# UNIVERSITY OF SOUTHAMPTON

Use of the Dilution Technique to Determine Microzooplankton Herbivory in Three Contrasting Oceanic Systems.

By

Elaine Susan Fileman

Thesis submitted for the degree of Master of Philosophy

School of Biological Sciences

November 2000

This thesis is dedicated to my mother *Beryl Edwards* who passed away 28th July 2001.

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

SCHOOL OF BIOLOGICAL SCIENCES

Master of Philosophy

# USE OF THE DILUTION TECHNIQUE TO DETERMINE MICROZOOPLANKTON HERBIVORY IN THREE CONTRASTING OCEANIC SYSTEMS

by Elaine Susan Fileman

The dilution technique has been used during both laboratory experiments and in the field in three contrasting oceanic systems: the northeast Atlantic, Bellingshausen Sea and Arabian Sea. Laboratory dilution grazing studies, using *Oxyrrhis marina* as the predator and *Dunaliella tertiolecta* as prey, have demonstrated a significant linear relationship between apparent phytoplankton growth and dilution factor. The daily turnover of prey by *Oxyrrhis* ranged between 45 and 95%. Growth of prey was constant at all dilutions demonstrating that phytoplankton growth was density independent. Studies carried out during summer along a transect in the northeast Atlantic between 60°N and 47°N showed microzooplankton to consume between 0.5 and 17mg phytoplankton C $m^{-3}$ $d^{-1}$. This was equivalent to between 288 and 589 mg C $m^{-2}$ $d^{-1}$ being grazed in the mixed layer. Microzooplankton grazing impact was highest at the southerly end of the transect. During the spring-bloom period in 1990, microzooplankton grazed between 28 and 78% and between 26 and 55% of the daily $^{14}C$ primary production in May and June respectively. The microzooplankton community was dominated by small cells (<20µm). In the Bellingshausen Sea, microzooplankton consumed between 0.04 and 31.21 mg C $m^{-3}$ $d^{-1}$, and were estimated to graze between 21 and 3260 mg C $m^{-2}$ $d^{-1}$ in the pack ice and open water station respectively. In the Arabian Sea, microzooplankton grazed between 1 and 17 mg C $m^{-3}$ $d^{-1}$. In the mixed layer microzooplankton grazed between 161 and 415 mg C $m^{-2}$ $d^{-1}$ during the SW monsoon and between 110 and 407 mg C $m^{-2}$ $d^{-1}$ during the intermonsoon period. Substantial grazing of phytoplankton biomass by the microzooplankton occurred in all three oceanic systems. Variations in the grazing and phytoplankton growth rates can be attributed to differences in the phytoplankton and microzooplankton communities encountered. Results demonstrate a tight coupling between growth of prey and consumption for the northeast Atlantic and Bellingshausen Sea.

# List of Contents

# List of Tables

# List of Figures

# Acknowledgements

My thanks go to my two supervisors Professors Michael Sleigh and Peter Burkill. Without the help and encouragement from Prof Sleigh this work would not have been completed and I am extremely grateful to him for this. My thanks also go to Tony John, for many helpful discussions concerning identification during the early BOFS years, and Dr Ray Leakey, for his continued enthusiasm and motivation. Finally thanks go to my husband Tim, for encouraging me to 'finish the job'.

The research described within this thesis was supported primarily through the NERC-BOFS (Natural Environment Research Council – Biogeochemical Ocean Flux Study) community research project based at Plymouth Marine Laboratory (NERC, 1989-1992). This included a NERC Special Topic award GST/02/375 and Aquatic Life Sciences grant GR3/8469. MOD/DRA Joint Grant TQ/10/3/2 funded research in the Arabian Sea.

# 1. INTRODUCTION

## *1.1 What are microzooplankton?*

Microzooplankton are microscopic, phagotrophic animal forms, which were originally classified as those organisms which pass through a 200$\mu$m mesh (Dussart 1965; Beers & Stewart 1967). However, since then, Sieburth et al 1978, proposed the use of size as a classification criterion whereby the microzooplankton are defined as heterotrophic organisms between 20 and 200$\mu$m and those from 2 to 20$\mu$m are termed the nanoplankton. For the purpose of this study, the term 'microzooplankton' will refer to the whole heterotrophic protozoan and metazoan assemblage, from 2 to 200$\mu$m. The term heterotrophic nanoplankton (HNAN) will be used to define heterotrophic nano- and dinoflagellates in the 2 to 20$\mu$m size category and heterotrophic microplankton (HMIC) will refer to all heterotrophic forms which are not heterotrophic nanoplankton (Figure 1.1). The HMIC can be further sub-divided into the protistan heterotrophic microplankton (PHMIC) and the metazoan heterotrophic microplankton (MHMIC).

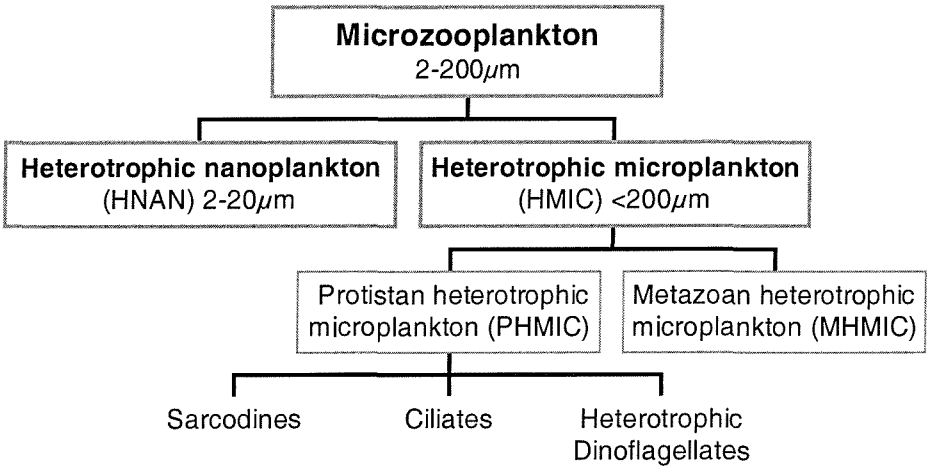



Figure 1.1: Organisation chart showing different groupings within the microzooplankton

There is a tremendous taxonomic diversity within the microzooplankton. The HMIC contains protistan taxa, which can be categorised into one of several groups (heterotrophic dinoflagellates, ciliates and sarcodines) as well as metazoan taxa. They have ubiquitous distribution, ingest a wide variety of food types and are in turn consumed by larger animals. As well as being capable of controlling algal and bacterial production, they have high growth rates, high growth efficiencies and have a major impact on nutrient cycling. Short generation times mean that they are able to

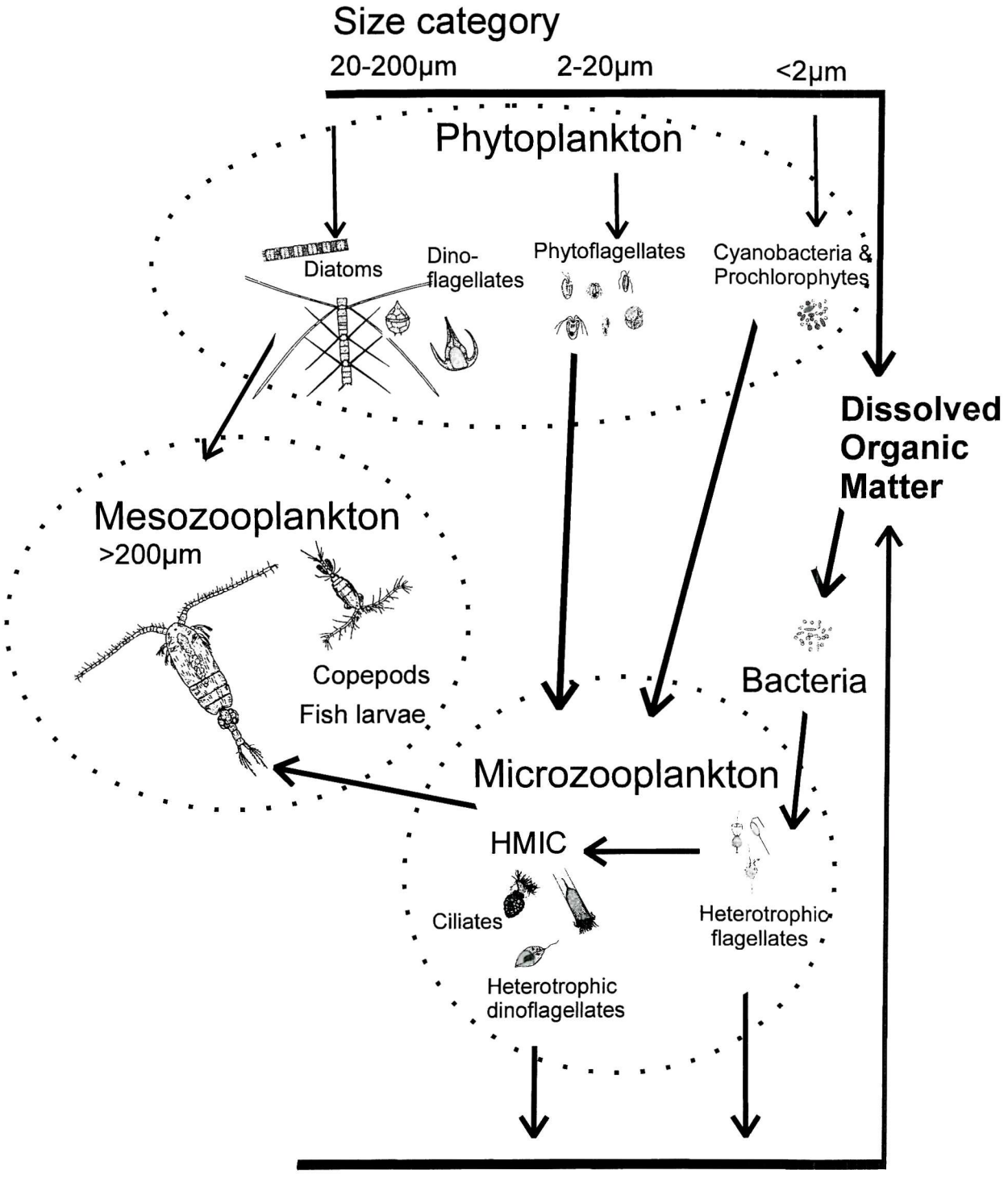
respond rapidly to changes in the availability of food, which gives them a distinct advantage over larger organisms with longer, more complex life cycles. Efforts to study the trophic role of these organisms are complicated by the fact that they exhibit a variety of complex trophodynamic interactions. The diets of small flagellates seem to be restricted to bacteria-sized particles (Sherr et al 1984). Most marine planktonic ciliates feed on prey that is about 10 times smaller in size (Jonsson 1986). Heterotrophic dinoflagellates are raptorial feeders capable of feeding on prey similar to, or greater than, their own body size (Jacobsen & Andersen 1986, Hansen 1992). Larger dinoflagellates have been found to feed on chain forming diatoms, other dinoflagellates, flagellates and ciliates (Jacobsen & Anderson 1986, Gaines & Elbrachter 1987). Studies on smaller dinoflagellates suggest that they may compete with ciliates for nanoplankton prey (Bjørnsen & Kuparinen 1991, Strom 1991). Many oligotrich ciliates exhibit mixotrophy (Stoecker 1991) and some can function as primary producers because they contain functional autotrophic endosymbionts (Laval-Pueto & Rassoulzadegan 1988). Grazing by metazoans on ciliates has also been reported and can be significant (Stoecker & Sanders 1985, Fessenden & Cowles 1994, Nielsen & Kiorboe 1994).

*1.2 History*

The study of microzooplankton dates back as far as 1676, with the first mention of marine protozoa in a publication by Van Leeuwenhoek (1677). Then during the late 1800s and early 1900s taxonomic studies were carried out by researchers such as Hensen 1911, Kofoid 1897, Lohmann 1903, Fauré-Fremiet 1924 and Kofoid & Campbell 1929. Lohmann 1908, 1920 was the first person to consider estimations of microzooplankton population structure and abundance in marine trophic ecology and he suggested that if protozoans consumed half their own body volume per day they could remove approximately 40% of daily production (Lohmann 1908). Despite the earlier recognition that these tiny organisms exist, most oceanographic and limnological methods adopted during early expeditions through to the late 1950s were designed for sampling and fixing larger metazoa and were inadequate for most of the smaller and more delicate protozoa. Interest in microzooplankton was revived in the 1960s with the collection of quantitative data on the distribution and abundance patterns of planktonic protozoa (e.g. Zeitzschel 1967, 1969; Beers & Stewart 1967, 1969, 1970,1971). It was only in the 1970s that progress began to be made in understanding the dynamic role of microzooplankton in the marine food web. There was a general trend towards the study of ecosystems as functional

entities and it became important to identify the role of all components of the ecosystem rather than just the large organisms. Prior to the 1970's, studies of diatoms and the fate of these large primary producers through simple food chains dominated marine pelagic ecology. Developments in methodologies and techniques led to closer inspection of the microbial community and it soon became clear that nanophytoplankton (<10$\mu$m) generally dominated both the biomass and productivity of the total phytoplanktonic assemblage. Copepods, which had been thought of as the dominant herbivore in the sea, were shown to be poorly adapted for the capture and ingestion of the smaller phytoplankton and it was the microzooplankton that were shown to be important consumers of phytoplankton in marine planktonic communities (Heinbokel 1978, Heinbokel & Beers 1979). Pomeroy (1974) focussed the attention on the significance of micro-organisms as transformers of energy and materials in marine waters and this lead to the development of the microbial loop hypothesis (Azam et al 1983). In this hypothesis, a significant proportion of the photosynthetic carbon is released as dissolved organic material (DOM). The heterotrophic bacteria then take up DOM and these, in turn, are grazed by the flagellates, which are in turn grazed by the larger heterotrophic microplankton (Figure 1.2). The amount of phytoplankton production being channelled through this loop depends on the composition of both the phytoplankton and the food web. It was unclear whether the production of the 'microbial loop' is efficiently transferred into the traditional food chain or whether it was simply consumed internally by respiration. By grazing small particles the microzooplankton actively incorporate particles that might otherwise form a biological "sink" in the ocean. However, their activities can also be seen as a "link" in that pico- and nanoautotrophs are channelled into the food web, with the microzooplankton then forming a food for higher trophic levels such as copepods and fish larvae.

Figure 1.2: The functional role of microzooplankton in the pelagic food web.



Research into the microbial food web advanced considerably. In the 1980s more evidence emerged that microzooplankton are important grazers of bacteria, autotrophic picoplankton and nanoplankton (Capriulo & Carpenter 1980, Landry & Hassett 1982, Sherr et al 1984, Sherr et al 1986, Davis & Sieburth 1984, Porter et al 1985, Stoecker & Evans 1985, Verity 1985, Jonsson 1986). Application of direct measurements of grazing by microzooplankton in the field, in a variety of environments (e.g. Burkill et al 1987, Paranjape 1987 and Gifford 1988) support earlier theoretical studies.

Today it is widely accepted that microzooplankton are important in fundamental ecological processes such as energy flow and nutrient regeneration in aquatic waters. Not only are they important consumers of bacteria, picoplankton and nanoplankton but they are also an important food source for larger zooplankton (Sheldon et al 1986) and even larval fish (Stoecker & Govoni 1984). Information on the ecology of protistan and metazoan groups is crucial to the construction of models of carbon cycling, energy flow and the remineralisation of essential nutrients e.g. phosphorus and nitrogen.

*1.3 The Carbon Cycle*

The oceans play a crucial role in the global carbon cycle by acting as a net sink for atmospheric carbon dioxide ($CO_2$) thereby slowing the rate at which $CO_2$ accumulates in the atmosphere. It is thought that the oceans have taken up a major part of the $CO_2$ produced by the burning of fossil fuels (Watson et al 1990). However, the exact amount of $CO_2$ presently in the oceans, together with the method by which it is removed from the atmosphere, and the processes by which carbon is transferred within the water column, are uncertain. Carbon dioxide can be incorporated into the ocean through the photosynthetic activities of the phytoplankton. This drawdown of $CO_2$ has been shown to be strongly correlated with the abundance of phytoplankton (Watson et al 1991) and is, therefore highly variable. On a global scale, an estimated 40GT of carbon is incorporated annually into oceanic phytoplankton via primary production. A major route in the transport of carbon out of the surface waters into deep water would be through the sedimentation of biological particles (McCave 1975). However, the magnitude of this "vertical flux" will depend on the composition of the phytoplankton community (Michaels & Silver 1988). It has been suggested that short food chains involving larger phytoplankton cells result in a higher proportion of sedimented carbon than those based on small phytoplankton, which cannot be

grazed by copepods (Cushing 1989). The additional trophic level introduced by microzooplankton grazing on small phytoplankton before being eaten by mesozooplankton results in a greater proportion of the fixed carbon being lost through respiration and excretion. The efficiency of the foodweb also affects the vertical flux of organic carbon. Discrete faecal pellets or aggregates which sink rapidly can result in a significant transfer of fixed carbon, derived from atmospheric $CO_2$, into the deep ocean. When small phytoplankton and microzooplankton dominate the water column productivity, significant vertical flux of carbon is unlikely. Some areas such as the North Atlantic are thought to be net sinks for $CO_2$ because of the combination of high *in situ* biological productivity together with the sedimentation of phytogenic material to deep water. The Arabian Sea, on the other hand, is thought to be a net source of $CO_2$ because respiratory activity and upwelling of $CO_2$-rich water results in release of more $CO_2$ than is fixed by photosynthesis. The importance of the Southern Ocean as a sink or source for atmospheric $CO_2$ is still in some doubt. Recent evidence now suggests that it is a sink, at least in summertime (Robertson & Watson 1995).

*1.4 Grazing*

Given the importance of microzooplankton grazing, it is important to have accurate methods to use in the field in order to make grazing measurements. Although fixation and enumeration of microzooplankton is relatively easy, there are still problems in measuring their grazing activity. There is a wealth of information on feeding rates for individual species of microzooplankton generated from laboratory studies (e.g. Heinbokel 1978, Rassoulzadegan 1982, Jonsson 1986, Stoecker et al 1983). It would be difficult and somewhat inaccurate however, to apply such variable, species-specific, rates to field populations, in order to quantify the impact of microzooplankton with respect to the whole microbial community. The major difficulty with determining microzooplankton grazing in the field is that it involves manipulation of small consumers that are of a similar size to their prey, share the same habitat and in many cases are very delicate. There are a number of observational and experimental approaches in use for assessing the grazing impact of microzooplankton all of which have their advantages and disadvantages e.g. selective metabolic inhibitors (Xiuren & Vaulot 1992), ingestion tracers such as microspheres or fluorescently labelled algae (Sherr et al 1987; Rublee & Gallegos 1989) and radioisotopes (Lessard & Swift 1985), food vacuole contents (Kopylov & Tumantseva 1987) size fractionation of natural communities (Rassoulzadegan &

Sheldon 1986) and the seawater dilution technique (Landry & Hassett 1982). There is no one definitive method and until there is, the key is to use existing methods and maximise their strengths and minimise any problems they may hold.

Since its introduction in 1982 by Landry & Hassett, the seawater dilution approach has been widely used in aquatic ecosystems as an experimental technique for estimating the growth rate of phytoplankton and the grazing impact of microzooplankton (e.g. Landry et al 1984, Campbell & Carpenter 1986, Paranjape 1987, Burkill et al 1987, Gifford 1988, Landry et al 1993, Verity et al 1993a, Burkill et al 1993a ). The technique has also been applied to assess bacterivory (e.g. Kirchman et al 1982, Landry et al 1984, Ducklow & Hill 1985 and Geider 1989).

Over the years the methodology and interpretation of results from dilution experiments have improved following closer scrutiny and critical review of this experimental method (e.g. Fuhrman & Bell 1985, Li & Dickie 1985, Gallegos 1989, Li 1990, Evans & Paranjape 1992). There has been much discussion in the literature about the potential responses of phytoplankton and grazers to dilution, and the effect these responses might have on experimental results and their interpretation (e.g. Gifford 1988, Andersen et al 1991, Landry et al 1993, Neuer & Cowles 1994, Landry et al 1995, Lessard & Murrell 1998). Despite its shortcomings, the dilution technique seems to be the best method available to estimate the actual community grazing impact since the method leaves the grazer and food species composition unchanged and handling can be kept to a minimum.

The magnitude of the role that microbial consumers play as herbivores in pelagic ecosystems and their role in determining the fate of primary production in marine biogeochemical cycles, has focussed attention on the activities of these assemblages within the context of the Joint Global Ocean Flux Study, JGOFS (SCOR 1990). In 1989 the Biogeochemical Ocean Flux Study (BOFS) was set up as a UK contribution within the framework of JGOFS, to investigate the natural controls on atmospheric $CO_2$ levels in two of the most important areas of the global ocean- the Atlantic and the Southern Oceans. The primary aim of the BOFS programme was to improve understanding of the biogeochemical processes influencing the dynamics of carbon cycling in the ocean. It was an interdisciplinary study that encompassed a major programme of cruises in the North Atlantic from 1989 –1991. An extension to BOFS funding meant that the work could be continued with a study

of the biogeochemistry of the Ice Edge in the Southern Ocean in 1992. The work presented in this thesis forms a contribution to a NERC Special Topic award made to Prof MA Sleigh & Dr PH Burkill within the BOFS programme: GST/02/375 "Distribution and trophic transformation by microzooplankton of single biogenic particles in the North-Eastern Atlantic" which was later extended to include the Southern Ocean and a subsequent Aquatic Life Sciences grant GR3/8469 'Seasonal studies of microzooplankton community structure and function in oceanic carbon cycling'. Further work was carried out in the Arabian Sea during 1994.

The purpose of the work presented here was to:

- learn and develop existing methodology and test the assumptions of the dilution approach in order to verify its use in the field;
- make direct measurements of the herbivorous activity of microzooplankton in the field which would then allow a quantitative assessment of the role of microzooplankton in regulating phytoplankton stocks;
- compare the role of microzooplankton within three contrasting oceanographic regimes.

The relationship between oceanographic processes and the transformation and vertical flux of carbon from surface waters cannot be determined without an understanding of the standing stocks of the planktonic populations within the system. Therefore one further aim of this work was the acquisition of data on the biomass of the microzooplankton assemblages in each of the study areas.

## CHAPTER 2:

## LABORATORY EXPERIMENTS & METHODOLOGY

### 2.1 *Microzooplankton herbivory using the dilution technique: Theory*

*"A simple manipulation of a complex food web"- Gallegos 1989*

The experimental 'dilution technique' is based on the determination of phytoplankton growth in a successive dilution series. The dilution series is made up of the natural microbial community in conjunction with particle-free seawater from the same sample, such that the successive dilutions decrease the encounter rate between predator and prey under otherwise identical conditions. This allows the phytoplankton to achieve highest relative multiplication in the more dilute concentrations of microorganisms where grazing pressure is most reduced. The main advantage of the dilution technique over other methods of determining grazing is that it provides information about the dynamics of the whole phytoplankton community, which other methods cannot and requires relatively little manipulation of the community. The dilution technique also yields estimates of both the specific growth rate of the phytoplankton and specific grazing rate of the microzooplankton. The experiments are simple in concept and execution although extreme care must be taken during manipulation of the community to reduce handling mortality. The main disadvantage of this technique is that it involves three critical assumptions, which under certain circumstances may be violated and in practice sometimes prove difficult to verify.

The basic principle of the dilution approach is outlined below (Figure 2.1). The apparent growth rate of phytoplankton is estimated in a series of containers in which sampled water is diluted with filtered water from the same location. Incubations are carried out over a period of 24 hours and sub-samples taken at the beginning and end of experiments. Phytoplankton growth can be determined using one of a number of different analytical techniques. For example changes in the bulk chlorophyll (Landry & Hassett 1982, Gifford 1988), size-fractionated chlorophyll (Verity et al 1993a), pigments determined by High Performance Liquid Chromatography (Burkill et al 1987, McManus & Ederington-Cantrell 1992), flow cytometry (Landry et al 1995) or cell counts from microscopy (Landry et al 1984, Weisse & Scheffel-Möser 1990).

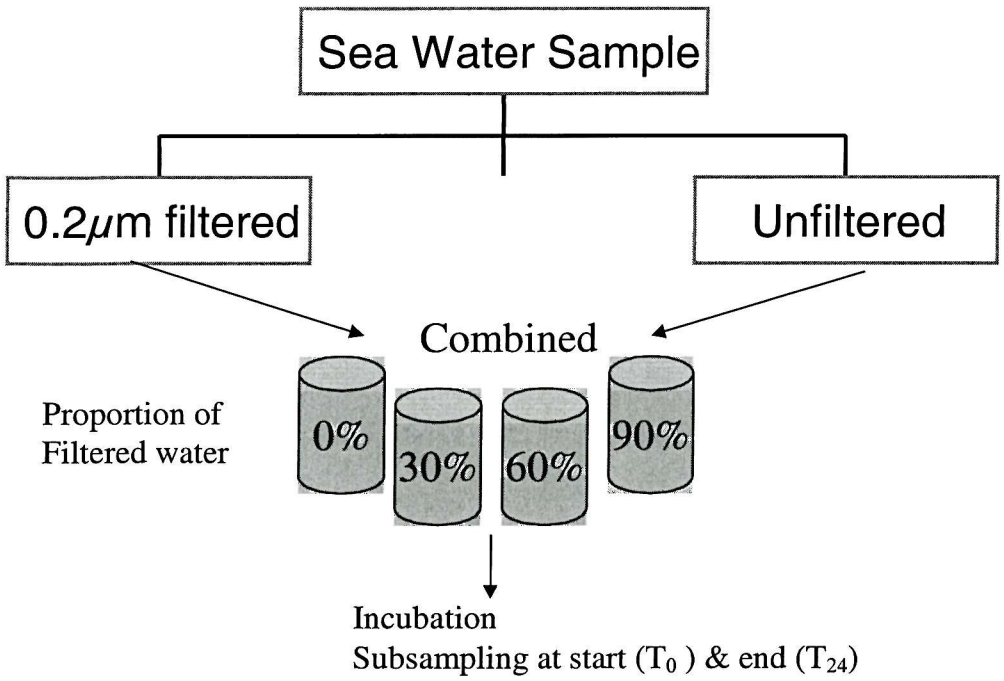



Figure 2.1 Schematic diagram showing the different steps of the dilution technique

There are three critical assumptions that must be satisfied for the dilution technique to be valid. Essentially it must be assumed that:: -

(1) Phytoplankton growth is density independent in the dilution series such that specific growth rates are not affected by dilution. To satisfy this assumption, dissolved nutrients must remain non-limiting, or equally limiting, to growth at all dilutions during the incubation.

(2) The probability of a phytoplankton cell being grazed is 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.

(3) Phytoplankton growth with time is exponential and can be represented by the equation:

$$P_t = P_0 \, e^{(k-g)\,t}$$

Where $P_0$ and $P_t$ are the prey concentrations initially and at time t, and k and g are the specific rates of prey growth and mortality due to grazing, respectively.
The net rate of population change ($r_n$), under natural conditions (i.e. undiluted), is:

$$r_n = k-g = 1 / t. \ln ( P_t / P_0 )$$

From the assumptions above, specific growth rates of prey should not be affected by dilution, but the specific mortality rates (which are density dependent) will be. Therefore in any diluted population, the net rate of change in the prey population ($r_d$) will be:

$$r_d = k-d \, . \, g = 1 / t. \ln ( P_t / P_0 )$$

Where d is the concentration of predators and prey relative to that in the undiluted population. From this, 'g' and 'k' may be determined either graphically or algebraically. In the graphical determination the relationship between observed growth rate of phytoplankton and relative concentration of prey should yield a linear negative slope, where 'g' may be determined as the gradient and 'k' as the intercept of the Y-axis (Figure 2.2).

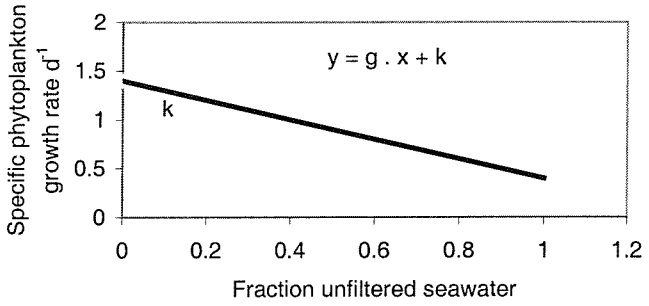



Figure 2.2: Typical dilution experiment plot produced when growth rate of phytoplankton and relative concentration of prey, measured here as chlorophyll, yields a significant linear regression.

Algebraically, 'g' may be determined provided 'k' is known. 'k' may be determined either experimentally or graphically.

$$g = [ \{1 / t . \ln ( P_t / P_0 )\} - k ] / d$$

In the absence of any predators, when d = 0,

$$k = 1 / t . \ln ( P_t / P_0 )$$

$$\text{Thus } g = \{ [ 1/t . ( P_t / P_0 ) ]_{d=d} - [1/t . \ln ( P_t / P_0 )] \} / \ d_{d=0}$$

Having determined 'k' and 'g', the quantity of prey grazed by the predators, either in terms of the rate of grazing or the mass of pigment grazed, can be calculated by multiplying the rate of turnover (r t o) by the cell concentration in the undiluted sample.

$$r t o = 1 - e^{-g}$$

(where g = the specific growth rate of mortality defined above).

Violation of either of the above assumptions could lead to difficulties in interpretation of the data:

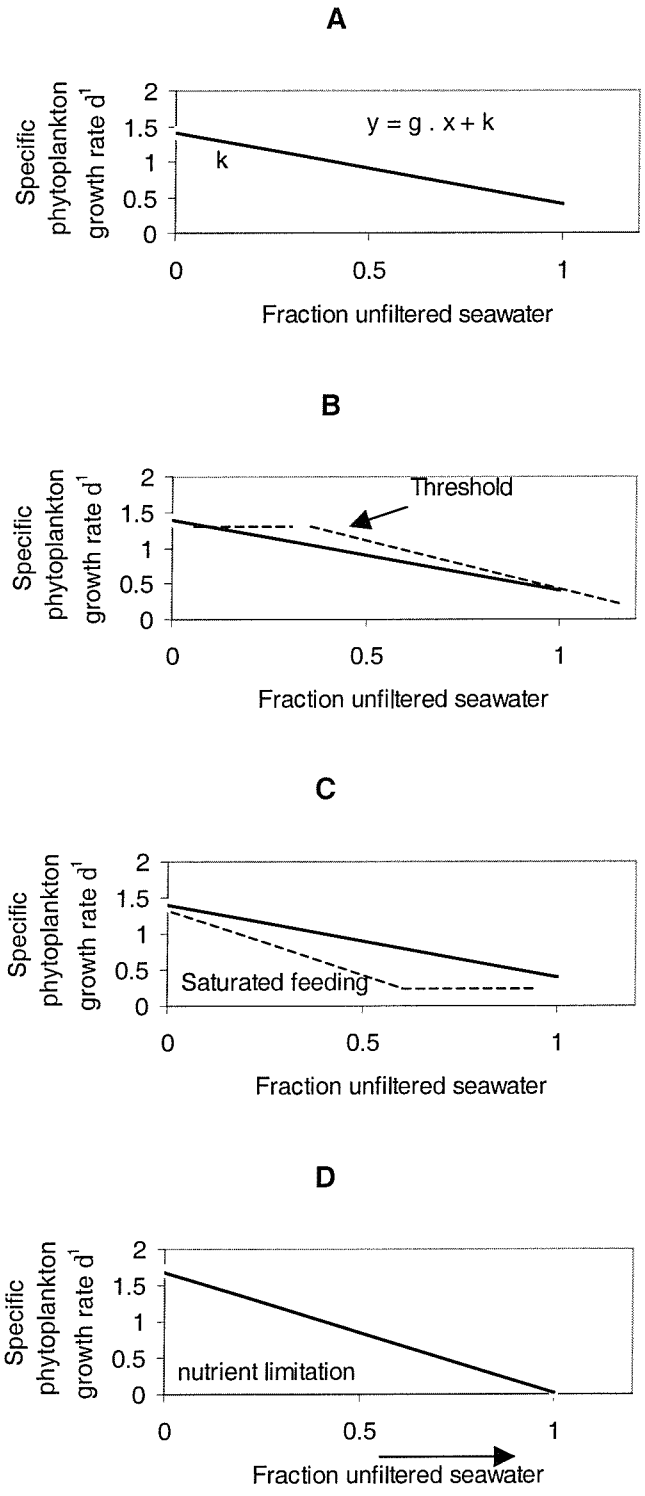



Figure 2.3: Dilution plots showing typical linear regression analysis (A), non-linear effects due to threshold feeding (B), saturated feeding (C) or nutrient limitation (D).

- The 'ideal' situation occurs when the relationship between observed growth rate of phytoplankton and relative concentration of prey yields a linear negative slope, where g is determined as the gradient and k as the intercept of the Y axis (Figure 2.3 A).
- Differential nutrient availability could result in greater phytoplankton growth rates at lower dilutions. This would exaggerate the slope of the regression line and overestimate grazing mortality (Figure 2.3 D). Nutrient depletion during incubation could lead to an underestimate of growth rate, but providing depletion occurs to the same degree at all dilutions grazing should not be affected.
- Contaminants in the filtered seawater used in the setup of the experiment may negatively affect phytoplankton growth and hence lead to underestimation of grazing or even show negative grazing.
- In environments with high phytoplankton concentrations the natural levels of available food may be so high that grazer ingestion rates become saturated. This may result in non-linear relationships, particularly where saturated feeding only occurs at the highest prey densities used in experiments (Figure 2.3 C). The result would be an underestimation of phytoplankton growth and grazing mortality. On the other hand, by diluting out prey when ambient food concentrations are very low, microzooplankton may reduce their feeding effort whereby a threshold feeding response occurs. As a result grazing would be under-estimated (Figure 2.3 B).
- If initial dilution levels are used as the independent variable rather than the relative density of grazers then the grazing rate could be significantly under- or overestimated depending on whether grazer abundance decreased or increased during the experimental incubation.

The consequences of violating these assumptions are further discussed in Landry & Hassett (1982), Ducklow & Hill (1985), Tremaine & Mills (1987), Gifford (1988), Gallegos (1989), Evans & Paranjape (1992), and Lessard & Murrell (1998). The basic theory of the dilution technique remains the same for experiments carried out both in the laboratory and in the field. However, methodology may vary according to conditions and facilities available and improvements have been made over time with increase in experience. Specific grazing methodology in the field will be dealt with in individual Chapters dealing with field data. Lab methodology will be covered in this chapter.

## 2.2 Materials and Methods

Initial laboratory studies were carried out in order to gain familiarity with and develop the dilution technique and to test some of the hypotheses of the dilution approach prior to use in the field. The experiments were aimed primarily at addressing Assumption 1 and 2 in section 2.1. Before this work could be done, it was necessary to gain familiarity with some experimental/analytical procedures which would be required to run dilution experiments successfully.

### 2.2.1 *Culture Procedures*

Before experiments could be set up, laboratory cultures of prey and predators had to be established. Four algal cultures were acquired from the Plymouth Culture Collection, (Plymouth Marine Laboratory) *Dunaliella tertiolecta*, *Tetraselmis suecica*, *Rhodomonas* sp, and *Phaeodactylum tricornutum*. A culture of the predatory heterotrophic dinoflagellate *Oxhyrris marina* Dujardin, was acquired from G Tarran (PML). Cultures were grown in F/2 enriched seawater culture medium (Guillard, 1975) in 250ml sterile Erlenmeyer glass flasks. Cultures were sub-cultured regularly to maintain healthy populations. This was carried out by pipetting a small amount of culture into a clean culture flask that contained fresh culture medium. Cultures were kept in constant light at a temperature of 16°C. A Coulter Multisizer was used routinely to determine concentrations and size distributions of cells in culture and determine their suitability for use in experiments.

### 2.2.2 *Coulter Multisizer II*

The Multisizer is a rapid, accurate particle counting and sizing device, which can be used to determine cell volume and size distribution. The Multisizer determines the number of particles in a conductive liquid by monitoring changes in electrical impedence (current + charge) as particles pass between 2 platinum electrodes. The larger the particles the greater the impedance.

### 2.2.3 *Predator & Prey*

The colourless heterotrophic dinoflagellate *Oxhyrris marina* Dujardin, has been widely used in laboratory grazing studies (e.g. Fuller 1990; Tarran 1992; Goldman et al 1989; Flynn & Fielder 1989) and was also chosen as a suitable predator for these experiments. *Oxyrrhis* is a simple dinoflagellate to culture, which is highly versatile, being capable of grazing a wide variety of phytoplankton species (Droop 1959, Dodge & Crawford 1974).

The cell is characterised as having an elongated ovoid structure (Fig 2.4) which is slightly flattened laterally and has a rounded anterior and an asymmetric posterior which projects to the left. It measures 20 to 30 $\mu$m in length and 15 to 20$\mu$m in width. The cell has no clear sulcus or girdle but two flagella arise from the posterior end from either side of a tentacular lobe. It has a single nucleus and contains no chloroplasts (Dodge & Crawford 1971a, b). *Oxyrrhis* is considered a raptorial feeder (Fenchel 1987) and like many heterotrophic dinoflagellates appears to seek out its prey, using its transverse flagellum for capture, before ingestion (Jacobson & Andersen 1986; Gaines & Elbrachter 1987; Tarran 1992). The chlorophyte alga *Dunaliella tertiolecta* was chosen as a suitable prey for *Oxyrrhis.*

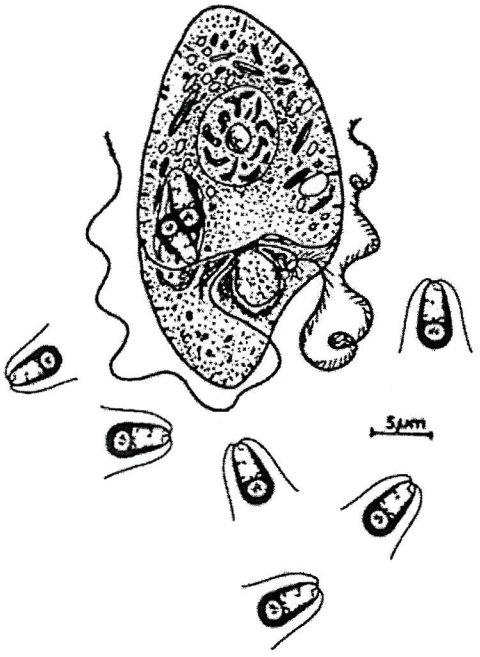



Figure 2.4: Diagramatic representation of the ventral view of the protozoan predator, *Oxyrrhis marina* (taken from Dodge & Crawford 1971a) and its algal prey *Dunaliella tertiolecta.*

### *2.2.4 Measurement of chlorophyll*

The concentration of chlorophyll in phytoplankton provides a measure of phytoplankton biomass. The method used to determine chlorophyll concentration depends upon the type of data required, the equipment and time available. The most commonly used methods for routine determination of chlorophyll concentration in oceanography are spectrophotometry and fluorometry. However, for precise determination of pigment

composition high-pressure liquid chromatography (HPLC) is required. This method requires collection of a larger sample volume, expensive equipment and more time and technical expertise in chromatographic techniques. The basic method for measurement of chlorophyll is summarised below (Figure 2.5).

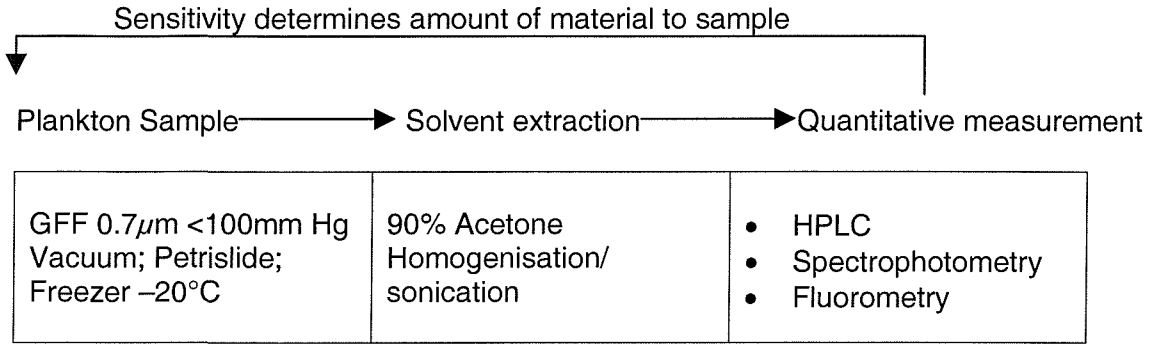



Figure 2.5: Summary of the basic method for chlorophyll analysis.

There are a number of individual techniques adopted for collection and analysis of chlorophyll samples. In this study, for the initial laboratory and field studies, fluorometry was used for the determination of chlorophyll concentration because it is 2-3 times more sensitive than spectrophotometric methods and has the advantage that it is simple and inexpensive to carry out. In 1965, Holm-Hansen et al (1965) introduced an acidification step into the fluorometric method, which enabled chlorophyll-a to be distinguished from degradation products e.g. phaeophytin and phaeophorbide. The concentration of these phaeopigments can be high in natural waters and would lead to an overestimate in chlorophyll-a if not accounted for. The following methodology was adopted:

- A known volume of sample was filtered onto 25mm Whatman Glass Fibre filters (GFF) using a vacuum pump at low pressure <100mm Hg. Chlorophyll was either extracted immediately or the filters were placed into petrislides and frozen for subsequent analysis. To extract chlorophyll, filters were placed in 5-10mls 90% acetone, they were homogenised until the filter was a soft pulp, centrifuged, and the clear remaining liquid poured into a fluorometer tube and fluorescence was measured. Care was taken at all times to keep chlorophyll samples in the dark because, once extracted, chlorophyll degrades rapidly in natural light.

- Measurements of chlorophyll were taken before and after addition of 1-2 drops of dilute hydrochloric acid, using a fluorometer built at Plymouth Marine Laboratory (PML) (Aiken, 1981). The high sensitivity of the fluorometer was necessary in order to detect the small changes in chlorophyll concentration which are likely to occur in dilution experiments carried out in the field. Before the fluorometer could be used it had to be calibrated. The fluorometer was calibrated at regular intervals throughout the study. The concentration of chlorophyll was determined using the equations described below.

During later field work i.e. post 1990, 0,2$\mu$m polycarbonate filters were used instead of GFFs and, rather than homogenisation, filters were extracted for 24 hours in acetone at –20 °C in a freezer.

2.2.5 *The Calibration procedure*

Calibration was carried out using the methods in Strickland & Parsons (1972). Chl-*a* standard was obtained from Sigma Chemical Company. The standard was dissolved in 90% acetone and its concentration measured using a spectrophotometer. The concentration of the standard was determined using the following equation:

$$\text{Chl-}a = A/E \times l$$

Where A= absorbance, E= extinction coefficient for Chl-*a* in 90% acetone at 664nm (87.67g $l^{-1}$ $cm^{-1}$) and l= path length (cm).

A series of dilutions were prepared from the standard stock solution. Fluorometer readings, measured in volts by a Multimeter, were taken before and after acidification by the addition of 1-2 drops of 10% HCl. Using these readings the relationship between Chl-*a* concentration, as determined by spectrophotometry and fluorometry can be determined. A linear calibration factor ($K_x$) was determined for each 'scale' on the fluorometer from the slope of this relationship. The acidification coefficient (Fm) was calculated by averaging the ratio of the unacidified and acidified readings ($F_0/F_a$) of pure Chlorophyll-*a*.

*Calculation of results:-*

Extracted chlorophyll samples were transferred to a fluorometer measurement tube. Readings were made on the most suitable scale setting, before and after acidification

with 1-2 drops 10% HCl. The concentration of chlorophyll-*a* in a sample can be calculated using the following equation:

$$Chl\ (\mu g\ l^{-1}) = (F_m / F_m\text{-}1) \times (F_0 - F_a) \times K_x \times (vol_{ex} / vol_{filt})$$

Where:
$F_m$ = acidification coefficient (F0/Fa) for pure Chl a
$F_0$ = reading before acidification
$F_a$ = reading after acidification
$K_x$ = door factor from calibration calculations
$Vol_{ex}$ = volume extracted
$Vol_{filt}$ = volume filtered

It is also possible to determine the concentration of phaeopigments in the sample using the following:-

$$Phaeo = (F_m / F_m\text{-}1) \times [(F_m \times F_a) - F_0] K_x - vol_{ex}$$

2.2.6 *Comparison of Chlorophyll concentration: Spectrophotometry v fluorometry?*
To determine whether the estimates of chlorophyll obtained by fluorometric methods were accurate, a simple comparison was carried out between estimates of chlorophyll concentration determined by spectrophotometry and fluorometry. Four algal cultures were used to determine their pigment content. Using the methods described above, two replicates of a known volume of culture was filtered and the pigments extracted. Half of each sample was measured using a spectrophotometer the other half using the fluorometer and the results were compared.

The amount of pigment present in samples measured by spectrophotometry was calculated using the following equations (Jeffrey & Humphrey, 1975):-

Ca (Chlorophyll *a*) = $11.85\ E_{664} - 1.54\ E_{647} - 0.08\ E_{630}$

Cb (Chlorophyll b) = $21.03\ E_{647} - 1.67\ E_{664} - 2.66\ E_{630}$

Cc (Chlorophyll c) = $24.52\ E_{630} - 1.67\ E_{664} - 7.60\ E_{647}$

Fluorometric measurements were determined as described in section 2.5.

2.2.7 *A test of the micro-extraction technique*

Phinney & Yentsch (1985) described a novel phytoplankton chlorophyll analysis technique, which eliminated the need for sample filtration. A short investigation was

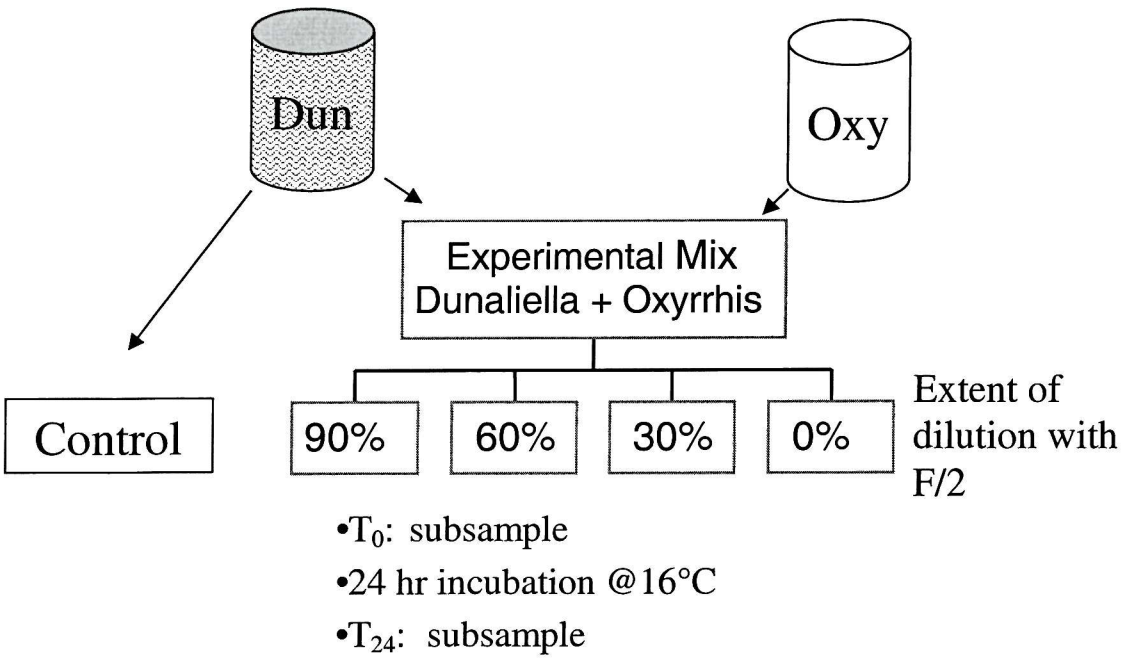
carried out in the lab to determine whether this technique might be a more suitable method for chlorophyll extraction. Five separate dilutions of *Dunaliella* culture were made up (2, 4, 10, 20 & 50%) in 50ml NUNCLON bottles. Triplicate sub-samples of 2-10mls from each dilution were filtered onto GF/Fs, extracted with 10mls acetone and after 6 hours their fluorescence was read.

At the same time triplicate sub-samples of 1.5ml were taken from each dilution, placed in a centrifuge tube with 8.5ml of 90% acetone. Tubes were stored in the dark at room temperature for 6 hours (Phinney & Yentsch, 1985). After the 6-hour period, samples were centrifuged and fluorescence read before and after acidification.

### 2.2.8 *Dilution Experiments in practice*

Three dilution experiments were carried out in the laboratory using *Oxyrrhis marina* and *Dunaliella tertiolecta* as the predator and prey. Prior to each experiment the concentration of each culture was determined using the Multisizer and the experimental mix made up using different predator: prey ratios. Experiments were carried out using 50ml NUNCLON bottles. The predator/ prey experimental mix was diluted out using clean F/2 culture medium in order to make up the dilution series ranging from 90, 60, 30 and 0% dilution in triplicate (Figure 2.6).

Figure 2.6: Schematic diagram showing main steps of the laboratory experimental procedure. Dun= *Dunaliella tertiolecta*; Oxy= *Oxyrrhis marina*



For each experiment 12 bottles (3 for each concentration) were set up containing only *Dunaliella* , these acted as a control and were treated in the same way as the experimental bottles. Experimental bottles were incubated for a period of 24 hours in a constant temperature culture room and were all kept an equal distance from the light source. Experiments were carried out at a temperature of 16°C in constant light. At the beginning of each experiment and at each time T, Multisizer measurements of total abundance of *Oxyrrhis* and *Dunaliella* were made from each bottle and triplicate sub-samples taken for determination of chlorophyll concentration. Basic estimates of phytoplankton growth ($\mu$ $d^{-1}$) and grazing mortality (m, $d^{-1}$) were calculated according to the linear relationship derived using relative chlorophyll concentration and relative prey density as the X -axis independent variables.

Although dilution experiments are designed to determine the community grazing impact of microzooplankton, measurements obtained from these experiments can be used to determine additional information on the average food concentration, ingestion rate and volume clearance rate per grazer. These can be calculated using the equations of Frost (1972) which have been adapted by Heinbokel (1978) to allow for the growth of grazers during the incubation.

The following measurements were determined: -

- initial food concentration (both cells $ml^{-1}$ and Chl a $\mu g\ l^{-1}$)
- $N_0$ - initial number of grazer in each bottle (cells $ml^{-1}$)
- incubation time
- final food concentration (both cells $ml^{-1}$ and Chl a $\mu g\ l^{-1}$)
- $N_t$ - final grazer concentration (cells $ml^{-1}$)

In order to determine the specific growth rate, $\mu$ $h^{-1}$, *Oxyrrhis* was assumed to be growing exponentially at a constant rate throughout the incubation time.

a) $$\mu = \frac{\ln (N_t) - \ln (N_0)}{t}$$

The average cell density of *Oxyrrhis* (N) was calculated using the following equation which allows for *Oxyrrhis* growth during the incubations:

b) $$N = \frac{Nt - No}{\ln (N_t) - \ln (N_0)}$$

The clearance rate CR, defined as the volume of water cleared of food per predator per unit of time (ml *Oxyrrhis*$^{-1}$ h$^{-1}$), was calculated as:

c) $$CR = \frac{\ln (P_t / P_0)}{T \times (N_0 + N_t)/2}$$

From this the ingestion rate (I), defined as the amount of food ingested per unit time per predator, in this case number of *Dunaliella* ingested per *Oxyrrhis* per hour was calculated using:

d) $$I = CR \times (P_0 + P_t)/2$$

Relationships between cell concentration and ingestion rate, clearance rate and growth rate can be detected by plotting the respective rates against the average food concentration for each bottle in the three experiments. Such a relationship is known as a functional response. In the absence of any limiting factor predator feeding rate will increase linearly with food concentration. However, limits to feeding rate do exist e.g. time taken to ingest or digest food (Fenchel 1980) and when these limits are reached feeding will not increase further with increase in food abundance. Feeding responses of this kind have been described for ciliates feeding on both bacteria and phytoplankton prey (e.g. Laybourn & Stewart 1975; Fenchel 1982) and dinoflagellates feeding on phytoplankton prey (e.g. Fuller 1990; Strom 1991; Hansen 1992).

*2.2.9 Feeding experiments with a tintinnid ciliate and a dinoflagellate prey.*

Tintinnids, in particular the genus *Favella*, are often associated with blooms of dinoflagellates (e.g. Blackbourn 1974) and through lab experiments have been shown to selectively prey on dinoflagellates (Stoecker et al 1981). Two time-course experiments were carried out in the lab using stock populations of the tintinnid, *Favella sp*, which had been maintained on cultures of the photosynthetic dinoflagellate *Heterocapsa triquetra.* The tintinnids culture was obtained from D.Stoecker at Woods Hole Oceanographic Institute, and had originally been isolated from Perch Pond, Falmouth, Massachussetts, USA. The algal culture was obtained from the Plymouth Culture Collection. A series of 5 food concentrations were set up in sterile NUNCLON bottles and 20-30 tintinnids were carefully micro-pipetted into each experimental bottle. A control of only *Heterocapsa* cells was also set up. Bottles were rotated regularly throughout the incubation to maintain mixing of predator and prey. Experiments were performed at a temperature of 17°C in the dark for either 30 or 48 hours. At the end of the incubation, the density of prey in the control and experimental bottles was determined using Multisizer cell counts. Experimental bottles were then fixed in 10% Lugol's iodine, the contents settled overnight, and the total number of *Favella sp.* in each bottle determined by inverted microscopy and the Utermöhl counting method (Utermöhl, 1958). The hourly specific growth rate of *Favella sp.* was determined using equation (a) described above. Clearance and ingestion rates were also calculated as described above. It was assumed that the growth of *Heterocapsa sp.* was exponential for the duration of the experiments.

## 2.3 RESULTS

### 2.3.1 *Comparison of Chlorophyll concentration: Spectrophotometry v fluorometry*

The results generated from this comparison demonstrate reasonably good agreement between chlorophyll concentration determined by spectrophotometry and fluorometry.

| Culture | Spectrophotometry mg Chl-*a* $m^{-3}$ | Fluorometry mg Chl-*a* $m^{-3}$ |
|---|---|---|
| *Dunaliella tertiolecta* | 242 (29)<br>312 | 319 (33)<br>237 |
| *Rhodomonas sp* | 580 (0)<br>580 | 527 (19)<br>574 |
| *Phaeodactylum tricornutum* | 133 (12)<br>104 | 114 (6)<br>99 |
| *Tetraselmis sueica* | 63 (2)<br>59 | 53 (0)<br>54 |

Table 2.1: Comparison of spectrophotometric and fluorometric analyses of 4 algal cultures; Standard Error of mean shown in brackets.

No significant differences (student t-test p=>0.1) were evident between spectrophotometric and fluorometric measurements of chlorophyll concentrations for the four algal cultures. The mean length of each algal species was measured to give an indication of relative cell size and using Multisizer cell concentrations for each culture, the chlorophyll content of each cell was determined (Table 2.2).

| Culture | Mean Length $\mu$m | Mean conc. (Cells $ml^{-1}$) | Mean Chlorophyll-*a* Content (ng $cell^{-1}$) |
|---|---|---|---|
| *Dunaliella tertiolecta* | 6.9 | 110,000 | 2.5 |
| *Rhodomonas sp* | 9.0 | 100,000 | 5.63 |
| *Phaeodactylum tricornutum* | 7.0 | 80,000 | 1.41 |
| *Tetraselmis sueica* | 8.0 | 80,000 | 0.72 |

Table 2.2: Mean lengths and cellular chlorophyll-a content for the 4 algal cultures.

2.3.2 *A test of the micro-extraction technique*

Chlorophyll measurements made using the micro-extraction technique showed that significantly higher levels of chlorophyll were detected using this method. The difference in the two datasets was most pronounced at higher chlorophyll concentrations (Figure 2.7).

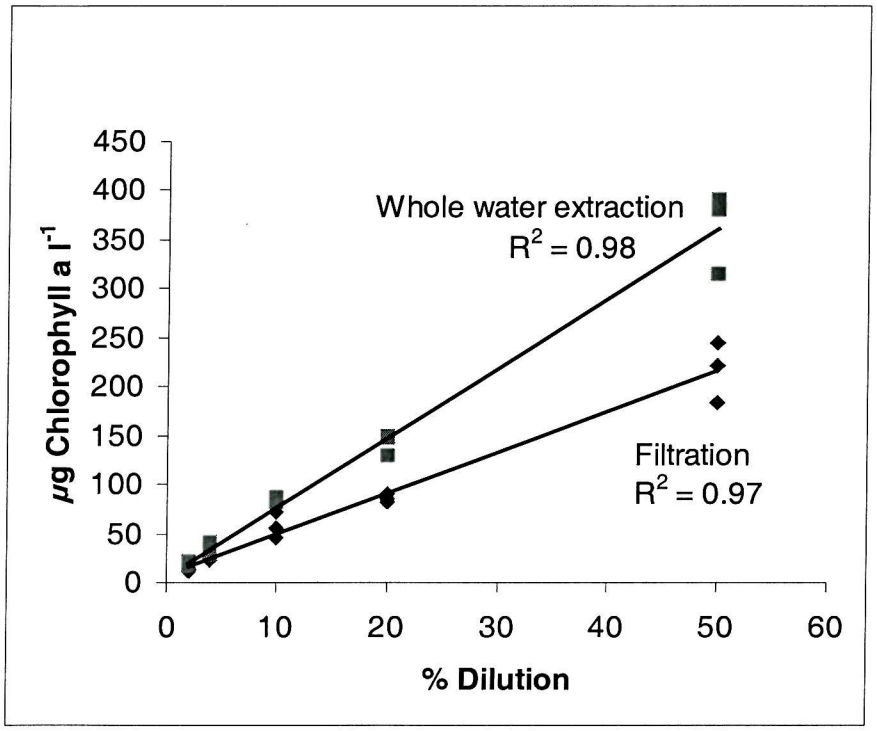
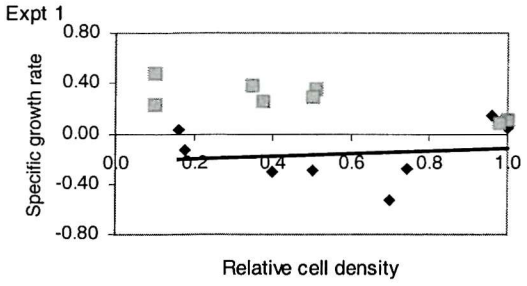


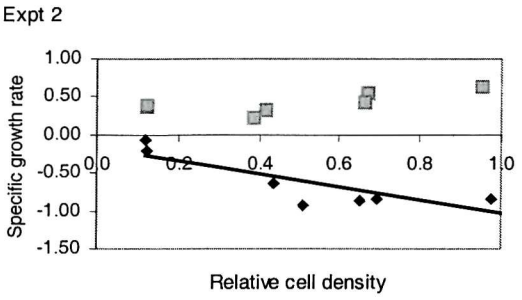

Figure 2.7: Comparison of filtration versus whole water extraction technique for measuring chlorophyll concentration by fluorometry.

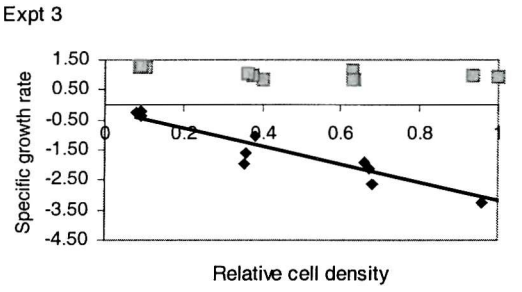
2.3.3 *Grazing by Oxyrrhis on Dunaliella*

The grazing response between *Oxyrrhis* and *Dunaliella* was linear in all 3 experiments with the exception of Experiment 1 where prey cell density was used as the X-axis variable. Here the undiluted bottles showed unexpected high growth in cell numbers and the grazing relationship was therefore insignificant (Figure 2.8).

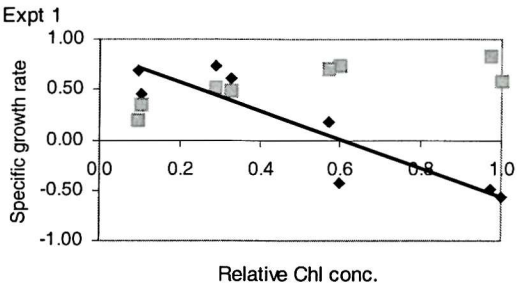
Multisizer

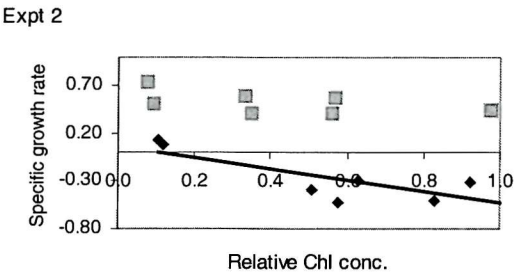



Fluorometry



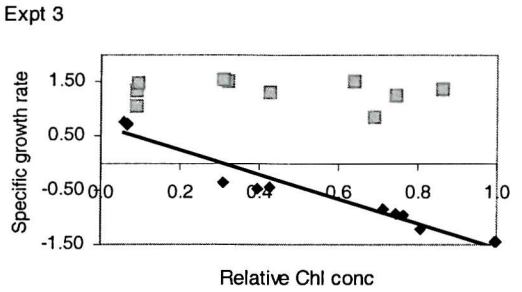




Figure 2.8: Dilution plots showing linear relationships between i) relative prey cell density and specific prey growth rate and ii) relative chlorophyll concentration against specific prey growth. ◆ = experimental results; ■ = *Dunaliella* control.

| | Cell density | | | | | Pigment content | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Expt | k ($d^{-1}$) | g ($d^{-1}$) | $r^2$ | P | Turnover % $d^{-1}$ | k ($d^{-1}$) | g ($d^{-1}$) | $R^2$ | P | Turnover % $d^{-1}$ |
| 1 | -0.227 | 0.106 | NS | NS | - | 0.854 | -1.42 | 0.82 | <0.001 | 76 |
| 2 | -0.175 | -0.86 | 0.73 | <0.001 | 58 | 0.007 | -0.60 | 0.67 | <0.02 | 45 |
| 3 | 0.20 | -2.95 | 0.86 | <0.001 | 95 | 0.70 | -2.26 | 0.95 | <0.001 | 90 |

Table 2.3: Summary of results from dilution experiments carried out in the lab using *Oxyrrhis marina* and its prey *Dunaliella tertiolecta*. k = phytoplankton growth coefficient; g = grazing coefficient ; $r^2$ = coefficient of variation, NS = not significant.

*Oxyrrhis* grazing rates (g) determined from cell density measurements were similar to those determined using chlorophyll concentration and ranged from 0.6 to 2.95 $d^{-1}$ (Table 2.3). The differences between apparent growth rate of *Dunaliella* determined by the two methods were greater, with the highest apparent growth rates being determined by fluorometry. This suggests that the prey cellular chlorophyll content increased but cell densities had not increased accordingly.

Growth of *Dunaliella* in the Control bottles was constant for all dilutions for experiments 2 and 3 but not experiment 1 where cell density in the undiluted bottles was lower than expected after the 24 hour incubation (Figure 2.8). This latter result is puzzling, one possible explanation for this might be that cells became nutrient limited in the undiluted treatment resulting in poor/reduced cell growth. The results determined for experiments 2 and 3 are evidence that prey growth rates were not affected by dilution and therefore verifies Landry & Hassett's first assumption as described in section 2.1, that phytoplankton growth is density independent.

### 2.3.4: *Growth, clearance and ingestion rates of Oxyrrhis*

The mean specific growth rate of *Oxyrrhis* in the undiluted treatments during these experiments ranged from 0.8 to 1.15 $d^{-1}$ that was equivalent to doublings of 0.6 to 0.8 $day^{-1}$ (Table 2.4).

| Experiment | Mean $\mu$ $d^{-1}$ | Doublings $day^{-1}$ | Clearance Rate $\mu l$ $cell^{-1}$ $d^{-1}$ | Ingestion Rate Cells $Oxy^{-1}d^{-1}$ |
|---|---|---|---|---|
| 1 | 1.15 | 0.79 | $1.69 \times 10^{-4}$ | 31 |
| 2 | 1.05 | 0.73 | $2.66 \times 10^{-4}$ | 21 |
| 3 | 0.80 | 0.55 | $5.27 \times 10^{-4}$ | 12 |
| **Mean** | **1.0** | **0.69** | **$3.21 \times 10^{-4}$** | **21** |

Table 2.4: The mean specific growth rate ($\mu$), population doubling times, mean clearance and ingestion rates of *Oxyrrhis* during 3 dilution grazing experiments.

Daily *Oxyrrhis* growth rates obtained from the three experiments for different concentrations of food have been pooled. These data have been plotted against food concentration in order to investigate the functional response of *Oxyrrhis* feeding on *Dunaliella.* Results demonstrate an increase in growth rate with increase in food concentration, levelling off at a concentration around 50,000 cells $ml^{-1}$ that was equivalent to 40-50 mg Chl $m^{-3}$ (Figure 2.9).

Clearance rates for *Oxyrrhis* ranged between $1.2 \times 10^{-4}$ and $7.57 \times 10^{-4}$ $\mu l$ $cell^{-1}$ $d^{-1}$ and there were no trends in the data when plotted against food concentration. Mean clearance rates determined for the undiluted experimental bottles were higher for experiments 2 & 3 than for experiment 1 (Table 2.4).

Ingestion rates of *Oxyrrhis* averaged between 1.6 and 1.9 *Dunaliella* cells per *Oxyrrhis* $h^{-1}$. Unlike rates of growth, ingestion rates of *Oxyrrhis* ($\mu$g Chl/pred/$h^{-1}$) did not decrease at high food concentrations instead there was a positive linear relationship with food concentration (Figure 2.10). Ingestion rates were similar but lower in experiments 1 and 2 and higher in experiment 3. It is possible that this difference in ingestion rates could be due to food quality. Further experiments would be required to determine whether ingestion is limited at higher food concentrations.

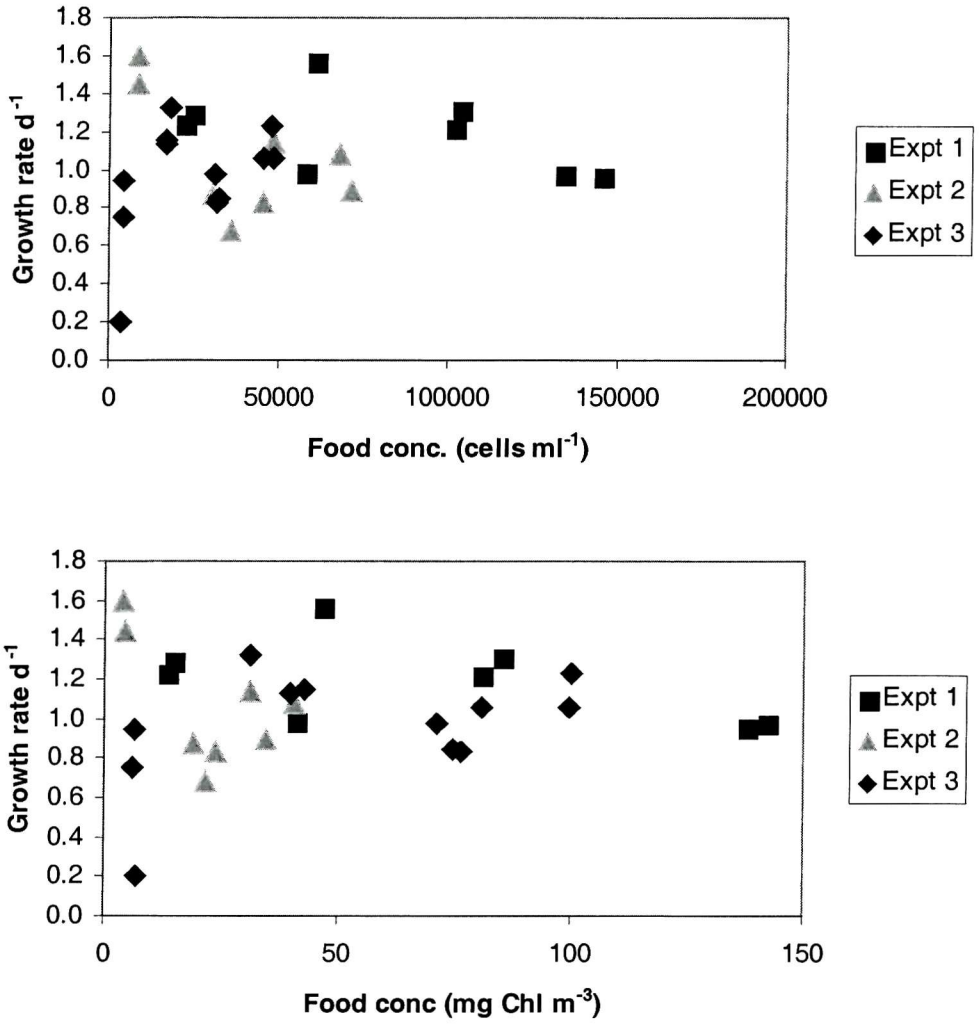



Figure 2.9: Growth rate of *Oxyrrhis marina* as a function of food concentration for three laboratory grazing experiments.

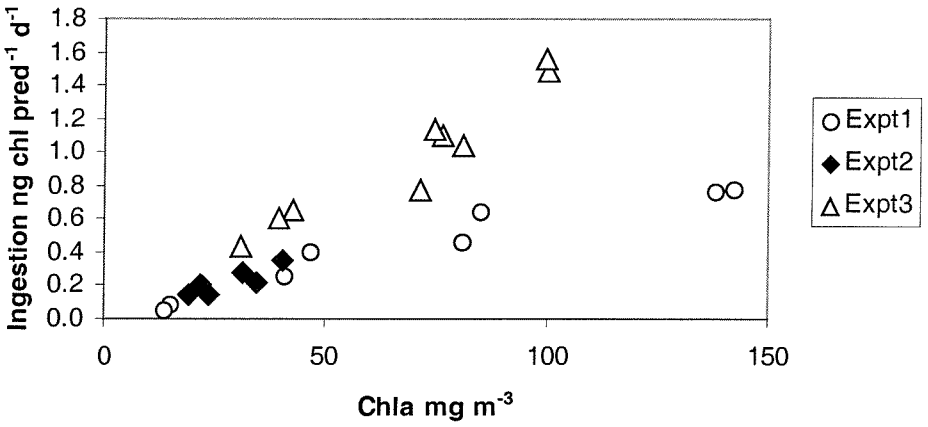



Figure 2.10: Quantity of Chlorophyll Ingested by *Oxyrrhis* during the three laboratory grazing experiments

2.3.5 *Growth, clearance and ingestion of Favella sp.*

*Favella* growth rates ranged from 0.1 to 1.0 $d^{-1}$. Highest growth rates were found when food concentrations were around 2,600 cells $ml^{-1}$. At higher food concentrations *Favella* growth rates decreased slightly. During these experiments *Favella* consumed between 2 and 45 *Heterocapsa* cells $tintinnid^{-1}$ $h^{-1}$. The volume swept clear by *Favella* ranged between 0.1 and 15$\mu$l $tintinnid^{-1}$ $h^{-1}$. The maximum clearance rate was achieved when *Heterocapsa* density was around 2,000 cells $ml^{-1}$.

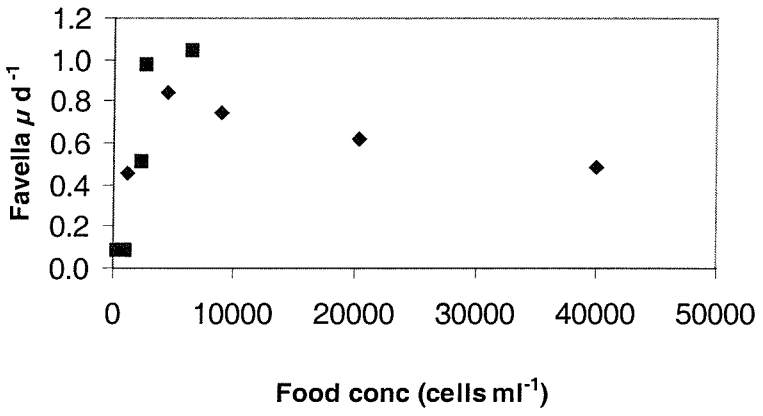



Figure 2.11: Growth rate of *Favella sp* as a function of food concentration for two laboratory experiments.

## 2.4 DISCUSSION

### 2.4.1 *Spectrometry v fluorometry*

Following the results of the comparison of fluorometric measurements with spectrophotometric, fluorometry was chosen as the most suitable method for the analysis of bulk chlorophyll for the dilution experiments. Not only is this method quick and easy to use but the high sensitivity of the fluorometer allows detection of small changes in the chlorophyll concentration, whilst not having to filter large volumes of seawater.

### 2.4.2 *Micro-extraction technique*

Current methods for the routine measurement of chlorophyll require concentration of material on filters. The disadvantages of this are mainly time taken to filter, mechanical damage of cells under vacuum (Platt et al 1983) and the fact that very small sized particles such as prochlorophytes could pass through the filter undetected. Increased sensitivity of fluorescence measurements would mean that chlorophyll could be measured in the field on unfiltered whole water samples. It is clear from the data presented in figure 2.7 that whole-water extraction gave a higher chlorophyll measurement than filtration. The most likely reason for this difference is mechanical damage of *Dunaliella* cells during filtration and subsequent loss of pigment during the extraction process. However, whilst this method has been demonstrated to be suitable for work with laboratory cultures where concentration of chlorophyll is high, there are limitations to using this technique routinely in the field (e.g. small sample volume). Further tests would be required before this method could be confidently adopted in the field.

### 2.4.3 *Grazing rates*

The results of the laboratory experiments performed here show close similarities in rates of grazing determined by both the cell count methods and chlorophyll measurement. However, measurements of the growth coefficient (k) were not similar suggesting that the increased growth in chlorophyll concentrations was not matched by an increase in cell density. This may be because there is a time delay between *Dunaliella* growth and actual cell division.

It is important when carrying out dilution incubations that light levels mimic the *in situ* light levels as closely as possible so as to avoid any changes in pigment content per cell during the incubation. Changes of this kind could lead to inaccurate estimates of growth and in some instances of grazing. Although it is unlikely that changes in cellular pigment content occurred due to incorrect light levels during these laboratory experiments it is an important consideration when performing experiments in the field. This point will be discussed further in Chapter 4 in relation to field data.

The heterotrophic dinoflagellate *Oxyrrhis marina* has been used as a model organism in this study to determine whether the dilution technique is a suitable method for using in the field for estimating microzooplankton herbivory. Grazing experimental results demonstrate that *Oxyrrhis* populations actively graze on *Dunaliella* cells suggesting that heterotrophic dinoflagellates may play an important role in grazing small phytoplankton in the field.

2.4.4 *Growth, Clearance & Ingestion rates of Oxyrrhis*

Growth rates of *Oxyrrhis* ranged between 0.8 and 1.15 $d^{-1}$. These estimates are similar to that reported by Goldman et al (1989) of 0.8 $d^{-1}$ for *Oxyrrhis* feeding on *Dunaliella tertiolecta.* The data also compare well with other growth rates from the literature (e.g Strom 1991; Hansen 1992) and are within the range of growth rates determined for dinoflagellates reported in Hansen et al (1997), but are lower than that reported by Fuller (1990). Growth rates of 0.9 $d^{-1}$ from lab studies using another heterotrophic dinoflagellate *Gymnodinium* sp, have been reported by Jakobsen & Hansen (1997). Growth rates determined for *Oxyrrhis* demonstrate the ability of these protozoa to grow at comparable rates to their autotrophic prey and faster than their autotrophic counterparts. Many small protozoa are reported in the literature to be capable of growing 2-3 times faster than their prey (Fenchel 1968, 1982; Goldman & Caron 1985). This could be a feature which they have adopted in order to exploit food supply in a patchy environment such as that found in natural waters.

Clearance rates determined for this study compare well with those determined for *Oxyrrhis* from other studies by Goldman et al (1989) and are within the ranges reported for other dinoflagellates such as Levandowsky & Kaneta (1987) (cited in Hansen et al 1997). Clearance rates in this study remained fairly constant with increasing food concentration. This would be expected because the food concentration was below that at which the maximum ingestion rate was obtained. If food concentrations went above

that at which maximum ingestion was achieved, clearance rates would become limited by the amount of time taken to ingest or digest food particles (Fenchel 1980).

What is the significance of these data in relation to assumptions of the dilution technique? Data from this study have been used to test whether a threshold or saturated feeding response had occurred during the experimental incubations. There is no indication from the data of a grazing threshold response. This situation occurs when the food concentration is below a level at which it is energetically unrewarding for a grazer to continue attempting to gather food (Frost 1975). The combined results of grazing experiments carried out between *Oxyrrhis* and *Dunaliella* demonstrate a reduction in growth rate of the predator when food concentrations exceeded $5 \times 10^4$ cells $ml^{-1}$ indicating that a saturated growth response would occur in the undiluted treatments. However, this was not matched by saturation in feeding rates. It is common for grazing rates generally to show a linear increase with relative food concentration until a threshold level is reached above which the predators grazing rate does not increase. This is probably because the volume of particles ingested has either blocked the mouth and the rate at which particles can be phagocytosed is limiting (Fenchel, 1980). This is known as the saturation concentration. If this situation arose during dilution experiments, the result would be a non-linear grazing response as shown in Figure 2.4 C and this would lead to an underestimate of the grazing rate. Since this situation did not occur in these experiments one can be confident that assumption 2, that grazers feed at a constant rate, as described in section 2.1 has been verified and that estimates of grazing are not biased by this. As with this study, Tarran (1992) also found that over the range of concentrations used in his experiments on *Oxyrrhis* and *Dunaliella* saturated feeding was not reached. Fuller (1990), on the other hand, also using *Oxyrrhis* and *Dunaliella,* found saturation concentrations below $8 \times 10^4$ cells $ml^{-1}$. In contrast, Goldman et al (1989) reported a cessation in growth and grazing when prey cell numbers fell below the threshold concentration of $5 \times 10^4$ cells $ml^{-1}$. This difference could be due to the conditions in which *Oxyrrhis* were kept prior to experiment setup, or perhaps a difference in the strain of *Oxyrrhis* used, or it's prey or both. If *Oxyrrhis* cells were in a starved state they could potentially graze *Dunaliella* at concentrations below $5 \times 10^4$ cells $ml^{-1}$. In this study and that of Fuller (1990), *Oxyrrhis* cultures were allowed to grow until food cells in the cultures had become depleted as far as possible so that the number of residual food cells would not be significant. Differences encountered between

measurements of *Oxyrrhis* growth rates in dilution experiments, as discussed in section 2.4.3, could also be explained by a semi-starvation state of original *Oxyrrhis* stock. It would be interesting to carry out further experimentation in the future on the effects of starvation on both *Oxyrrhis* growth and grazing rates.

2.4.5 *Growth, ingestion and clearance rates of Favella*

It is known that in laboratory feeding experiments with tintinnids using algal monocultures as food, ingestion rates of the tintinnids typically increase with increase in food concentration until a maximum rate is obtained (Heinbokel 1978). Data from experiments in this study suggest that maximum ingestion rates were reached at prey abundance <10,000 cells $ml^{-1}$. Data from this study are within the ranges reported in the literature for clearance, ingestion and growth rates of *Favella* feeding *on Heterocapsa triquetra* (Table 2.5), although estimates of ingestion sometimes exceeded those of other studies. It is, however, known that tintinnid species such as *Favella* may exceed their digestive capacity when food is abundant (Fuller 1990). This may explain the higher rates recorded here.

| | **Clearance rate** | **Ingestion rate** | **Growth rate** |
|---|---|---|---|
| | $\mu l$ tintinnid$^{-1}$ $h^{-1}$ | Cells tintinnid$^{-1}$ $h^{-1}$ | $d^{-1}$ |
| This study | 0.1 - 15 | 2 - 45 | 0.09 – 0.97 |
| Stoecker & Guillard (1982) | 7.7 – 22.8 | 1.3 – 9.1 | - |
| Stoecker, Davis & Provan (1983) | - | - | 0.016 – 0.058 |
| Stoecker (1984) | - | 2.5 | - |
| Stoecker & Evans (1985) | 1 – 11 | 2 - 15 | - |
| Aelion & Chisholm (1985) | - | 2 – 5 | <0.2 – 0.8 |

Table 2.5: Comparison of clearance, ingestion and growth rates of *Favella sp.* grazing on *Heterocapsa triquetra* with measurements from other laboratory studies.

These studies provide evidence that *Favella* may be an important species in controlling dinoflagellate blooms. It has also been shown that this tintinnid selectively grazes dinoflagellates in preference to other protozoan taxa such as ciliates (Stoecker et al 1981).

*Concluding remarks*

It would be difficult to draw any hard and fast conclusions from just a handful of experiments; however these preliminary investigations have provided a sound basis for taking the dilution technique forward on a wider scale in the field. In this study there was no evidence of non-linear feeding, which could arise if there is a progressive de-coupling between phytoplankton growth and mortality due to grazing, with dilution. Prey growth in control bottles was found to be constant at all dilutions therefore demonstrating that prey growth was density independent. These studies have proved useful not only in testing two of the hypotheses of the dilution approach and providing estimates of the population growth and mortality rates, but also in providing information regarding the feeding rates and growth of typical microbial grazers.

## CHAPTER 3

*Biogeochemical Ocean Flux Study, 1989: The North Atlantic*

One of the characteristics of the North Atlantic Ocean are large seasonal variations in phytoplankton biomass, the most prominent of these seasonal events being the spring bloom. The bloom begins in subtropical waters around 20-30°N latitude at the onset of boreal heating and stratification in late March and proceeds towards polar waters reaching 60-70°N by late June (Riley 1957, Colebrook 1982). These blooms have been witnessed by satellite imagery (Esaias et al 1986) and have been described as "the largest biological signal on the planet" (Lewis 1989). However, they have not been studied at sea on a large scale due to the immense task involved in covering such a large span of time and space by intensive research. Early knowledge of the plankton ecology of the North Atlantic spring bloom is limited to the larger phytoplankton which has been collected by the Continuous Plankton Recorder (CPR) survey (Colebrook 1979). Observations on the plankton of the north Atlantic (Colebrook 1984) have demonstrated latitudinal differences between areas in the region of 34-65°N; between 44 and 60°N, the bloom appears to be under-exploited by grazing, whilst in areas outside this range grazing appears to be an important process. It has been suggested that a large part of the phytoplankton would sink directly out of surface waters at the end of the spring bloom (Billet et al 1983). However the CPR surveys do not sample the smaller phytoplankton species effectively. Further observations have shown that phytoplankton in the <10$\mu$m size fraction is often numerically dominant in the North Atlantic (Murphy & Haugen 1985, Li & Wood 1988) and tend to dominate in the more stratified conditions encountered during late spring and summer when irradiance is higher. Under such circumstances one would expect microzooplankton herbivory to be important as phytoplankton growth is likely to be more dependent on recycled nutrients. Information on the grazing pressure of microzooplankton in the northeast Atlantic was scarce. However, similar studies were carried out in parallel with the present work during 1989 and later in 1991 by American colleagues who have since reported on microzooplankton herbivory in this area e.g. Verity et al 1993a, Gifford et al 1995.

The Biogeochemical Ocean Flux Study (BOFS) was an interdisciplinary community research project involving a large number of participants from 14 institutions within the UK. The project began with a major programme of cruises in the North Atlantic during 1989. A series of cruises were organised on the NERC research vessel *RRS Discovery* and formed the UK's contribution to the Joint Global Ocean Flux Study (JGOFS) North Atlantic Bloom Experiment (NABE). The emphasis of the NABE was

on the development and fate of the spring bloom of phytoplankton along the 20°W meridian. During the UK North Atlantic Pilot Study the study transect at 20°W was sampled on three consecutive cruises on *RRS Discovery*. The first cruise in May sampled at 47°N for 7 days then sampled a transect from 47 °N to 59°N with detailed sampling at 52°N and 56°N. The second cruise sampled the same region in June /July but began at 60°N and worked south. The third cruise took place in July/August and followed the same route as the first cruise in May.

One berth was allocated onboard *RRS Discovery* on the second of the above cruises for microzooplankton grazing studies. Participation in this cruise programme was my first opportunity to test the use of the dilution technique in the field. Data generated from the 1989 cruises are presented here in the form of 2 papers, the first on microzooplankton herbivory and the second on the microzooplankton community structure. The grazing work was important in that the resulting data were required to integrate with other studies and formed part of a modelling paper by Taylor et al (1995) (Appendix 1) and a further paper presenting a synthesis of the changes occurring in the community structure associated with bloom evolution by Weeks et al (1993) (Appendix 2). Reports on some of the other components of the carbon cycle, studied by colleagues during these cruises, have been published in the same volume of Deep-Sea Research.

The following published papers were included in the bound thesis. These have not been digitised due to copyright restrictions, but the links are provided.

P.H. Burkill, E.S. Edwards, A.W.G. John and M.A. Sleigh (1993)

**Microzooplankton and their herbivorous activity in the north-eastern Atlantic Ocean**

Deep Sea Research II: Vol. 40, Nos. 1-2, pp.479-493

http://dx.doi.org/10.1016/0967-0645(93)90028-L

M.A. Sleigh, E.S. Edwards, A.W.G. John and P.H. Burkell (1996)

**Microzooplankton community structure in the north-eastern Atlantic: trends with latitude, depth and date, between May and early August**

J. Mar. Biol. Ass. UK: Vol. 76, No. 2, pp.287-296

http://dx.doi.org/10.1017/S0025315400030551

## CHAPTER 4:
## The BOFS 1990 Lagrangian Study

### 4.1 Introduction

*4.1.1 Background*

The main objective of the BOFS 1990 Lagrangian Experiment was to study the development of the spring bloom in the northeast Atlantic from well-mixed winter to stratified summer conditions. A Lagrangian study (i.e. one which repeatedly samples the same body of water), involving two consecutive cruises on board *RRS Charles Darwin*, was undertaken between 1 and 20 May and between 28 May and 15 June in the vicinity of 47-50°N 15-20°W. A drifting buoy, attached to a sub-surface drogue at 20m, was released at 49°N 19°W, an area in which initially there was no evidence of a seasonal thermocline or phytoplankton bloom development. The site was also within an anticyclonic eddy identified from a large scale SeaSoar survey (Savidge et al 1992). Serial observations of basic physical, chemical and biological variables together with a wide range of biological rate process measurements were made close to the drifting system as it moved in a south-easterly direction. Full details of the cruises, the drogue, its movement and the hydrographic environment are reported by Savidge et al (1992). As one component of this multi-disciplined study I measured the changes in abundance and biomass of the microzooplankton community and rates of herbivorous grazing in the water column over the course of the spring bloom in this area of the northeast Atlantic. Other investigations of relevance to this work were carried out by Tarran (analysis of picoplankton by flow cytometry), Sieracki et al (1993) (heterotrophic nanoplankton), Stoecker et al (1994) (autotrophic and heterotrophic microplankton), and Verity et al (1993b) (heterotrophic dinoflagellates). In addition to this work the vertical structure of the microzooplankton community was determined from fixed samples collected during the cruises, of which I carried out all computational analyses as described below.

### 4.2 Methods

*4.2.1 Grazing*

Dilution experiments to determine microzooplankton grazing were performed on 22 occasions during the study period. For each experiment 60l of water were collected in acid-cleaned 30l Go-flo bottles pre-dawn from a depth of either 10 or 25m. Water was carefully pre-screened through a 200$\mu$m mesh to exclude any larger predators. Dilution series were prepared with 0, 30, 60 and 90% of 0.2$\mu$m filtered seawater from the same

collection, in triplicate 2-litre acid-cleaned clear polycarbonate bottles. For all experiments, bottles were incubated in either a Gallenkamp laboratory incubator set at ambient light and temperature levels or using an *in situ* rig positioned at the required depth of either 10 or 25m. Incubations were carried out for a period of 24 hours. Sub-samples were taken at time $T_0$ and $T_{24}$ for i) chlorophyll analysis, these were taken in triplicate from each experimental bottle and filtered onto GF/Fs ii) heterotrophic microplankton community determination, these were taken from each dilution and fixed in 1% Lugols iodine and iii) for nutrient analysis, 50mls were taken from $T_0$ and $T_{24}$ undiluted bottles. During one experiment chlorophyll samples were taken at 8, 12, 16 and 24 hrs to determine whether or not there was a diel pattern to growth and grazing. Chlorophyll was extracted with 90% acetone and after homogenisation samples were left at –20°C overnight and measured using a highly sensitive fluorometer (Aiken, 1981). Fixed Lugol's samples were stored cool and in the dark for later laboratory analysis by microscopy. Paul McCardle, Queen's University Belfast, carried out nutrient analyses on board ship using the RVS Chemlab Auto-Analyser system. Microzooplankton grazing was determined from measurements of the apparent growth rate of phytoplankton estimated according to Landry & Hassett (1982) as described in section 2.1. Estimates of the grazing coefficient 'g' were converted to carbon using a conversion factor of 32 (Morales et al 1991). Microzooplankton grazing rates were compared to parallel measurements of primary production determined using the $^{14}C$ method by Phil Boyd, Queen's University of Belfast in May and Alan Pomroy, Plymouth Marine Laboratory, in June. The proportion of primary production grazed by the microzooplankton was determined by using the following equation:

$$\% \text{ primary production grazed} = \frac{\text{turnover x chlorophyll carbon}}{\text{primary production.}} \times 100$$

*4.2.2 The Microzooplankton Community*

Microzooplankton biomass samples were collected in conjunction with the grazing experiments. Water was collected using the CTD from 9 depths within the top 300m of the water column. Two complementary techniques are required for the quantification of microzooplankton biomass. The heterotrophic microplankton including the nanociliates were quantified by settlement microscopy. The smaller HNAN (including heterotrophic

nanodinoflagellates) which cannot be distinguished from autotrophic forms by ordinary light microscopy, were enumerated by epifluorescence microscopic analysis of stained samples on microscope slides. 500mls of water sample were fixed in 1% acid Lugol's solution and stored in amber glass jars away from direct sunlight, as Lugol's solution is photosensitive. In addition, a small volume (20-50mls) of the same water sample was fixed in 0.3% glutaraldehyde for the determination of nanoplankton abundance. These latter samples were dual-stained with diamidino-2-phenylindole (DAPI, final concentration 5$\mu$g ml$^{-1}$) and proflavine (final concentration 5$\mu$g ml$^{-1}$) and then filtered onto 0.4$\mu$m (for nanoflagellates) and 0.8$\mu$m (for nanodinoflagellates) black polycarbonate filters. The filters were mounted onto microscope slides using a small drop of 'Resolve' immersion oil and frozen at –20°C for subsequent analysis by fluorescence microscopy. 450ml water samples were fixed in 2% (final concentration) formaldehyde buffered with hexamethylamine, a fixative which allows the differentiation of plastidic and non-plastidic cells using epifluorescence microscopy (Stoecker et al 1989). These samples were stored at 4°C in the dark until enumerated.
Peter Verity, Mike Sieracki and their co-workers at Bigelow Laboratory and Skidaway Institute, USA respectively, carried out analyses of nanoplankton samples. Diane Stoecker and co-workers at Horn Point Environmental Laboratory, USA, carried out plastidic and non-plastidic microplankton determinations. Tony John, Plymouth Marine Laboratory, carried out analyses of vertical profile samples whilst I determined the abundance and biomass of microzooplankton in all experimental samples.

The methods for analysis of experimental Lugol's samples and vertical profile Lugol's samples are exactly the same. Prior to analysis, Lugol's samples were gently mixed and subsamples of between 20 and 100mls were concentrated for a minimum of 16 hours in sedimentation chambers. To quantify heterotrophic microzooplankton biomass samples were analysed by inverted microscopy (Utermöhl, 1958). Each sample was examined at a magnification of x300 using an Olympus IMT-2 inverted microscope fitted with phase contrast. All ciliate grazers and heterotrophic dinoflagellates (>20$\mu$m) were enumerated and identified. By observing live samples at sea using fluorescence microscopy, it was possible to determine the absence or presence of chlorophyll within cells and this enabled the distinction of heterotrophic dinoflagellates from phototrophic forms. Reference was also made to Lessard & Swift (1986) and Lebour (1925). All ciliates were assumed to be heterotrophic. Each organism was categorised into one of 5 groups:

heterotrophic dinoflagellates (>20$\mu$m), aloricate oligotrich ciliates, tintinnids, other ciliates and "others" which included metazoan nauplii and sarcodines. The outline of each protistan cell counted was traced with a computer "mouse" linked to a PML image analysis system. Information on the name, area and maximum length of each cell was stored electronically and used to compute cellular biovolume according to the equations below. All cells were assumed to be ellipsoid or semi-ellipsoid in shape where:

$$\text{Volume of an Ellipsoid/semi-ellipsoid } \mu m^3 = \frac{\frac{8}{3\pi} \times \text{area}^2}{\text{length}}$$

The carbon content of each cell was determined using an appropriate carbon conversion factor; for ciliates this was 0.19 pg C $\mu m^{-3}$ (Putt & Stoecker, 1989) and for dinoflagellates 0.14 pg C $\mu m^{-3}$ (Lessard, 1991). The biomass of each organism was summed and divided by the number of mls settled to determine the amount of carbon present in pg $ml^{-1}$, and was converted to express results as $\mu$g C $l^{-1}$. Sarcodine biomass was calculated using a conversion factor of 0.10 pg $\mu m^{-3}$ (Michaels et al 1995). The biomass of metazoan nauplii was calculated assuming a naupliar carbon content of 60ng C per individual (Verity et al 1993a).

## 4.3 Results

### *4.3.1 Description of environmental conditions*

Despite the study area being characterised by a complex hydrographic pattern, including eddies, discontinuities and warm and cold intrusions, the general characteristics of a spring bloom were observed (Savidge et al 1992). During the first part of the cruise low cloud and misty conditions prevailed resulting in low irradiance. From 12 May onwards less cloudy conditions were encountered leading to increased irradiances. Mean sea-surface temperature increased gradually from 11.5 to 16.0°C and was accompanied by an increase in stratification. A seasonal thermocline developed at a depth of 20-35m. Nitrate concentrations in the surface mixed layer decreased slowly but steadily from 6$\mu$M $l^{-1}$ on 1 May to 3$\mu$M $l^{-1}$ by 13 May. This decreasing trend was consistent with an increase in chlorophyll concentrations which, at a depth of 5m, rose from 1.2 $\mu$g $l^{-1}$ on 1 May to 3.7$\mu$g $l^{-1}$ at the peak of the bloom in mid-May (Barlow et al 1993). At this time the water appeared milky with a green background colouration, this bloom coincided with satellite data showing bands of high reflectivity in the area (Savidge et al 1992). The dominant

phytoplankton species was identified as the 5$\mu$m diatom, *Nanoneis haslae* (Derek Harbour pers comm.) Following this there was a subsequent decline in chlorophyll concentration by 19 May. At the same time nitrate concentrations decreased from 4$\mu$M $l^{-1}$ to <0.5 $\mu$M $l^{-1}$.

The phytoplankton was dominated by nanophytoplankton and not microplankton as had been hypothesised for a spring bloom period. A rapid uptake of silicate between 13 and 17 May indicated that the major growth of the phytoplankton was dominated by diatoms; this was confirmed by taxonomic observations (Derek Harbour pers comm.) Size-fractionated chlorophyll measurements showed a dominance of the 1-5 $\mu$m size fraction with the picoplankton averaging less than 10% of the phytoplankton. Water column integrated primary production steadily increased reaching a peak of 1914 mg C $m^{-2}$ $d^{-1}$ on 17 May (Savidge et al 1995). In June, the post-bloom phase, the phytoplankton population was dominated by prymnesiophytes (Barlow et al 1993).

*4.3.2 Grazing*

Examples of the results of 6 dilution experiments are shown in Figure 4.1. A significant linear grazing response was recorded in 15 out of the 22 dilution experiments. Results of all dilution experiments are summarised in Table 4.1. The phytoplankton growth coefficient 'k' ranged from 0.1 to 0.6 $day^{-1}$. The grazing coefficient 'g' ranged from 0.2 to 0.5 $day^{-1}$. Growth and grazing coefficients were variable and there were no clear trends in the data. It is interesting that the lowest phytoplankton growth occurred on and after the 19 May, which coincided with a drop in ambient nutrient concentrations. The daily turnover of chlorophyll standing stocks ranged from 22 to 37% in May and from 16 to 40% in June.

Table 4.1: Summary of phytoplankton specific growth rate (k) and grazing mortality (g) determined from dilution experiments in the Northeast Atlantic in May and June 1990. Chl-*a* = initial chlorophyll concentration, $NO_3$ = nitrate concentration; $r^2$ = correlation coefficient of regression analysis, ns = not significant, no asterisk- P<0.1, *- p<0.05, **- p<0.01, *** -p<0.005, **** -p<0.001; % turnover = daily turnover of chlorophyll standing stock; - indicates that no measurements were made.

| Date | Depth (m) | Temp °C | Chl-*a* mg $m^{-3}$ | $NO_3$ $\mu M$ $T_0$ | $NO_3$ $\mu M$ $T_{24}$ | k $d^{-1}$ | g $d^{-1}$ | r2 | % Turnover |
|---|---|---|---|---|---|---|---|---|---|
| 1/5 | 10 | 12.3 | 1.47 | - | - | 0.322 | -0.45 | 0.70** | 36 |
| 3/5 | 25 | 12.3 | 1.30 | 6.3 | - | 0.132 | -0.30 | 0.44ns | |
| 4/5 | 10 | 12.5 | 1.30 | - | - | 0.574 | -0.28 | 0.99**** | 25 |
| 5/5 | 25 | 12.3 | 1.24 | 6.31 | 6.01 | 0.286 | -0.34 | 0.78*** | 29 |
| 7/5 | 10 | 12.7 | 2.13 | 4.91 | 4.58 | 0.219 | -0.34 | 0.86**** | 29 |
| 8/5 | 10 | 12.7 | 1.32 | 4.36 | - | 0.181 | -0.29 | 0.58* | 25 |
| 9/5 *in situ* | 10 | 12.7 | 1.89 | 4.26 | 4.29 | 0.347 | -0.46 | 0.93**** | 37 |
| 9/5 | 10 | 12.7 | 1.89 | 4.26 | 4.29 | 0.493 | -0.46 | 0.77*** | 37 |
| 11/5 | 25 | 12.9 | 1.15 | 6.52 | 6.42 | 0.277 | -0.37 | 0.56* | 31 |
| 13/5 | 25 | 12.7 | 2.10 | 3.77 | 3.01 | 0.224 | -0.34 | 0.85**** | 29 |
| 15/5 | 10 | 13.4 | 3.70 | - | 2.77 | 0.049 | -0.06 | 0.06 ns | |
| 17/5 | 10 | 13.4 | 3.50 | - | - | 0.344 | -0.18 | 0.24 ns | |
| 19/5 | 25 | 13.8 | 1.80 | - | - | 0.007 | -0.25 | 0.67 | 22 |
| 28/5 | 25 | 14.3 | 1.80 | - | - | 0.491 | -0.15 | 0.14 ns | |
| 30/5 | 25 | 14.9 | 1.80 | - | | 0.044 | -0.26 | 0.37 ns | |
| 1/6 | 10 | 14.5 | 2.36 | 0.51 | 0.49 | 0.395 | -0.50 | 0.71** | 39 |
| 3/6 | 10 | 15.2 | 1.42 | - | - | 0.281 | -0.11 | 0.21 ns | |
| 5/6 | 25 | 14.7 | 1.30 | 2.57 | 2.57 | 0.219 | -0.27 | 0.65* | 23 |
| 8/6 | 25 | 14.7 | 1.20 | 0.63 | 0.65 | 0.120 | -0.20 | 0.78*** | 18 |
| 9/6 | 25 | 14.7 | 0.41 | - | - | 0.05 | -0.628 | 0.40 ns | |
| 11/6 | 10 | 15.3 | 1.20 | 0.26 | 0.06 | 0.079 | -0.18 | 0.75*** | 16 |
| 13/6 | 10 | 15.6 | 0.50 | 0.41 | 0.34 | 0.535 | -0.50 | 0.75*** | 40 |
| 15/6 | 10 | 15.6 | 0.50 | - | - | 0.186 | -0.42 | 0.48 | 34 |

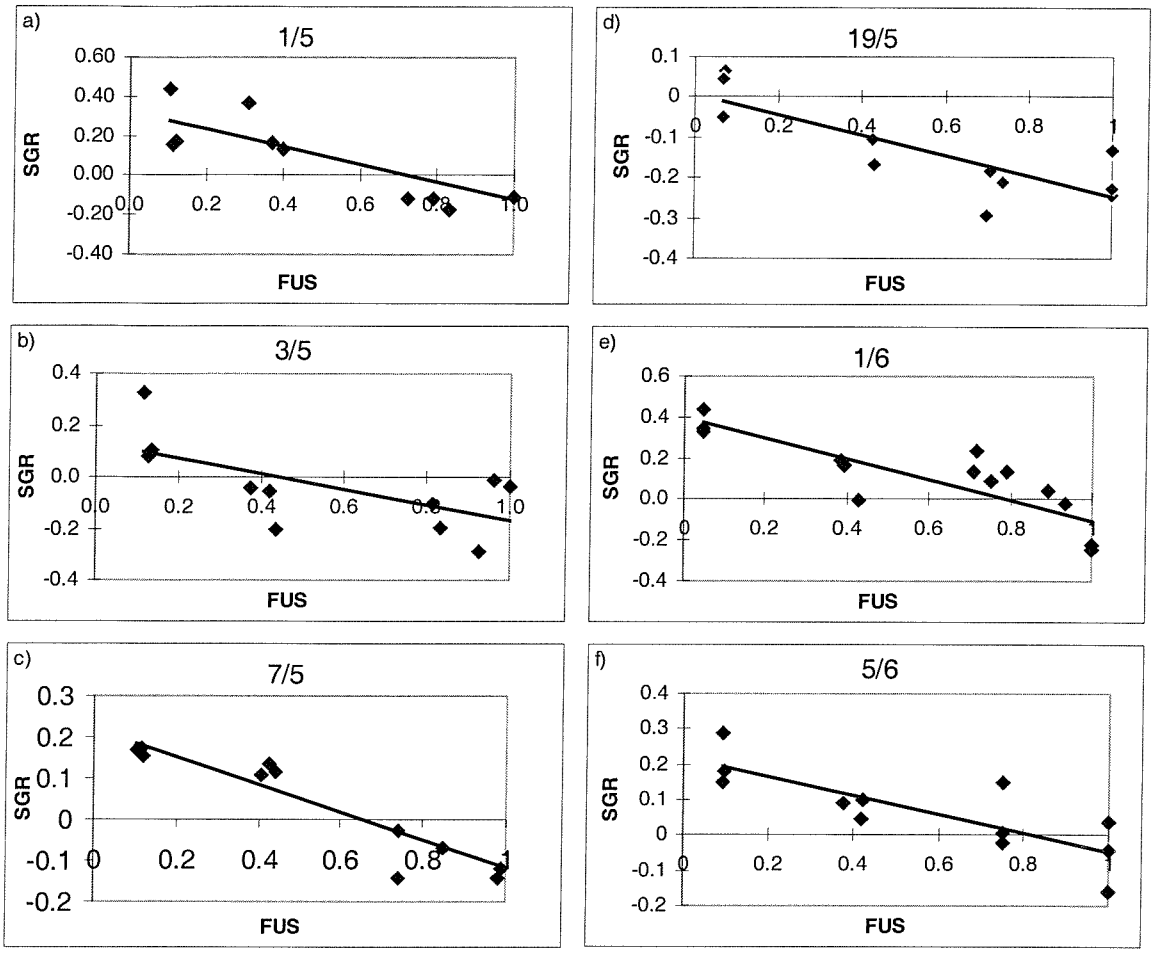



Figure 4.1: Examples of Dilution plots from microzooplankton grazing experiments: SGR = specific growth rate, FUS = fraction undiluted seawater. Line through data points = linear regression.

The proportion of the $^{14}C$ primary production grazed by the microzooplankton per day ranged between 28 and 78% during May with highest grazing impact occurring during early May but not coincident with the peak of the phytoplankton bloom in mid-May. During June, microzooplankton grazing was lower with between 26 and 55% of the primary production being removed daily (Figure 4.2). Microzooplankton herbivory was positively correlated to chlorophyll concentration ($r^2$= 75%, p=<0.001) and $^{14}C$ primary production ($r^2$=52%, p=<0.05).

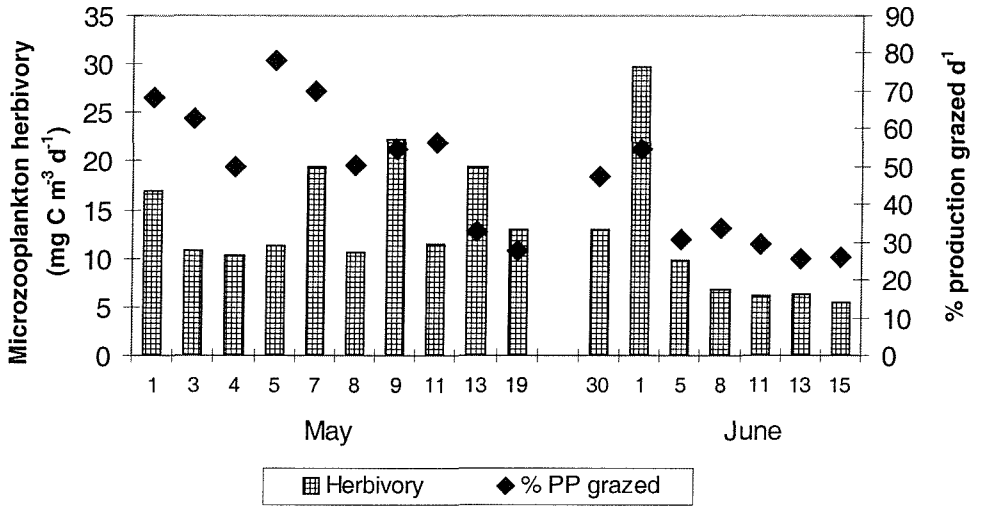



Figure 4.2: Microzooplankton grazing impact and the proportion of Primary production grazed daily in surface waters (assuming C:Chl ratio of 32).

*4.3.2.1 Diel Experiment*

Results of the diel experiment suggest that microzooplankton grazing was highest during the day when phytoplankton growth was also highest and decreased during the night (Table 4.2). Over the first 12 hours microzooplankton turned over between 74 and 81 % of the phytoplankton but during the night the turnover decreased to between 25 and 50%.

| Time | 'k' | 'g' | $r^2$ | % T |
|---|---|---|---|---|
| *8 hours* | 1.18 | -1.355 | 0.94 | 74 |
| *12 hours* | 1.60 | -1.625 | 0.93 | 81 |
| *16 hours* | 0.94 | -0.696 | 0.94 | 50 |
| *24 hours* | 0.57 | -0.283 | 0.99 | 25 |

Table 4.2 Apparent growth rate (k) and grazing rate (g) determined from a diel dilution experiment carried out on 4 May in the northeast Atlantic $r^2$= correlation coefficient of linear regression T = daily turnover of chlorophyll stock

4.3.3 *The Microzooplankton Community*

*4.3.3.1 Abundance and biomass*

Cell concentrations and biomass for the main heterotrophic microplankton groups recorded in one of the undiluted bottles for each dilution experiment are shown in Table 4.3. a & b.

| Date | Abundance Cells $l^{-1}$ | | | | |
|---|---|---|---|---|---|
| | **Oligotrich ciliates** | **Tintinnid Ciliates** | **Other Ciliates** | **>20$\mu$m HDinos** | **Metazoan nauplii** |
| 1/5/1990 | 35048 | 0 | 571 | 1048 | 95 |
| 3/5/1990 | 30857 | 336 | 381 | 2952 | 667 |
| 4/5/1990 | 11381 | 268 | 1572 | 4645 | 0 |
| 5/5/1990 | 9540 | 265 | 1060 | 2385 | 50 |
| 8/5/1990 | 14700 | 0 | 49 | 1100 | 400 |
| 9/5/1990 | 16550 | 50 | 200 | 1100 | 150 |
| 11/5/1990 | 6161 | 649 | 2378 | 8755 | 108 |
| 13/5/1990 | 4600 | 100 | 150 | 950 | 250 |
| 15/5/1990 | 4312 | 0 | 64 | 1352 | 303 |
| 19/5/1990 | 2450 | 0 | 100 | 700 | 300 |
| 30/5/1990 | 2500 | 3750 | 400 | 1100 | 150 |
| 1/6/1990 | 4500 | 19800 | 200 | 800 | 100 |
| 3/6/1990 | 3418 | 22650 | 0 | 475 | 0 |
| 5/6/1990 | 2700 | 7000 | 0 | 850 | 0 |
| 8/6/1990 | 3900 | 7100 | 100 | 3300 | 100 |
| 11/6/1990 | 3850 | 1000 | 350 | 600 | 100 |
| 13/6/1990 | 5800 | 200 | 100 | 3000 | 100 |
| 15/6/1990 | 3650 | 300 | 350 | 4050 | 100 |

Table 4.3 a) Abundance of five heterotrophic microplankton groups present in undiluted experimental bottles for 18 out of the 22 dilution experiments carried out.

Initially the concentration of HMIC was very high, >35,000 cells $l^{-1}$ and this was due to large numbers of small oligotrich ciliates. However, cell concentrations decreased by 50% by 3 May (Figure 4.3). Interestingly, this decreasing trend continued until the end of the May cruise. During June, HMIC abundance was highest around 1-3 June and showed a decreasing trend until the end of the study. There are no clear trends in the abundance of metazoa, ideally larger sample volumes would be required in order to have accurate measurements of metazoan abundance. However, the data available from experimental bottles do indicate that metazoan nauplii were abundant in some

grazing experiments with abundance ranging from 0 to 667 individuals $l^{-1}$ (Table 4.3a). The overall trend in PHMIC biomass shows a gradual decrease from 16 mg C $m^{-3}$ on 1 May to 2 mg C $m^{-3}$ on 19 May with the exception of 8 and 9 May when biomass reached maximum values of between 20 and 22 mg C $m^{-3}$. During June PHMIC biomass was less variable ranging from 2.4 to 5.8 mg C $m^{-3}$ (Figure 4.3). MHMIC biomass ranged from 0 to 40 mg C $m^{-3}$ during May and was lower and less variable in June averaging 5 mg C $m^{-3}$; in a number of experiments the biomass of MHMIC exceeded that of PHMIC (Table 4.3b).

| Date | Biomass mg C $m^{-3}$ | | | | |
|---|---|---|---|---|---|
| | **Oligotrich ciliates** | **Tintinnid ciliates** | **Other Ciliates** | **>20$\mu$m HDinos** | **Metazoa nauplii** |
| 1/5/1990 | 16.06 | 0 | 0.035 | 0.357 | 6 |
| 3/5/1990 | 11.94 | 0.34 | 0.02 | 2.09 | 40 |
| 4/5/1990 | 8.44 | 1.16 | 0.204 | 3.442 | 0 |
| 5/5/1990 | 5.40 | 1.62 | 0.113 | 1.519 | 3 |
| 7/5/1990 | n/a | n/a | n/a | n/a | n/a |
| 8/5/1990 | 19.81 | 0 | 0.003 | 0.407 | 24 |
| 9/5/1990 | 21.58 | 0.00 | 0.007 | 0.267 | 9 |
| 11/5/1990 | 2.07 | 1.78 | 0.716 | 1.340 | 6 |
| 13/5/1990 | 4.26 | 0.316 | 0.648 | 0.459 | 15 |
| 15/5/1990 | 2.00 | 0 | 0.000 | 1.678 | 18 |
| 19/5/1990 | 1.37 | 0 | 0.007 | 0.348 | 18 |
| 30/5/1990 | 1.40 | 0.36 | 0.006 | 0.646 | 9 |
| 1/6/1990 | 2.84 | 1.89 | 0.012 | 0.751 | 6 |
| 3/6/1990 | 1.14 | 2.16 | 0.000 | 0.557 | 0 |
| 5/6/1990 | 1.28 | 0.67 | 0.000 | 1.385 | 0 |
| 8/6/1990 | 1.12 | 0.68 | 0.000 | 2.479 | 6 |
| 11/6/1990 | 1.78 | 0.25 | 2.241 | 0.576 | 6 |
| 13/6/1990 | 3.84 | 0.05 | 0.003 | 1.939 | 6 |
| 15/6/1990 | 1.21 | 0.08 | 0.034 | 1.284 | 6 |

Table 4.3 b) Biomass of five heterotrophic microplankton groups present in undiluted experimental bottles for 18 out of the 22 dilution experiments carried out.

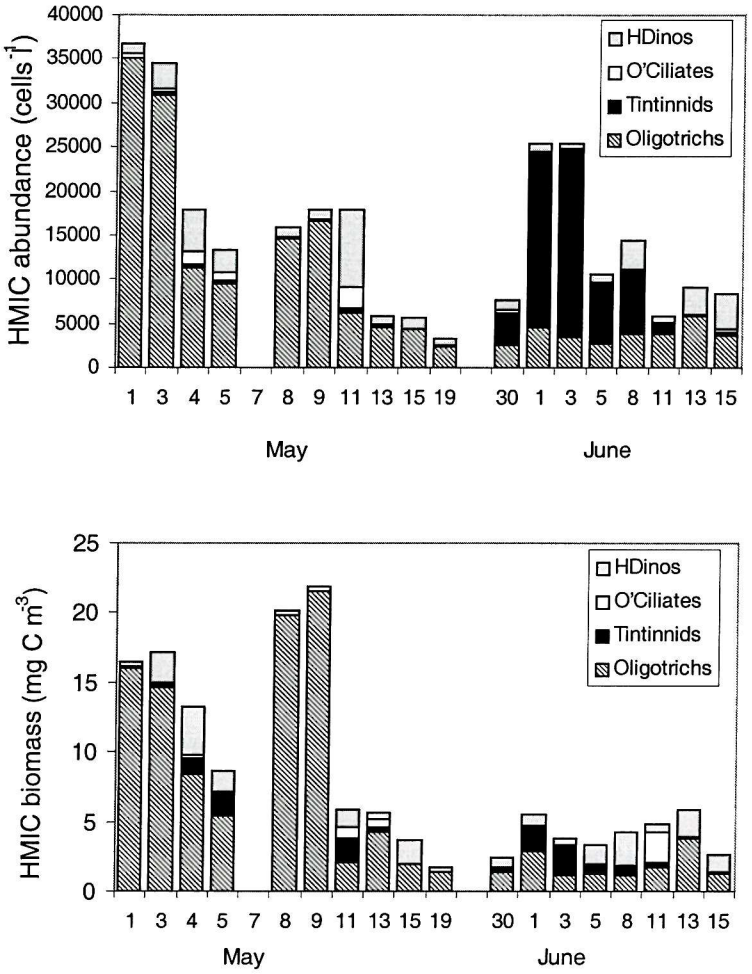



Figure 4.3 Changes in heterotrophic microplankton (HMIC) abundance and biomass during May and June 1990

*4.3.2.2 Community composition of PHMIC*

During May the PHMIC community was numerically dominated by oligotrich ciliates, typically small <20$\mu$m *Strombidium* spp. Oligotrich abundance ranged from 2,450 to 35,048 cells $l^{-1}$ (Table 4.3a). Tintinnids and 'other ciliates' comprised only a small proportion of the total microzooplankton abundance. The situation was very different during June when tintinnids were numerically the most abundant. On 3 June tintinnids comprised 70% of the total abundance (Figure 4.4). The tintinnid responsible for the high abundance of this group was a *Salpingella* sp. which reached maximum abundance of 22,650 cells $l^{-1}$.

Oligotrichs and heterotrophic dinoflagellates increased in importance towards the end of the study in mid-June.

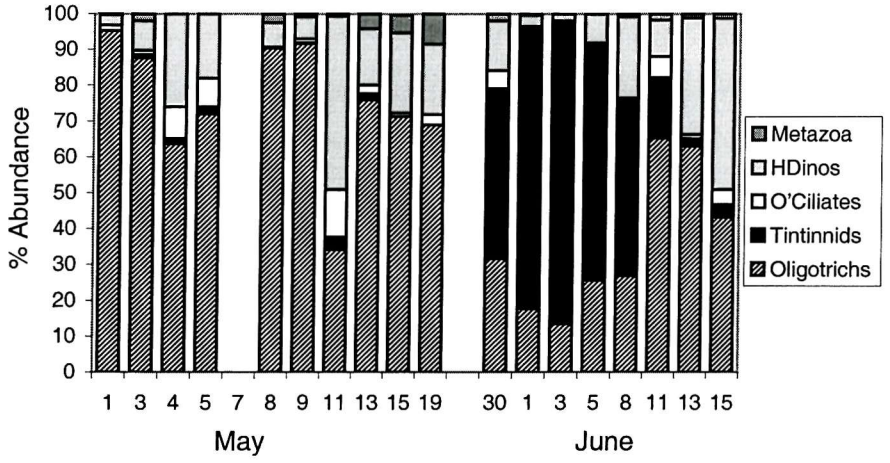



Figure 4.4 -The contribution of heterotrophic microplankton groups to total cell concentration

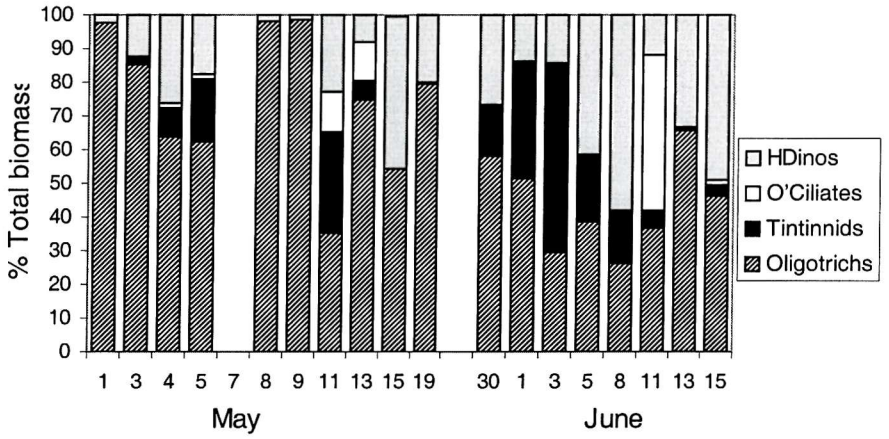



Figure 4.5 -The contribution of heterotrophic microplankton groups to total HMIC biomass.

Biomass of PHMIC followed a similar trend to abundance (Figure 4.5). Oligotrich ciliates comprised a high percentage of the total biomass in May but heterotrophic dinoflagellates and tintinnids formed a greater contribution in early June. On the 11 June over one third of the biomass was due to 'Other ciliates', although their abundance remained low. This was due to the presence of a large unidentified ciliate with a maximum length of >50$\mu$m.

*4.3.2.3 Size distribution*

There was an overall increasing trend in the mean cell volume, and thus carbon content, of heterotrophic microplankton cells. Cellular carbon increased from 0.4 pg C cell$^{-1}$ on 1

May to a maximum of approx 1.2 pg C cell$^{-1}$ being reached on 8 to 15 May. At the end of May and in early June, cell carbon decreased to 0.2 to 0.4 pg C cell$^{-1}$ (Figure 4.6).

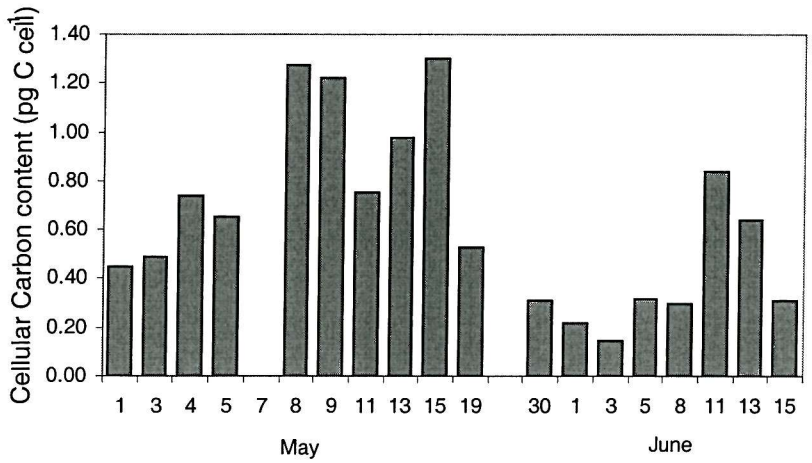



Figure 4.6 Heterotrophic Microplankton cell carbon during may and June 1990.

Heterotrophic microplankton were categorised into four size classes according to their maximum length and the mean frequency was calculated for May and June. The results show that during both May and June the cells of <20$\mu$m in length dominated with a frequency of over 10,000 cells l$^{-1}$. The proportion of cells above 40$\mu$m in length was much greater in May than in June. Cells with a maximum length >80$\mu$m were rare (Figure 4.7).

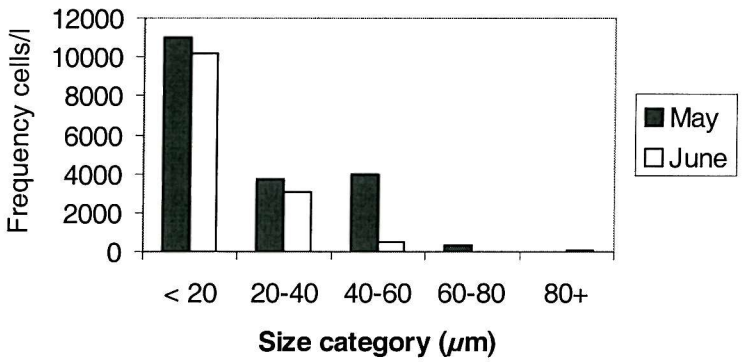



Figure 4.7 Heterotrophic microplankton size distribution during May and June 1990.

Figure 4.8 a) and b) demonstrate how the size distribution of heterotrophic microplankton changed with time during May and June. The most striking change occurred in early May when there was a dramatic decrease in the abundance of <20$\mu$m

oligotrichs. This was followed by a rise in the 40-60$\mu$m-size class. During June abundance of oligotrichs was much lower, <5,000 cells $l^{-1}$ and the increase in <20$\mu$m sized cells in early June was due to high numbers of small *Salpingella*-like tintinnids.

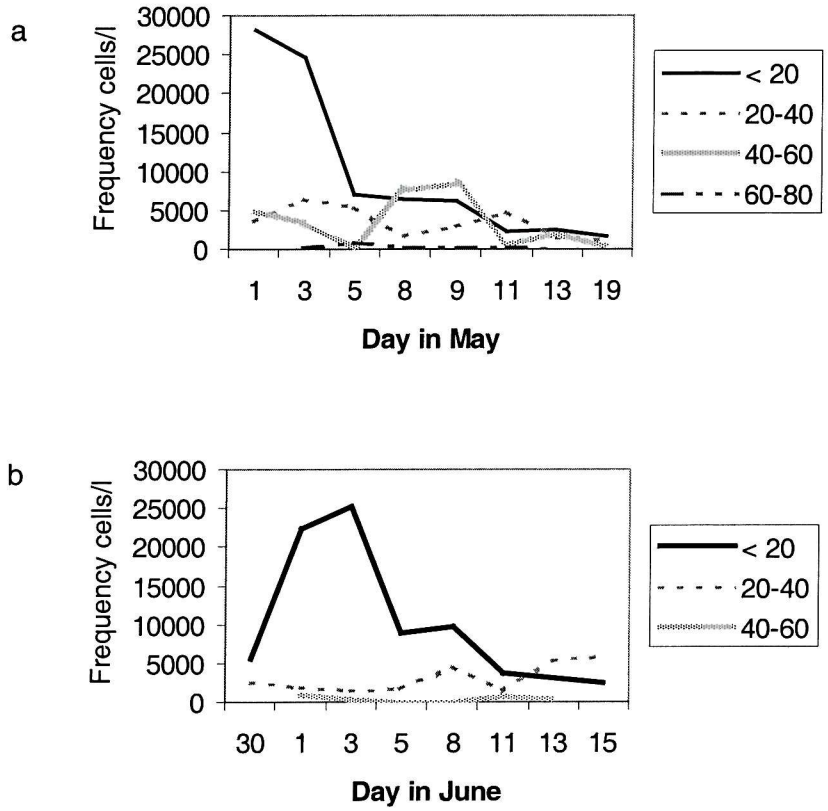



Figure 4.8a & b Changes in the size distribution of heterotrophic microplankton during May and June 1990

*4.3.2.4 Vertical structure*

The vertical structure of heterotrophic microplankton communities was determined on 7 occasions in May and on only 2 occasions during June. The data show differences in the heterotrophic community with depth. This was quite pronounced on 13 and 15 May when oligotrichous ciliates clearly dominated in the top 20m but at 30-40m, tintinnid ciliates formed a significant proportion of the biomass (Figure 4.9). Heterotrophic dinoflagellates comprised a higher proportion of the biomass at 40m depth than at 10m. Biomass of metazoan nauplii has not been included with these data. Depth integrated PHMIC biomass varied from 157 to 488 mg C $m^{-2}$ during May and 253 to 478 mg C $m^{-2}$

in June. These data were compared to standing stocks of other heterotrophic components of the community and the results are shown in Table 4.4

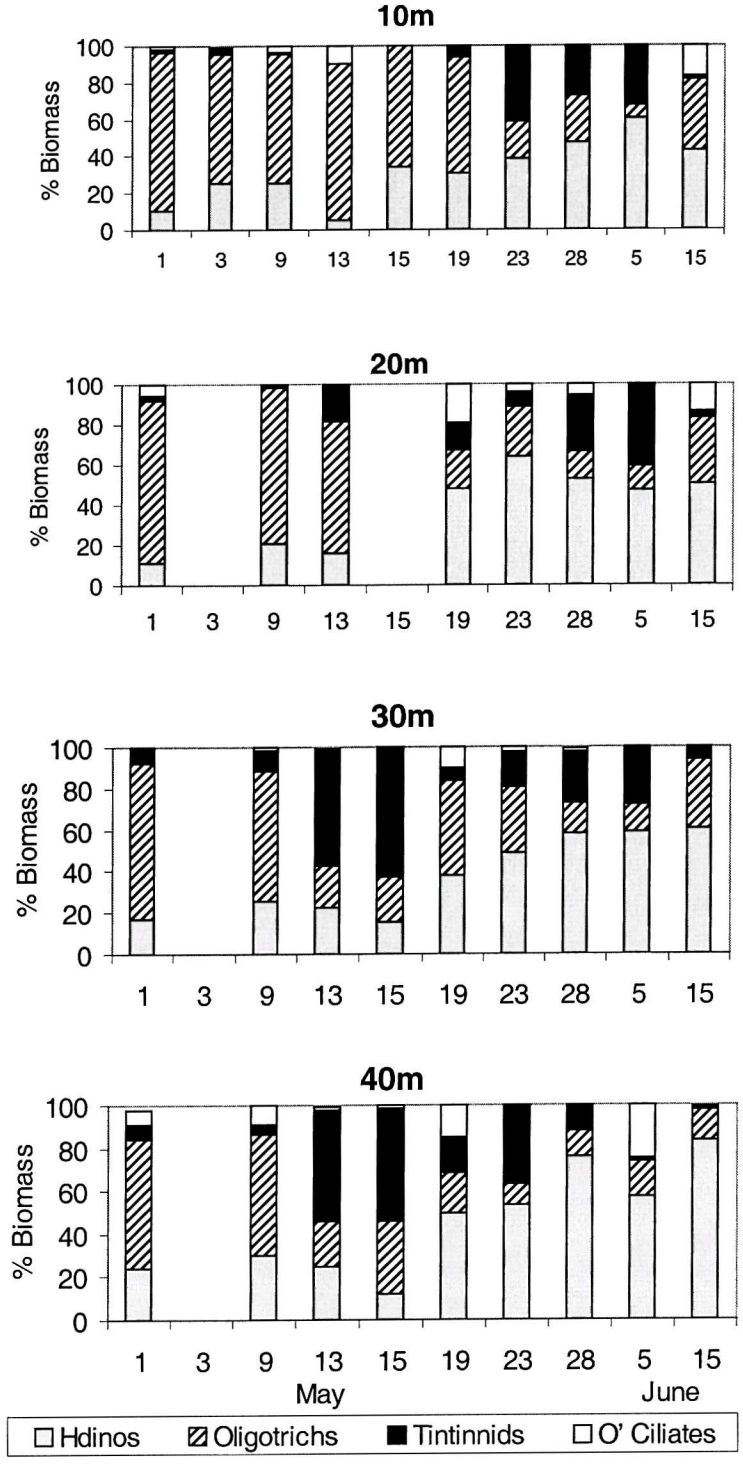



Figure 4.9 The contribution of the four main PHMIC groups to total PHMIC biomass between 10 and 40m.

| | Depth integrated biomass mg C $m^{-2}$ | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1/5 | 9/5 | 19/5 | 23/5 | 28/5 | 5/6 | 15/6 |
| HNAN * | 334 | 345 | 336 | n.d | 278 | 297 | 662 |
| HDINOS <20$\mu$m # | 415 | 230 | 440 | n.d | 380 | 308 | 120 |
| PHMIC | 488 | 784 | 157 | 165 | 232 | 253 | 478 |
| MHMIC | 183 | 144 | 72 | 279 | 195 | 78 | 69 |
| TOTAL HETEROTROPHS 2-200$\mu$m | 1420 | 1503 | 1005 | | 1085 | 936 | 1329 |

*data provided by Mike Sieracki published in Stoecker et al 1994.

# data from Verity et al 1993.

Table 4.4 Biomass composition of total microzooplankton community during May and early June 1990. Values were calculated by integrating measurements made in the top 30m. n.d = no data.

## 4.4 Discussion

This study has illustrated how the microzooplankton can respond to changes in phytoplankton standing stocks associated with the spring bloom in the Northeast Atlantic.

*4.4.1 Grazing*

The microzooplankton grazing and phytoplankton growth coefficients measured from dilution experiments in 1990 indicate that the grazing impact of microzooplankton on total phytoplankton chlorophyll was between 16 and 40% of chlorophyll standing stock, which was equivalent to between 26 and 78 % of primary production being removed by the microzooplankton daily. Grazing impact in surface waters was greater during early May. These observed grazing rates are consistent with other rates measured during similar studies conducted in the same area of the North Atlantic e.g. 37-100% of the primary production was grazed daily during the 1989 spring bloom at 47°N (Verity et al 1993a). Clearly calculations of this nature are highly dependent on the choice of C:Chl ratio. In this study a C:Chl ratio of 32 has been used, Verity et al (1993) used 40 and Fasham et al (1999) used 50. If a ratio of 40 is applied to the data in this study, the range of primary production grazed increases to between 32 and 98%.

Estimates of phytoplankton growth rate determined during May of between 0.1 and 0.6 $day^{-1}$, although within the ranges reported for oceanic areas (e.g. Strom & Strom 1996; Verity et al 1996), are lower than those reported for the northeast Atlantic spring bloom in May 1989 (Verity et al, 1993a). It is possible that estimates of 'k' determined by shipboard incubations of dilution experiments could be biased if light levels are not realistic. For these experiments, the Gallenkamp incubator used has a set lighting regime and although every effort was made to ensure that light levels were as close as possible to ambient levels, it may not have been possible to accurately match the changes in irradiance during May. Matching irradiance levels was also a problem for incubation of water collected from 25m. To alleviate this problem one could assume that the plankton populations were similar at 10 and 25m depths and therefore all grazing rates are equivalent to an incubation depth of 10m. One consequence of different irradiance levels, would be a change in the cellular chlorophyll content as a result of photoadaptation (McManus, 1995). Such changes in chlorophyll $cell^{-1}$ during incubations would be misinterpreted as 'growth' or 'mortality' of phytoplankton but are in fact unrelated to actual changes in abundance or biomass of the phytoplankton assemblage. In addition, the incubation of samples at a light intensity different to *in situ* conditions could affect the photosynthetic rate of the phytoplankton in the incubated bottles and therefore result in growth rates that are not indicative of *in situ* rates. In an attempt to determine whether levels were over- or underestimated, incubations for dilution experiment 7 were carried out using an *in situ* rig. This involved the attachment of 12 experimental bottles to a 'dexian' cube, bottles were held in place with jubilee clips. The cube was attached to the primary productivity rig and incubated at the same depth from which the experimental water had been collected. Interestingly estimates for 'g' were exactly the same for each of the incubation methods (Table 4.1) but the estimate of phytoplankton growth was lower '*in situ*'. As this result is only for one experiment, further comparisons on a larger number of samples will need to be carried out in the future to determine the significance of this data. However, it must be borne in mind that phytoplankton growth measured in dilution experiments that were incubated in the Gallenkamp incubator may be overestimated perhaps by as much as 30%.

*4.4.1.2 Saturated feeding & the 3-point method*

The phytoplankton growth response in the dilution experiments in most cases was linear with the fraction of undiluted seawater (see Figure 4.1). In a few cases where the

response appeared to be non-linear the question arose as how best to interpret the data. Non-linear responses have been observed in cases of saturated feeding such as that described by Gallegos (1989) for eutrophic waters. The question is whether this non-linear response found in the northeast Atlantic is due to saturated feeding or some other factor. There were four occasions where dilution curves indicated saturated feeding by the microzooplankton (three of which are shown in Figure 4.1 a, b & d), and the '3-point' method of Gallegos (1989) has been applied. With this method, only the most dilute treatments are used to extrapolate for phytoplankton growth at the y-intercept. The 3-point method relies upon the apparent growth in the 2 most dilute treatments; thereby avoiding any biases which may be caused by saturated grazing at high food concentrations.

| **Date** | **Depth (m)** | **g $d^{-1}$** | **g $d^{-1}$** | **k $d^{-1}$** | **k $d^{-1}$** | **$r^2$** | **$r^2$** | **T % $d^{-1}$** | **T % $d^{-1}$** | **% PP grazed $d^{-1}$** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ***LR*** | ***3-pt*** | ***LR*** | ***3-pt*** | ***LR*** | ***3-pt*** | ***LR*** | ***3pt*** | ***3 pt*** |
| 01/05 | 10 | -0.445 | -0.625 | 0.322 | 0.376 | 0.70 | 0.75 | 36 | 47 | 89 |
| 03/05 | 25 | -0.299 | -0.491 | 0.132 | 0.187 | 0.44 | 0.69 | 25 | 39 | 94 |
| 09/05 | 10 | -0.456 | -0.583 | 0.347 | 0.391 | 0.93 | 0.99 | 37 | 44 | 66 |
| 19/05 | 25 | -0.254 | -0.387 | 0.007 | 0.043 | 0.67 | 0.84 | 22 | 32 | 39 |

Table 4.5: Comparison of dilution experiment rate estimates ($d^{-1}$) obtained using different computation methods. 'g'= grazing coefficient; 'k' = growth coefficient ; $r^2$ = correlation coefficient of regression analysis; T = turnover per day ; LR = linear regression ; 3-pt = 3-point method (Gallegos 1989)

The correlation coefficient increased for all 4 experiments when the 3-point method was adopted. Use of the '3-point method' in this instance, demonstrates that values of 'g' and to a lesser extent 'k', would be underestimated in cases of non-linearity arising from saturated feeding (Table 4.5). As a result there would be an increase in the daily turnover of chlorophyll standing stocks by between 19 and 56%. This would result in an increase in the total primary production grazed by between 11 and 31% $d^{-1}$. Gallegos (1989) found non-linearity in dilution experiments to occur as a result of saturated feeding in eutrophic conditions, similarly it has been documented for Chesapeake Bay, USA (McManus & Ederington-Cantrell 1992). Other authors, James & Hall (1998) also

found non-linearity in their data, particularly in winter when initial prey concentrations were low. They concluded that their non-linear response was unlikely to be due to threshold or saturated feeding or nutrient depletion. In this study the chlorophyll concentration was between 1 and 2 $\mu g\ l^{-1}$, therefore is saturated feeding the real reason for this non-linear effect? Or in this case is it due to nutrient deficiency? In this study, nutrient limitation is unlikely to have occurred, results of nutrient analyses from several experiments showed that nutrients remained abundant in the undiluted bottles at the end of the 24-hour incubations (Table 4.1). The 3-point method would only be a solution to nutrient deficiency if nutrients became depleted in the undiluted treatments, but were still available throughout the incubation for all other dilutions.

The results demonstrate how the use of the 3-point method would allow a more accurate estimation of grazing rates under conditions of saturated grazing. Evans & Paranjape (1992) caution this method because reduced numbers of observations could lead to loss of precision with regard to linear regression based estimates and high natural variability. The data presented here, however, do not appear to support this.

### *4.4.2 The microzooplankton community*

#### *4.4.2.1 Abundance & biomass*

During the 1990 Lagrangian study abundance and biomass of microzooplankton were high and there were differences in the structure of the communities compared to studies in 1989 (Verity et al 1993b, Stoecker et al 1994). This reflects the differences in the phytoplankton community encountered between the two years. It is interesting that the maximum microzooplankton biomasses found during this study do not coincide with the peak in the spring bloom chlorophyll concentrations. This could be due to the composition of the phytoplankton community. It is known from microscopic analysis that in 1990 the <20$\mu$m pennate diatoms were an important component of the phytoplankton community (Stoecker et al 1994) and that this was dominated by a small 5$\mu$m species, *Nanoneis haslae*. This dominance by diatoms is further confirmed from pigment analyses by Barlow et al (1993), who found that 70% of the phytoplankton community was made up of diatoms in surface waters during May. It is generally recognised that the preferred food for oligotrichous ciliates are nanoflagellates (Rassoulzadegan et al 1988) and that most ciliates ingest food particles 10 times smaller than themselves (Hansen et al 1994). Heterotrophic dinoflagellates, on the other hand, are able to capture and engulf their prey, which is often larger than themselves and in some cases can be up to

5 times their own body volume (Hansen & Calado, 1999). However, in this study, the abundance of these large heterotrophic dinoflagellates was very low, the size distribution data clearly show that during May the majority of cells were <20$\mu$m in length and microscopic analysis has shown that this community was dominated by oligotrichous ciliates. Data from Sieracki show that heterotrophic nanoflagellate biomass in surface waters in May was consistently between 9 and 12 mg C $m^{-3}$, this was considerably lower than that found in June. Information from size–fractionated chlorophyll measurements shows that the nano-sized fraction of the phytoplankton population decreased and the >5$\mu$m fraction of the chlorophyll increased at this time (Savidge et al 1992). Data from Verity et al (1993b) on heterotrophic dinoflagellates show that they were extremely abundant in 1990 with concentrations gradually decreasing during mid-May. However, the average size of heterotrophic nanodinoflagellates increased and so the biomass of this group remained fairly constant at a mean of 6 mg C $m^{-3}$. Heterotrophic nanodinoflagellates feed mainly on nanoflagellates, they therefore can potentially compete with planktonic ciliates for food (Bjørnsen & Kuparinen, 1991; Strom 1991). One scenario could therefore be that the nanoflagellates were being grazed by the oligotrichous ciliates and heterotrophic dinoflagellates leaving the pennate diatoms potentially ungrazed allowing them to bloom.

Although the protozoa dominated the heterotrophic microplankton assemblage the contribution of the metazoa to the total microzooplankton herbivorous activity could have been significant. No estimates of naupliar feeding rates were made during this Lagrangian study. However, colleagues experimentally determined copepod herbivory and concluded that this represented consumption of on average <10% of the primary production (Morales et al 1993). Concentrations of metazoan nauplii in experimental bottles were similar to those reported by Verity et al (1993a). Metazoan nauplii which pass through a 200$\mu$m mesh, are known to graze both auto- and heterotrophic prey and in these experiments were present in high enough concentrations to remove substantial numbers of heterotrophic microplankton (Stoecker & Egloff, 1987; Stoecker et al 1990). Many nauplii are efficient grazers on cells as small as 4-5$\mu$m (Paffenhöfer, 1984; Berggreen et al 1988). Final concentrations of heterotrophic cells at the end of 24-hour incubations have only been determined for 3 experiments but these data show that the % contribution of oligotrichous ciliates to total abundance dropped by 0-14% during the 24-hour incubation period. Unfortunately there is no data on the abundance of HNAN at

this time. Further analyses would need to be carried out to test the significance of this data, but considering that *in situ* concentrations of oligotrichous ciliates showed a general decreasing trend, the chance that they were being cropped by larger organisms such as metazoan nauplii is fairly high.

Two papers have been published using results from the analyses of heterotrophic nanoflagellates, nanodinoflagellates and plastidic versus non-plastidic microzooplankton (Verity et al 1993b and Stoecker et al 1994). These studies demonstrate the striking differences between the microbial communities observed during the spring bloom in 1989 and 1990.

*4.4.2.2 Community composition*

Protozoa dominated the depth-integrated biomass of microzooplankton (Table 4.4) although for some experimental incubations Metazoan biomass exceeded that of the Protozoa (Table 4.3b). Protozoan assemblages during the first half of the study were dominated by members of the sub-class Choreotrichia (orders Oligotrichida and Choreotrichida). This is in agreement with results reported from open ocean (Stoecker et al 1989; Sime-Ngando et al 1992) and coastal (Smetacek 1981, Dolan 1991) waters. Concentrations and biomass of ciliated protozoa were found to be higher than during the summer in 1989 when heterotrophic dinoflagellates dominated the microzooplankton community (Burkill et al 1993a). The study carried out in the North Atlantic by Stoecker et al (1994) for which samples were collected by myself on the same day and from the same depth but from consecutive casts, show quite different results, table 4.6.

| | | Abundance cells $l^{-1}$ | | |
|---|---|---|---|---|
| | | 1-12 May | 13-19 May | 28-15 June |
| This study | Oligotrichs | 22612 (49%) | 3605 (31%) | 3350 (27%) |
| | Tintinnids | 224 (104%) | 105 (55%) | 7582 (112%) |
| Stoecker et al (1994) | Oligotrichs | 4049 (53%) | 807 (28%) | 1106 (55%) |
| | Tintinnids | 134 (83%) | 167 (72%) | <20 |

Table 4.6: Numerical abundance of planktonic ciliates during and after the North Atlantic spring bloom in 1990, a comparison with the study by Stoecker et al (1994). Coefficients of variation for samples taken on different dates within each interval are given in brackets.

Whilst tintinnid abundance is not too dissimilar for May clearly the high abundance of tintinnids for June was not found in Stoecker et al's analyses. Oligotrich abundance in my samples was almost an order of magnitude higher in May and three times higher in June than those of Stoecker et al The major difference between the two sets of data is the method of fixation. In this study samples were fixed in 1% acid Lugol's solution, whereas for Stoecker et al, samples were fixed in 2% final concentration hexamethylamine-buffered formaldehyde. Although Lugol's iodine is a poor fixative to use if you want to look at taxonomically important structures or chlorophyll autofluorescence, it does appear to be the best for short-term preservation of ciliate populations for the estimation of community abundance and biomass (Leakey et al 1994). However, Stoecker et al accounted for oligotrich loss due to fixation by formaldehyde by multiplying their counts by an empirically determined factor of 1.5. One other factor to explain this discrepancy between data could be the small <20μm oligotrich fraction. It is not clear whether these were included in analyses by Stoecker et al Even so, this does not explain the low tintinnid abundance for June and I can only suggest that perhaps these small *Salpingella*–like tintinnids are left unrecognisable when fixed in formaldehyde.

*4.4.2.3 Size distribution*

It is clear that the microzooplankton community was dominated by cells smaller than 40µm and a high proportion of those cells being even < 20µm in length. During May, the occurrence of small cells was most pronounced. This evidence suggests that during this time the metazoan nauplii were cropping the larger ciliates.

**Summary**

Interpretation of a seasonal pattern during the 1990 spring bloom is complicated by the complex mesoscale interactions that are typical of the region (Savidge et al 1992, Lochte et al 1993). The possibility of mixing of plankton populations from adjacent waters cannot be ruled out. It is known that the BOFS 1990 study was not strictly Lagrangian for the whole duration of the 2 cruises due to the loss of the drogue attached to the drifting Argos buoy, probably during June. However, there appears to be a coherent time series of data and this suggests that at least for the first part of the study in May, it was Lagrangian. It is clear that microzooplankton were important during this study with high abundance, biomass and rates of herbivory. Microzooplankton were found to graze between 26 and 78% of daily primary production and these results are surprisingly similar to those found during the same period and area in 1989, despite differences in the trophic structure of the microbial communities. Omnivory and carnivory among microzooplankton groups has been discussed, and would affect estimates of individual microzooplankton grazing estimates, but would not affect estimates of herbivory by the microzooplankton community as a whole. However, omnivory could partially control the trophic dynamics and structure of microzooplankton communities (Stoecker & Evans, 1985).

When meso- and microzooplankton herbivory are summed, it appears that a significant fraction of the phytoplankton community is grazed in surface waters leaving only a small proportion of the primary production available for export or as a food resource for larger herbivores. This conclusion is in keeping with that of Fasham et al 1999, whose size-structured ecosystem model showed that the estimated downward particulate organic carbon flux was less than 5% of the gross primary production.

## CHAPTER 5

*The Southern Ocean*

The Southern Ocean is a complex environment characterised by low temperatures, extreme variation in irradiance levels and high nutrient concentrations. Satellite imagery has shown that the Southern Ocean exhibits areas of extremes of productivity, with some areas with low productivity despite high nutrient levels whilst elsewhere there are high levels of productivity, as seen at the Marginal Ice Zone. The Marginal Ice Zone is in fact the most dynamic microbial habitat in the Southern Ocean, characterised by short periods of intense biological activity. As the ice melts, phytoplankton which are present in the ice are released providing food for microzooplankton grazers in the plankton beneath. The melting ice has low salinity and stratification of the water column occurs, which can lead to massive blooms of phytoplankton. It is not yet known to what extent carbon and energy are transferred to higher trophic levels, recycled, or exported from surface waters (Vincent, 1988).

The traditional view of a large "net" phytoplankton (diatom)- metazoan "net" zooplankton (krill)- higher predator planktonic food chain present in Antarctic waters tends to persist. However, it is now becoming clear that there is an abundant and active autotrophic and heterotrophic microbial community (Garrison et al 1991), suggesting that the trophic structure of the Southern Ocean may be similar to that of other oceans where protozoans are commonly the dominant grazers (Capriulo, 1990).

The Bellingshausen Sea, west of the Antarctic peninsula, has been under-studied. In the austral spring-summer 1992 a study entitled 'STERNA', was carried out using two British Royal Research Ships, *James Clark Ross* and *Discovery.* This was the first major process-orientated study of the marginal ice zone of this area. The main aim of the study was to investigate the timing and mechanisms behind phytoplankton bloom development in the marginal ice zone and to quantify associated biogeochemical fluxes. RRS *James Clark Ross* carried out process studies at stations along a transect which encompassed a range of regimes from thick pack ice through to open water, whilst RRS *Discovery* carried out a detailed physical and biogeochemical survey in open water of the marginal ice zone to the north.

Microzooplankton grazing work as well as an investigation of the structure of the microzooplankton community was carried out on RRS *James Clark Ross.* I was responsible for the collection of microzooplankton samples, running of dilution grazing experiments, all analysis of samples, including bacteria, and work up of related data.

The results from this study have been published as two papers, which formed contributions to two Special Issue volumes, the first, Burkill, Edwards & Sleigh (1995) in Deep-Sea Research, the second, Edwards, Burkill & Sleigh (1998) in the Journal of Marine Systems. Contributions were also made to a modelling paper by Murphy et al (1998).

The following published papers were included in the bound thesis. These have not been digitised due to copyright restrictions, but the links are provided.

P.H. Burkill, E.S. Edwards and M.A. Sleigh (1995)

**Microzooplankton and their role in controlling phytoplankton growth in the marginal ice zone of the Bellingshausen Sea**

Deep Sea Research II: Vol. 42, Nos. 4-5, pp.1277-1290

http://dx.doi.org/10.1016/0967-0645(95)00060-4

E.S. Edwards, P.H. Burkill and M.A. Sleigh (1998)

**Microbial community structure in the marginal ice zone of the Bellingshausen Sea**

Journal of Marine Systems: Vol. 17, Nos. 1-4, pp.87-96

http://dx.doi.org/10.1016/S0924-7963(98)00031-1

## CHAPTER 6

*The Arabian Sea*

The Arabian Sea is subject to a range of biogeochemical conditions within relatively close spatial scales, including eutrophic, oligotrophic, upwelling, downwelling and reduced oxygen environments (Burkill et al 1993b). It is a well-known example of a monsoon-reversal system. Because of the regular pronounced seasonal variability of biological production associated with the seasonally reversing monsoonal wind pattern (Yoder et al 1993), the Arabian Sea was chosen as an area for a biogeochemical process-orientated study.

From June through to September (Southwest monsoon) winds are predominantly from the southwest. During November through to March the winds reverse coming predominantly from the northeast (Northeast monsoon). The winds parallel to the coast during the southwest monsoon result in Ekman divergence from the coast and produce a region of coastal upwelling that is characterised by enhanced nutrient conditions and high biological productivity. During the Southwest Monsoon chlorophyll-*a* concentrations in the surface 20m can reach 4 mg $m^{-3}$ or more (Banse & McClain 1986). Diatoms often dominate the phytoplankton in upwelling areas (reviewed in Malone 1980) and it is thought that many of these diatoms sink ungrazed or are incorporated into sinking faecal pellets. In the absence of monsoonal forcing, the phytoplankton community is dominated by small phytoplankton, and chlorophyll concentrations are more consistent with those in typical oligotrophic tropical oceans, <0.1 mg $m^{-3}$ (Brock et al 1993). The differences in the size structure of the planktonic food web have important implications for understanding variability in the amount of primary production exported from the euphotic zone as particulate organic flux (Eppley & Peterson 1979, Michaels & Silver 1988, Peinert et al 1989).

'ARABESQUE' was an international programme of upper-ocean biogeochemistry in the Arabian Sea, which was conducted during two contrasting seasons, the end of the southwest monsoon and the inter-monsoon /northeast monsoon transition period. The project formed the main UK contribution to the JGOFS Arabian Sea Process Study. The primary aim of ARABESQUE was to describe the routes and quantify the rates of key biogeochemical processes involving plankton communities. Two major international research cruises to the area were carried out on board the RRS Discovery. The main study area for the two cruises was a 1590km offshore transect from Masira Bay on the Omani coast across the Arabian Sea to an oceanic station situated at 08°N, 67°E.

Data, which I generated from the ARABESQUE programme, are presented here in the form of a paper on the herbivorous impact of microzooplankton in the Arabian Sea during and after the SW Monsoon. As part of this work I also carried out analyses of the heterotrophic nanoplankton community and with these data I contributed to a further paper (Stelfox et al 1999 - Appendix 3).

Elaine S. Edwards, Peter H. Burkill and Claire E. Stelfox

**Zooplankton herbivory in the Arabian Sea during and after the SW monsoon, 1994**

Deep Sea Research II: Vol. 46, Nos. 3-4, pp.843-863

http://dx.doi.org/10.1016/S0967-0645(98)00130-1

## CHAPTER 7: DISCUSSION

The dilution technique is a useful tool for estimating phytoplankton growth rates and microzooplankton grazing rates from natural communities. An increasing wealth of information on grazing by microzooplankton has accumulated since the introduction of the technique in 1982 (references in Fig 1 in Dolan et al 2000). When the present study began, microzooplankton grazing dynamics had not been studied extensively in oceanic areas, although the importance of microzooplankton grazing was recognised. Nowadays, the microzooplankton are accepted as being important, if not the most significant, grazers on phytoplankton populations in many oceanic systems (e.g. Paranjape 1990, Strom 1991, Verity et al 1993a, Gifford et al 1995, Froneman & Perissinotto 1996, Verity et al 1996, Reckermann & Veldhuis 1997, James & Hall 1998, Lessard & Murrell 1998, Landry et al 1998, Stelfox-Widdicombe et al *in press*). The data presented in this study provide more evidence in support of this.

In this study, I have demonstrated use of the dilution technique, to determine rates of microzooplankton grazing both in the laboratory, using cultures, and in the field in three different oceanographic areas. Since the beginning of this study the methodology used has improved and developed. Perhaps the most significant of these changes was the shift from use of a laboratory Gallenkamp incubator for 24 hour incubations to using an *in situ* rig, allowing ambient levels of light and temperature to be matched as closely as possible.

### 7.1 Verification of the critical assumptions of the dilution technique

In previous chapters, I have discussed the importance of validating the three assumptions associated with the dilution technique (as described in Chapter 2) in order to achieve accurate estimates of microzooplankton grazing rates. In practice, this is difficult to do, particularly because of the time-consuming nature of these experiments. But, without acceptable justification of these assumptions, the whole technique can be seriously questioned. In the theory section of Chapter 2 I have summarised the difficulties which can arise when interpreting dilution data if the assumptions are not valid. In chapter 4, I have used field data to demonstrate the consequences of non-linear feeding, which could result from an over-abundant food supply, such as found in eutrophic waters. Results such as these, if considered to be linear, could lead to an

underestimated grazing rate. In situations where saturated feeding is suspected, a dilution experiment protocol should have sufficient dilute incubations (e.g. up to 95%) in order to resolve the phytoplankton growth rate. The microzooplankton net growth rate should also be measured.

### 7.2 Comparison of field data

The results presented in this study demonstrate the importance of microzooplankton herbivory in the Northeast Atlantic, the Bellingshausen Sea and the Arabian Sea and in this chapter I will compare the results obtained from the three areas. Data generated from these studies compare well with other studies carried out in similar areas (Table 7.1).

During the summer of 1989 in the Northeast Atlantic, the grazing impact by microzooplankton was higher in nanophytoplankton dominated waters at 47°N than at 60°N where larger phytoplankton comprised up to 90% of the total phytoplankton production (Joint et al 1993). Over the course of the 1990 spring bloom microzooplankton grazing impact was similar to levels found in 1989 by Verity et al (1993a) even though the phytoplankton communities at that time were quite different.

In the Arabian Sea, microzooplankton herbivorous impact was higher during the intermonsoon, when the phytoplankton was dominated by cyanobacteria and prochlorophytes (Tarran et al 1999) than during the SW monsoon, when the phytoplankton comprised a mixture of diatoms, autotrophic nanoflagellates and cyanobacteria. Grazing rates reported here for the Arabian Sea are lower than those found for both the North Atlantic and the Bellingshausen Sea. One possible explanation for this might be that in the Arabian Sea, the mesozooplankton are exerting a greater grazing pressure on the microzooplankton which then lessens their impact on the phytoplankton. Certainly microzooplankton have been shown to comprise a significant proportion of a copepod's diet (e.g. Verity & Paffenhöfer 1996; Fessenden & Cowles 1994; Irigoien et al 1998; Batten et al in press). In the Arabian Sea, Smith et al (1998) found a high biomass of epipelagic zooplankton during the SW monsoon in the area of upwelling, and standing stocks of mesozooplankton determined by Stelfox et al (1999) were significantly higher than those found in 1989 in the North Atlantic (Morales et al 1991). In the Northeast Atlantic at 47°N, microzooplankton standing stocks (excluding the HNF contribution, which can be significant) were four times higher than

mesozooplankton standing stocks whilst in contrast, Stelfox et al (1999) found that mesozooplankton standing stocks were similar to and at times higher than the microzooplankton stocks in the Arabian Sea.

### 7.3 Microzooplankton herbivory as a function of phytoplankton growth

Microzooplankton, through their small size, can respond rapidly to changes in their food supply, usually small phytoplankton cells. In doing so they are able to maintain close coupling between production and consumption in the water column. However, vertical flux of particles from the euphotic zone to the deep ocean has been observed as a result of phytoplankton blooms in surface waters. The fact that these blooms occur means that the tight trophic coupling between microzooplankton and their prey is not always predominant. A dominance of large bloom-forming diatoms which may not be grazed effectively by the microzooplankton would lead to a greater export of production to the deep ocean either through the direct sinking of large phytoplankton cells or channeling through mesozooplankton faecal pellet production and/or vertical migration. Where a large proportion of the primary production is grazed by the microzooplankton less material would be available for sinking out of surface waters (e.g. Michaels & Silver, 1988). Therefore grazing by microzooplankton can strongly influence the phytoplankton community composition. The percentage of phytoplankton production passing through the microbial loop depends on the composition of the food web, including that of the phytoplankton.

One way to determine whether there is a close relationship between microzooplankton herbivory and phytoplankton production is to compare phytoplankton growth (k) with phytoplankton mortality due to grazing (g) as determined from dilution experiments. By combining all the field data generated from this study it is clear that on average there is a fairly good correlation between phytoplankton growth and mortality with some experiments showing a 1:1 relationship (Figure 7.1).

Table 7.1: Comparison of grazing impact of microzooplankton on phytoplankton standing stock and primary production determined by dilution experiments

| **Area** | **Season** | **Chl-a Conc $\mu g\ l^{-1}$** | **% Chi standing stock removed** | **% primary production removed** | **Source** |
|---|---|---|---|---|---|
| Northeast Atlantic 47°N | Late spring bloom<br>Spring bloom | 0.4-1.7<br>0.5-2.4 | 2-45<br>16-47 | 39-115<br>26-78 | This study |
| Northeast Atlantic 47°N | Late spring bloom | 3.0-4.0 | 43-53 | 37-100 | Verity et al (1993a) |
| Northeast Atlantic 60°N | Spring post- bloom<br>Late Summer | 0.6-2.9<br>1.4-2.27 | - | 100<br>37-53 | Gifford et al (1995) |
| Northeast Atlantic 60°N<br><br>37°N | <br>Coccolithophore bloom<br>Summer | <br>0.94-1.26<br>0.05-0.07 | <br>59-77<br>23-43 | <br>-<br>- | Stelfox-Widdicombe et al 2000 |
| Bellingshausen Sea | MIZ Summer | 0.05-1.4 | 3-40 | 21-271 | This study |
| South Atlantic | Sub-tropical convergence winter | 0.2-0.9 | 30-51 | 56-69 | Froneman & Perissinotto (1996) |
| South Island, New Zealand | Winter<br>Spring | 0.1-0.6<br>0.2-2 | 10-92<br>4-57 | 71-194<br>20-126 | James & Hall 1998 |
| Arabian Sea | SW Monsoon | 0.17-0.51 | 14-49 | 4-29 | This study |
| Arabian Sea | Inter-monsoon | 0.09-0.28 | 11-49 | 24-60 | This study |
| Arabian Sea | NE Monsoon | 0.25-1.04 | 38 | 67 | Reckermann & Veldhuis (1997) |
| Arabian Sea | NE Monsoon<br>Intermonsoon | 0.1-1.4<br>0.1-1.4 | 10-56<br>15-50 | 22-69<br>31-207 | Caron & Dennett (1999) |
| Arabian Sea | SW Monsoon<br>NE Monsoon | 0.2-2.4<br>0.2-0.8 | 45<br>45 | -<br>- | Landry et al (1998) |

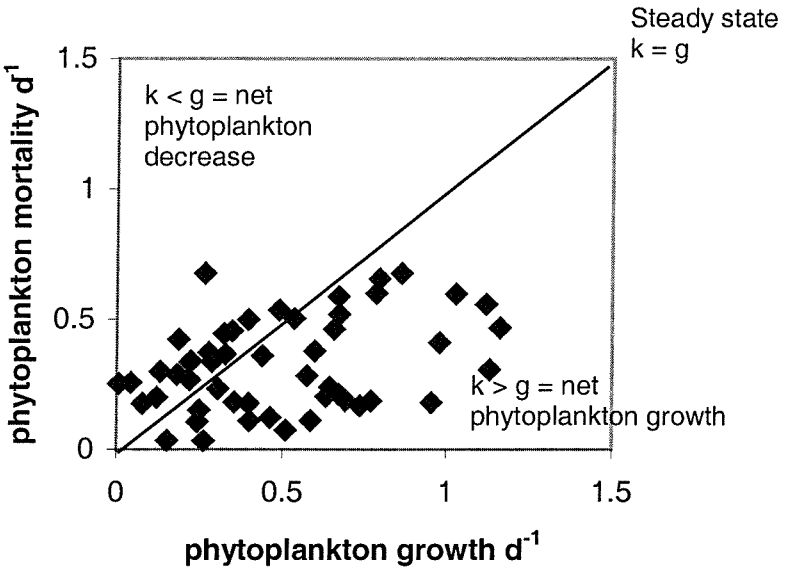



Figure 7.1 Relationship between specific growth coefficient and grazing mortality rate for phytoplankton, as measured in dilution experiments carried out in the North Atlantic in 1989, 1990, in the Bellingshausen Sea and the Arabian Sea. The diagonal line indicates the line of one-to-one correspondence between growth and mortality rates.

I also compared phytoplankton growth and mortality estimates from experiments carried out in the North Atlantic with those from the Arabian Sea and the Southern Ocean separately (Figure 7.2).

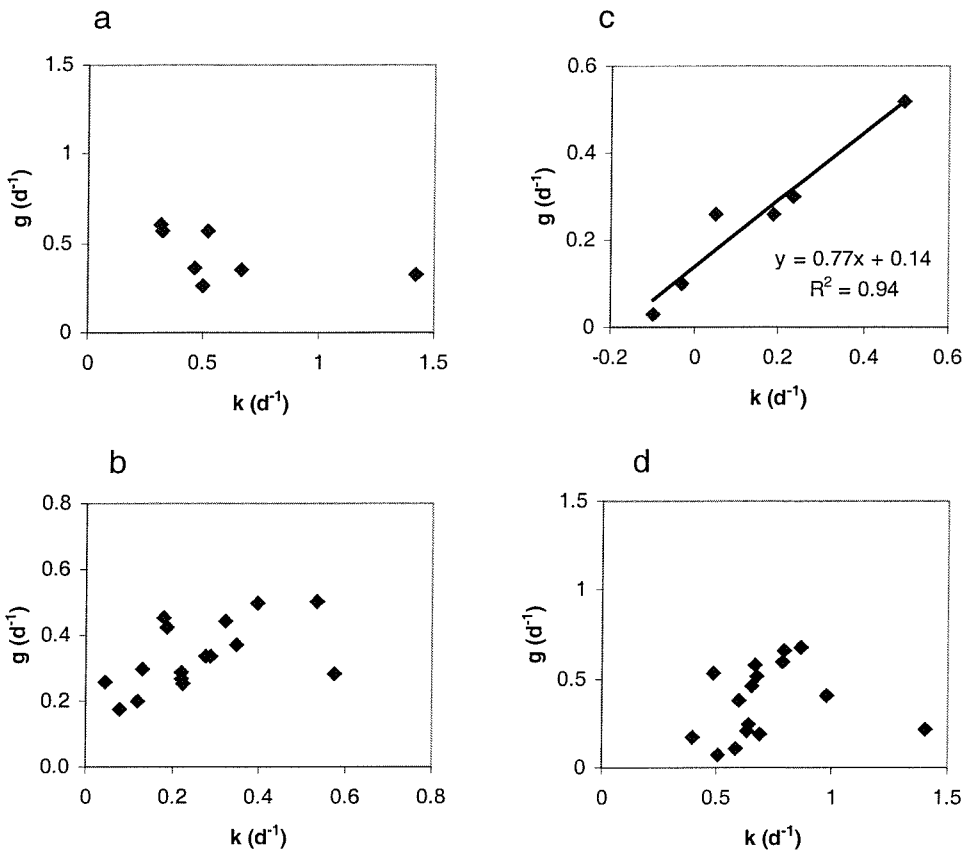



Figure 7.2: Relationship between specific growth coefficient (k) and grazing mortality rate (g) for phytoplankton, as measured in dilution experiments. a = Northeast Atlantic 1989; b = Northeast Atlantic 1990; c = Bellingshausen Sea; d = Arabian Sea

*North Atlantic*

The results show that for the North Atlantic in 1989 the correlation observed between phytoplankton growth rate and mortality was very poor (Figure 7.2a) but for 1990 several experiments showed an almost 1:1 correspondence between mortality and growth, with grazing exceeding growth in most other experiments (Figure 7.2b). These data fit in well with other studies of the North Atlantic, despite these studies being carried out in different seasons. In Figure 7.3, 1989 and 1990 North Atlantic data have been pooled with those of Verity et al (1993a) from the North Atlantic May 1989 spring bloom period at 47°N, and Gifford et al (1995) from stratified summer conditions at 59°N in 1991.

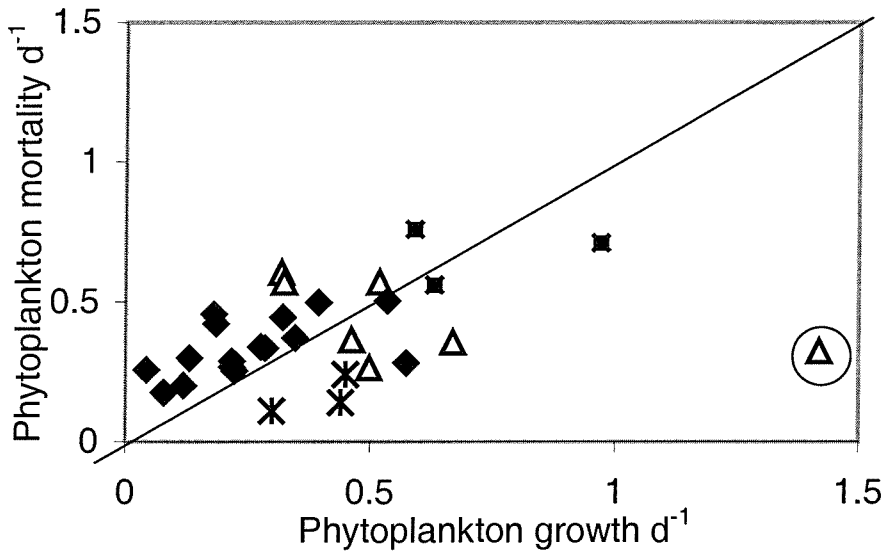



Figure 7.3: Comparison of phytoplankton growth and phytoplankton mortality determined from dilution experiments performed in this study in 1989 and 1990 and from Verity et al (1993a) and Gifford et al (1995). (△) = data from 60°N station, summer 1989; ◆ = 1990; △ =1989; ✳ = Gifford (1995); ✖ = Verity et al (1993).

A good correlation between phytoplankton growth rates and mortality was often observed (Figure 7.3) with many experiments showing a near 1:1 correspondence between phytoplankton growth and grazing by microzooplankton. These data suggest that for the North Atlantic in 1989 (this study and Verity et al 1993), the 1990 spring bloom (this study) and to a lesser extent summer 1991 (Gifford et al 1995) coupling between microzooplankton herbivory and production was close. During May 1989, following a micro-diatom bloom in late April, the phytoplankton community was composed of abundant, small phytoflagellates (Sieracki et al 1993). Later in the summer in 1989, the phytoplankton community at 60°N shifted from being dominated by >5m diatoms to dinoflagellates and 1-5$\mu$m prymnesiophytes (Weeks et al 1993). This shift in phytoplankton community composition is reflected in the data from this study. The one experiment carried out in surface waters at 60°N, showing high phytoplankton growth but low grazing, indicates that there was net growth of phytoplankton at this station. In 1990, a nano-diatom bloom occurred and small phytoflagellates, mostly prymnesiophytes, were also very abundant (Savidge et al 1995). In Chapter 4, I discussed the possibility

that phytoplankton growth estimates for 1990 might have been overestimated. If this is so, the ratio between phytoplankton growth and grazing is more likely to fall below the steady state line. This would suggest that some net growth of phytoplankton occurred during that time and this is backed up by evidence that a diatom bloom occurred which did not coincide with a maximum grazing impact by the microzooplankton.

*The Southern Ocean*

Both the Southern Ocean and the Northeast Atlantic are regions of high seasonality in phytoplankton concentrations, marked by a seasonal phytoplankton bloom (Banse, 1992). I have demonstrated that for the North Atlantic close coupling between phytoplankton growth and consumption can exist, such as when nano-sized diatoms produce a diatom bloom or where abundance of nanoflagellates is high. During the Bellingshausen Sea study in 1992, the <20$\mu$m phytoplankton, mostly autotrophic nanoflagellates, dominated at the ice stations whilst diatoms, up to 99% of which were <20$\mu$m, dominated in open water stations (Edwards et al 1998). A significant linear relationship was observed between phytoplankton growth rates and mortality (Figure 7.2c) although only in one experiment was the result close to a steady state. These data suggest that despite a large proportion of the phytoplankton population comprising diatoms, particularly at the open-water stations, the ratio of grazing to phytoplankton growth in surface waters was high and indicates a system where little material would be available for export.

*The Arabian Sea*

The relationship between phytoplankton growth and grazing mortality for experiments carried out in the Arabian Sea was more variable than for the North Atlantic and Southern Ocean studies (Figure 7.2). Data from a large proportion of the experiments occurred below the one-to-one correspondence line indicating net growth of phytoplankton. The ratios between mortality and growth varied spatially but there was no clear trend to suggest that lower ratios were encountered in upwelling diatom-dominated areas and higher ratios at oligotrophic stations. The range of mortality to growth ratios was similar for both SW Monsoon and Intermonsoon cruises but the mean ratio was higher during the SW Monsoon (0.55 ± 0.29) than the Intermonsoon (0.35 ± 0.20). These results are surprising. Caron and Dennett (1999) report a similar situation where rates of phytoplankton growth consistently exceeded mortality during the NE Monsoon.

However, one might expect there to be strong trophic coupling between microzooplankton and phytoplankton particularly in the oligotrophic stations of the Arabian Sea. In contrast to data presented here, Landry et al 1998, found that for open-ocean oligotrophic stations, a good balance was observed between phytoplankton growth and microzooplankton grazing losses. They concluded that here the system was based on strong microbial coupling and remineralisation. Consistent with this was a dominance of photosynthetic bacteria and picoeukaryotic algae. At stations with higher nutrient concentrations and where larger phytoplankton dominated, Landry et al 1998 found substantial surplus phytoplankton growth providing conditions to support a large biomass of mesozooplankton. A similar situation is reported by Reckermann & Veldhuis (1997). In the oligotrophic Sargasso Sea, there was a balanced relationship between growth and grazing during the decline of the spring bloom (Lessard & Murrell, 1998). Dilution experiment studies carried out in the present study are from one depth and one point in time. Variations in the balance between growth and consumption are likely to occur and may partly explain these differences. It is also possible that an increased number of trophic levels within the microzooplankton community could suppress microzooplankton herbivorous impact on the phytoplankton. In such a situation one could expect to find HNAN grazing autotrophic picoplankton, small ciliates and heterotrophic dinoflagellates grazing on HHAN, and larger ciliates and dinoflagellates grazing on smaller ciliates and so forth. Caron & Dennett (1999) suggest that changes in the composition of the microzooplankton assemblage may also tend to obscure any relationships between the data.

### 7.4 Future work

The routine addition of nutrients to experimental bottles is one factor that has not been explored during this work, although some nutrient addition was carried out in Arabian Sea experiments. Other studies have shown the importance of nutrient addition, in particular in areas where nutrient levels are known to be low (e.g. Landry & Hassett 1982, Landry et al 1995, Landry et al 1998). One major concern with addition of nutrients is that the phytoplankton growth coefficients are artificially elevated and therefore are not true representatives of *in situ* growth (Landry & Hassett, 1982). Some studies have also shown that nutrient addition can have detrimental effects on delicate protozoan grazers (Gifford 1988, Lessard & Murrell 1998). However, in situations where bottle containment

or dilution would result in nutrient depletion during incubation, nutrient addition would be appropriate to satisfy assumption 1 (Chapter 2). In the future, it will be important to investigate the effect that nutrient addition might have on all dilution treatments during incubation experiments.

One further point to be explored is the effect of dilution on the growth rates of grazers as this could affect grazing rate estimates. A recent study (Dolan et al 2000) found that within a dilution series considerable apparent growth and mortality can occur among ciliates. Dolan's study argues that grazing patterns from dilution experiments can be different from natural communities due to changes in the grazer community during an experiment.

There are several other variations to the dilution protocol that can be made depending on what the scientific questions might be. The water collected for all experiments performed during this study was pre-screened through a 200$\mu$m mesh to exclude larger predators. It would be possible to run two dilution experiments in parallel, one excluding the mesozooplankton, the other including it, to determine the effect of larger predators on microzooplankton herbivory. One of the drawbacks of the experimental protocol used in this study is that it was not possible to distinguish between grazing on different size fractions of the phytoplankton community. Therefore, in addition to experiments above, size fractionation experiments are also possible where grazing is determined for instance, for the <20$\mu$m size fraction only (e.g. Verity et al 1993a) allowing the microzooplankton grazing impact on different phytoplankton size classes to be determined.

### 7.5 Summary

It is critical that the three important assumptions of the dilution technique can be justified for any dilution experiments carried out. The results of dilution experiments carried out in this study indicate that there was substantial grazing of phytoplankton biomass by microzooplankton in the three ocean areas studied. These results provide further evidence of the importance of microzooplankton as primary grazers in marine ecosystems. The magnitude of phytoplankton growth and grazing varied between the three oceans and this can be attributed to differences in the phytoplankton communities encountered. Estimates of microzooplankton grazing and phytoplankton growth from the North Atlantic study are in good agreement with data from similar studies by other

workers and demonstrate a tight coupling between growth and consumption. Net growth of phytoplankton seemed to occur in the North where a micro- diatom bloom dominated. From the North Atlantic datset it can be concluded that when small phytoplankton dominate, grazing by microzooplankton is most significant. The data indicate a similar situation ie. tight coupling, for the Bellingshausen Sea. However, in contrast, in the oligotrophic Arabian Sea where small picoplankton dominated the phytoplankton community, phytoplankton growth exceeded losses due to microzooplankton grazing.

## BIBLIOGRAPHY

Aelion C M & S W Chisholm (1985) Effect of temperature on growth and ingestion rates of *Favella* sp. Journal of Plankton Research 7 (6): 821-839.

Aiken J (1981) A chlorophyll sensor for automatic, remote, operation in the marine environment. Marine Ecology Progress Series 4: 235-239

Andersen T, A K Schartau & E Paasche (1991) Quantifying external and internal nitrogen and phosphorus pools supplied through remineralisation, in coastal marine plankton by means of a dilution technique. Marine Ecology Progress Series 69:67-80.
Azam F, T Fenchel , J G Field, J S Gray, L A Meter-Reil and F Thingstad (1983) The ecological role of water-column microbes in the sea. Marine Ecology Progress Series 10: 257-263.

Barlow R G, R F C Mantoura, M A Gough & T W Fileman (1993) Pigment signatures of the phytoplankton composition in the northeastern Atlantic during the 1990 spring bloom. Deep-Sea research II 40 (1/2): 459-477.

Batten S D, E S Fileman & E Halvorsen (in press) the contribution of microzooplankton to the mesozooplankton diet in an upwelling filament off Galicia. Progress in Oceanography.

Banse K (1992) Grazing, temporal changes of phytoplankton concentrations, and the microbial loop in the open sea. In: Falkowski P G, Woodhead A D (eds) *Primary productivity and biogeochemical cycles in the sea.* Plenum, New York, p409-440.

Banse K & C R McClain (1986) Winter blooms of phytoplankton in the Arabian Sea as observed by the coastal zone color scanner. Marine Ecology Progress Series 34: (3) 201-211

Beers J R & Stewart G L (1967) Microzooplankton in the euphotic zone at five locations across the California Current. Journal of the Fisheries research Board of Canada 24: 2053-2068.

Beers J R & Stewart G L (1969) The vertical distribution of microzooplankton and some ecological observations. Journal du Conseil International l' Exploration de la Mer 33: 30-44.

Beers J R & Stewart G L (1970) Numerical abundance and estimated biomass of microzooplankton. In: *The ecology of the Plankton off La Jolla, California, in the period*

*April through to September, 1967* (part VI), J D H Strickland, editor, Bulletin Scripps Institute of Oceanography 17, 67-87.

Beers J R & Stewart G L (1971) Microzooplankters in the plankton communities of the upper waters of the eastern tropical Pacific. Deep-Sea Research 18: 861-883.

Berggreen E, B Hansen & T Kiørboe (1988) Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: Implications for determination of copepod production. Marine Biology 99:341-352.

Billet D S M, R S Lampitt, A L Rice & R F C Mantoura (1983) Seasonal sedimentation of phytoplankton to the deep-sea benthos. Nature 302: 520-522.

Bjørnsen P K & J Kuparinen (1991) Growth and herbivory by heterotrophic dinoflagellates in the Southern Ocean studied by microcosm experiments. Marine Biology 109: 397-405.

Blackbourn D J (1974) The feeding biology of tintinnid protozoa and some other inshore microzooplankton. Ph.D. thesis. University of British Columbia (Vancouver), 224 pp.

Brock J C, S Sathyendranath & T Platt (1993) Modelling the seasonality of subsurface light and primary production in the Arabian Sea. Marine Ecology Progress Series 101: 209-221.

Burkill PH, Mantoura RFC, Llewellyn CA, Owens NJP (1987) Microzooplankton grazing and selectivity of phytoplankton in coastal waters. Marine Biology 93: 581 - 590.

Burkill P H, Edwards ES, John AWG, Sleigh MA (1993a). Microzooplankton and their herbivorous activity in the northeastern Atlantic Ocean. Deep-Sea Research II 40 (1/2): 479-493

Burkill P H, R J G Leakey, N J P Owens & R F C Mantoura (1993b). *Synechococcus* and its importance to the microbial foodweb of the northwestern Indian Ocean. Deep-Sea Research II 40(3): 773-782.

Campbell L & E J Carpenter (1986) Estimating the grazing pressure of heterotrophic nanoplankton on *Synechococcus spp.* Using the seawater dilution and selective inhibitor techniques. Marine Ecology Progress Series 33: 121-129.

Capriulo G M (1990) Feeding related ecology of marine protozoa. In: Ecology of Marine protozoa. G M Capriulo (ed), Oxford University Press, pp 186-259.

Capriulo G M & E J Carpenter (1980) Grazing by 35-202$\mu$m microzooplankton in Long Island Sound. Marine Biology 56: 319-326.

Caron D A & M R Dennett (1999) Phytoplankton growth and mortality during the 1995 Northeast Monsoon and Spring Intermonsoon in the Arabian Sea. Deep-Sea Research II 46: 1665-1690.

Colebrook J M (1979) Continuous Plankton Records: seasonal cycles of phytoplankton and copepods in the North Atlantic Ocean and the North sea. Marine Biology 51: 23-32.

Colebrook J M (1982) Continuous plankton records: seasonal variations in the distribution and abundance of plankton in the North Atlantic Ocean and North Sea. Journal of Plankton Research 4: 435-462.

Colebrook J M (1984) Continuous plankton records: Relationship between species of phytoplankton and zooplankton in the seasonal cycle. Marine Biology 83: 313-323

Cushing D H (1989) A difference in structure between ecosystems in strongly stratified waters and in those that are only weakly stratified. Journal of Plankton Research 11: 1-13.

Davis P G & J A Sieburth (1984) Estuarine and oceanic microflagellate predation of actively growing bacteria: Estimation by frequency of dividing-divided bacteria. Marine Ecology Progress Series 19: 237-246

Dolan J R (1991) Guilds of ciliate microzooplankton in the Chesapeake Bay. Estuarine Coastal and Shelf Science 33: (2) 137-152

Dolan J R, C L Gallegos & A Moigis (2000) Dilution effects on microzooplankton in dilution grazing experiments. Marine Ecology Progress Series 200: 127-139.

Droop M R (1959) A note on some physical conditions for cultivating *Oxyrrhis marina*. Journal of the Marine Biological Association, UK 38: 599-604.

Dodge J D & R M Crawford (1971a) Fine structure of the dinoflagellate *Oxyrrhis marina* I. The general structure of the cell. Protistologica VII: 295-304.

Dodge J D & R M Crawford (1971b) Fine structure of the dinoflagellate *Oxyrrhis marina* II. The Flagellar System. Protistologica VII: 399-409.

Dodge J D & R M Crawford (1974) Fine structure of the dinoflagellate *Oxyrrhis marina*. III. Phagotrophy. Protistologica, 10: 239-244.

Ducklow H W & Hill S M (1985) The growth of heterotrophic bacteria in the surface waters of warm core rings. Limnology & Oceanography 30:329-259.

Dussart B.M. (1965) Les différentes catégories de plancton. Hydrobiologia 26: 72-74.

Edwards E.S, P H Burkill, & M A Sleigh (1998). Microbial community structure in the marginal ice zone of the Bellingshausen Sea. Journal of Marine Systems 17(1): 87-96.

Eppley R W & B J Peterson (1979) Particulate organic carbon flux and planktonic new production in the deep ocean. Nature 282: 677-680.

Esaias W E, G C Feldman, C R McClain & J A Elrod (1986) Monthly satellite-derived phytoplankton pigment distribution for the North Atlantic Ocean Basin. Eos 67: 835-837.

Evans G T & M A Paranjape (1992) Precision of estimates of phytoplankton growth and microzooplankton grazing when the functional response of grazers may be nonlinear. Marine Ecology Progress Series 80: 285-290.

Fasham M J R, P W Boyd & G Savidge (1999) Modeling the relative contributions of autotrophs and heterotrophs to carbon flow at a Lagrangian JGOFS station in the Northeast Atlantic: The importance of DOC. Limnology & Oceanography 44(1): 80-94.

Fauré-Fremiet E (1924) Contribution à la connaissance des infusiores planktoniques. Bulletin Biologique de la France et de la Belgique 19 Supplement 6:1-171.

Fenchel T (1968) The ecology of marine microbenthos III. The reproductive potential of ciliates. Ophelia 14: 317-326.

Fenchel T (1980) Suspension feeding in ciliated protozoa functional response and particle size selection. Microbial Ecology 6: 1-11.

Fenchel T (1982) Ecology of heterotrophic microflagellates. 2. Bioenergetics and growth.

Marine Ecology Progress Series 8: 225-231

Fenchel, T. (1987). Ecology of protozoa: *The biology of free-living phagotrophic protists.* Springer-Verlag.

Fessenden L & T J Cowles (1994) Copepod predation on phagotrophic ciliates in Oregon coastal waters. Marine Ecology Progress Series 107: 103-111.

Flynn K J & Fielder J (1989) Changes in intracellular and extracellular amino acids during the predation of the chlorophyte *Dunaliella primolecta* by the heterotrophic dinoflagellate *Oxyrrhis marina* and the use of the glutamine/glutamate ratio as an

indicator of nutrient status in mixed populations. Marine Ecology Progress Series 53: 117-127.

Froneman P W and R Perissinotto (1996) Structure and grazing of the microzooplankton communities of the Subtropical Convergence and a warm-core eddy in the Atlantic sector of the Southern Ocean. Marine Ecology Progress Series 135: 237-245.

Frost B W (1972) Effects of size and concentration of food particles on the feeding behaviour of marine planktonic copepod *Calanus pacificus*. Limnology & Oceanography 17: 805-815.

Frost B W (1975) A threshold feeding behaviour in Calanus pacificus. Limnology & Oceanography 20: 263-266.

Fuhrman J A & T M Bell (1985) Biological considerations in the measurement of dissolved free amino acids in seawater and implications for chemical and microbiological studies. Marine Ecology progress Series 25: 13-21.

Fuller A K R (1990) The grazing and growth rates of some marine protozoa measured in batch and continous culture with particular reference to the heterotrophic dinoflagellate *Oxyrrhis marina*. Ph.D. University of London, 296 pp.

Gaines G & M Elbrachter (1987) Heterotrophic nutrition. In Taylor F J R (ed), *The Biology of Dinoflagellates*. Blackwell Scientific Publications, Oxford pp 224-268.

Gallegos C L (1989) Microzooplankton grazing on phytoplankton in the Rhode River, Maryland: nonlinear feeding kinetics. Marine Ecology Progress Series 57: 23-33.

Garrison D L, K R Buck, & M M Gowing. (1991) Plankton assemblages in the ice edge zone of the Weddell Sea during the Austral winter. Journal of Marine Systems 2: 123-130.

Geider RJ (1989) Use of radiolabeled tracers in dilution grazing experiments to estimate bacterial growth and loss rates. Microbial Ecology 17: 77-87.

Gifford D J (1988) Impact of grazing by microzooplankton in the Northwest Arm of Halifax Harbour, Nova Scotia. Marine Ecology Progress Series 47: 249-258.

Gifford D J, L M Fessenden P R Garrahan & E Martin (1995) Grazing by microzooplankton and mesozooplankton in the high-latitude North Atlantic Ocean: Spring versus summer dynamics. Journal of Geophysical Research 100 (4), 6665-6675.

Goldman J C & D A Caron (1985) Experimental studies on an omnivorous microflagellate: Implications for grazing and nutrient regeneration in the marine microbial food chain. Deep-Sea Research 32: 899-915

Goldman J C, M R Dennett & H Gordin (1989) Dynamics of herbivorous grazing by the heterotrophic dinoflagellate *Oxyrrhis marina*. Journal of plankton Research 11(2): 391-407.

Guillard R R L (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith W L and M Chanley (eds), *Culture of Marine Invertebrate* Animals. Plenum Press, New York, p.29-60.

Hansen P J (1992) Prey size selection, feeding rates and growth rate dynamics of heterotrophic dinoflagellates with special emphasis on *Gyrodinium spirale*. Marine Biology 114: 327-334.

Hansen B, P K Bjørnsen & P J Hansen (1994) The size ratio between planktonic predators and their prey. Limnology & Oceanography 39: 395-403.

Hansen P J, P K Bjørnsen & B Hansen (1997) Zooplankton grazing and growth: scaling within the 2-2000$\mu$m body range. Limnology & Oceanography 42: 687-704.

Hansen P J & A J Calado (1999) Phagotrophic mechanisms and prey selection in free-living dinoflagellates. Journal of Eukaryotic Microbiology 46(4): 382-389.

Hensen V (1911) Das Leben im Ozean nach Zahlüngen seiner Bewohner. Ergebnisse der Plankton-Expedition der Humbolt-Stifung, Bd. V. O., pp1-406 + Tabellen.

Heinbokel J F (1978) Studies on the functional role of tintinnids in the Southern California Bight. 1 Grazing and growth rates in laboratory cultures. Marine Biology 47: 177-189.

Heinbokel J F & Béers J R (1979) Studies on the functional role of tintinnids in the southern California Bight III. Grazing and impact of natural assemblages. Marine Biology 52: 23-32.

Holm-Hansen O, C J Lorenzen, R W Holmes & J D H Strickland (1965) Fluorometric determination of chlorophyll. Journal du Conseil International pour l' Exploration de la Mer 30: 3-15.

Irigoien X, R Head, U Klenke, B Meyer-Harms, D Harbour, B Niehoff, H-J Hirche & R Harris (1998) A high frequency time series at weathership M, Norwegian Sea, during the

1997 spring bloom: feeding of adult female *Calanus finmarchicus*. Marine Ecology Progress Series 172: 127-137.

Jacobson D M & Anderson D M (1986) Thecate heterotrophic dinoflagellates: feeding behaviour and mechanisms. Journal of Phycology 22: 249-258.

Jakobsen H H & P J Hansen (1997) Prey-size selection, growth and grazing responses of a small heterotrophic dinoflagellate *Gymnodinium* sp. And a ciliate *Balanion comatum*: a comparative study. Marine Ecology Progress Series 158: 75-86.

James M R & J A Hall (1998) Microzooplankton grazing in different water masses associated with the Subtropical Convergence round the South Island, New Zealand. Deep-Sea Research 45: 1689-1707.

Jeffrey S W & G F Humphrey (1975) New spectrophotometric equations for determining Chlorophylls a, b, $c_1$ and $c_2$ in higher plants, algae and natural phytoplankton. Biochemie und Physiologie der Pflanzen 167: 191-194.

Joint I R, A Pomroy, G Savidge, P Boyd (1993) Size fractionated primary productivity in the North East Atlantic in Spring 1989. Deep-Sea Research II 40: 423-440.

Jonsson P R (1986) Particle size selection, feeding rates and growth dynamics of marine planktonic oligotrichous ciliates (Ciliophora: Oligotrichina). Marine Ecology Progress Series 33:265-277.

Kirchman D , H Ducklow & R Mitchell (1982) Estimates of bacterial growth from changes in uptake rates and biomass. Applied Environmental Microbiology 44: 1296-1307.

Kofoid C A (1897) On some important sources of error in the plankton method. Science 6: 829-832.

Kofoid C A and A S Campbell (1929) *Marine and Freshwater Ciliata*. University of California Publications in Zoology, volume 34, pp1-403. University of California Press.

Kopylov A I & N I Tumantseva (1987) Analysis of the contents of tintinnid food vacuoles and evaluation of phytoplankton production off the Peru coast. Oceanology 27: 343-347.

Landry M R & Hassett R P (1982) Estimating the grazing impact of marine micro-zooplankton. Marine Biology 67: 283-288.

Landry M R, Haas L W and Fagerness V.L (1984) Dynamics of microbial plankton communities: experiments in Kaneohe Bay, Hawaii. Marine Ecology Progress Series 16: 127-133.

Landry M R, B C Monger & K E Selph (1993) Time-dependency of microzooplankton grazing and phytoplankton growth in the Subarctic Pacific. Progress in Oceanography 32: 205-222

Landry M R, J Constantinou & J Kirshtein (1995) Microzooplankton grazing in the central equatorial Pacific during February and August, 1992. Deep-Sea Research II 42: 657-671.

Landry M R, S L Brown, L Campbell, J Constantinou, H Liu (1998) Spatial patterns in phytoplankton growth and microzooplankton grazing in the Arabian Sea during monsoon forcing. Deep-Sea Research II 45: 2353-2368.

Laval-Pueto M & F Rassoulzadegan (1988) Autofluorescence of marine planktonic Oligotrichina and other ciliates. Hydrobiologia 159: 99-110.

Laybourn J E M & J M Stewart (1975) Studies on consumption and growth in the ciliate *Colpidium campylum* Stokes. Journal of Animal Ecology 44: 165-174.

Leakey R J G, P H Burkill & M A Sleigh (1994) A comparison of fixatives for the estimation of abundance and biovolume of marine planktonic ciliate populations. Journal of Plankton Research 16 (4): 375-389.

Lebour M V (1925) The dinoflagellates of northern seas. The Marine Biological Association of the United Kingdom. 250pp

Leeuwenhoeck A (1677) Letters written by another but signed by Leeuwenhoeck; London, Royal Society. Phil Trans Vol XII no 133: 821-831.

Lessard E J & E Swift (1985) Species-specific grazing rates of heterotrophic dinoflagellates in oceanic waters, measured with a dual-label radioisotope technique. Marine Biology 87: 289-296.

Lessard E J & M C Murrell (1998) Microzooplankton herbivory and phytoplankton growth in the northwestern Sargasso Sea. Aquatic Microbial Ecology 16: 173-188.

Lessard, E.J. (1991). The trophic role of heterotrophic dinoflagellates in diverse marine environments. Marine Microbial Food Webs 5: 49-58.

Levandowsky M & P Kaneta (1987) Behaviour in dinoflagellates. In: F J R Taylor (ed) *The Biology of Dinoflagellates*. Botanical Monographs 21: Blackwell.

Lewis M R (1989) The variegated ocean: a view from space. New Scientist 1685: 37-40.

Li W K W (1990) Particles in "particle-free" seawater: growth of ultraphytoplankton and implications for dilution experiments. Canadian Journal of Fisheries and Aquatic Sciences, 47: 1258-1268.

Li W K W & P M Dickie (1985) Growth of bacteria in seawater filtered through 0.2$\mu$m Nuclepore membranes: implications for dilution experiments. Marine Ecology Progress Series 26: 245-252.

Li W K W & A M Wood (1988) Vertical distribution of North Atlantic ultraphytoplankton: analysis by flow cytometry and epifluorescence microscopy. Deep-Sea Research 35: 1615-1638.

Lochte K, H W Ducklow, M J R Fasham & C Steinen (1993) Plankton succession and carbon cycling at 47°N 20°W during the JGOFS North Atlantic Bloom Experiment. Deep Sea Research II 40 (1/2): 91-114.

Lohmann H (1903) Neue Untersuchungen uber den Reichtum des meeres an plankton und über die Brauchbakeit der verschiedenen Fangmethoden. Wiss. Meeresunters. (Abt. Kiel) 10: 1-88.

Lohmann H (1908) Untersuchungen zur Festellung des vollständigen Gehaltes des Meeres an Plankton. Wiss. Meeresunters. (Abt. Kiel) 10: 131-370.

Lohmann H (1920) Die Bevölkerung des Ozeans mit Plankton nach den Ergebnissen der zentrifugenfänge während der Ausreise de "Deutschland" 1911. Zugleich ein Beitrag zur Biologie des Atlantischen Ozeans. Arch. Biontol. Berl. 4: 1-617.

Malone TC (1980) Size-fractionated primary productivity of marine phytoplankton. In: Falkowski PG (ed) *Primary Productivity in the Sea*, Plenum Press,New York, pp301-319.

McCave I N (1975) Vertical fluxes of particles in the oceans. Deep-Sea Research 22: 491-502.

McManus G B (1995) Phytoplankton abundance and pigment changes during simulated in situ dilution experiments in estuarine waters: possible artifacts caused by algal light adaptation. Journal of Plankton Research 17 (8): 1705-1716.

McManus G B & M C Ederington-Cantrell (1992) Phytoplankton pigments and growth rates, and microzooplankton grazing in a large temperate estuary. Marine Ecology Progress Series 87:77-85.

Michaels A F & M W Silver (1988) Primary production, sinking fluxes and microbial foodweb. Deep-Sea Research 35: 473-490.

Michaels A F, D A Caron, N R Swanberg, F A Howse and C M Michaels (1995) Planktonic sarcodines (Acantharia, Radiolaria, Foraminifera) in surface waters near Bermuda: abundance, biomass and vertical flux. Journal of Plankton Research 17: 131-163.

Morales CE, A Bedo, R P Harris & P Tranter (1991) Grazing of copepod assemblages in the North-east Atlantic- the importance of the small size fraction. Journal of Plankton Research 13: (2) 455-472.

Morales C E, R P Harris, R N Head & P R G Tranter (1993) Copepod grazing in the oceanic northeast Atlantic during a 6 week drifting station: the contribution of size classes and vertical migrants. Journal of Plankton Research 15 (2): 185-211.

Murphy L S & E M Haugen (1985) The distribution and abundance of phototrophic ultraplankton in the North Atlantic. Limnology & Oceanography 30: 47-58.

Murphy E J, P W Boyd, R J G Leakey, A Atkinson, E S Edwards, C Robinson, J Priddle, S J Bury, D B Robins, P H Burkill, G Savidge, N J P Owens, D Turner (1998). Carbon flux in ice-ocean-plankton systems of the Bellingshausen Sea during a period of ice retreat. Journal of Marine Systems: 17(1), 207-227

Neuer S. & T.J. Cowles (1994) Protist herbivory in the Oregon upwelling system. Marine Ecology Progress Series 113: 147-162.

Nielsen T G & T Kiorboe (1994) Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. 2. Ciliates. Limnology & Oceanography 39 (3): 508-519

Paffenhöfer G A (1984) Food ingestion by the marine planktonic copepod *Paracalanus* in relation to abundance and size distribution of food. Marine Biology 80: 328-333.

Paranjape M A (1987) Grazing by microzooplankton in the eastern Canadian Arctic in summer 1983. Marine Ecology Progress Series 40: 239-246.

Paranjape M A (1990) Microzooplankton herbivory on the Grand Bank (Newfoundland, Canada): A seasonal study. Marine Biology 107: 321-328

Peinert R, von Bodungen, & V S Smetacek (1989) Food web structure and loss rate. In: Productivity of the Ocean: present and past, W H Berger, V S Smetacek and G Wefer, editors, Dahlem Konferenzen, pp. 35-48.

Phinney D A and C S Yentsch (1985) A novel phytoplankton chlorophyll technique: toward automated analysis. Journal of Plankton Research 7 (5): 633-642.

Platt T, D V Subba Rao & B Irwin (1983) Photosynthesis of picoplankton in the oligotrophic ocean. Nature 310: 702-704.

Pomeroy L (1974) The ocean's food web: a changing paradigm. Bioscience 24: 499-504.

Porter K G, E B Sherr, B F Sherr, M Pace & R W Sanders (1985) Protozoa in planktonic food webs. Journal of Protozoology 32 (3): 409-415.

Putt M & D K Stoecker (1989). An experimentally determined carbon:volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnology & Oceanography 34: 1097-1103.

Rassoulzadegan F (1982) Feeding in marine planktonic protozoa. Annals de l' Institute Oceanographique, Paris (Nouv Ser) 58 : 191-206.

Rassoulzadegan F & R W Sheldon (1986). Predator-prey interaction of nano-zooplankton and bacteria in an oligotrophic marine environment. Limnology & Oceanography 31(5): 1010-1021.

Rassoulzadegan F, M Laval-Pueto & R W Sheldon (1988) Partitioning the food ration of marine ciliates between pico- and nanoplankton. Hydrobiologia 159:75-88.

Reckermann M & M J W Veldhuis (1997) Trophic interactions between picophytoplankton and micro- and nanozooplankton in the western Arabian Sea during the NE monsoon 1993. Aquatic Microbial Ecology 12: 263-273.

Riley G A (1957) Phytoplankton of the north central Sargasso Sea, 1950-52. Limnology & Oceanography 2: 252-270.

Robertson J & A J Watson (1995) A summertime sink for atmospheric carbon dioxide in the Southern Ocean between 88°W and 80°E. Deep-Sea Research II 42 (4-5): 1081-1092.

Rublee P A & C L Gallegos (1989) Use of fluorescently labelled algae (FLA) to estimate microzooplankton grazing. Marine Ecology Progress Series 51: 221-227.

Savidge G, D R Turner, P H Burkill, A J Watson, M V Angel, R D Pingree, H Leach, K J Richards (1992). The BOFS 1990 Spring Bloom Experiment:: Temporal evolution and spatial variability of the hydrographic field. Progress in Oceanography 29: 235-281.

Savidge G, P Boyd, A Pomroy, D Harbour & I Joint (1995) Phytoplankton production and biomass estimates in the northeast Atlantic Ocean, May-June 1990. Deep-Sea Research 42 (1): 599-617.

SCOR (1990) JGOFS Science Plan. JGOFS Report No. 4. Scientific Committee on Oceanic Research.

Sherr B F, E B Sherr & S Y Newell (1984) Abundance and productivity of heterotrophic nanoplankton in Georgia coastal waters. Journal of Plankton Research 6 (1): 195-202

Sherr E B, B F Sherr, R D Fallon & S Y Newell (1986) Small aloricate ciliates as a major component of the marine heterotrophic nanoplankton. Limnology & Oceanography 31: 177-183.

Sherr B F, E B Sherr & R D Fallon (1987) Use of monodispersed fluorescently-labelled bacteria to estimate *in situ* protozoan bacterivory. Applied and Environmental Microbiology 53: 958-965.

Sheldon R W, P Nival & F Rassoulzadegan (1986). An experimental investigation of a flagellate-ciliate-copepod food chain with some observations relevant to the linear biomass hypothesis. Limnology & Oceanography 31: 184-188.

Sieburth J A, V Smetacek & J Lenz (1978) Pelagic ecosystem structure: heterotrophic compartments of the plankton and their relationship to plankton size fractions. Limnology & Oceanography 23: 1256-1263.

Sieracki M E, P G Verity, D K Stoecker (1993). Plankton community response to sequential silicate and nitrate depletion during the 1989 North Atlantic spring bloom. Deep- Sea Research II 40: 213-225.

Sime-Ngando T, K Juniper, & A Vézina (1992). Ciliated protozoan communities over Cobb seamount: increase in biomass and spatial patchiness. Marine Ecology Progress Series 89: 37-51.

Smetacek V (1981) The annual cycle of protozooplankton in Kiel Bight. Marine Biology 63:1-11.

Smith S L, M R Roman, I Prusova, K F Wishner, M M Gowing, L A Codispoti, R T barber, J marra & C Flagg (1998). Seasonal response of zooplankton to monsoonal reversals in the Arabian Sea. Deep-Sea Research II 45: 2369-2403.

Stelfox, C E, P H Burkill, E S Edwards, R P Harris, & M A Sleigh. (1999). The structure of zooplankton communities, in the 2 to 2000 um size range, in the Arabian Sea during and after the SW Monsoon, 1994. Deep-Sea Research II 46 (3/4): 815-842.

Stelfox-Widdicombe, C E, E S Edwards, P H Burkill and M A Sleigh (2001 in press) Microzooplankton grazing activity in the temperate and sub-tropical NE Atlantic: Summer 1996. Marine Ecology Progress Series.

Stoecker D K (1984) Particle production by planktonic ciliates. Limnology & Oceanography 29: 930-940

Stoecker D K (1991) Mixotrophy in marine planktonic ciliates: physiological and ecological aspects of plastid-retention by oligotrichs. In: Reid P C, C M Turley & P H Burkill (eds*) Protozoa and their role in marine processes*, pp 161-179. Springer-Verlag, Berlin.

Stoecker D K & R R L Guillard (1982) Effects of temperature and light on the feeding rate of *Favella* sp. (ciliated Protozoa, suborder Tintinnina). Ecology of Marine Planktonic Protozoa, Villefranche-sur-Mer (France), May 1981. Marine Pelagic Protozoa and Microzooplankton Ecology. North Atlantic Treaty Organisation 58: 309-318

Stoecker D K & Govoni J J (1984) Food selection by young larval gulf menhaden (*Brevoortia patronus*). Marine Biology 80: 299-306.

Stoecker D K & G T Evans (1985) Effects of protozoan herbivory and carnivory in a microplankton food web. Marine Ecology Progress Series 25: 159-167.

Stoecker D K & D A Egloff (1987) Predation by *Acartia tonsa* Dana on planktonic ciliates and rotifers. Journal of Experimental Marine Biology & Ecology 110: 53-68.

Stoecker D K, R R L Guillard & R M Kavee (1981) Selective Predation by *Favella ehrenbergii* (Tintinnina) on and among dinoflagellates. Biological Bulletin of the Marine Biological Laboratory, Woods Hole 160: 136-145.

Stoecker D K, L Davis & A Provan (1983) Growth of *Favella* sp. (Ciliata, Suborder Tintinnina) and other microzooplankters in cages incubated *in situ* and comparison to growth *in vitro*. Marine Biology 75: 293-302.

Stoecker D K, A Taniguchi & A E Michaels (1989) Abundance of autotrophic, mixotrophic and heterotrophic planktonic ciliates in shelf and slope waters. Marine Ecology Progress Series 50: 241-254.

Stoecker D K, J McDowell & J M Capuzzo (1990) Predation on protozoa: its importance to zooplankton. Journal of Plankton Research 12 (5): 891-908.

Stoecker D K, M E Sieracki, P G Verity, A E Michaels, E Haugen, P H Burkill, & E S Edwards. (1994). Nanoplankton and protozoan microzooplankton during the JGOFS North Atlantic bloom experiment: 1989 and 1990. Journal of the Marine Biological Association of the United Kingdom 74(2): 427-443.

Strickland J D H & Parsons T R (1972) Fluorometric determination of chlorophylls. In: A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada 167: 201-203.

Strom S (1991) Growth and grazing rates of the herbivorous dinoflagellate *Gyrodinium* sp. from the open subarctic Pacific Ocean. Marine Ecology Progress Series 78: 103-113.

Strom S L & M W Strom (1996) Microplankton growth, grazing and community structure in the northern Gulf of Mexico. Marine Ecology Progress Series 130: 229-240.

Tarran G A (1992) Aspects of the grazing behaviour of the marine dinoflagellate *Oxyrrhis marina*, Dujardin. PhD University of Southampton.

Tarran, G.A., P H Burkill, E S Edwards, E M S Woodward (1999). Phytoplankton community structure in the Arabian Sea during and after the SW monsoon, 1994. Deep Sea Research II 46 (3/4): 655-676

Tremaine S C & A L Mills (1987) Tests of the critical assumptions of the dilution method for estimating bacterivory by microeukaryotes. Applied Environmental Microbiology 53: 2914-1282.

Utermöhl H (1958) Zur Vervolkommung der Quantitativen Phytoplanktonmethodik. Mitteilungen der Internationalen Vereinigung für Limnologie 9: 1-38

Verity P G (1985). Grazing, respiration, excretion, and growth-rates of tintinnids. Limnology & Oceanography 30 (6): 1268-1282 1985

Verity P G & G A Paffenhöfer (1996) On assessment of prey ingestion by copepods. Journal of Plankton Research 18: 1767-1779.

Verity P G, D K Stoecker, M E Sieracki & J R Nelson (1993a) Grazing, growth and mortality of microzooplankton during the 1989 North Atlantic spring bloom at 47°N, 18°W. Deep-Sea Research 40 (9): 1793-1814.

Verity P G, D K Stoecker, M E Sieracki, P H Burkill, E S Edwards, C R Tronzo (1993b). Abundance, biomass and distribution of heterotrophic dinoflagellates during the North Atlantic spring bloom. Deep Sea Research II 40(1/2): 227-244.

Verity, P.G., D K Stoecker, M E Sieracki, & J R Nelson (1996). Microzooplankton grazing of primary production at 140°W in the equatorial Pacific. Deep Sea Research II 43 (4-6): 1227-1255

Vincent W F (1988) Microbial Ecosystems of Antarctica. Cambridge University Press, 304pp.

Watson R T, H Rhode, H Oeschger & U Siegenthaler (1990) Greenhouse gases and aerosols. In: *Climate Change: the IPCC scientific assessment*, J T Houghton, G J Jenkins and J J Ephramus, (eds), Cambridge University Press, Cambridge, pp 5-40.

Watson A J, C Robinson, J E Robinson, P J Le B Williams and M R Fasham (1991) Spatial variability in the sink for atmospheric carbon dioxide in the North Atlantic. Nature 350: 50-53.

Weeks A, M H Conte, R P Harris, A Bedo, I Bellan, P H Burkill, E S Edwards, D S Harbour, H Kennedy, C Llewellyn, R F C Mantoura, C E Morales, A J Pomroy, & C M Turley. (1993). The physical and chemical environment and changes in community structure associated with bloom evolution: the Joint Global Flux Study North Atlantic bloom experiment. Deep-Sea Research II 40(1/2): 347-368.

Weisse T & U Scheffel-Möser (1991). Uncoupling the microbial loop, growth and grazing loss rates of bacteria and heterotrophic nanoflagellates in the North Atlantic. Marine Ecology Progress Series 71: 195-205

Xiuren & Vaulot (1992) Estimating *Synechococcus spp.* growth rates and grazing pressure by heterotrophic nanoplankton in the English Channel and the Celtic Sea. Acta Oceanologica sinica 11:255-273

Yoder J A, C R McClain, G C Feldman, W E Esaias (1993). Annual cycles of phytoplankton chlorophyll concentrations in the global ocean: a satellite view. Global Biogeochemical Cycles 7:181-193.

Zeitzschel B (1967) Die Bedeutung der Tintinnen als Gleid der Nahrungskette. Hellgoländer Meeresunters 15: 589-601.

Zeitzschel B (1969) Tintinnen des westlichen Arabischen Meeres, ihre Bedeutung als Indikatoren für Wasserkörper und Gleid der Nahrungskette. "*Meteor*" Forsch. Ergebn. D (4): 47-101.

# Appendices

Appendix 1: Taylor A H, D S Harbour, R P Harris, P H Burkill & E S Edwards 1993. Seasonal succession in the pelagic ecosystem of the North Atlantic and the utilisation of nitrogen. Journal of Plankton Research 15 (8): 875-891.

Appendix 2: Weeks A, M H Conte, R P Harris, A Bedo, I Bellan, P H Burkill, E S Edwards, D S Harbour, H Kennedy, C Llewellyn, R F C Mantoura, C E Morales, A J Pomroy, C M Turley. 1993. The physical and chemical environment and changes in community structure associated with bloom evolution: the Joint Global Flux Study North Atlantic bloom experiment. Deep Sea Research II 40(1/2): 347-368.

Appendix 3: Stelfox, C E, P H Burkill, E S Edwards, R P Harris, & M A Sleigh. 1999. The structure of zooplankton communities, in the 2 to 2000 µm size range, in the Arabian Sea during and after the SW Monsoon, 1994. Deep-Sea Research II 46 (3/4): 815-842

The following published papers were included in the bound thesis. These have not been digitised due to copyright restrictions, but the links are provided.

A.H. Taylor, D.S. Harbour, R.P. Harris, P.H. Burkill and E.S. Edwards (1993)

**Seasonal succession in the pelagic ecosystem of the North Atlantic and the utilization of nitrogen**

Journal of Plankton Research: Vol. 15, No. 8, pp.875-891

http://dx.doi.org/10.1093/plankt/15.8.875

A. Weeks, M.H. Conte, R.P. Harris, A. Bedo, I. Bellan, P.H. Burkill, E.S. Edwards, D.S. Harbour, H. Kennedy, C. Llewellyn, R.F.C. Mantoura, C.E. Morales, A.J. Pomroy and C.M. Turley (1993)

**The physical and chemical environment and changes in community structure associated with bloom evolution: the Joint Global Flux Study North Atlantic Bloom Experiment**

Deep Sea Research II: Vol. 40, Nos. 1-2, pp.347-368

http://dx.doi.org/10.1016/0967-0645(93)90021-E

Claire E. Stelfox, Peter H. Burkill, Elaine S. Edwards, Roger P. Harris and Michael A. Sleigh (1999)

**The structure of zooplankton communities, in the 2 to 2000 um size range, in the Arabian Sea during and after the SW monsoon, 1994**

Deep Sea Research II: Vol. 46, pp.815-842

http://dx.doi.org/10.1016/S0967-0645(98)00129-5