemblage was dominated by aloricate ciliates, primarily oligotrichs (species of *Strombidium* and *Strombilidium*), although a few tintinnids were also observed. At station 2 the dominant grazer was the heterotrophic dinoflagellate *Gyrodinium* spp. Tintinnids were more important here than at other stations, and small oligotrichs ($12 \times 12 \mu\text{m}$) were also present. The autotrophic ciliates *Myrionecta rubra* and *Laboea strobila* were evident in low concentrations at all stations. Examples of the protozoans seen in the samples are presented in Plate 1 (a-h).

Phytoplankton standing stocks are also represented in Table 1. Station 2 had the largest phytoplankton concentration, with a mixed assemblage of diatoms and dinoflagellates. Stations 6, 12 and X comprised mainly autotrophic flagellates of 3-8 μm length, although the diatom *Amphora* sp. and various dinoflagellates were also present.

Table 1. Biomass of microzooplankton grazers and pico- (<2 μm), nano- (20-2 μm) and netplankton (300-20 μm)

Stations	Microzooplankton		Phytoplankton ($\mu\text{g C l}^{-1}$)			
	N l ⁻¹	$\mu\text{g C l}^{-1}$	pico	nano	net	Total < 300
2	14 726	38.13	18	260	291	569
6	7 797	5.53	41	89	11	141
12	21 041	10.60	19	75	26	120
X	11 884	3.45	24	100	23	147



Microzooplankton grazing

Growth (k) and grazing (g) coefficients for the different prey categories, as well as the correlation coefficient (r) of the relationship between apparent growth and dilution factor, are presented in Table 2. Picoplankton were heavily grazed at all stations, with only station 6 microzooplankton exhibiting any grazing of the nanoplankton fraction.

Algal growth coefficients in the $< 2 \mu\text{m}$ fraction ranged from 0.09 h^{-1} at station 12 to 0.15 h^{-1} at station 2, while grazing coefficients ranged from 0.08 h^{-1} to 0.13 h^{-1} at the same stations. The algal growth coefficient for the nanoplankton community at station 6 was the lowest recorded in the study, at 0.05 h^{-1} .

The average filtration rates for the mixed species assemblages of microzooplankton ranged from 3.62 to 12.68 ($\bar{x}=8.71$) $\mu\text{l ind.}^{-1}\text{h}^{-1}$ (Table 3). These figures agree well with those of Capriulo & Carpenter (1980) and Burkhill et al. (1987) who found that natural communities of ciliates (mostly oligotrichs and tintinnids) exhibited average filtration rates of 1.3-84.7 ($\bar{x}=26.7$) $\mu\text{l ind.}^{-1}\text{h}^{-1}$ and 1-11 $\mu\text{l ind.}^{-1}\text{h}^{-1}$, respectively. Laboratory feeding studies by Capriulo (1982) yielded filtration rates by tintinnids of 2-65 $\mu\text{l ind.}^{-1}\text{h}^{-1}$, while Rassoulzadegan (1982) showed that the ciliate *Lohmaniella spiralis* filtered between 2 and 9 $\mu\text{l ind.}^{-1}\text{h}^{-1}$ when feeding on natural particulates.

However, since the microzooplankton assemblage is made up of species of varying size, a more accurate representation of filtration rate is expressed in terms of consumer body carbon (Table 3). This reveals different trends in filtration rates between stations. The rates are likely to be overestimated slightly, since only protozoa larger than $9 \times 12 \mu\text{m}$ were counted as grazers.

Table 2. Linear regression of apparent phytoplankton growth versus dilution factor. k = algal growth coefficient; g = grazing coefficient; r = correlation coefficient

Phytoplankton	Coefficient	Station 2	Station 6	Station 12	Station X
<2 μm	k h ⁻¹	0.15 ± 0.01	0.13 ± 0.02	0.09 ± 0.01	0.09 ± 0.04
	g h ⁻¹	0.13 ± 0.02	0.08 ± 0.03	0.08 ± 0.02	0.10 ± 0.07
	r	0.99 ± 0.01	0.87 ± 0.02	0.95 ± 0.01	0.73 ± 0.04
2-20 μm	k h ⁻¹	-	0.05 ± 0.05	-	-
	g h ⁻¹	-	0.10 ± 0.07	-	-
	r	-	0.68 ± 0.04	-	-

Table 3. Microzooplankton filtration and ingestion rates on phytoplankton. F_1 = filtration rate per individual microzooplankton; F_2 = biomass specific filtration rate; I_1 = biomass specific ingestion rate; I_2 = microzooplankton community filtration rate

Phytoplankton	Station	F_1 $\mu\text{l ind.}^{-1} \text{h}^{-1}$	F_2 $\text{ml } (\mu\text{g C}_{\text{ind}})^{-1} \text{h}^{-1}$	I_1 $\text{ng C } (\mu\text{g C}_{\text{ind}})^{-1} \text{h}^{-1}$	I_2 $\mu\text{g C l}^{-1} \text{h}^{-1}$
< 2 μm	2	8.87	3.43	61.65	2.35
	6	10.02	14.12	584.73	3.23
	12	3.62	7.18	137.90	1.46
	X	8.36	28.75	690.05	2.38
2 - 20 μm	2	-	-	-	-
	6	12.68	17.89	1591.77	8.80
	12	-	-	-	-
	X	-	-	-	-

Table 4. Day-time and night-time grazing impact of the microzooplankton assemblage, assuming a 12:12 light:dark cycle.

Potential production, $P_p = (P_0 e^k) - P_0$, where P_0 is initial standing stock

Stations	Initial <2 μm standing stock grazed 12 h ⁻¹ (%)	Potential production of <2 μm chlorophyll ($\mu\text{g C l}^{-1}$ 12 h ⁻¹)	Potential <2 μm production grazed 12 h ⁻¹ (%)	Initial <300 μm standing stock grazed 12 h ⁻¹ (%)
2	156.72	322.77	8.74	4.92
6	93.72	497.80	7.79	102.48
12	91.32	76.90	22.80	14.64
X	119.16	99.56	28.73	19.56

Microzooplankton ingestion rates were maximal at station 6 where both the pico- and nanoplankton were exploited (Table 3). The population grazing rate (I_2) on picoplankton was remarkably constant between stations at $1.5-3.2 \mu\text{g C l}^{-1}$ (Table 3). At these rates microzooplankton removed 91-157% of the initial $<2 \mu\text{m}$ standing stock over a 12 h period (Table 4). However, because of the high growth rates of this community, microzooplankton grazers were capable of removing only 8-29% of the potential $<2 \mu\text{m}$ production per 12 h period. The size structure of the phytoplankton community has an important influence on the grazing impact of microzooplankton. At the netplankton dominated station 2, 5% of the total chlorophyll standing stock was grazed during the night, while 15-102% was grazed at the pico- and nanoplankton dominated stations 6, 12 and X. The impact on the potential production of the total $<300 \mu\text{m}$ phytoplankton community in this study could not be calculated since growth rates measured for the different dilutions were erratic, yielding insignificant regressions.

DISCUSSION

This study suggests that competition between microzooplankton and mesozooplankton for phytoplankton prey is minimal in that the former appeared to graze mainly $<2 \mu\text{m}$ chlorophyll. Undoubtedly, much of this fraction is composed of cyanobacteria. Cyanobacteria were largely overlooked by Azam et al. (1983) in their microbial loop hypothesis. However, since they are generally larger than heterotrophic bacteria, cyanobacteria are likely to be cleared more effectively by ciliates (Fenchel 1980b). Ciliate bacterivory by-passes at least one step in the bacteria-flagellate-ciliate microbial loop, making the transfer of energy to higher order consumers more efficient (Sherr &

Sherr 1987). There have been a number of reports of ciliates ingesting cyanobacteria (Sherr et al. 1986a, Rassoulzadegan et al. 1988).

In the present study we have no data for protozoan grazing on heterotrophic bacteria. However, Hagström et al. (1988) modelled a system in which primary production was dominated by cyanobacteria and found that flagellates consumed a much larger fraction of cyanobacteria than heterotrophic bacteria. According to their model the main organic flux route is cyanobacteria carbon into bacterivores, although a substantial part of heterotrophic bacteria is concomitantly consumed.

Other authors have reported that although ciliates are in general likely to take smaller food particles than copepods, there may at certain times be an overlap of prey selection in a size range $>4 \mu\text{m}$. Rassoulzadegan et al. (1988) examined the food size range selected by 13 species of Tintinnia and 18 species of Oligotrichina, and concluded that ciliates between $30 \mu\text{m}$ and $50 \mu\text{m}$ take 70% of their ingested ration as nanoplankton, while larger ciliates ($>50 \mu\text{m}$) take nanoplankton almost exclusively. In fact, Smetacek (1981) noted that many protozoans, primarily heterotrophic dinoflagellates and ciliates, sampled from the Kiel Bight, contained ingested netplankton cells ($>20 \mu\text{m}$). Although tintinnids seem only able to ingest particles 40-45% of their oral diameter (Heinbokel 1978b, Rassoulzadegan & Etienne 1981), aloricate ciliates can adapt their shape to that of ingested particles, with the result that they and other flexible protozoa are capable of ingesting cells their own size and even larger (MacKinnon & Hawes 1961). This was the case at our station 2, where the athecate dinoflagellate *Gyrodinium* spp. was observed with ingested *Thalassiosira* spp. cells of $20\text{-}40 \mu\text{m}$ diameter. The normal size of this dinoflagellate was $10\text{-}15 \mu\text{m}$ diameter (see Plate 1, h-j). Thus although chlorophyll measurements failed to reveal any grazing in the $20\text{-}300 \mu\text{m}$ size class, ingestion of phytoplankton in this size class did occur.

The rôle of dinoflagellates in microzooplankton grazing may have been overlooked during the years of debate about ciliate and nanoflagellate grazing. Heterotrophic forms comprise half of all dinoflagellates and the majority of the athecate forms (Gaines & Elbrachter 1987). In fact, the first record of mixotrophic phagotrophy, the ingestion of food particles by chlorotic dinoflagellates, was for a *Gyrodinium* species, *G. fissum* Levander, from the Baltic Sea (Levander 1894), although this record has not been subsequently verified (Gaines & Elbrachter 1987). As early as 1921 Kofoid and Swezy had identified 15 *Gyrodinium* species with ingested food bodies, and various other authors have added to this list, bringing the number to 22 (see review by Gaines & Elbrachter 1987). Most recently, Smetacek (1981) and Nöthig & von Bodungen (1989) have photographed *Gyrodinium* spp. with ingested pennate diatoms.

Even the more rigid thecate dinoflagellates have been found to contain ingested food bodies (Bursa 1961, Dodge & Crawford 1970), but the impact of dinoflagellates as grazers may have been underestimated if based solely on such observations, since the ability to digest prey extracellularly may be ubiquitous. *Gymnodinium fungiforme* feeds by attaching to its prey and ingesting cytoplasm or body fluids through a highly extensible peduncle (Spero & Morée 1981, Spero 1982), while Gaines & Taylor (1984) observed that *Protoperidinium conicum* extrudes a feeding "veil" to surround prey, which is subsequently digested extracellularly. Such mechanisms allow dinoflagellates to prey upon organisms many times larger than themselves.

This study indicates that microzooplankton grazers can have a significant impact on phytoplankton standing stocks. Grazing rates represented about 5% of phytoplankton biomass under bloom conditions and 15-102% ($\bar{x}=46\%$) in post-bloom conditions. Capriulo & Carpenter (1980) found that microzooplankton in Long Island Sound

removed 11-41% of the chlorophyll *a* standing stock per day, while Burkhill et al. (1987) recorded that 30 and 65% of the algal standing stocks in Carmarthen Bay and the Celtic Sea, respectively, were grazed daily. Landry & Hasset (1982) reported a grazing impact of 6 to 24% of phytoplankton standing stock and 17 to 52% of production per day in coastal waters off Washington. Similarly, Beers & Stewart (1971) estimated a microzooplankton impact of 7-52% of primary production in the Southern California Bight, while Verity (1986) found that 62% of the $< 10 \mu\text{m}$ and $< 5 \mu\text{m}$ chlorophyll *a* production was removed annually.

Although the clearance rates of microzooplankton are generally much lower than those of copepods, because of their greater abundance, the population clearance rates for microzooplankton may be higher (Capriulo & Carpenter 1980, Lessard & Swift 1985). Capriulo & Ninivaggi (1982) calculated that when the phytoplankton in Long Island Sound was dominated by small forms ($6 \mu\text{m}$ ESD), 6-120x more biomass could be removed by microzooplankters (specifically tintinnids) than by copepods. In the present study copepods removed 18.6% of the phytoplankton standing stock at station 2 and 0.1-2.0% ($\bar{x}=1.1\%$) at stations 6, 12 and X (see Chapter 3). Therefore, although microzooplankton only removed one third of the biomass removed by copepods during bloom conditions, they were able to remove 10-136x more biomass than the copepods during post-bloom conditions.

This example illustrates the potential impact microzooplankton might have in structuring phytoplankton communities in aged upwelled waters. This increased importance of microzooplankton grazing on aged waters correlates well with their proposed rôle in nutrient regeneration (Glibert 1982, Probyn 1985) and the shift from new to regeneration based production during bloom succession.

CHAPTER 3

COPEPOD GRAZING

INTRODUCTION

The debate surrounding the rôle of the microbial loop (Azam et al. 1983) as a source or sink of organic matter in the pelagial (Ducklow et al. 1986, Sherr et al. 1987) has precipitated a resurgence of interest in the grazing of protozoans by metazoans (Sherr et al. 1986b). Clearly this link between protozoans and metazoans is critical to the transfer of nano- and picoplankton production to food chains leading to pelagic fish. Copepods cannot ingest particles less than 5 μm in size effectively (Boyd 1976, Nival & Nival 1976, Sherr et al. 1986b); however, protozoans are capable of ingesting these smaller particles in both the nanoplankton (Jonsson 1986, Verity 1986, Rassoulzadegan et al. 1988) and picoplankton (Sherr et al. 1986a, Sherr & Sherr 1987; Rassoulzadegan et al. 1988) size range. In the absence of small protozoans, therefore, this transfer would be interrupted.

Many of the earlier studies employed gut content or faecal pellet analysis to determine the natural diets of copepods (Mullin 1966, Harding 1974, Turner & Anderson 1983). These studies revealed that tintinnid protozoans were indeed ingested by copepods. However, little attention was given to the more ubiquitous soft-bodied aloricate ciliates, which would not be preserved in the guts or faecal pellets. In fact, the lorica of tintinnids may serve to reduce capture of the animal (Capriulo et al. 1982), an idea supported by Stoecker & Egloff (1987), who reported that the estuarine calanoid copepod *Acartia tonsa* demonstrated a preference for aloricate ciliates over loricate species.

Most feeding studies have been conducted in the laboratory and have made use of monocultures of either phytoplankton or ciliates at concentrations much higher than those found in the natural environment. Only two studies to date have examined the

quantitative importance of protozoans to copepod diet in natural microplankton assemblages. Gifford & Dagg (1988) found that microzooplankton (primarily aloricate oligotrich ciliates) accounted for between 3 and 41% of the total carbon ingested ration of *Acartia tonsa*, depending on environmental conditions. However, Tiselius (1989) reported that ciliate carbon was always less than 10% and generally less than 1% of total carbon ingestion by *Acartia clausi* and *Centropages hamatus*. Typical concentrations of aloricate ciliates in the coastal waters of the Kattegat, Skagerrak and the Baltic were, however, low compared to other studies.

The present study is the first to examine the extent of copepod feeding on microzooplankton in the Southern Benguela upwelling region, using natural assemblages of copepods and microplankton.

METHODS

Copepod feeding experiments were carried out at four stations during a three day cruise off the Cape Peninsula, two at night (stations 2 and 12) and two during the day (stations 6 and X).

Experimental seawater was collected from a depth of 2 m with a Rosette sampler and prescreened through a 300 μm mesh to remove larger zooplankton. Mesozooplankton were collected with a drifting 300 μm plankton net (Peterson et al. 1990) from about 4 m and a representative assemblage, including adults and juveniles, was transferred to an experimental bottle. Incubations were performed in 1 l diffusion chambers equipped with 0.1 μm Nuclepore filters, allowing for gaseous and nutrient exchange. The chambers were kept in deck boxes cooled with flowing surface seawater and exposed to

75% incident light levels. Each experiment consisted of two experimental bottles with different concentrations of copepods in each, and a control bottle with no copepods. Day-time incubations were carried out over 4½ to 6 hours, while night-time incubations lasted 11 to 14 hours.

At the beginning of each experiment 200 ml of the experimental water were preserved with 10% Lugol's iodine for microzooplankton enumeration, and 100 ml duplicates of <300, <20 and <2 μm size fractionated seawater were filtered onto Whatman GF/F filters and frozen immediately for chlorophyll analysis. At the end of the experiment this was repeated for each of the bottles and the copepods were filtered onto pre-heated (500 °C for 3 hours) Whatman GF/F filters for CHN analyses.

The natural biomass of mesozooplankton at each station was determined from vertically-towed Bongo net (300 μm mesh) hauls over the upper 30 or 50m of the water column. The zooplankton were preserved in formalin and transported back to the laboratory, where they were counted and identified.

Back in the laboratory 100 ml subsamples of the Lugol's-preserved microzooplankton assemblage were cleared with thiosulphate, stained with Rose Bengal and filtered onto 3 μm Millipore filters. These were then mounted with hydroxypropyl methacrylate to make permanent slides (Crumpton 1987) and the microzooplankton enumerated under a Nikon Alphaphot YS light microscope. Volumes of the individual cells were calculated from linear dimensions. A volume:carbon ratio of 0.19 $\text{pg C } \mu\text{m}^{-3}$ was used for protozoans (Putt & Stoecker 1989).

Chlorophyll *a*, corrected for phaeopigments, was measured fluorometrically in 90% acetone extracts (Holm - Hansen et al. 1965). Phytoplankton carbon was then estimated

from a carbon:chlorophyll ratio of 30 for picoplankton (Joint & Pomroy 1986) and 50 for net- and nanoplankton (Ryther et al. 1971, Pitcher 1988).

CHN analyses of the copepods were performed on a Heraeus CHN analyser. Feeding rates are expressed in terms of copepod body carbon to compensate for the different species assemblage at each station. Clearance and ingestion rates of chlorophyll and microzooplankton carbon were calculated using Frost's (1972) equations, but with the modification of using initial cell concentration instead of mean concentration, as suggested by Marin et al. (1986).

RESULTS

The dominant zooplankton species present at each of the stations sampled are shown in Table 1. It is evident that the smaller species, such as *Oithona* spp., *Paracalanus parvus*, *Ctenocalanus vanus* and *Clausocalanus arcuicornis*, were equally abundant in the euphotic zone during the day and night. However, the larger species, *Calanus australis*, *Centropages brachiatus* and *Calanoides carinatus*, were present in greater numbers at the night-time stations, 2 and 12, indicating diel migration (Peterson et al. 1990). There were remarkably few copepods at station X, despite the fact that the total carbon available as food was comparable with the other stations, as shown in Table 2.

Station 2 was characterized by a high chlorophyll concentration (Table 2), with the phytoplankton composed of a mixed assemblage of diatoms and dinoflagellates. It also had the highest concentration of protozoan carbon, at $16.68 \text{ ug C l}^{-1}$, but this was attributed not to ciliates, which accounted for only 2.14 ug C l^{-1} , but to a large heterotrophic dinoflagellate, *Gyrodinium* spp., of approximately $50 \times 20 \text{ }\mu\text{m}$, which

Table 1. The dominant zooplankton species present in the upper waters ($z = 30-50$ m) at the time of the study. Adults include females, males and C4 and C5 copepodite stages.

Dominant Zooplankton		No. m^{-3}			
		St 2	St 6	St 12	St X
<i>C. brachiatus</i>	Adults	487	40	224	58
	Juv.	861	52	482	104
<i>C. carinatus</i>	Adults	419	212	1221	108
	Juv.	91	32	45	4
<i>C. australis</i>	Adults	215	4	78	6
	Juv.	193	0	45	0
<i>P. parvus</i>					
<i>C. vanus</i>		2085	1348	1165	346
<i>C. arcuicornis</i>					
<i>Oithona</i>		805	616	526	137
Euphausiid larvae		193	260	90	31

Table 2. Concentrations of protozoan carbon and nano- (2-20 μm) and net- (300-20 μm) phytoplankton, regarded as being available for copepod consumption.

Stations	Available Carbon ($\mu\text{g l}^{-1}$)				
	Protozoans < 10 000 μm^3	Phytoplankton nano	Phytoplankton net	Total	Protozoans % of total
2	16.68	260	291	567.68	2.94
6	2.95	89	11	102.95	2.87
12	7.38	75	26	108.38	6.81
X	1.89	100	23	124.89	1.51

were frequently observed to contain ingested *Thalassiosira* spp. cells of $\sim 25 \mu\text{m}$ diameter.

At the other stations, most of the phytoplankton was made up of species in the 2-20 μm size class. However, most of the chlorophyll in this size class was probably due to autotrophic flagellates of 3-8 μm , which are likely to be too small to be ingested by copepods (Nival & Nival 1976, Paffenhöfer 1984). Although the ciliates at stations 6 and X were present in low concentrations, the ratio of phytoplankton to protozoan carbon was similar to that of station 2. Station 12 had the highest percentage of protozoans, composed of a variety of species from the families Strombidiidae and Strobilidiidae.

Clearance and ingestion rates of phytoplankton carbon by copepods at each station are compared in Table 3. For the stations where incubations were conducted at two concentrations of copepods we concentrated our interpretations on results obtained for the incubations with lower concentrations of copepods as these are more likely to represent the natural environment.

At stations 2, 6 and X phytoplankton in the 20-200 μm size class appeared to be cleared at similar rates, but because of the high standing stock of phytoplankton at station 2, considerably more was actually ingested here. At station 6 only the 20-200 μm size fraction was grazed, despite the fact that the available concentration in this fraction was only 9% of the total phytoplankton carbon $> 2 \mu\text{m}$. This would appear to indicate that copepods were selecting for larger prey species. This trend is also evident in station X, where the 20-200 μm fraction was grazed almost four times faster than the 2-20 μm fraction, although it comprises only 18% of the total phytoplankton $> 2 \mu\text{m}$, and hence contributes less as ingested carbon.

Table 3. Biomass specific filtration ($F_c = \text{ml cop}^{-1}\text{h}^{-1}$) and ingestion ($I_c = \text{ng C } (\mu\text{g C}_{\text{cop}})^{-1}\text{h}^{-1}$) rates by copepods on the three phytoplankton size classes. Results are presented for two different copepod concentrations

Size Class (μm)	Station 2				Station 6				Station 12				Station X			
	7 cop.l ⁻¹		14 cop.l ⁻¹		12 cop.l ⁻¹		25 cop.l ⁻¹		14 cop.l ⁻¹		52 cop.l ⁻¹		12 cop.l ⁻¹		21 cop.l ⁻¹	
	F _c	I _c	F _c	I _c	F _c	I _c	F _c	I _c	F _c	I _c	F _c	I _c	F _c	I _c	F _c	I _c
200 - 20	1.23	357.18	0.88	257.34	1.49	13.38	2.84	25.52	-	-	-	-	1.27	38.63	0.38	8.45
20 - 2	-	-	0.37	97.27	growth stimulation				-	-	-	-	0.34	33.30	0.06	5.48
< 2	growth stimulation				growth stimulation				0.21	3.87	0.24	4.33	growth stimulation			
Total Carbon Ingested	357.18		354.61		13.38		25.52		-		-		61.93		13.93	

In the experimental bottles with higher numbers of copepods, evidence of a shift in grazing pressure was observed (Table 3). At station 2 the copepods grazed the 20-200 μm fraction at a slower rate, but also cleared the 2-20 μm fraction at half this rate. At station X both fractions were cleared at a slower rate. However, at station 6 the copepods cleared the 20-200 μm fraction at the highest rate recorded for phytoplankton in this study, $2.84 \text{ ml } (\mu\text{g } C_{\text{cop}})^{-1} \text{ h}^{-1}$, which corresponds to $25.43 \text{ ml copepod}^{-1} \text{ h}^{-1}$, although little was ingested because of the low standing stock. They did not switch to grazing the lower size fraction, despite its higher concentration, which supports the theory of size selection.

In both incubations of station 12, containing 14 and 52 copepods l^{-1} respectively, no phytoplankton was grazed except in the $<2 \mu\text{m}$ size fraction. This is not attributed to copepods, however. Rather, it is possible that small flagellates and ciliates are relieved of grazing pressure through the removal of larger ciliates by copepods. These smaller protozoans would then be able to graze on the $<2 \mu\text{m}$ cyanobacteria and autotrophic flagellates at elevated rates. At the other stations the $<2 \mu\text{m}$ fraction exhibits growth stimulation: this could also be explained by the removal of ciliate predators by copepods.

Copepod grazing on protozoans is presented in Table 4. The ciliates were divided into five size classes, chosen on the basis of dominant species. The $<900 \mu\text{m}^3$ class comprised mainly $12 \times 12 \mu\text{m}$ ciliates, which were numerically dominant at all stations, reaching concentrations of 6000 l^{-1} . A conical $25 \times 20 \mu\text{m}$ ciliate, with a volume of $2600 \mu\text{m}^3$, was the only other species common to all stations in significant numbers. The autotrophic ciliate, *Myrionecta rubrum*, with an average volume of $5200 \mu\text{m}^3$, was observed at some stations, but because it was not present in large numbers, it was

included in a size category. Ciliates with a volume greater than $10\,000\ \mu\text{m}^3$ were excluded from rate measurements because they were not present in high enough numbers to be counted with any degree of confidence. For the same reason, phagotrophic dinoflagellates of more than $20\,000\ \mu\text{m}^3$ were also ignored. Examples of protozoans from the designated size classes are shown in Plate 1, Chapter 2.

There were very few ciliates, other than those in the $<900\ \mu\text{m}^3$ class, present at station 2. These small ciliates grew rapidly since they were not ingested by copepods (Table 4). However, the largest ciliates, in the same size range as the smaller class of *Gyrodinium* spp., were grazed fairly heavily. The copepods demonstrated obvious selection for the largest protozoa available to them, the $10\,000 - 20\,000\ \mu\text{m}^3$ dinoflagellates, with clearance rates of $4.12\ \text{ml} (\text{ug } C_{\text{cop}})^{-1}\text{h}^{-1}$.

At station 6 the $<900\ \mu\text{m}^3$ size class was grazed, albeit weakly. The initial biomass of this class was higher than that of the next two classes, which were not grazed, but not as high as the classes greater than $2600\ \mu\text{m}^3$, which were cleared at an average rate of $0.79\ \text{ml} (\text{ug } C_{\text{cop}})^{-1}\text{h}^{-1}$.

The ciliate assemblage of station 12 was composed of a large variety of species, but the $<900\ \mu\text{m}^3$ and the $2600\ \mu\text{m}^3$ ciliates were numerically most important. The clearance rate for the abundant $2600\ \mu\text{m}^3$ species was slightly faster than that for larger species, which may indicate recognition and selection of that species.

Station X was an exception in that the standing stock of the $<900\ \mu\text{m}^3$ ciliates was so high that the copepods ingested more carbon out of this class than the $2600\ \mu\text{m}^3$ class, despite being cleared at half the rate (Table 4).

Table 4. Biomass specific filtration ($F_C = \text{ml cop}^{-1}\text{h}^{-1}$) and ingestion ($I_C = \text{ng C } (\mu\text{g } C_{\text{cop}})^{-1}\text{h}^{-1}$) rates by copepods on protozoans at two different copepod concentrations. At station 6, the 25 copepod l^{-1} incubation bottle was spoiled.

Cell volume (μm^3)	Station 2		Station 6		Station 12		Station X							
	7 cop. l^{-1}		14 cop. l^{-1}		14 cop. l^{-1}		52 cop. l^{-1}		12 cop. l^{-1}		21 cop. l^{-1}			
	F_C	I_C	F_C	I_C	F_C	I_C	F_C	I_C	F_C	I_C	F_C	I_C		
<u>Ciliates</u>														
< 905	-	-	-	-	0.04	0.02	0.12	0.17	0.13	0.18	0.78	0.76	0.12	0.12
905 - 2618	-	-	-	-	-	-	-	-	-	-	0.37	0.06	-	-
2618	-	-	-	-	-	-	0.33	0.20	0.39	0.24	1.40	0.56	1.04	0.42
2618 \leq 5236	-	-	1.25	0.23	0.76	0.81	0.22	0.41	0.63	1.16	-	-	0.16	0.03
5236 - 10000	1.32	2.00	0.80	1.21	0.82	1.02	0.18	0.48	0.47	1.21	-	-	-	-
<u>Flagellates</u>														
< 10000	0.92	5.06	-	-	-	-	-	-	-	-	-	-	-	-
10000 - 20000	4.12	37.21	0.71	6.44	-	-	-	-	-	-	-	-	-	-
Total	44.27		7.88		1.85		1.26		2.79		1.38		0.57	

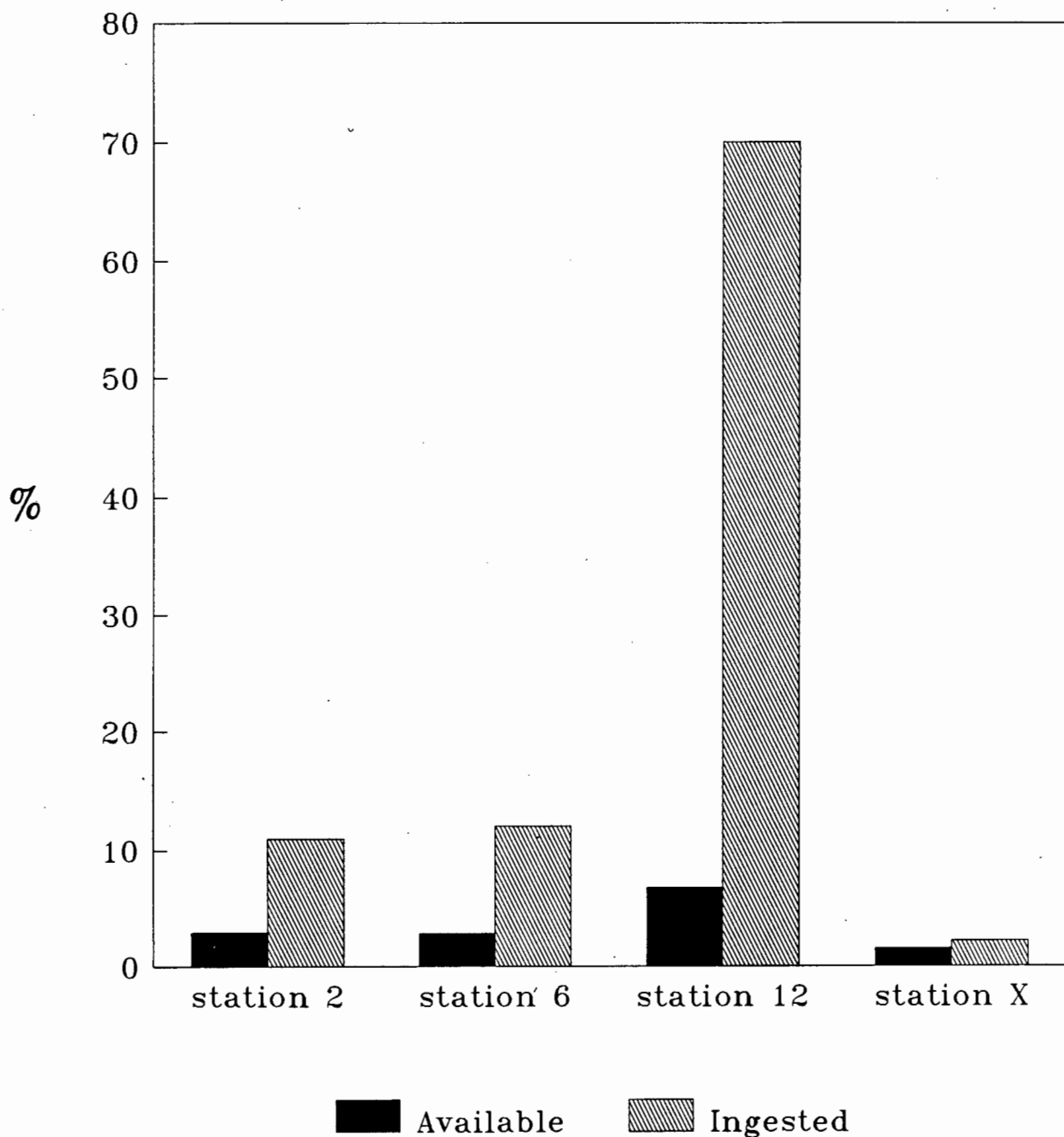


Fig. 1. Bar graphs showing the percentage of protozoan carbon regarded as being available for consumption, and the percentage of protozoan carbon ingested by copepods

The average total ingested carbon for stations 6, 12 and X was only about 3% of that of station 2, at $44.27 \text{ ng C (ug } C_{\text{cop}})^{-1} \text{ h}^{-1}$, owing to the ingestion of the large dinoflagellates which were only present at this station.

As for phytoplankton grazing, experiments using higher concentrations of copepods indicated slight changes in feeding behaviour. At station 2, the copepods started clearing the $2600 \leq 5200 \mu\text{m}^3$ size class as well as the original classes, which were cleared at a much slower rate. No data were available for station 6, but at station 12 the ciliates $>2600 \mu\text{m}^3$ were cleared at a faster rate than the $2600 \mu\text{m}^3$ species. This may be due to copepods clearing ciliates likely to provide a higher carbon source, rather than wasting energy finding the dominant species. At station X the copepods also appeared to switch to clearing larger ciliates, with the result that most of the carbon ingested originated from the $2600 \mu\text{m}^3$ species.

DISCUSSION

Our results indicate that protozoans account for a highly variable portion of copepod diet, ranging from 4% to almost 100%. Although clearance rates of phytoplankton and ciliates over the size ranges were similar, when the percentage of carbon available as protozoans is compared to the percentage ingested, as in Figure 1, it appears that copepods show a preference for protozoans. At station 12, for example, ciliates comprised only 7% of the total available carbon $>2 \mu\text{m}$, but almost 100% of ingested carbon. Direct microscope counts of phytoplankton at station 12 did, in fact, reveal low rates of ingestion for the diatom *Amphora* sp., which were not evident from chlorophyll

measurements. Accounting for this grazing of *Amphora* sp. results in ciliates contributing 70% of ingested carbon.

This apparent selection of protozoans is evident at all stations. However, the results may be biased in that much of the available phytoplankton carbon may have been due to autotrophic flagellates, 3-8 μm in length, too small to be ingested by copepods (Nival & Nival 1976, Paffenhöfer 1984). Also, many of the ciliates counted may have been autotrophic, and would thus have been included in all chlorophyll measurements of available and ingested carbon. Stoecker et al. (1989) estimated that 50% of ciliates in the euphotic zone may contain chlorophyll, while Laval-Peuto & Rassoulzadegan (1988) found that 41% of all Oligotrichina species they observed displayed strong autofluorescence. However, even once we had recalculated our ingestion rates with the assumption that 50% of the ciliate biomass was chlorotic and 50% of the phytoplankton in the 2-20 μm size class was "unavailable", the trends illustrated in Figure 1 were still evident, although to a lesser degree.

There appear to be numerous advantages of carnivory for copepods, related to higher food qualities of animal prey. Corner et al. (1976) showed that assimilation efficiencies of *Calanus helgolandicus* were higher during carnivorous feeding, while Berk et al. (1977) found that the survival of copepods was significantly longer if ciliates were added as a food source. More recently, Stoecker & Egloff (1987) measured higher egg production rates when *Acartia tonsa* was fed ciliates and rotifers, as opposed to phytoplankton.

It would thus be highly beneficial for a copepod to be able to perceive differences in food quality of prey items, and numerous studies have reported this ability (Paffenhöfer & Van Sant 1985, Poulet & Marsot 1978, Cowles et al. 1988, Wiadnyana &

Rassoulzadegan 1989). The C:N ratio appears to be the most important food quality variable affecting egg production (Ambler 1986) or ingestion, with the copepods maximising cellular protein and nitrogen intake (Libourel Houde & Roman 1987, Cowles et al. 1988).

These differences in food quality may be detected by copepods because of larger "microzones" or steeper chemical gradients surrounding higher quality food items (Libourel Houde & Roman 1987, Cowles et al. 1988). Moreover, Paffenhöfer & Van Sant (1985) and Jonsson & Tiselius (1990) have proposed that moving ciliates could also be detected through mechanoreception, by distorting the flow patterns around the copepods' antennae.

However, although these data indicate selection for protozoans, these trends could also be attributed to the copepods selecting food items in direct proportion to their abundance, *providing* they are of an adequate size. As mentioned previously, much of the phytoplankton at stations 6, 12 and X was probably too small to be effectively ingested. The reason that *Gyrodinium* spp. was grazed heavily in station 2 may have been because these cells were in the same size range as some of the larger phytoplankton species. Gifford & Dagg (1988) found that microzooplankton only accounted for large portions of total ingested carbon when the phytoplankton community was composed almost entirely of particles $<5 \mu\text{m}$. In addition, various authors have demonstrated that copepods "track" food concentrations, ingesting particles of the greatest relative abundance (Poulet 1973, 1978, Huntley 1981, Landry 1981, Conley & Turner 1985, Turner & Tester 1989).

The ability to take advantage of alternative food items will be highly beneficial in a pulsed upwelling environment, such as the Southern Benguela, where animals are

exposed to extremely variable quantities and qualities of food. Diatom blooms induced by high nitrate concentrations during upwelling are followed by stratified, nutrient depleted waters characterized by small phytoplankters, high dissolved organic matter (DOM) production rates and complicated microbial pathways. It is probable that different copepod species, although omnivorous, have different degrees of preference for phytoplankton versus microzooplankton prey. This may be related to the swimming behaviours of both the predators and their prey (Paffenhöfer & Knowles 1980, Conley & Turner 1985). Indeed, Jonsson & Tiselius (1990) recorded the lowest rates of ingestion for the fast swimming *Myrionecta rubrum*, when comparing capture efficiencies by *Acartia tonsa* on three species of ciliates.

Our results indicate that an almost purely ciliate diet, as at our station 12, is unlikely to sustain a large copepod community. The total carbon ingested was only 0.5% of that at station 2, an example of the classical grazer food web thought to typify upwelling environments. Short-lived "blooms" of ciliates do occur (Tiselius 1989), and under these conditions carnivory may be able to support substantial copepod production. Although the concentration of ciliates at station 12 appears to be at the higher end of the range reported by other authors for coastal and shelf environments (see Tiselius 1989, Table 1), it is unknown what constitutes bloom concentrations for the Southern Benguela, because of a paucity of microzooplankton data. However, the ability of copepods to exploit protozoan biomass, even when not unusually high, may constitute an important survival strategy for copepods during the lean times between netplankton diatom blooms.

CHAPTER 4

BUDGETING THE FLUX OF NITROGEN

INTRODUCTION

It has recently become clear that a large proportion of primary production occurs in the small size classes $<10 \mu\text{m}$ or even $<2 \mu\text{m}$ (Bienfang & Takahashi 1983, Furnas 1983a, Li et al. 1983, Platt et al. 1983, Sellner 1983). Although these small phototrophs appear to be quantitatively more important in oligotrophic environments, a number of studies have shown that they can account for a substantial fraction of primary production in temperate shelf seas (Joint et al. 1986, Harrison & Wood 1988, Probyn 1990). It follows that these organisms are also important assimilators of dissolved nitrogen in the pelagial (Nalewajko & Garside 1983, Probyn 1985, Koike et al. 1986, Harrison & Wood 1988).

Since many pico- and nanophytoplankton species are too small to be efficiently consumed by mesozooplankton grazers (Nival & Nival 1976), the protozoan grazers of the microbial loop are the likely intermediaries in the transfer of energy and nutrients to higher trophic levels. In this respect the Oligotrichine ciliates (including tintinnids and oligotrichs) are regarded as the most important because of their primarily herbivorous feeding (Beers & Stewart 1970, 1971, Beers et al. 1975, 1980, Heinbokel 1978a, 1987b, Smetacek 1981, Stoecker et al. 1981). Indeed, copepods have been shown to ingest ciliates (Berk et al. 1977, Stoecker & Egloff 1987, Gifford & Dagg 1988), but little information exists on how efficiently primary production is disseminated through this "link". The "link or sink" debate (Ducklow et al. 1986, Sherr et al. 1987) concentrated on the scavenging of dissolved organic matter (DOM) by bacteria and their subsequent ingestion by bacterivores, thereby returning energy to the main food chain. The transfer of primary production in the small size classes was largely ignored; furthermore, carbon rather than nitrogen was used as the "currency" of trophic transfer.

If the microbial loop is a carbon sink, it nevertheless serves an important rôle in nitrogen recycling within the euphotic zone, since flagellates and other microzooplankton excrete nitrogen in the form of ammonium and urea (Glibert 1982, Probyn 1987). In stratified, oligotrophic environments, regenerated nitrogen may be the only source of nitrogen available to primary producers.

The aim of this chapter was to trace the pathways of nitrogen assimilated by primary producers to microzooplankton and mesozooplankton by measuring nitrogen flow in the microbial and classical food webs. The coupling between these two systems will reveal to what extent microbial pathways serve as a link or sink in the pelagial, as well as examining their respective rôles in nitrogen recycling.

METHODS

Experiments were carried out during a cruise in the Southern Benguela upwelling region in April 1989. Nitrogen uptake by phytoplankton and excretion by microzooplankton and copepods were measured using ^{15}N isotope dilution techniques, as detailed in Chapter 1. Herbivorous microzooplankton (mainly oligotrichine ciliates) grazing on phytoplankton was quantified by predator:prey dilution experiments (Chapter 2), while natural species assemblages of copepods were incubated in ambient seawater to examine grazing rates on phytoplankton and microzooplankton (Chapter 3).

It is important to note that nanoflagellates were omitted from microzooplankton grazer size classes, since only protozoans larger than $9 \times 12 \mu\text{m}$ were enumerated (see Methods, Chapter 2). In addition, rates of ingestion of picoplankton are based solely on chlorophyll containing particles, even though heterotrophic bacteria may have

contributed to nitrogen assimilation by this size class. As such picoplankton assimilation rates will be overestimated by an unknown extent. Recent evidence, however, does suggest that heterotrophic bacteria will assimilate amino acids in preference to ammonium (Kirchman et al. 1989). Thus their contribution to nitrogen assimilation in the present study was probably small.

All ingestion rates were converted into nitrogen terms. Table 1 shows the carbon:chlorophyll and C:N ratios used for the different size fractions of phytoplankton and the protozoans.

Table 1: Conversion factors used in the budget

Prey type	C:chl	C:N	reference
Picoplankton	30		Joint & Pomroy 1986
Net/nanoplankton	50		Ryther et al. 1971, Pitcher 1988
Protozoans		4	Putt & Stoecker 1989 (<i>S. spiralis</i> 3-4) Børnsheim & Bratbak 1987 (<i>Monas</i> 4.5)
Picoplankton		4.6	Taguchi & Laws 1989
Net/nanoplankton		6.6	Redfield 1934

RESULTS

Flow diagrams representing the flux of nitrogen in the euphotic zone are presented in Figures 1a-d. In each diagram the width of the arrows is proportional to the magnitude of nitrogen flow in $\mu\text{g N l}^{-1}\text{h}^{-1}$; however, the scale is not consistent between figures. The data used for the flow diagrams are presented in Table 2.

The flow diagrams and Table 3 indicate that for stations 6, 12 & X most of the nitrogen flux was channelled through microbial pathways, while at station 2 the copepods ingested relatively more phytoplankton than the protozoans. The grazing pressure on picophytoplankton by protozoans was remarkably constant at the four stations ($\bar{x}=0.513 \mu\text{g N l}^{-1}\text{h}^{-1} \pm 0.154$ $p \leq 0.05$). At most of the stations the microbial loop was an efficient recycler of nitrogen; only at station 2 was the excreted nitrogen only 15% of the ingested nitrogen. At the day-time stations (6 & X) microzooplankton excretion exceeded uptake, while at stations 2 & 12 microzooplankton excretion could not account for all NH_4^+ uptake.

Despite the fact that the nanoplankton size class usually comprised the major share of phytoplankton biomass, in most cases copepods ingested more nitrogen from the netplankton size class. This is because much of the nanoplankton consisted of flagellates of 3-8 μm length, which are too small to be effectively ingested. Direct microscope counts were used in the calculation of grazing rate on phytoplankton at station 12.

Protozoans made up a highly variable component of the copepod diet. At stations 2 & X protozoans contributed only 3.5% of nitrogen ingested by copepods, while at station 6 protozoans comprised 18% of this ration. However, at station 12, where the plankton

Table 2. Data used for the flow diagrams, Fig. 1a-d. All rates are expressed as $\mu\text{g N l}^{-1}\text{h}^{-1}$. Biomass of the phytoplankton size fractions are expressed as $\mu\text{g chl l}^{-1}$

Stations	2	6	12	X
Netplankton biomass	5.83	0.18	0.53	0.45
Nanoplankton biomass	5.20	1.78	1.51	0.61
Picoplankton biomass	0.60	1.38	0.61	0.80
NO_3^- uptake by netplankton	0.1456	0.3320	0.0602	0.2674
NO_3^- uptake by nanoplankton	0.1512	0.000	0.0602	0.0154
NO_3^- uptake by picoplankton	0.0406	0.4032	0.1680	0.3696
NH_4^+ uptake by netplankton	0.1148	0.1400	0.0000	0.2912
NH_4^+ uptake by nanoplankton	0.1358	0.1820	0.4214	0.3038
NH_4^+ uptake by picoplankton	0.0658	0.3136	0.2702	0.7294
Protozoan grazing on picoplankton	0.5110	0.7030	0.3180	0.5180
Protozoan grazing on nanoplankton	-	1.3340	-	-
Copepod grazing on protozoa	0.2734	0.0045	0.0066	0.0014
Copepod grazing on netplankton	1.337	0.0200	0.0017	0.0170
Copepod grazing on nanoplankton	-	-	-	0.0200
Protozoan NH_4^+ excretion	0.0756	0.8260	0.3080	3.5140
Copepod NH_4^+ excretion	0.0238	-	0.0420	-

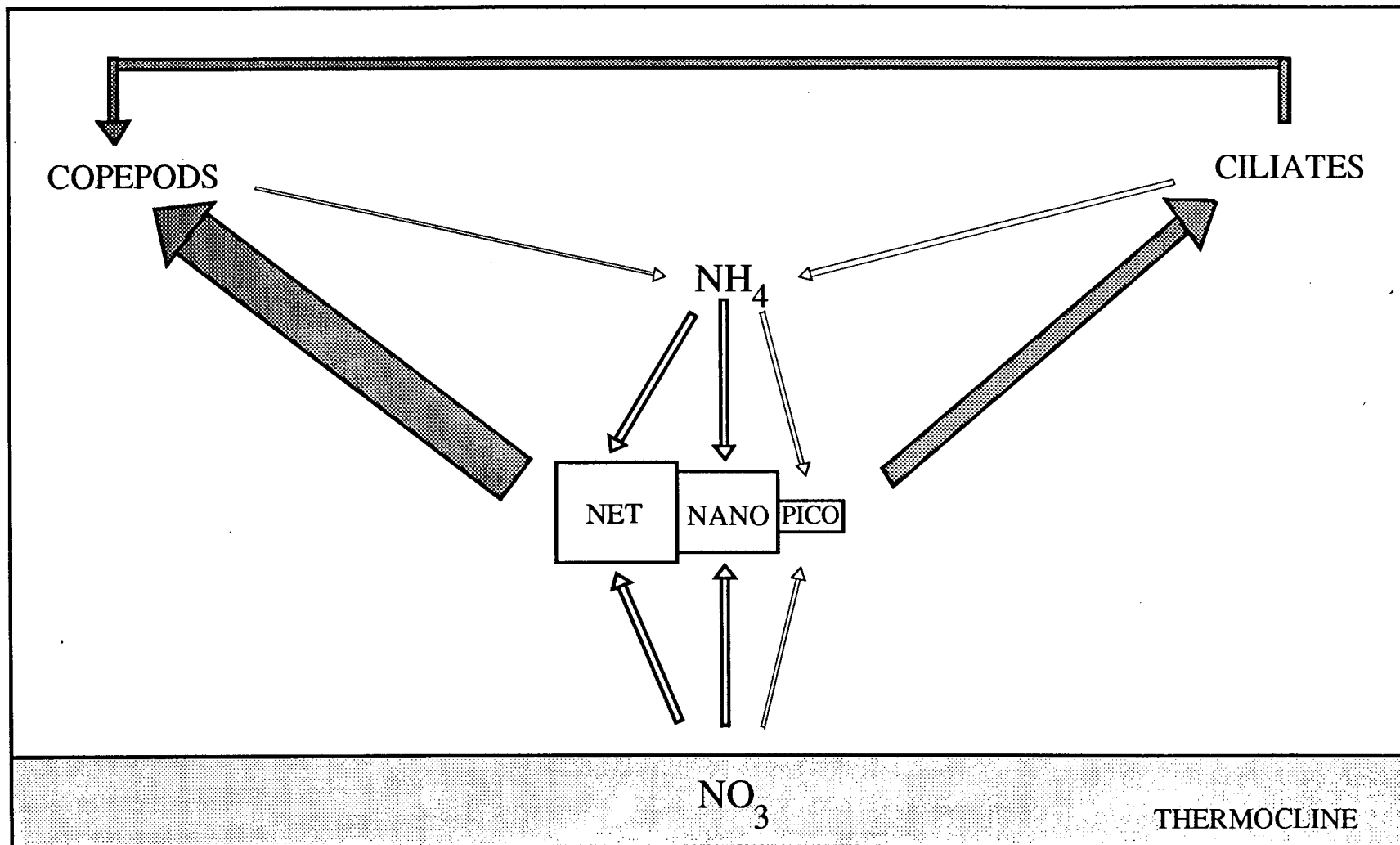


Fig. 1a. Measured nitrogen flux at station 2. Microplankton compartments (net, nano, pico) are based on mean Chl concentrations. Shaded arrows represent predator-prey trophic transfers and clear arrows represent dissolved N fluxes. Arrow width represents relative flux.

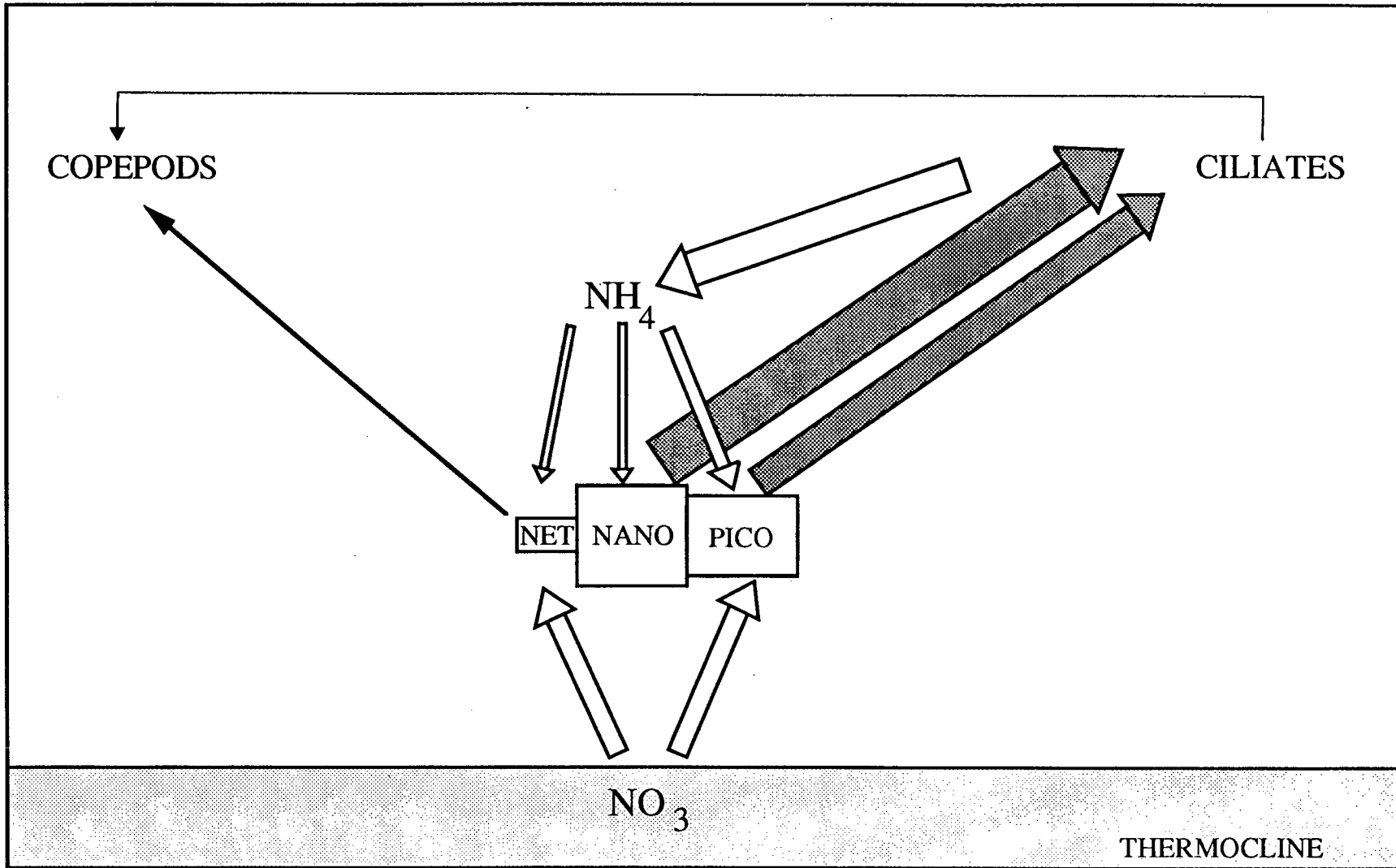


Fig. 1b. Measured nitrogen flux at station 6. See Fig. 1a for explanation of diagram

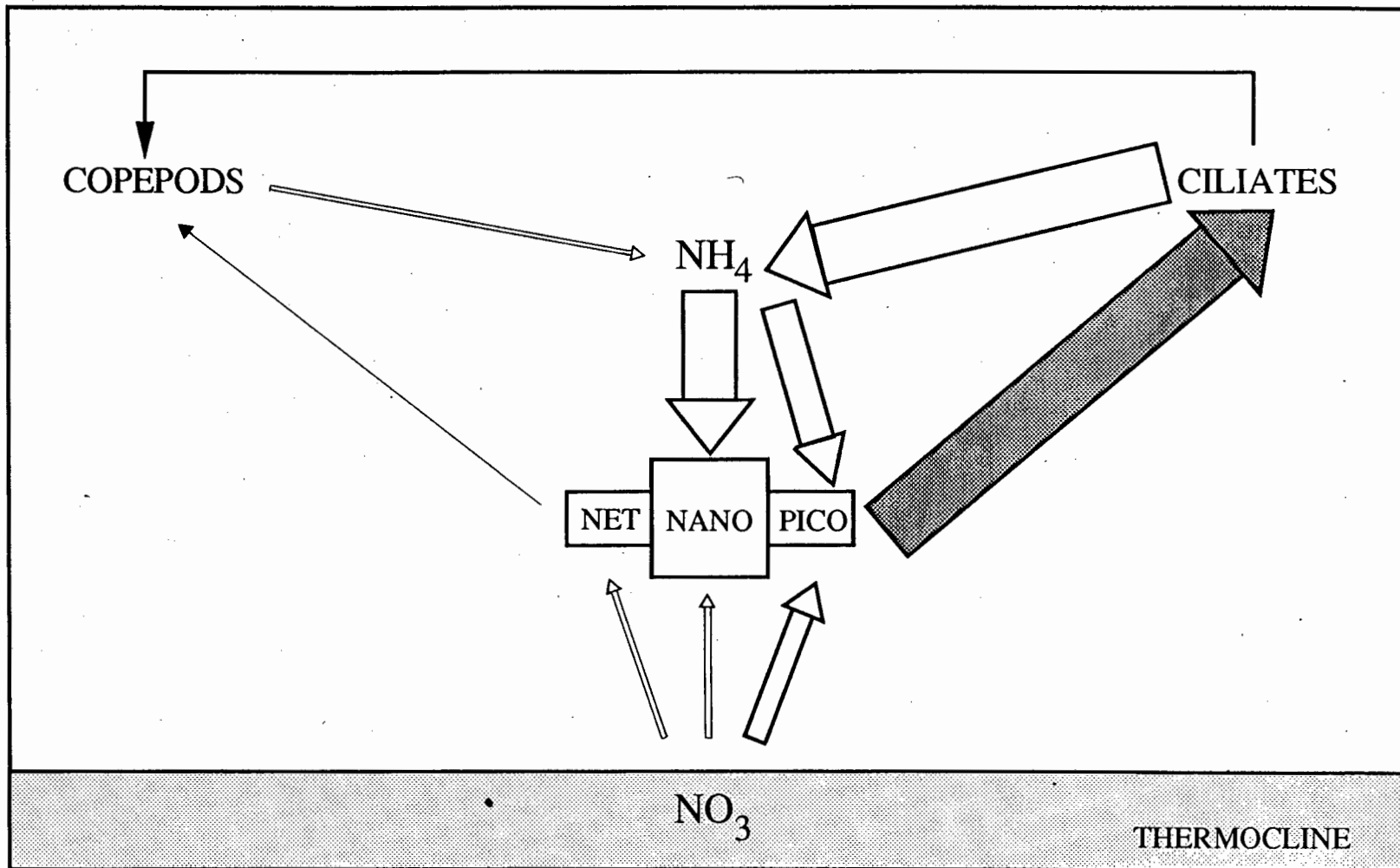


Fig. 1c. Measured nitrogen flux at station 12. See Fig.1a for explanation of diagram

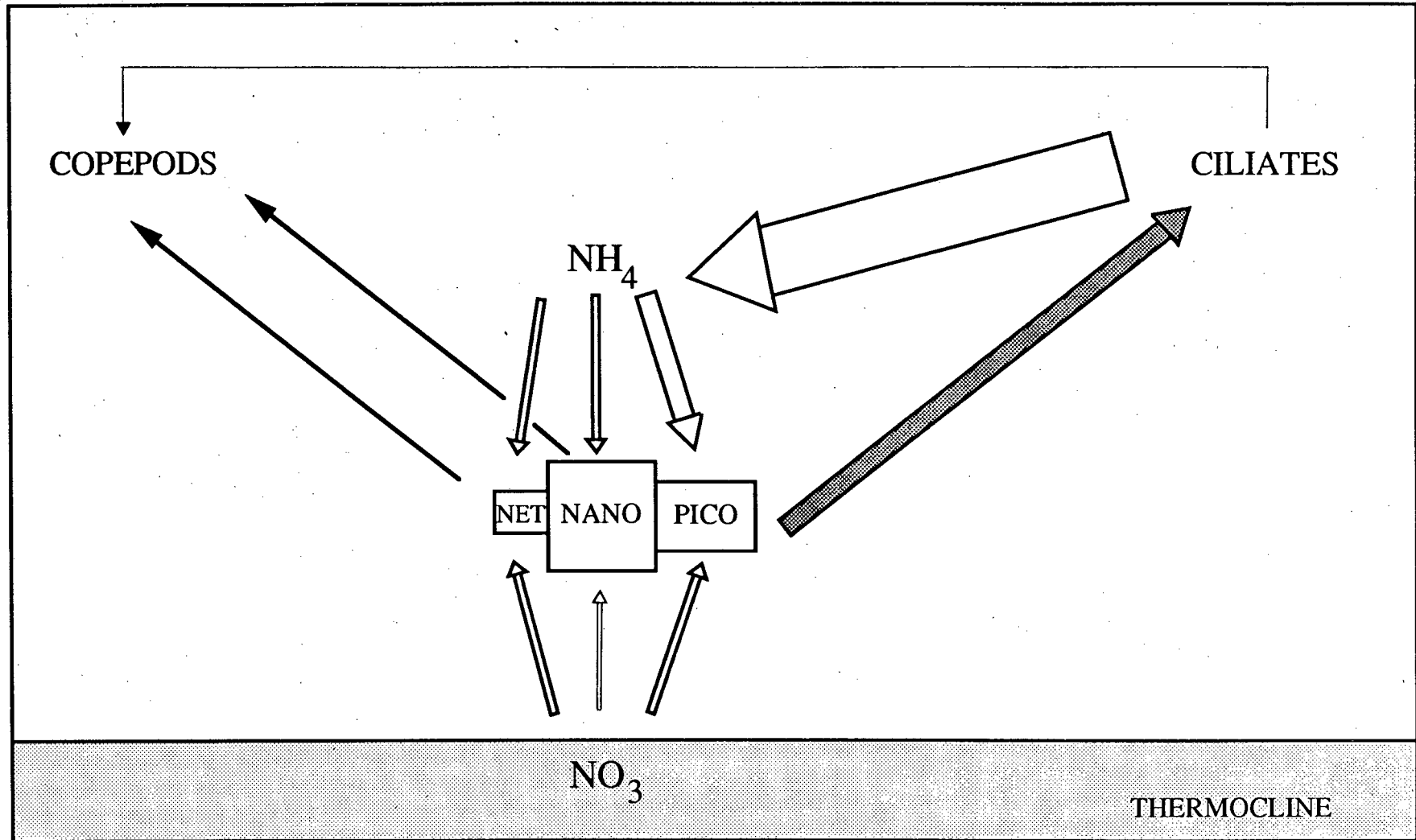


Fig. 1d. Measured nitrogen flux at station X. See Fig. 1a for explanation of diagram

Table. 3. The relative importance of copepods and protozoans as consumers of primary nitrogen production

Stations	Primary N production ($\mu\text{g N l}^{-1}$)	Copepod consumption (%)	Protozoan consumption (%)
2	0.654	203	78
6	1.361	1.5	150
12	0.980	0.2	32
X	1.977	1.9	26

assemblage was dominated by oligotrichous ciliates, 80% of the copepod diet consisted of protozoans. However, the total ingested ration at this station was only 0.6% of that at station 2, and it is unlikely that such a diet could support a large production potential.

DISCUSSION

In the present study station 2 represents a typical classical food chain, with 203% of the primary nitrogen production being ingested by copepods at night. At the other stations copepods consumed only 0.2-2% of the primary nitrogen production and the majority of the production (26-150%) was channelled through microbial pathways. The fact that ingestion exceeds production by such a large factor at station 2 is an indication of the fall-off in netphytoplankton activity at night (Chapter 1) and the dielily-migrant nature of the larger species of copepods (Peterson et al. 1990). The proportion of phytoplankton production ingested by copepods is likely to be considerably altered when considered over a 24 hour period. Unfortunately, no day-time copepod grazing rates are available for a netplankton dominated assemblage such as was present at station 2.

Our results indicate that only 0.3-9.5% ($\bar{x}=3\%$) of the nitrogen ingested by protozoans is subsequently transferred to copepods. Microbial pathways thus appear to have a minor rôle in the transfer of organic matter to higher trophic levels, their function being mainly the regeneration of nutrients for primary producers. Protozoans do nevertheless repackage food items too small to be ingested by copepods (Sherr & Sherr 1988). In addition, copepods have a higher assimilation efficiency (Corner et al. 1976) and egg production rate (Stoecker & Egloff 1987) when feeding carnivorously.

Therefore under certain environmental conditions, such as when the available phytoplankton is too small to be effectively ingested, protozoans may make up an important part of the copepod diet (Robertson 1983, Gifford & Dagg 1988). Protozoans contributed 80% of the copepod ingested nitrogen ration at station 12 in this study, where the planktonic assemblage was dominated by oligotrichous ciliates.

Omnivory is likely to be extremely advantageous to copepods in a pulsed ecosystem such as the Southern Benguela upwelling system. Diatom blooms induced by high nitrate concentrations during upwelling are followed by stratified, nutrient depleted waters characterized by small phytoplankters, high DOM production rates and complicated microbial pathways. Thus the relative importance of the classical food chain versus the microbial loop will alternate depending on the size structure of the planktonic assemblage. Similar observations were made by Andersen (1988) in the North Bering/Chukchi seas, Nielsen & Richardson (1989) in the North Sea, and Kiørboe et al. (1990) in the Skagerrak.

The existence of a classical food chain as opposed to a microbial loop type food chain will have important ramifications on the extent of organic flux to the benthos. Large cells such as diatoms have rapid sinking rates, and a large part of any bloom may sink out of the euphotic zone before being ingested by copepods (Dagg et al. 1982, Falkowski et al. 1988, Nielsen & Richardson 1989). Small flagellates and ciliates are able to maintain their position in the euphotic zone through their swimming activity, as well as having slower sinking rates.

Furthermore, the flux of organic matter to the benthos through faecal sinking will also be affected by the type of food chain. It was not possible to measure faeces production in this study, but Small et al. (1983) estimated that faecal pellet flux could account for

up to 41% of the total flux out of the euphotic zone. Microzooplankton faeces are likely to remain suspended in the water and may be remineralized within the euphotic zone (Sieburth et al. 1978, Antia et al. 1980), but copepod faeces will rapidly sink to the benthic waters. The structure of the food chain may in addition affect the sinking rates of these faeces, since Bienfang (1980) suggested that a diatom diet will result in faecal pellets of a greater density than those associated with a flagellate diet. Moreover, if a faecal pellet egestion of 25% of dietary phytoplankton nitrogen and 10% of dietary microzooplankton nitrogen is assumed for copepods (Probyn et al. 1990), faecal pellet production in the present study is calculated to be 0.001-0.009 $\mu\text{g N l}^{-1}$ for stations 6, 12 and X, and 0.339 $\mu\text{g N l}^{-1}$ for the diatom-dominated station 2. This lends support to the concept of greater faecal flux in classical rather than microbial loop type food chains.

The relative importance of the classical versus microbial loop type food chain in the Southern Benguela upwelling region is likely to be seasonal. The upwelling - generated blooms of spring and summer will support classical food chains, while the stratified, nutrient-depleted waters during the rest of the year may give rise to complicated microbial pathways. Further work needs to be carried out on the trophic transfer to higher order consumers when microbial loop type food chains predominate.

REFERENCES

- Albright, L.J., Sherr, E.B., Sherr, B.F., Fallon, R.D. (1987). Grazing of ciliated protozoa on free and particle-attached bacteria. *Mar. Ecol. Prog. Ser.* 38: 125-129
- Ambler, J.W. (1986). Effect of food quantity and quality on egg production of *Acartia tonsa* Dana from East Lagoon, Galveston, Texas. *Estuar. coast. Shelf Sci.* 23: 183-196
- Andersen, P. (1988) The quantitative importance of the "microbial loop" in the marine pelagic: a case study from the North Bering/Chuckchi Seas. *Arch. Hydrobiol., Beih. Ergebn. Limnol.* 31: 243-251
- Antia, N.J., Berland, B.R., Bonin, D.J. (1980). Proposal for an abridged nitrogen turnover cycle in certain marine planktonic systems involving hypoxanthine-guanine excretion by ciliates and their reutilization by phytoplankton. *Mar. Ecol. Prog. Ser.* 2: 97-103
- Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Riel, L.A., Thigstad, F. (1983). The ecological rôle of water-column microbes in the sea. *Mar. Ecol. Prog. Ser.* 10: 257-263
- Båmstedt, U. (1985). Seasonal excretion rates of macrozooplankton from the Swedish west coast. *Limnol. Oceanogr.* 30: 607-617
- Beers, J.R., Reid, F.M.H., Stewart, G.L. (1975). Microplankton of the North Pacific Central Gyre. Population structure and abundance, June, 1973. *Int. Revue ges. Hydrobiol.* 60: 607-638
- Beers, J.R., Reid, F.M.H., Stewart, G.L. (1980). Microplankton population structure in southern California nearshore waters in late spring. *Mar. Biol.* 60: 607-683
- Beers, J.R., Stewart, G.L. (1970). The ecology of the plankton off LaJolla, California in the period April-Sept., 1967. Part VI. Numerical abundance and estimated biomass of microzooplankton. *Bull. Scripps Instn Oceanogr. (New Ser.)* 17: 67-87

- Beers, J.R., Stewart, G.L. (1971). Microzooplankton in the plankton communities of the upper waters of the eastern tropical Pacific. *Deep Sea Res.* 18: 861-883
- Berk, S.G., Brownlee, D.C., Heinle, D.R., Kling, H.J., Colwell, R.R. (1977). Ciliates as a food source for marine planktonic copepods. *Microb. Ecol.* 4: 27-40
- Bienfang, P.K. (1980). Herbivore diet affects fecal pellet settling. *Can. J. Fish. Aquat. Sci.* 37: 1352-1357
- Bienfang, P.K., Takahashi, M. (1983). Ultraplankton growth rates in a subtropical ecosystem. *Mar. Biol.* 76: 213-218
- Bidigare, R.R. (1983). Nitrogen excretion by zooplankton. In: Carpenter, E.J., Capone, D.G. (ed.) *Nitrogen in the marine environment*. Academic Press, New York, p. 385-409
- Blackburn, T.H. (1979). Method for measuring rates of NH_4^+ turnover in anoxic marine sediments, using a $^{15}\text{N-NH}_4^+$ dilution technique. *Appl. Environ. Microbiol.* 37: 760-765
- Børnsheim, K.Y. (1984). Clearance rates of bacteria-sized particles by freshwater ciliates, measured with monodisperse fluorescent latex beads. *Oecologia (Berl.)* 63: 286-288
- Børnsheim, K.Y., Bratback, G. (1987). Cell volume to cell carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. *Mar. Ecol. Prog. Ser.* 36: 171-175
- Boyd, C.M. (1976). Selection of particle sizes by filter-feeding copepods: A plea for reason. *Limnol. Oceanogr.* 21: 175-180
- Burkhill, P.H., Mantoura, R.F.C., Llewellyn, C.A., Owens, N.J.P. (1987). Microzooplankton grazing and selectivity of phytoplankton. *Mar. Biol.* 93: 581-590
- Bursa, A.S. (1961). The annual oceanographic cycle at Igloodik in the Canadian Arctic. II. The phytoplankton. *J. Fish. Res. Bd. Can.* 18: 563-615

- Campbell, L., Carpenter, E.J. (1986). Estimating the grazing pressure of heterotrophic nanoplankton on *Synechococcus* spp. using the seawater dilution and selective inhibitor techniques. Mar. Ecol. Prog. Ser. 33: 121-129
- Caperon, J., Schell, D., Hirota, J., Laws, E. (1979). Ammonium excretion rates in Kaneohe Bay, Hawaii, measured by a ^{15}N isotope dilution technique. Mar. Biol. 54: 33-40
- Capriulo, G.M. (1982). Feeding of field collected tintinnid microzooplankton on natural food. Mar. Biol. 71: 73-86
- Capriulo, G.M., Carpenter, E.J. (1980). Grazing by 35 to 202 μm microzooplankton in Long Island Sound. Mar. Biol. 56: 319-326
- Capriulo, G.M., Carpenter, E.J. (1983). Abundance, species composition and feeding impact of tintinnid microzooplankton in Central Long Island Sound. Mar. Ecol. Prog. Ser. 10: 277-288
- Capriulo, G.M., Gold, K., Okuba, A. (1982). Evolution of the lorica in tintinnids: a possible selective advantage. Annls Inst. océanogr., Paris 58(S): 319-324
- Capriulo, G.M., Ninivaggi, A. (1982). A comparison of the feeding activities of field collected tintinnids and copepods fed identical natural particle assemblages. Annls Inst. océanogr., Paris 58(S): 325-334
- Carpenter, E.J., Dunham, S. (1985). Nitrogenous nutrient uptake, primary production and species composition of phytoplankton in the Carmans River estuary, Long Island, N.Y. Limnol. Oceanogr. 30: 513-526
- Conley, R.J., Turner, J.T. (1985). Omnivory by the coastal marine copepods *Centropages hamatus* and *Labidocera aestiva*. Mar. Ecol. Prog. Ser. 21: 113-120
- Corner, E.D.S., Head, R.N., Kilvington, C.C., Pennycuik, L. (1976). On the nutrition and metabolism of zooplankton. X. Quantitative aspects of *Calanus helgolandicus* feeding as a carnivore. J. mar. biol. Ass. U.K. 56: 345-358

- Cowles, T.J., Olson, R.J., Chisholm, S.W. (1988). Food selection by copepods: discrimination on the basis of food quality. *Mar. Biol.* 100: 41-49
- Crompton, W.G. (1987). A simple and reliable method for making permanent mounts of phytoplankton for light and fluorescence microscopy. *Limnol. Oceanogr.* 32: 1154-1159
- Cuhel, R.L., Ortner, P.B., Lean, D.R.S. (1984). Night synthesis of protein by algae. *Limnol. Oceanogr.* 29: 731-744
- Cynar, F.J., Sieburth, J. McN. (1986). Unambiguous detection and improved quantification of phagotrophy in apochlorotic nanoflagellates using fluorescent microspheres and concomitant phase contrast and epifluorescence microscopy. *Mar. Ecol. Prog. Ser.* 32: 61-70
- Dagg, M.J., Vidal, J., Whitley, T.E., Iverson, R.L., Goering, J.J. (1982). The feeding, respiration, and excretion of zooplankton in the Bering Sea during a spring bloom. *Deep Sea Res.* 29: 45-63
- Dodge, J.D., Crawford, R.M. (1970). The morphology and fine structure of *Ceratium hirundinella* (Dinophyceae). *J. Phycol.* 6: 137-149
- Ducklow, H.W., Purdie, D.A., Williams, P.J. LeB., Davies, J.M. (1986). Bacterioplankton: a sink for carbon in a coastal marine plankton community. *Science* 232: 865-867
- Dugdale, R.C., Goering, J.J. (1967). Uptake of new and regenerated forms of nitrogen in primary productivity. *Limnol. Oceanogr.* 12: 196-206
- Eppley, R.W., Peterson, B.J. (1979). Particulate organic matter flux and planktonic new production in the deep ocean. *Nature, Lond.* 282: 677-680
- Eppley, R.W., Renger, E.H., Venrick, E.L., Mullin, M.M. (1973). A study of plankton dynamics and nutrient cycling in the central gyre of the North Pacific Ocean. *Limnol. Oceanogr.* 18: 534-551

- Eppley, R.W., Rogers, J.N., McCarthy, J.J. (1969). Half-saturation constants for the uptake of nitrate and ammonium by marine phytoplankton. *Limnol. Oceanogr.* 14: 912-920
- Falkowski, P.G., Flagg, C.N., Rowe, G.T., Smith, S.L., Whittedge, T.E., Wirick, C.D. (1988). The fate of a spring phytoplankton bloom: export or oxidation. *Cont. Shelf Res.* 8: 457-484
- Fenchel, T. (1980a). Suspension feeding in ciliated protozoa: Functional response and particle size selection. *Microb. Ecol.* 6: 1-11
- Fenchel, T. (1980b). Suspension feeding in ciliated protozoans: Feeding rates and their ecological significance. *Microb. Ecol.* 6: 13-25
- Fenchel, T. (1982). Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as consumers of bacteria.
- Fiedler, R., Proksch, G. (1975). The determination of nitrogen-15 by emission and mass spectrometry in biochemical analysis: A review. *Anal. Chim. Acta* 78: 1-62
- Frost, B.W. (1972). Effects of size and concentration of food particles on the feeding behaviour of the marine planktonic copepod, *Calanus pacificus*. *Limnol. Oceanogr.* 17: 805-815
- Furnas, M.J. (1983a). Community structure, biomass and productivity of size-fractionated summer phytoplankton populations in lower Narragansett Bay, Rhode Island. *J. Plankton Res.* 5: 637-655
- Furnas, M.J. (1983b). Nitrogen dynamics in lower Narragansett Bay, Rhode Island. I. Uptake by size-fractionated phytoplankton populations. *J. Plankton Res.* 5: 657-676
- Gaines, G., Elbrachter, M. (1987). Heterotrophic nutrition. In: Taylor, F.J.R. (ed.) *The biology of dinoflagellates*. Blackwell Scientific, Oxford, p. 224-268
- Gaines, G., Taylor, F.J.R. (1984). Extracellular digestion in marine dinoflagellates. *J. Plankton Res.* 6: 1057-1061

- Gallegos, C.L. (1989). Microzooplankton grazing on phytoplankton in the Rhode River, Maryland: nonlinear feeding kinetics. *Mar. Ecol. Prog. Ser.* 57: 23-33
- Gast, V. (1985). Bacteria as a food source for microzooplankton in the Schlei Fjord and Baltic Sea with special reference to ciliates. *Mar. Ecol. Prog. Ser.* 22: 107-120
- Gifford, D.J. (1985). Laboratory culture of marine planktonic oligotrichs (Ciliophora: Oligotrichida). *Mar. Ecol. Prog. Ser.* 23: 257-267
- Gifford, D.J. (1988). Impact of grazing by microzooplankton in the Northwest Arm of Halifax Harbour, Nova Scotia. *Mar. Ecol. Prog. Ser.* 47: 249-258
- Gifford, D.J., Dagg, M.J. (1988). Feeding of the estuarine copepod, *Acartia tonsa* Dana: carnivory vs. herbivory in natural microplankton assemblages. *Bull. mar. Sci.* 49: 458-468
- Glibert, P.M. (1982). Regional studies of daily, seasonal and size fraction variability in ammonium remineralization. *Mar. Biol.* 70: 209-222
- Glibert, P.M., Biggs, D.C., McCarthy, J.J. (1982a). Utilization of ammonium and nitrate during austral summer in the Scotia Sea. *Deep Sea Res.* 29: 837-850
- Glibert, P.M., Goldman, J.C., Carpenter, E.J. (1982b). Seasonal variations in the utilization of ammonium and nitrate by phytoplankton in Vineyard Sound, Massachusetts, USA. *Mar. Biol.* 70: 237-249
- Glibert, P.M., Lipschultz, F., McCarthy, J.J. (1982c). Isotope dilution models of uptake and remineralization of ammonium by marine plankton. *Limnol. Oceanogr.* 27: 639-650
- Glover, H.E., Prézelin, B.P., Campbell, L., Wymans, M., Garside, C. 1988. A nitrate-dependent *Synechococcus* bloom in surface Sargasso Sea water. *Nature* 331: 161-163
- Goldman, J.C., Caron, D.A. (1985). Experimental studies on an omnivorous microflagellate: implications for nutrient regeneration in the marine microbial food chain. *Deep Sea Res.* 32: 899-915

- Hagström, A., Azam, F., Andersen, A., Wikner, J., Rassoulzadegan, F. (1988). Microbial loop in an oligotrophic pelagic marine ecosystem: possible rôle of cyanobacteria and nanoflagellates in the organic fluxes. *Mar. Ecol. Prog. Ser.* 49: 171-178
- Harding, G. C.H. (1974). The food of deep-sea copepods. *J. mar. biol. Ass. U.K.* 54: 141-155
- Harris, E. (1959). The nitrogen cycle in Long Island Sound. *Bull. Bingham oceanogr. Coll.* 17: 31-65
- Harrison, W.G. (1978). Experimental measurements of nitrogen remineralization in coastal waters. *Limnol. Oceanogr.* 23: 684-694
- Harrison, W.G. (1980). Nutrient regeneration and primary production in the sea. In: Falkowski, P.G. (ed.) *Primary productivity in the sea*. Plenum Press, New York, p. 433-460
- Harrison, W.G., Douglas, D., Falkowski, P., Rowe, G., Vidal, J. (1983). Summer nutrient dynamics of the Middle Atlantic Bight: Nitrogen uptake and regeneration. *J. Plankton Res.* 5: 539-556
- Harrison, W.G., Head, E.H.H., Conover, R.J., Longhurst, A.R., Sameoto, D.D. (1985). The distribution and metabolism of urea in the eastern Canadian Arctic. *Deep Sea Res.* 32: 23-42
- Harrison, W.G., Wood, L.J.E. (1988). Inorganic nitrogen uptake by marine picoplankton: evidence for size partitioning. *Limnol. Oceanogr.* 33: 468-475
- Heinbokel, J.F. (1978a). Studies on the functional rôle of tintinnids in the Southern California Bight. I. Grazing and growth rates in laboratory cultures. *Mar. Biol.* 47: 177-189
- Heinbokel, J.F. (1978b). Studies on the functional rôle of tintinnids in the Southern California Bight. II. Grazing rates of field populations. *Mar. Biol.* 52: 23-32

- Holm-Hansen, O., Lorenzen, C., Holmes, R.W., Strickland, J.D.H. (1965). Fluorometric determination of chlorophyll. *J. Cons. Cons. Int. Explor. Mer.* 30: 3-15
- Huntley, M. (1981). Nonselective, nonsaturated feeding by three calanid copepod species in the Labrador Sea. *Limnol. Oceanogr.* 26: 831-842
- Jawed, M. (1969). Body nitrogen and nitrogenous excretion in *Neomysis rayii* Murdoch and *Euphausia pacifica* Hansen. *Limnol. Oceanogr.* 14: 748-754
- Joint, I.R., Owens, N.J.P., Pomroy, A.J. (1986). Seasonal production of photosynthetic picoplankton and nanoplankton in the Celtic Sea. *Mar. Ecol. Prog. Ser.* 28: 251-258
- Joint, I.R., Pomroy, A.J. (1983). Production of picoplankton and small nanoplankton in the Celtic Sea. *Mar. Biol.* 77: 19-27
- Joint, I.R., Pomroy, A.J. (1986). Photosynthetic characteristics of nanoplankton and picoplankton from the surface mixed layer. *Mar. Biol.* 92: 465-474
- Jonsson, P.R. (1986). Particle size selection, feeding rates and growth dynamics of marine planktonic oligotrichous ciliates (Ciliophora: Oligotrichina). *Mar. Ecol. Prog. Ser.* 33: 265-277
- Jonsson, P.R., Tiselius, P. (1990). Feeding behaviour, prey detection and capture efficiency of the copepod *Acartia tonsa* feeding on planktonic ciliates. *Mar. Ecol. Prog. Ser.* 60: 35-44
- Kaufman, Z.G., Lively, J.S., Carpenter, E.J. (1983). Uptake of nitrogenous nutrients by phytoplankton on a barrier island estuary: Great South Bay, New York. *Estuar. coast. Shelf Sci.* 17: 483-493
- Kiørboe, T., Kaas, H., Kruse, B., Møhlenberg, F., Tiselius, P., Ærtebjerg, G. (1990). The structure of the pelagic food web in relation to water column structure in the Skagerrak. *Mar. Ecol. Prog. Ser.* 59: 19-32

- Kirchman, D.L., Keil, R.G., Wheeler, P.A. (1989). The effect of amino acids on ammonium utilization and regeneration by heterotrophic bacteria in the subarctic Pacific. *Deep Sea Res.* 36: 1763-1776
- Kofoed, C.A., Swezy, O. (1921). The free-living unarmoured dinoflagellata. *Mem. Univ. Calif.* 5: 1-538
- Koike, I., Holm-Hansen, O., Biggs, D.G. (1986). Inorganic nitrogen metabolism by Antarctic phytoplankton with special reference to ammonium cycling. *Mar. Ecol. Prog. Ser.* 30: 105-116
- Koroleff, F. (1983). Determination of nutrients. In: Grasshoff, K., Ehrhardt, M., Kremling, K. (ed.) *Methods of seawater analysis*. Verlag Chemie, Weinheim, p. 125-187
- Kuenzler, E.J., Stanley, D.W., Koenings, J.P. (1979). Nutrient kinetics of phytoplankton in the Pamlico River, North Carolina. Water Research Institute of the University of North Carolina, Project No. B-092-NC
- La Roche, J. (1983). Ammonium regeneration: its contribution to phytoplankton nitrogen requirements in a eutrophic environment. *Mar. Biol.* 75: 231-240
- Landry, M.R. (1981). Switching behaviour between herbivory and carnivory by the marine planktonic copepod *Calanus pacificus*. *Mar. Biol.* 65: 77-82
- Landry, M.R., Haas, L.W., Fagerness, V.L. (1984). Dynamics of microbial plankton communities: experiments in Kaneohe Bay, Hawaii. *Mar. Ecol. Prog. Ser.* 16: 127-133
- Landry, M.R., Hassett, R.P. (1982). Estimating the grazing impact of marine microzooplankton. *Mar. Biol.* 67: 283-288
- Larsson, U., Hagström, A. (1982). Fractionated phytoplankton primary production, exudate release, and bacterial production in a Baltic eutrophication gradient. *Mar. Biol.* 67: 57-70

- Laval-Peuto, M., Rassoulzadegan, F. (1988). Autofluorescence of marine planktonic oligotrichina and other ciliates. *Hydrobiologia* 159: 99-110
- Laws, E. (1984). Isotope dilution models and the mystery of the vanishing ^{15}N . *Limnol. Oceanogr.* 29: 379-386
- Le Bouteiller, A. (1986). Environmental control of nitrate and ammonium uptake by phytoplankton in the Equatorial Atlantic Ocean. *Mar. Ecol. Prog. Ser.* 30: 167-179
- Lessard, E.J., Swift, E. (1985). Species-specific grazing rates of heterotrophic dinoflagellates in oceanic waters, measured with a dual-label radioisotope technique. *Mar. Biol.* 87: 289-296
- Levander, K. (1894). Materialien zur kenntniss der wasserfauna in der umgebung von helsnigfors mit besonderer berücksichtigung der meeresfauna. I. Protozoa. *Acta Soc. Fauna Flora fennica* 12: 43-50
- Li, W.K.W., Subba Rao, D.W., Harrison, W.G., Smith, J.C., Cullen, J.J., Irwin, B., Platt, T. (1983). Autotrophic picoplankton in the tropical ocean. *Science* 219: 292-295
- Libourel Houde, S.E., Roman, M.R. (1987). Effects of food quality on the functional ingestion response of the copepod *Acartia tonsa*. *Mar. Ecol. Prog. Ser.* 40: 69-77
- Longhurst, A.R., Harrison, W.G. (1988). Vertical nitrogen flux from the oceanic photic zone by diel migrant zooplankton and nekton. *Deep Sea Res.* 35: 881-889
- MacIsaac, J.J., Dugdale, R.C. (1969). The kinetics of nitrate and ammonia uptake by natural populations of marine phytoplankton. *Deep Sea Res.* 16: 45-57
- MacIsaac, J.J., Dugdale, R.C. (1972). Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. *Deep Sea Res.* 19: 209-232
- MacKinnon, O.L., Hawes, R.S.J. (1961). An introduction to the study of protozoa. Oxford Univ. Press, Oxford.

- Malone, T.C. (1980). Size fractionated primary productivity of marine phytoplankton. In: Falkowski, P.G. (ed.) Primary productivity in the sea. Plenum Press, New York, p. 301-320
- Marin, V., Huntley, M.E., Frost, B. (1986). Measuring feeding rates of pelagic herbivores: analysis of experimental design and methods. *Mar. Biol.* 93: 49-58
- McCarthy, J.J., Taylor, R.W., Tart, J.L. (1977). Nitrogenous nutrition of the plankton in Chesapeake Bay. 1. Nutrient availability and phytoplankton preferences. *Limnol. Oceanogr.* 22: 996-1011
- McCarthy, J.J., Wynne, D., Berman, T. (1982). The uptake of dissolved nitrogenous nutrients by Lake Kinneret (Israel) microplankton. *Limnol Oceanogr.* 27: 673-680
- McManus, G.B., Fuhrman, J.A. (1986). Bacterivory in seawater studied with the use of inert fluorescent particles. *Limnol. Oceanogr.* 31: 420-426
- Mullin, M.M. (1966). Selective feeding by calanoid copepods from the Indian Ocean. In: Barnes, H. (ed.) Some contemporary studies in marine science. George Allen and Unwin, London, p. 545-554
- Murphy, L.S., Haughen, E.M. (1985). The distribution and abundance of phototrophic ultraplankton in the North Atlantic. *Limnol. Oceanogr.* 30: 47-58
- Nalewajko, C., Garside, C. (1983). Methodological problems in the simultaneous assessment of photosynthesis and nutrient uptake in phytoplankton as functions of light intensity and cell size. *Limnol. Oceanogr.* 28: 591-597
- Nicolajsen, H., Møhlenberg, F., Kiørboe, T. (1983). Algal grazing rate by the planktonic copepods *Centropages hamatus* and *Pseudocalanus* sp.: diurnal and seasonal variation during the spring phytoplankton bloom in the Oresund. *Ophelia* 22: 15-31
- Nielsen, T.G., Richardson, K. (1989). Food chain structure of the North Sea plankton communities: seasonal variations of the rôle of the microbial loop. *Mar. Ecol. Prog. Ser.* 56: 75-87

- Nival, P., Nival, S. (1976). Particle retention efficiencies of an herbivorous copepod, *Acartia clausi* (adult and copepodite stages): effects on grazing. *Limnol Oceanogr.* 21: 24-38
- Nöthig, E-M., Bodungen, B. von (1989). Occurrence and vertical flux of faecal pellets of probably protozoan origin in the southeastern Weddell Sea (Antarctica). *Mar. Ecol. Prog. Ser.* 56: 281-289
- Owens, N.J.P., Mantoura, R.F.C., Burkhill, P.H., Howland, R.J.M., Pomroy, A.J., Woodward, E.M.S. (1986). Nutrient cycling studies in Carmarthen Bay: phytoplankton production, nitrogen assimilation and regeneration. *Mar. Biol.* 93: 329-342
- Paasche, E., Kristiansen, S. (1982). Ammonium regeneration by microzooplankton in the Oslofjord. *Mar. Biol.* 69: 55-63
- Pace, M.L., Bailiff, M.D. (1987). Evaluation of a fluorescent microsphere technique for measuring grazing rates of phagotrophic microorganisms. *Mar. Ecol. Prog. Ser.* 40: 185-193
- Packard, T.T. (1973). The light dependence of nitrate reductase in marine phytoplankton. *Limnol. Oceanogr.* 18: 466-469
- Paffenhöfer, G-A. (1984). Calanoid copepod feeding: grazing on small and large particles. In: Meyers, D.G., Strickler, J.R. (ed.) *Trophic interactions within aquatic ecosystems*. Am. Assoc. Adv. Sci. Select. Symp. 85. Westview, p. 75-95
- Paffenhöfer, G-A., Knowles, S.C. (1980). Omnivorousness in marine planktonic copepods. *J. Plankton Res.* 2: 355-365
- Paffenhöfer, G-A., Van Sant, K.B. (1985). The feeding response of a marine planktonic copepod to quantity and quality of particles. *Mar. Ecol. Prog. Ser.* 27: 55-65
- Paranjape, M.A. (1987). Grazing by microzooplankton in the eastern Canadian arctic in summer 1983. *Mar. Ecol. Prog. Ser.* 40: 239-246

- Peterson, W.T., Painting, S.J., Hutchings, L. (1990). Diel variations in gut pigment content, diel vertical migration and estimates of grazing impact for copepods in the southern Benguela upwelling region in October 1987. *J. Plankton Res.* 12: 259-281
- Pitcher, G.C. (1988). Mesoscale heterogeneities of the phytoplankton distribution in St. Helena Bay, South Africa, following an upwelling event. *S. Afr. J. mar. Sci.* 7: 9-23
- Platt, T., Subba Rao, D.V., Irwin, B. (1983). Photosynthesis of picoplankton in the oligotrophic ocean. *Nature, Lond.* 301: 702-704
- Porter, K.G., Sherr, E.B., Sherr, B.F., Pace, M., Sanders, R.W. (1985). Protozoa in planktonic food webs. *J. Protozool.* 32: 415-423
- Poulet, S.A. (1973). Grazing of *Pseudocalanus minutus* on naturally occurring particulate matter. *Limnol. Oceanogr.* 18: 564-573
- Poulet, S.A. (1978). Comparison between five coexisting species of marine copepods feeding on naturally occurring particulate matter. *Limnol. Oceanogr.* 23: 1126-1143
- Poulet, S.A., Marsot, P. (1978). Chemosensory grazing by marine calanoid copepods (Arthropoda: Crustacea). *Science* 200: 1403-1405
- Probyn, T.A. (1985). Nitrogen uptake by size-fractionated phytoplankton populations in the southern Benguela upwelling system. *Mar. Ecol. Prog. Ser.* 22: 249-258
- Probyn, T.A. (1987). Ammonium regeneration by microplankton in an upwelling environment. *Mar. Ecol. Prog. Ser.* 37: 53-64
- Probyn, T.A., Lucas, M.I. (1987). Ammonium and phosphorous flux through the microplankton community in Agulhas Bank waters. In: Payne, A.I.L., Gulland, J.A., Brink, K.H. (ed.) *Benguela* 86. *S. Afr. J. mar. Sci.* 5: 209-221

- Probyn, T. A., Painting, S.J. (1985). Nitrogen uptake by size-fractionated phytoplankton populations in Antarctic surface waters. *Limnol. Oceanogr.* 30: 1327-1332
- Probyn, T. A., Waldron, H.N., James, A.G. (1990). Size-fractionated measurements of nitrogen uptake in aged upwelled waters: Implications for pelagic food webs. *Limnol. Oceanogr.* 35: 202-210
- Putt, M., Stoecker, D.K. (1989). An experimentally determined carbon:volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. *Limnol. Oceanogr.* 34: 1097-1103
- Rassoulzadegan, F. (1982). Dependence of grazing rate, gross growth efficiency, and the food size range on temperature in a pelagic oligotrichous ciliate, *Lohmanniella spiralis* Leeg., fed on naturally occurring particulate matter. *Annls Inst. océanogr.*, Paris 58: 177-184
- Rassoulzadegan, F., Etienne, M. (1981). Grazing rate of the tintinnid *Stenosomella ventricosa* (Clap. et Lach.) Jorg. on the spectrum of the naturally occurring particulate matter from a Mediterranean neritic area. *Limnol. Oceanogr.* 26: 2258-2270
- Rassoulzadegan, F., Laval-Peuto, M., Sheldon, R.W. (1988). Partitioning of the food ration of marine ciliates between pico- and nanoplankton. *Hydrobiologia* 159: 75-88
- Redfield, A.C. 1934. On the proportions of organic derivatives in seawater and their relation to the composition of plankton. In: James Johnstone Memorial Volume, Liverpool University Press, p. 176-192
- Rivier, A., Brownlee, D.C., Sheldon, R.W., Rassoulzadegan, F. (1985). Growth of microzooplankton: a comparative study of bacterivorous zooflagellates and ciliates. *Mar. Micro. Food Webs* 1: 51-60

- Robertson, J. R. (1983). Predation by estuarine zooplankton on tintinnid ciliates. *Estuar. coast Shelf Sci.* 16: 27-36
- Ryther, J.H., Menzel, D.W., Hulburt, E.M., Lorenzen, C.J., Corwin, N. (1971). Production and utilization of organic matter in the Peru coastal current. Anton Bruun Rep. No. 4, Texas A & M Press, College Station
- Sellner, K.G. (1983). Plankton productivity and biomass in a tributary of the upper Chesapeake Bay. I. Importance of size-fractionated phytoplankton productivity, biomass and species composition in carbon export. *Est. coast. Shelf Sci.* 17: 197-206
- Selmer, J-S. (1988). Ammonium regeneration in eutrophicated coastal waters of Sweden. *Mar. Ecol. Prog. Ser.* 44: 265-273
- Sherr, B.F., Sherr, E.B. (1984). Rôle of heterotrophic protozoa in carbon and energy flow in aquatic ecosystems. In: Klug, M.J., Reddy, C.A. (ed.) *Current perspectives in microbial ecology.* American Society for Microbiology, Washington, p. 412-423
- Sherr, B.F., Sherr, E.B., Newell, S.Y. (1984). Abundance and productivity of heterotrophic nanoplankton in Georgia coastal waters. *J. Plankton Res.* 6: 195-202
- Sherr, B.F., Sherr, E.B., Pedrós-Alió, C. (1989a). Simultaneous measurement of bacterioplankton production and protozoan bacterivory in estuarine water. *Mar. Ecol. Prog. Ser.* 54: 209-219
- Sherr, E.B., Rassoulzadegan, F., Sherr, B.F. (1989b). Bacterivory by pelagic choreotrichous ciliates in coastal waters of the NW Mediterranean Sea. *Mar. Ecol. Prog. Ser.* 55: 235-240
- Sherr, E.B., Sherr, B.F. (1987). High rates of consumption of bacteria by pelagic ciliates. *Nature, Lond.* 325: 710-711
- Sherr, E.B., Sherr, B.F. (1988). Rôle of microbes in pelagic food webs: A revised concept. *Limnol. Oceanogr.* 33: 1225-1227

- Sherr, E.B., Sherr, B.F., Albright, L.J. (1987). Bacteria: Link or sink? *Science* 235: 88-89
- Sherr, E.B., Sherr, B.F., Fallon, R.D., Newell, S.Y. (1986a). Small, aloricate ciliates as a major component of the marine heterotrophic nanoplankton. *Limnol. Oceanogr.* 31: 177-183
- Sherr, E.B., Sherr, B.F., Paffenhöfer, G-A. (1986b). Phagotrophic protozoa as food for metazoans: a "missing" trophic link in marine pelagic food webs? *Mar. Microb. Food Webs* 1: 61-80
- Sieburth, J. McN., Davis, P.G. (1982). The rôle of heterotrophic nanoplankton in the grazing and nurturing of planktonic bacteria in the Sargasso and Caribbean Sea. *Annl Inst. océanogr. Paris* 58(S): 285-296
- Sieburth, J. McN., Smetacek, V., Lenz, J. (1978). Pelagic ecosystem structure: heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnol. Oceanogr.* 23: 1256-1263
- Small, L.F., Fowler, S.W., Moore, S.A., LaRosa, J. (1983). Dissolved and fecal pellet carbon and nitrogen release by zooplankton in tropical waters. *Deep Sea Res.* 30: 1199-1220
- Smetacek, V. (1981). The annual cycle of the protozooplankton in Kiel Bight. *Mar. Biol.* 63: 1-11
- Smith, S.L. (1978). The rôle of zooplankton in the nitrogen dynamics of a shallow estuary. *Estuar. coast. Shelf Sci.* 7: 555-565
- Smith, S.L., Whitley, T.E. (1977). The rôle of zooplankton in regeneration of nitrogen in a coastal upwelling system off northwest Africa. *Deep Sea Res.* 24: 49-56
- Sorokin, Y.I. (1979). Zooflagellates as a component of the eutrophic and oligotrophic waters in the Pacific Ocean. *Oceanology* 19: 316-319

- Spero, H.J. (1982). Phagotrophy in *Gymnodinium fungiforme* (Pyrrhophyta): the peduncle as an organelle of ingestion. *J. Phycol.* 18: 356-360
- Spero, H.J., Morée, M.D. (1981). Phagotrophic feeding and its importance to the life cycle of the holozoic dinoflagellate, *Gymnodinium fungiforme*. *J. Phycol.* 17: 43-51
- Steele, J.H. (1974). The structure of marine ecosystems. Harvard Univ. Press, Cambridge, Massachusetts
- Stoecker, D.K., Egloff, D. (1987). Predation by *Acartia tonsa* Dana on planktonic ciliates and rotifers. *J. Exp. Mar. Biol. Ecol.* 110: 53-68
- Stoecker, D.K., Guillard, R.R.L., Kavee, R.M. (1981). Selective predation by *Favella ehrenbergii* (Tintinnina) on and among dinoflagellates. *Biol. Bull. mar, biol. Lab. Woods Hole* 160: 136-145
- Stoecker, D.K., Taniguchi, A., Michaels, A.E. (1989). Abundance of autotrophic, mixotrophic and heterotrophic planktonic ciliates in shelf and slope waters. *Mar. Ecol. Prog. Ser.* 50: 241-254
- Taguchi, S., Laws, E.A. (1989). Biomass and compositional characteristics of Kaneohe Bay, Oahu, Hawaii, phytoplankton inferred from regression analysis. *Pac. Sci.* 43: 316-331
- Tiselius, P. (1989). Contribution of aloricate ciliates to the diet of *Acartia clausi* and *Centropages hamatus* in coastal waters. *Mar. Ecol. Prog. Ser.* 56: 49-56
- Turner, J.T., Anderson, D.M. (1983). Zooplankton grazing during dinoflagellate blooms in a Cape Cod embayment, with observations of predation upon tintinnids by copepods. *P.S.Z.N.I. Mar. Ecol.* 4: 359-374
- Turner, J.T., Tester, P.A. (1989). Zooplankton feeding ecology: nonselective grazing by the copepods *Acartia tonsa* Dana, *Centropages velificatus* De Oliveira, and *Eucalanus pileatus* Giesbrecht in the plume of the Mississippi River. *J. exp. mar. Biol. Ecol.* 126: 21-43

- Vargo, G.A. (1979). The contribution of ammonia by zooplankton to phytoplankton production in Narragansett Bay. *J. Plankton Res.* 1: 75-84
- Verity, P.G. (1985). Grazing, respiration, excretion and growth rates of tintinnids. *Limnol. Oceanogr.* 30: 1268-1282
- Verity, P.G. (1986). Grazing of phototrophic nanoplankton by microzooplankton in Narragansett Bay. *Mar. Ecol. Prog. Ser.* 29: 105-115
- Walsh, J.J. (1983). Death in the sea: enigmatic phytoplankton losses. *Prog. Oceanogr.* 12: 1-86
- Wiadnyana, N.N., Rassoulzadegan, F. (1989). Selective feeding of *Acartia clausi* and *Centropages typicus* on microzooplankton. *Mar. Ecol. Prog. Ser.* 53: 37-45
- Williams, P.J. LeB. (1981). Incorporation of microheterotrophic processes into the classical paradigm of the planktonic food web. *Kieler Meeresforsch. (Sonderh.)* 5: 1-28