Microzooplankton herbivory and community structure

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INTRODUCTION

Microzooplankton, comprising protozoa and metazoa <200 µm in length, form a significant proportion of the total zooplankton biomass in oceanic environments and are important in controlling phytoplankton production. They play an important role as trophic intermediaries in pelagic food webs by acting as a link between phytoplankton and larger consumers such as copepods. Rates of grazing by microzooplanton on phytoplankton in natural waters may be estimated using the "dilution approach" first described by Landry and Hassett (1982). This technique provides information about the dynamics of the whole phytoplankton community and can be carried out routinely at sea.

The experimental technique is based on the determination of phytoplankton growth in a dilution series. This dilution series is made up of the natural community in conjunction with particle-free seawater. Phytoplankton growth is assumed to be density independent with specific growth rates that are constant under all conditions of dilution. To satisfy this assumption, dissolved nutrients must remain non-limiting, or equally limiting, to growth at all dilutions during the incubation. The probability of a phytoplankton cell being grazed is assumed to be dependent on the encounter frequency between predator and prey and that the grazers feed at a constant rate. Consequently there is a progressive uncoupling, between phytoplankton growth and mortality due to grazing, with dilution. It is also assumed that phytoplankton growth with time is exponential. The relationship between observed growth rate of phytoplankton and relative concentration of prey should yield a negative slope which corresponds to the magnitude of natural grazing mortality.

Microzooplankton abundance and biomass can be determined from analysis of fixed samples by means of inverted and epifluorescence microscopy. In this report we will deal only with the Protozoan component of the microzooplankton, this includes the heterotrophic nanoflagellates (HNF).

This project contributes to WP I, II and IV although the main focus of our efforts in this second year has been in WP I.

METHODS

Task I.4 (c), II.10.2, IV.2 Microzooplankton herbivory

During August 1998 we participated in the Work Package I cruise, *Charles Darwin CD114*. The cruise was divided into two Legs each a short-term 'Lagrangian' drift experiment. Leg 1 followed a patch of upwelled water as it moved south along the shelf; Leg 2 followed a filament that extended off the shelf. Conditions encountered between both Legs differed significantly. Leg 1 was characterised by high nutrients and elevated chlorophyll concentrations whilst on Leg 2 nutrient and chlorophyll levels were low. A total of 12 microzooplankton dilution grazing experiments were carried out during Legs 1 and 2 of *CD114*. Six experiments were carried out during each Lagrangian experiment. Experimental water was collected pre-dawn from a depth of 10 m using a 30-L Go-Flo bottle. Half of this water was gravity filtered through a 0.2–µm Gelman Suporcap filter which had been pre-soaked in Milli-Q water overnight. The remaining water was pre-screened using a 200 µm mesh bag to exclude larger predators. A series of dilutions were made up by gently combining the screened water with the filtered water in 1- or 2-litre polycarbonate bottles. To determine the potential effects of nutrient limitation three additional bottles of undiluted seawater sample were enriched with a nutrient mixture of 0.5 µM NH_4, 0.03 µM PO_4, 1.0 nM $FeSO_4$ and 0.1 nM $MnSO_4$. All incubations were carried out over a 24-hr period in an ambient temperature-cooled deck incubator screened to the 33% light level. Sub-samples were taken at T_0 and T_{24} for the determination of (*i*) chlorophyll concentration (*ii*) microzooplankton and HNF biomass and community structure and (*iii*) nutrient concentration.

Task I.4 (d), II.5.5, II.9.1, II.9.2 Microzooplankton community structure

Microzooplankton samples for the determination of abundance, biomass and community structure have been collected from 6 OMEX cruises. Heterotrophic nanoflagellates cannot be distinguished from autotrophic cells by ordinary light microscopy. Therefore, in order to determine the abundance, biomass and community structure of both the HNF and other protozoa (10-200 μm), samples must be treated using two different protocols.

Table 1: Summary of microzooplankton samples collected on OMEX cruises 1997-98

CRUISE	HNF	Other Protozoa
CD105	✓	✓
CD110		✓
Poseidon P237/1	✓	✓
Belgica BG9815		✓
Shtokman		✓
CD114	✓	✓

HNF: Approximately 30-50 ml of water sample were fixed in 0.5% glutaraldehyde, dual-stained with DAPI and proflavine (final concentration 5 μg ml^{-1}) and filtered onto 0.8 μm black polycarbonate filters. The filters were mounted onto slides and frozen until analysed in the laboratory by inverted fluorescence microscopy. The HNF comprise the heterotrophic nanoflagellates (HNAN) and the heterotrophic nanodinoflagellates (HNDINOS)

Protozoa 20-200 μm: 500 ml water samples were fixed in 1% acid Lugol's solution. These samples will be analysed at PML using inverted microscopy and image analysis for the determination of total abundance, biomass and species composition. Individual cells were categorised into one of 4 groups based on their morphological structure: heterotrophic dinoflagellates (HDINOS), aloricate oligotrich ciliates (OLIGOS), tintinnids (TINTINS) and 'Other Ciliates'. Measurements of each cell-type, determined by image analysis, have been used to determine cell volume, this in turn has been multiplied by the following volume to carbon conversion factors (literature values) to compute biomass.

Category	C:vol Conversion	Reference
HNAN	0.22 pg C $cell^{-1}$	Borshein and Bratbak (1987)
HDINOS and HNDINOS	0.14 pg C $cell^{-1}$	Lessard (1991)
OLIGOS	0.19 pg C $cell^{-1}$	Putt and Stoecker (1989)
Other ciliates and TINTINS	0.19 pg C $cell^{-1}$	Putt and Stoecker (1989)

Biomass values will be converted to standing stocks by integration through the surface mixed layer.

TASK SPECIFIC RESULTS AND DISCUSSION

Task I.4 Zooplankton and microbial cycling (*also for* ***Task IV.2, IV.3)***

Objectives: (*c*) To experimentally quantify the trophic impact of microzooplankton grazing on phytoplankton using short-term shipboard experiments (*d*) to quantify microzooplankton distribution and standing stocks associated with Lagrangian drift experiments

Task I.4 (c) Grazing rates in terms of the total amount of chlorophyll grazed per day have been determined from each experiment carried out on *CD114*. We have converted this to Carbon using a carbon: chlorophyll ratio of 68 (POC data from IIM). From this data we were able to calculate the proportion of primary production (PML-c) grazed by the microzooplankton daily shown in Figure 1. The impact of microzooplankton on primary production was most pronounced at the beginning of the shelf experiment, thereafter decreasing from 80% to <40% of the production being grazed daily at the

end of the drift experiment. Microzooplankton herbivory was much lower within the filament but the proportion of primary production consumed still in the region of 40% daily.

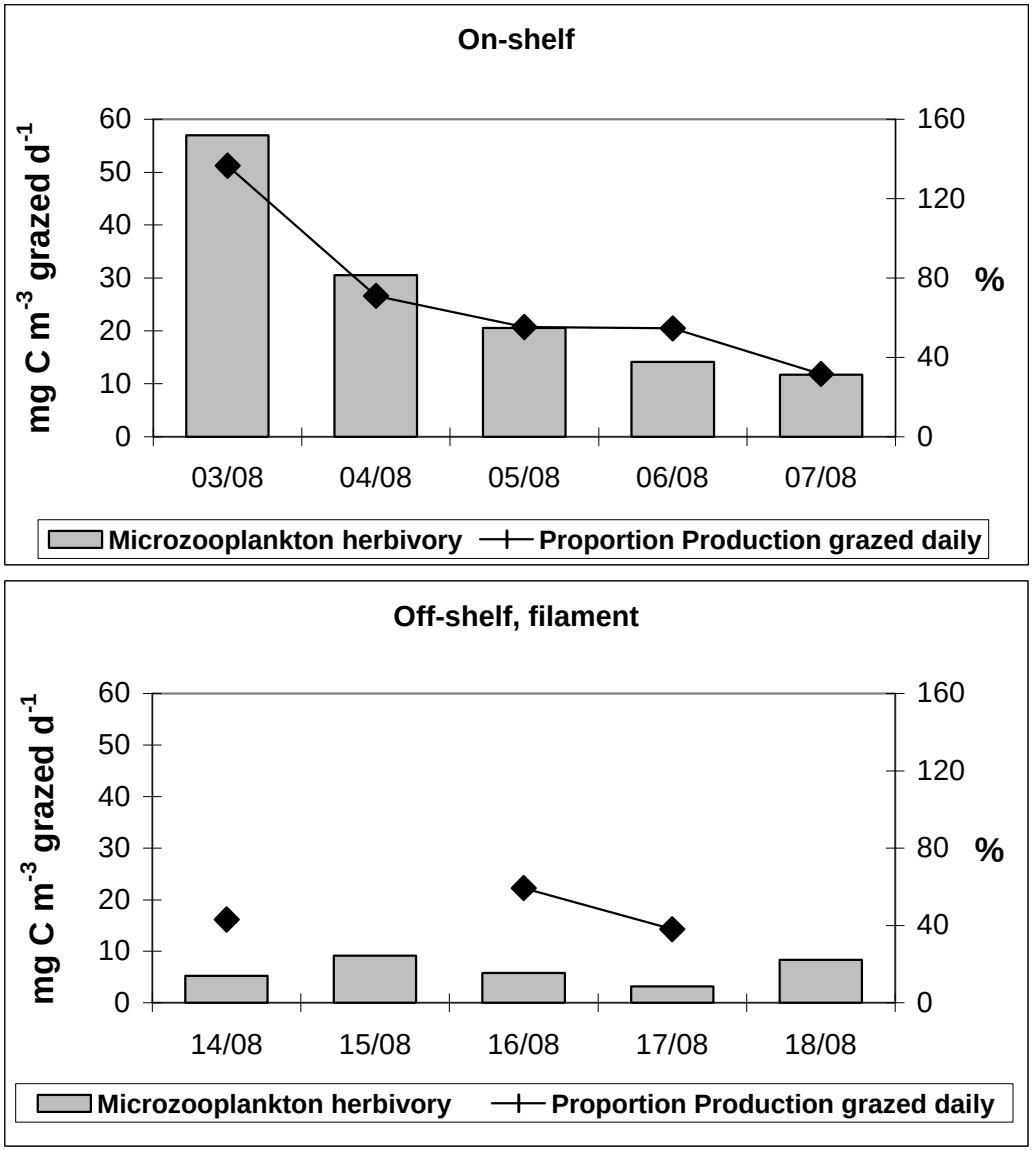

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Figure 1: The trophic impact of microzooplankton grazing on phytoplankton during short-term shipboard experiments during CD114.

Task I.4 (d) Water samples for the determination of microzooplankton abundance and biomass were collected from 8 depths within the top 200 m of the water column from 12 dawn CTD casts. All HNF samples have been analysed and analysis of Lugol's fixed samples from 5 and 10 m and some deeper samples is also complete.

The vertical profile of protozoan and HNF biomass on Day 1 during the shelf study (Figure 2) clearly shows maximum biomass at a depth of 10 m.

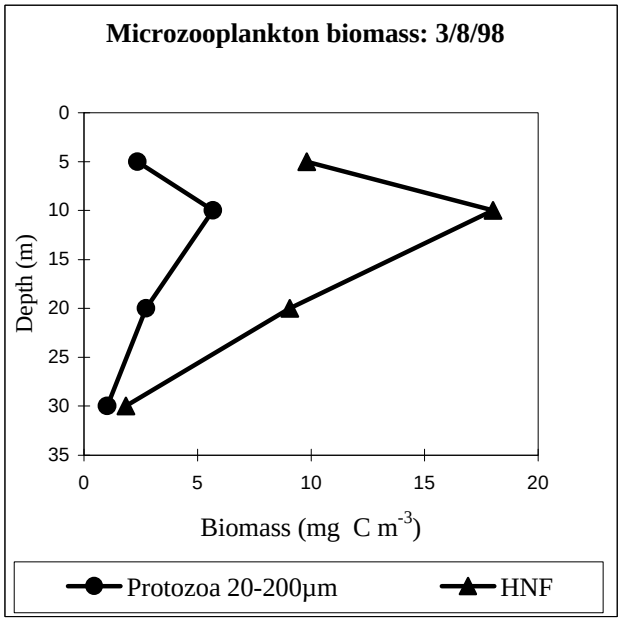

Figure 2: An example of the vertical distribution of microzooplankton biomass during Shelf Lagrangian experiment (CD114).

The microzooplankton community showed clear differences between the two 'Lagrangian' experiments. Microzooplankton abundance was high, particularly in surface waters on the shelf where concentration of protozoa (10-200 µm) ranged from 15,000 to 58,000 cells l^{-1} (mean 23095 cells l^{-1}) and concentration of HNF was between 200 and 700 cells ml^{-1} (mean 413 cells ml^{-1}). In the filament, HNF concentration averaged 248 cells ml^{-1} and mean protozoan concentration 3685 cells l^{-1}. Microzooplankton biomass increased from 16 to 44 mg C m^{-3} on the shelf but was much lower and less variable within the filament, around 10-12 mg C m^{-3} (Figure 3). It is interesting that the increasing trends in microzooplankton abundance and biomass do not match the decreasing grazing trend. This could be due to changes in the phytoplankton community and further analysis of the data should enable us to determine whether this is so.

The microzooplankton community during the shelf experiment, was dominated initially by HNF and HDINOS with OLIGOS and Other Ciliates increasing in importance with time and by 6/8 they comprised more than 50% of the biomass. One important finding during this study was the high contribution of the heterotrophic nanoflagellates to the total microzooplankton biomass. This was particularly pronounced within the filament where they comprised up to 90% of the total biomass. Tintinnids and other ciliates were very low in abundance in surface waters within the filament. The data also indicate that within the protozoan 10-200 µm size fraction, cells were larger, almost double, during the filament study. Further investigations into differences in size structure will be carried out in year 3.

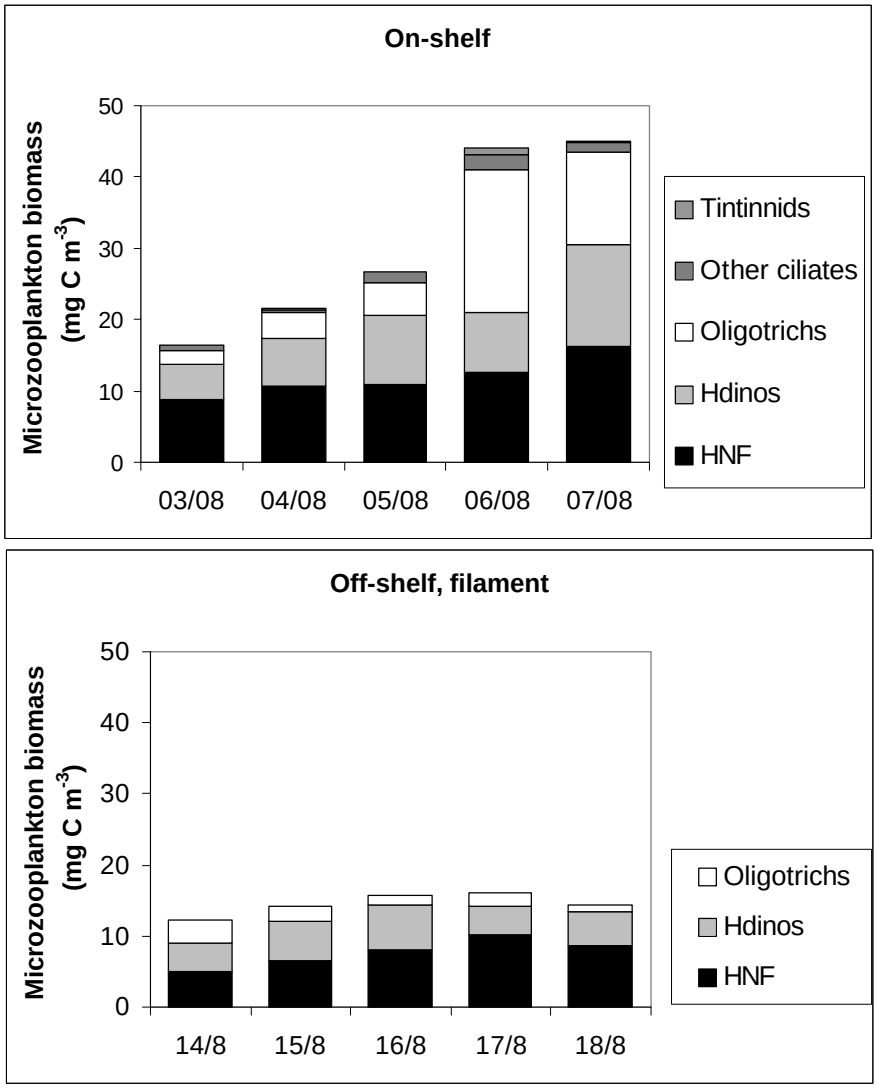

Figure 3: Average microzooplankton biomass in top 10 m of water column during Shelf and filament Lagrangian drift experiments, showing relative contributions of the different taxonomic groups to total biomass.

Task II.5 Source markers of particulate carbon

Task II.5.5 *Biomass carbon*
Objective: To convert or determine the biomasses of bacteria, phytoplankton, micro- and mesozooplankton into organic carbon inventories.

Microzooplankton data are being generated from 5 cruises. Integration of this data together with bacteria, phytoplankton and mesozooplankton datasets from relevant partners has been initiated. The following table has been drawn up in order to identify comparable datasets. In some instances data are available by other partners not specifically involved in this task.

Carbon Component	Partner	*CD105*	*Belgica BG9714*	*CD110*	*Poseidon*	*Belgica BG9815*	*Prof. Shtokman*
BACTERIA	UAL-a			✓		✓	✓
MICROZOOPLANKTON	PML-b	✓		✓	✓	✓	✓
MESOZOOPLANKTON	SOC		✓	✓			
	SAHFOS	Monthly tows within study area					19 UOv
PHYTOPLANKTON	PML-a/c	✓	IIM	✓ IIM	✓	✓	20 IEO

Table 2: Summary of datasets available from WP II cruises which will be used to estimate total biomass Carbon for ***Task II.5.5****.*

Task II.9 Microbial populations as pelagic sinks (*also* ***Tasks IV.2, IV.3)***

Task II.9.1 *Distribution of Bacteria and Microzooplankton*
Objective: To determine the seasonal 3-D distribution of bacteria and microzooplankton biomass in the upwelling regions of the Iberian upwelling system.

Microzooplankton samples (Lugol's fixed only) have been collected on a further 2 WP II cruises this year (*Belgica BG9815*, June/July - 16 stations N/P/S transects and *Prof. Shtokman*, Aug - 15 stations again N/P and S transects). Sample analysis by inverted microscopy has begun, initially concentrating on the P-transect. Not surprisingly, lowest biomass levels of <2 mgC m^{-3} have been recorded in January and highest biomass of almost 15 mgC m^{-3} in August. During March and August microzooplankton biomass was highest on the shelf, but this trend is not so evident during July, further analysis will be carried out to address this.

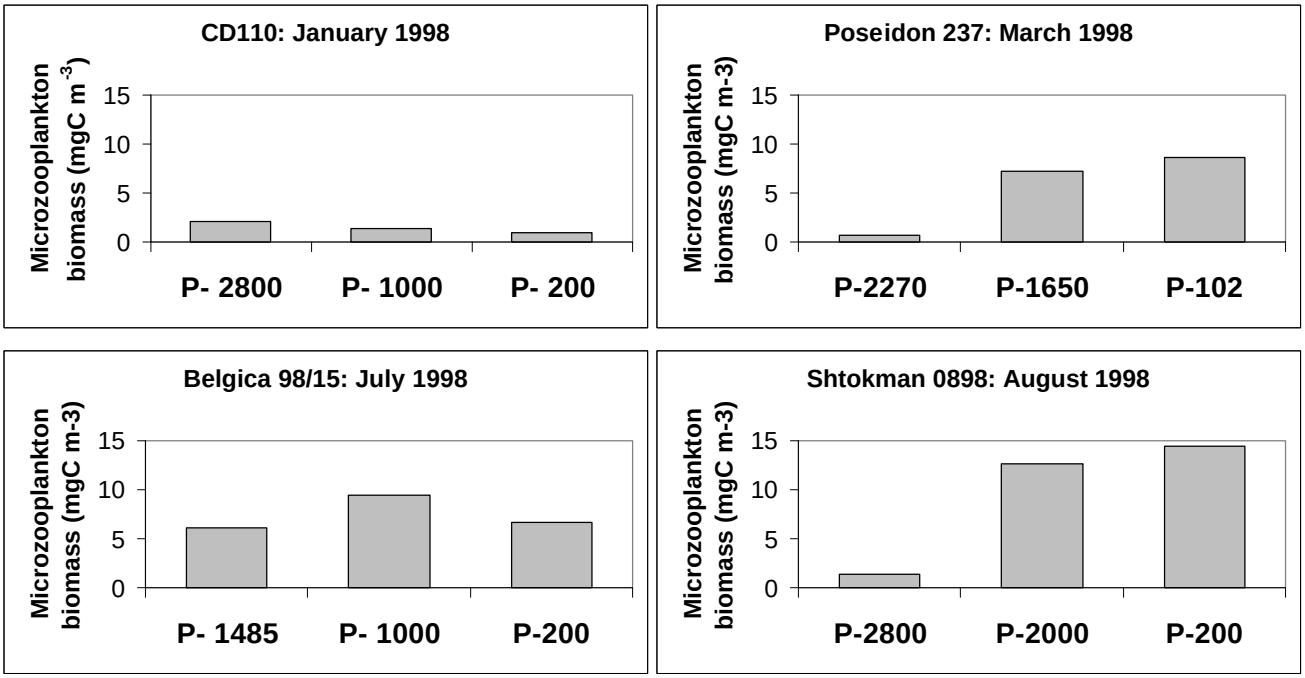

Figure 4: Seasonal study of microzooplankton biomass along the P transect, determined from lugol's fixed samples, collected from WP II cruises during 1998.

Protozoan Community Structure

Although there are slight differences within the 20-200 µm protozoan community in surface waters the most important taxonomic group was found to be the OLIGOS. This is typical for many marine protozoan communities. Further analysis will allow us to determine whether the community structure changes with depth and whether these changes are due to differences in the phytoplankton community.

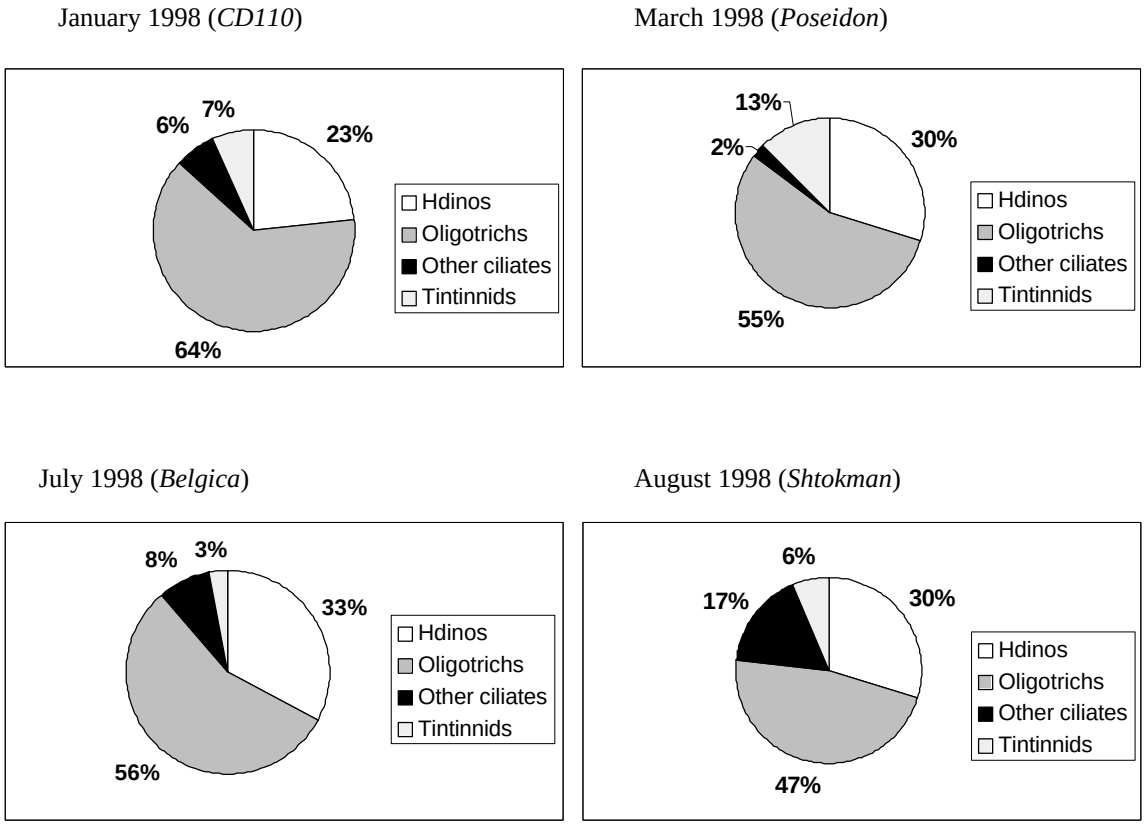

Figure 5: Pie charts showing the seasonal change in biomass of the different taxonomic components of the microzooplankton community in surface waters during 1998.

Analysis of 72 HNF samples collected during *Poseidon P237/1* has been completed and data on their abundance and biomass will be deposited with BODC by the end of July. Concentration of HNF in surface waters was found to be similar, with a mean of 391 cells ml^{-1} (± 65) at shelf stations with a water depth of <550 m, 391 cells ml^{-1} (± 116) at stations between 550 and 2000 m and 417 cells ml^{-1} (± 65) at offshore stations with water depths > 2000 m. Average HNF biomass for each station ranged from 10 to 30 mgC m^{-3} and was highest at station S1479. HNF comprised a high proportion of the total microzooplankton biomass. This was most pronounced at offshore stations (Figure 5).

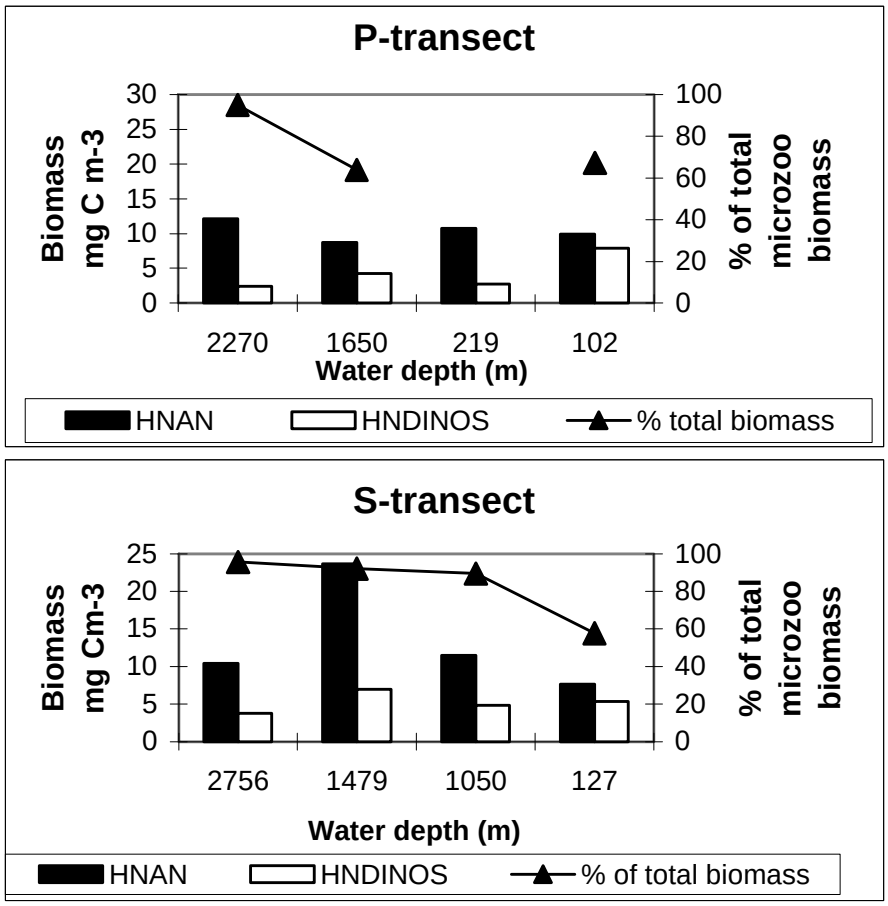

Figure 6: Mean HNF biomass along transects P and S during March 1998.

Task II.9.2 *Nitrogen and CO_2 Regeneration by Bacteria, Micro- and Mesozooplankton*
As for **Task II.9.1**, further samples have been collected and once analysed the respiratory role of microzooplankton will be determined using methods adopted in OMEX I.

FUTURE WORK
Much of our work over the next 12 months will involve analysis of Lugols fixed samples, although this is a lengthy process it is envisaged that a large proportion of the samples collected will be analysed and the data deposited at BODC by the end of Dec 1999. Results from completed HNF analysis will be carefully checked before submission to BODC by the end of July. With the aid of microscopic counts of predator densities, microzooplankton grazing rates will be interpreted, finalised and sent to BODC. These rate measurements will then be used to develop relationships which when applied to biomass data will provide indirect estimates of grazing for WP II **Task II.10.2**. One manuscript on WP I data to be included in the WP I Special Issue Publication is planned for submission at month 36.

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