The role of heterotrophic dinoflagellate and ciliate grazers in the food web at Helgoland Roads, North Sea

by

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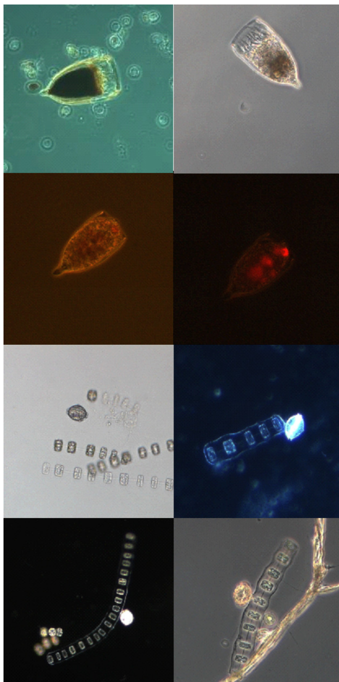
A thesis submitted in partial fulfilment

of the requirements for the degree of

Doctor of Philosophy

in Biology



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“What we know is a drop, what we don’t know is an ocean”

Isaac Newton

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INTRODUCTION

Understanding "classic" pelagic trophic interactions

Early concepts on trophic interactions in the marine pelagic realm often consisted of simple food chains (Figure 1). Web-like interactions including omnivory or loops, if at all described, were considered rare phenomena and thus neglected. The flow of energy was considered a one way flux from phytoplankton via herbivorous mesozooplankton through to small fish with large fish as the "top" predators (Steele, 1976). Organic matter leaving the food chain as detritus, dead cells or dead individuals, was thought to be remineralised by bacteria and re-incorporated, in the form of nutrients, into the food chain via autotrophic production. These simple ideas, although plausible at that time, were a considerable simplification of the actual predator-prey interactions in the plankton (Williams, 1981). Consequently, the first simple models of the food chain were not able to explain the total amount of pelagic fish production (Pomeroy, 1974, Steele, 1976).

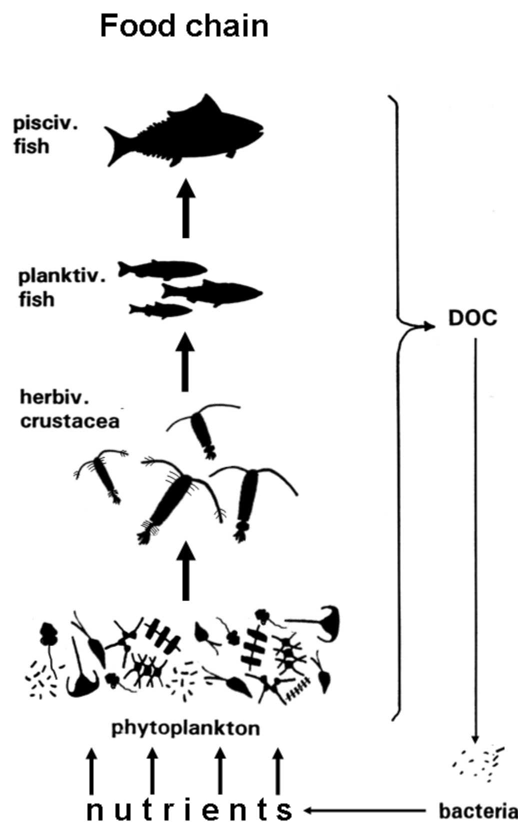

Figure 1: The classic pelagic food chain (after Sommer, 2005).

Pomeroy (1974) stated that “although the ocean food web has been investigated for more than a century, several recent discoveries indicate that the classical textbook description of a chain from diatoms through copepods and krill to fishes and whales may in fact be only a small part of the flow of energy”. Indeed, expanded food web models were found to be a better fit to reality and explained energy fluxes in the food web more accurately (Pace et al., 1984). We now know that pelagic trophic relationships constitute highly complex web-like interactions between members of various groups.

In addition, some consumers which were formerly classified as “top predators” are now known to be ingested by their “prey”, at least in some life-stages, e.g. during their larval or juvenile stages. This is the case, when, as an example, copepods feed on fish eggs or fish larvae (Turner et al., 1985, Yen, 1987). The closer food web interactions are studied, the more relationships in terms of cross-linkages and loopings appear, even where this has not been previously expected. Such studies are thus fundamental to dealing with our increasing demands upon the ocean (Pomeroy, 1974).

Although our knowledge of pelagic food web structures has improved during the second half of the last century, the role of many organisms in this pelagic food web, as well as interactions between them, are still poorly understood or as yet undiscovered.

Autotrophic phytoplankton forms the basis of the pelagic food web and the manner in which this resource is used by herbivores is decisive for the transport of energy to higher trophic levels such as fish (De Laender et al., 2010). Only recently the crucial role of microzooplankton organisms as the probably most important primary consumers in the ocean has been addressed (Landry & Calbet, 2004). In addition, microzooplankton is increasingly viewed as an irreplaceable food source for higher trophic levels (Stoecker, 1990, Montagnes et al., 2010). This group thus interacts with a wide range of trophic levels in the marine food web. However, despite years of research a lot of questions relating to microzooplankton are still unanswered.

The microzooplankton – long neglected phytoplankton grazers

Traditionally, planktonic crustaceans (copepods) were considered to be the main herbivores and meanwhile another group of phytoplankton grazers in the oceans has been overlooked for a long time: The microzooplankton.

The term microzooplankton refers to the size fraction of heterotrophic planktonic organisms between 20 and 200 µm (Sieburth et al., 1978). It consists of taxonomically diverse groups of protozoa (e.g. ciliates, dinoflagellates and other heterotrophic

flagellates) and metazoa (e.g. rotifers, nauplii and other planktonic larvae). However, the numerically most important components within this group are heterotrophic dinoflagellates and ciliates (Capriulo et al., 1991).

Microzooplankton only started receiving more attention when Azam et al. (1983) coined the term microbial loop. Where dissolved organic matter (DOM) released by phytoplankton is utilised by heterotrophic bacteria, heterotrophic nanoflagellates consume these bacteria and are in turn prey for microzooplankton organisms. Via this microbial loop energy in the form of DOM released by phytoplankton is returned to the main food chain (Azam et al. 1983).

Subsequent investigations showed that microzooplankton not only plays a significant role in transferring energy to higher trophic levels (Sherr et al., 1986) but that it can also consume up to 60–75% of the daily phytoplankton production (Landry & Calbet, 2004). Early continental shelf models including microzooplankton assumed that they only feed on phytoplankton fractions smaller than 60 µm (Pace et al., 1984). However, we now know that they have a broad food spectrum (Smetacek, 1981, Jeong, 1999) placing them in direct competition with copepods for bigger phytoplankton (Hansen, 1992, Aberle et al., 2007). Recent studies even show that dinoflagellates can be the most important grazers during diatom blooms (Sherr & Sherr, 2007). Irigoien et al. (2005) went one step further hypothesizing that phytoplankton blooms can only occur when microalgae are released from microzooplankton grazing pressure. This relationship was also experimentally shown by Sommer et al. (2005).

Only recently a growing number of studies have started to investigate the role of microzooplankton as phytoplankton grazers (Calbet & Landry, 2004, Fonda Umani et al., 2005, Irigoien et al., 2005, Putland & Iverson, 2007, Sherr & Sherr, 2007). Although their pivotal role as phytoplankton grazers especially during phytoplankton blooms has now been recognised, less is known about the functional diversity of microzooplankton.

Crucial for an understanding of the ecological role of microzooplankton is more research on its abundance, species composition, seasonal distribution and succession patterns as well as the biotic and abiotic factors influencing all of these aspects. Another blank area in our knowledge about microzooplankton concerns investigations on its capacity for food selectivity. Scarcely anything is known about the plasticity in microzooplankton food preferences and how this can influence bloom assemblages, both of the phytoplankton prey and the microzooplankton predators. Although fundamental to ecological considerations, to date, interactions within the microzooplankton community, e.g., competitive patterns or inter-specific predation

within the microzooplankton, have rarely been investigated. In addition, experiments on microzooplankton are hampered by the fragility of certain groups (Gifford, 1985). The methodological approaches for the investigation of these species have to be improved as the conservation of fragile species for and also in experiments is fundamental to the ability to answer questions about their ecology.

These examples, proxies for a whole list of unanswered questions, show that there is still a great need for further research on microzooplankton and its role in the marine food web. In the following paragraphs I will focus on the current knowledge on dinoflagellates and ciliates to give a brief insight into their ecology.

The most important microzooplankton groups: Dinoflagellates and ciliates

Dinoflagellates and ciliates are cosmopolitan groups in marine, freshwater, benthic and planktonic habitats. In the oceans they occur in such contrasting ecosystems as the eutrophic, turbid and shallow Wadden Sea, the oligotrophic tropical Pacific, the Mediterranean and the Polar Regions. Many species of both groups are known to be mixotrophic and their nutrition ranges from phototrophic with the ability to ingest organic particles, to phagotrophic with the additional ability to retain chloroplasts of their prey organisms (so-called ‘kleptochloroplasts’) and to use these for photosynthesis. Examples of mixotrophy among phototrophic species are the ciliate *Myrionecta rubra* (Johnson & Stoecker, 2005) and a variety of phototrophic dinoflagellates (Du Yoo et al., 2009); phagotrophs with the ability to retain chloroplasts are, e.g., the ciliate *Laboea strobila* (Stoecker et al., 1988) and the dinoflagellate genus *Dinophysis* (Carvalho et al., 2008). However, many species in both groups display a purely heterotrophic nutrition.

Dinoflagellates

Dinoflagellates span a large size range from 2 µm (*Gymnodinium simplex*) to 2 mm (*Noctiluca scintillans*) (Taylor, 1987), but the size of the majority lies within 20 to 200 µm thus belonging to the microzooplankton (Sieburth et al., 1978).

Today approximately 2500 living species of various morphologically highly variable genera of dinoflagellates have been described, of which roughly 40 – 60% are photosynthetic. However, among those dinoflagellates regarded as photosynthetic a growing number is found to be capable of taking up organic carbon (mixotrophy) and of active feeding (Du Yoo et al., 2009) (in this thesis the terms “dinoflagellates” and “heterotrophic dinoflagellates” refer to the same: Species capable of active feeding).

The remaining species are obligate heterotrophs, either free-living or intra- and extracellular parasites of different hosts (Margulis et al., 1990, Lee et al., 2000). Important photosynthetic dinoflagellates in the North Sea are, for example, several species of the genus *Ceratium, Prorocentrum* and *Scrippsiella* or the species *Akashiwo sanguinea, Lepidodinium chlorophorum* and *Torodinium robustum*. Important heterotrophic dinoflagellates are, e.g., *Gyrodinium* spp., *Protoperidinium* spp., the *Diplopsalis* group as well as the species *Noctiluca scintillans*.

General characteristics

The majority of dinoflagellates are motile. They swim by means of two flagella. One longitudinal flagellum extends out from the sulcal groove of the posterior part of the cell and propels the cell forward. One flattened flagellum lies in the cingulum, the groove that spans the cell's equator. The undulation of the flagella provides the ability to navigate and move forward. As a result of the action of the two flagella the cell spirals as it moves. The motility enables dinoflagellates to vertically migrate within their habitat and to pursue their prey organisms, as well as allowing them to concentrate in patches of high prey density.

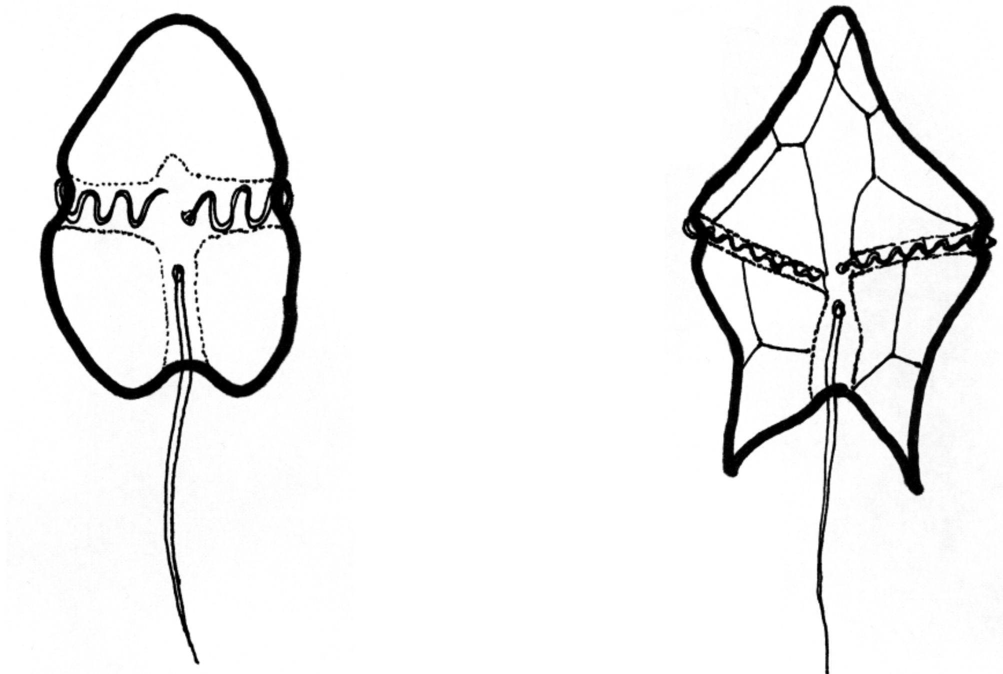

Figure 2: Two types of dinoflagellates. Left: A "naked", athecate dinoflagellate, right: An "armoured", thecate dinoflagellate showing typical cellulose plates (after Taylor, 1987).

Two major groups are found within the dinoflagellates: Naked or athecate cell forms (e.g. *Gyrodinium* spp., *Gymnodinium* spp., *Noctiluca scintillans*) and armoured or thecate cells with thecal plates made of cellulose (e.g. *Protoperidinium* spp., *Ceratium* spp., *Dinophysis* spp.) (Figure 2). The arrangement of the plates is used for species identification in thecate forms (Dodge, 1982, Tomas, 1996, Kraberg et al., 2010). Also characteristic of dinoflagellates is their large nucleus known as the *dinokaryon*. This contains chromosomes in a highly condensed form which do not decondense during the interphase of cell division. Vegetative cells of dinoflagellates grow by asexual cell division (Taylor, 1987). They display maximum specific growth rates up to ~2 d^{-1}, depending on the species, but in general a division rate about 0.5-1 d^{-1} is common.

Feeding strategies

Heterotrophic dinoflagellates are known to catch and consume prey by a variety of different feeding mechanisms (Schnepf & Elbrächter, 1992, Hansen & Calado, 1999). Many naked genera (Figure 3) are able to ingest whole intact prey cells via direct engulfment (e.g. *Gyrodinium* spp., *Gymnodinium* spp.) (Hansen, 1992). This strategy is widespread and has recently also been described for some thecate species (Jeong et al., 1999) (e.g. *Fragilidium* cf. *mexicanum*, *Peridiniella danica*). A common feeding strategy within thecate forms is pallium feeding (Figure 4): The prey is surrounded by a pseudopodium, the pallium, reaching out of the flagellar pore of the dinoflagellate's cell, and is digested outside the theca (*Protoperidinium* spp., the *Diplopsalis* group) (Jacobson & Anderson, 1986, Hansen & Calado, 1999).

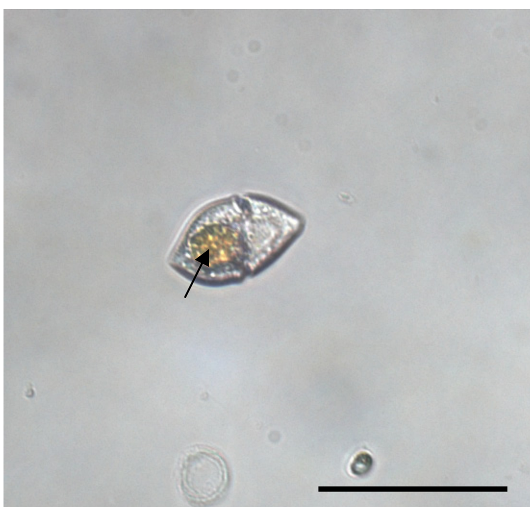

Figure 3: Direct engulfment: *Gyrodinium dominans* with an ingested *Scrippsiella trochoidea* cell (arrow). Scale bar 50 µm.

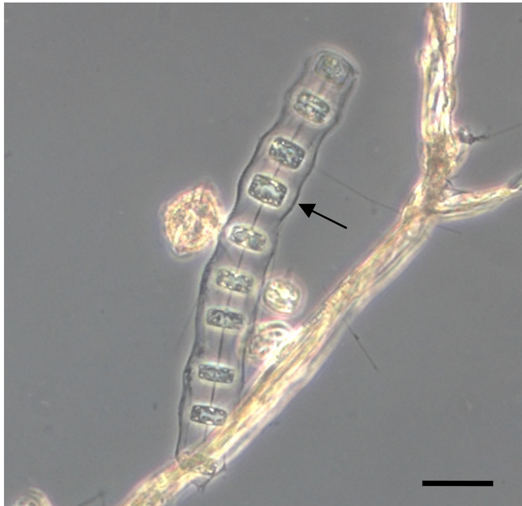

Figure 4: *Diplopsalis lenticula* digesting a *Thalassiosira rotula* chain in its pallium (arrow). Scale bar 50 µm.

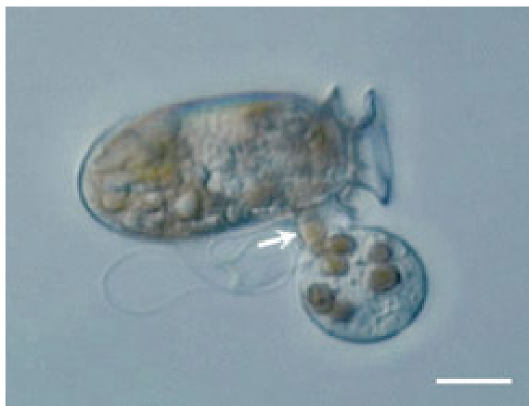

Figure 5: *Dinophysis acuminata* feeding on *Myrionecta rubra* with a peduncle (arrow). Scale bar 10 µm. Photo: Myung Gil Park (Park et al., 2006)

Another group of dinoflagellates takes up food by feeding tubes (Figure 5), used to pierce the prey cell and suck out its cytoplasm (e.g. *Amphidinium* spp., *Dinophysis* spp.). Two different types of feeding tubes have been described: Peduncle and phagopod (Schnepf & Elbrächter, 1992)

Food spectrum

A wide range of prey items are reported for dinoflagellates, including almost every kind of organic particle present in the marine habitat. Food particles range from bacteria to nanoflagellates, all size classes of microalgae, especially chain-forming diatoms, marine snow, microzooplankton as well as copepod eggs and even injured metazoans (Jeong, 1999). However, the prey used by a particular species of dinoflagellate most probably depends on different factors such as their size, chemo-attraction and swimming behaviour (Hansen, 1992). Laboratory determined predator:prey size ratios within heterotrophic dinoflagellates show that they can feed and grow on predator:prey size

ratios ranging between 5.2:1 and 0.15:1. Maximum growth is detected when dinoflagellates feed on prey approximately as big as themselves (Hansen, 1992, Naustvoll, 2000a, Naustvoll, 2000b).

Ciliates

Within the phylum Ciliata about 7500 living species are currently known from terrestrial soils, benthic and pelagic aquatic habitats. Planktonic ciliates consume a wide spectrum of particle sizes from bacteria to large diatoms and dinoflagellates, as well as other ciliates. They play a crucial role as herbivorous primary consumers in marine food webs (Urrutxurtu, 2003). A few ciliates can reach up to 2 mm in length, but most of them span the same size range as the dinoflagellates, i.e. 20-200 µm. Mixotrophy has been recognized as a common strategy in several ciliate genera and ciliates which retain kleptochloroplasts can constitute a large proportion of the ciliate assemblages in coastal waters (Stoecker et al., 1987). Several parasitic species are also known. *Myrionecta rubra* is the most important photosynthetic ciliate in North Sea waters and different *Strombidium* spp., e.g., *Laboea strobila* and *Strombidium capitatum*, are common mixotrophic ciliates in the North Sea. Examples with a purely heterotrophic nutrition are several species of the genera *Strombidium, Strobilidium* and *Favella* as well as other tintinnids.

General characteristics

With few exceptions, the typical features of a ciliate are the rows of ciliated organelles on the cell surface, known as kinities (during at least one stage of life). The arrangement of these kinities on the body surface, the ciliature, is distinct in most ciliates and conspicuous around the cell mouth or cytostome. The ciliature is used for classification (Agatha, 2004). Ciliates are capable of very fast movements and use their cilia to propel themselves forward through the water. At first glance two groups of ciliates can be distinguished (Figure 6): Tintinnids - ciliates with lorica (a shell, manly consisting of a cup shaped organic wall with or without agglutinated particles, e.g. *Favella* spp., *Tintinnopsis* spp.) and ciliates without lorica (e.g. *Strobilidium* spp., *Strombidium* spp.).
Characteristic for ciliates is their nuclear dualism in which the larger macronucleus is active, while the smaller micronucleus is the germ nucleus whose meiotic products are exchanged during sexual reproduction (Margulis et al., 1990, Lee et al., 2000). In contrast to the dinoflagellates, asexual reproduction takes place by ‘budding’ rather than simple cell division. Most ciliates display higher maximum specific growth rates

compared to dinoflagellates and generally, within similar size classes, growth rates are twice as high as those of dinoflagellates (up to > 2 d^{-1}) (Montagnes, 1996, Strom & Morello, 1998).

Figure 6: Left: A ciliate with a lorica (arrow), right: A ciliate without lorica (after Montagnes, 2003).

Feeding strategies

Ciliates collect food using different mechanisms (Tillmann, 2004). This includes suspension-feeding, where retention of relatively small food particles takes place by sieving feeding currents through the ciliated organelles arranged round the cytostome (e.g. *Euplotes* spp.) (Fenchel, 1980). Other common strategies are deposit feeding and active hunting (e.g. *Didinium* spp.) of motile or non-motile prey (Capriulo et al., 1991).

Food spectrum

In general, ciliates probably do not have as wide a food spectrum as phagotrophic dinoflagellates (Jonsson, 1986). The prey size of tintinnids is restricted by the width of their rigid lorica. Suspension feeding ciliates normally take up food particles in the bacterial size range (0.2-1µm) (Montagnes, 1996). Most ciliates (e.g. *Strombidium* spp., *Strobilidium* spp.) are reported to feed on different flagellates (Figure 7) but some also grow on small diatoms (Tillmann, 2004) (*Cyclotrichium* sp.). However, field

observations indicate that naked ciliates can feed on prey items that are similar in size or even larger than themselves (Smetacek, 1981, Gifford, 1985).

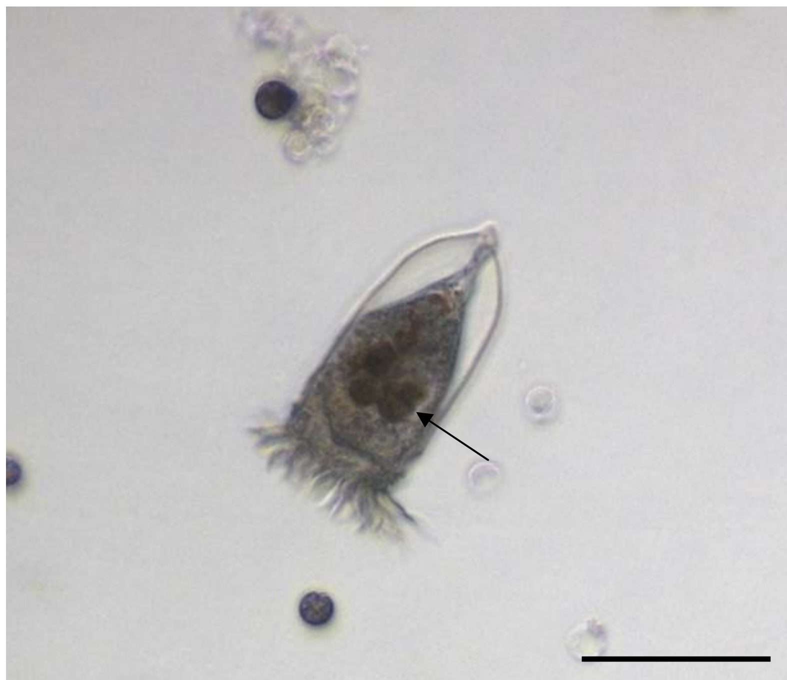

Figure 7: The tintinnid *Favella ehrenbergii* with ingested *Scrippsiella trochoidea* cells (arrow). Scale bar 100 µm.

Methods for estimating microzooplankton grazing

As microzooplankton species have received more attention in ecology, their role as grazers has repeatedly been investigated and different techniques to study their feeding habits have been established (Kivi & Setälä, 1995). Two types of methods can be distinguished: Direct and indirect measurement procedures. Direct approaches make use of stained or fluorescently-labelled artificial or natural food particles, which are recognizable in the grazers after ingestion and which can be detected using various microscopy techniques (Bernard & Rassoulzadegan, 1990, Christoffersen & Gonzalez, 2003). Related methods use radioactively-labelled food particles (Lessard & Swift, 1985) for the measurement of food-uptake. Indirect methods measure the decrease in the quantity of food particles (Frost, 1972, Rassoulzadegan & Etienne, 1981) or prey cell-pigments (Lionard et al., 2005) with time in the incubation media. Among those the most widely used method is the estimation of the *in situ* grazing of the whole microzooplankton community on natural assemblages of phytoplankton, called the

"seawater dilution technique" first established by Landry & Hasset in 1982. This technique is based on relatively short incubations of natural plankton assemblages at different dilution levels using sterile filtered seawater as diluent. When mesozooplankton is abundant it is frequently excluded by net screening to prevent trophic cascade effects due to extensive predation on microzooplankton (Fonda Umani et al., 2005). The dilution method is based upon three assumptions regarding the interactions among nutrients, phytoplankton and microzooplankton: (1) that the growth rate of phytoplankton is not limited by nutrients or phytoplankton density, (2) that phytoplankton grows exponentially during the experiments and (3) that microzooplankton shows linear consumption rates with respect to phytoplankton concentration. According to these theoretical considerations the consumption of a phytoplankton cell is merely a function of the rate at which it is encountered by microzooplankton predators, which should decrease according to dilution factor. The grazing rate of the microzooplankton community is estimated as the slope from the linear relationship between apparent phytoplankton growth *vs*. dilution level (Landry & Hassett, 1982, Landry, 1993). Its simplicity and the advantage of using barely manipulated grazer communities makes Landry & Hassett's dilution technique an elegant method for estimations of microzooplankton grazing. Consequently, this method has become standard in studies of *in situ* grazing rates of microzooplankton in pelagic systems (Landry & Calbet, 2004).

The dilution technique was chosen here as one important tool to characterise the *in situ* grazing of microzooplankton at Helgoland Roads during this study. Laboratory experiments on the grazing of typical microzooplankton species from the North Sea were carried out using primarily indirect grazing measurement procedures.

RESEARCH AIMS

The general aim of this study was to elucidate the role of heterotrophic dinoflagellates and ciliates at the base of the North Sea food web at Helgoland Roads. The North Sea around the island of Helgoland has long been recognized as a reference area for the whole German Bight and thus marine research has a long tradition on Helgoland. Continuous work-daily measurements of several abiotic parameters and phytoplankton counts are carried out since 1962 and result in one of the richest temporal marine data sets available - the Helgoland Roads time series (Wiltshire et al., 2010). The combination of its representative location and the availability of the long-term data set provide an excellent potential for marine research on Helgoland, especially in the field of food web interactions between planktonic organisms. This potential has been extensively utilised during this study. The detailed research aims during this thesis are given in the next paragraphs.

1. Before detailed studies of food web interactions were feasible baseline data on the actual species composition and seasonality of heterotrophic dinoflagellates and ciliates at Helgoland Roads were vital. Therefore, the first step was the set up of a continuous monitoring study, which enumerated both groups on a regular basis. This was fundamental to determine important “key” model species for establishing cultures which served for detailed laboratory investigations. Furthermore, the monitoring was aimed at revealing times in the year at which heterotrophic dinoflagellates and ciliates potentially play a key role as grazers and at which experiments on their grazing impact should best be conducted.

2. The second aim was to ascertain the most appropriate method to investigate the grazing impact of microzooplankton with focus on the determination of the *in situ* grazing rate of microzooplankton. Furthermore, this method was tested, weak points in methodology addressed and if necessary improvements were considered and evaluated experimentally.

3. These first two crucial steps were then followed by experimental studies to determine the importance of the *in situ* grazing impact of the microzooplankton in comparison to the impact of mesozooplankters, i.e. copepods, especially during phytoplankton blooms. According to the monitoring data, the timing of the grazing experiments was planned considering times during which microzooplankton grazers potentially play a key role.

Apart from the pure grazing impact of microzooplankton and copepods it was also an aim to investigate food selectivity patterns as well as the relationship between both groups.

4. A further important point of interest was the investigation of the relationships and inter-specific interactions between microzooplankton predators. In a first approach cultures of "key" model species of heterotrophic dinoflagellates and ciliates as well as their potential prey organisms were established. In a second approach these species served for detailed laboratory investigations in which the interactive patterns between different microzooplankton species were elucidated.

OUTLINE OF THE THESIS

Species composition of ciliates and dinoflagellates at Helgoland Roads

As a first approach a 2.5 year monitoring for species composition and succession of naturally occurring heterotrophic dinoflagellates and ciliates was established on a weekly basis at Helgoland Roads. This monitoring program served as a background to determine the seasonal distributions of dinoflagellate and ciliate species at Helgoland Roads and their relative importance to other organisms throughout the year. Results of this monitoring are reported in Manuscript I.

Improvement of the methodology of microzooplankton grazing experiments

There is a lot of debate on the loss of fragile microzooplankton species during manipulation of microzooplankton communities when setting up seawater dilution experiments and other grazing experiments. As this fragility could lead to a bias in microzooplankton composition, problematic for biodiversity considerations and *in situ* grazing studies, I investigated a potential improvement of the method for manipulating water samples containing microzooplankton communities. The resulting alternative "filling" technique is evaluated in comparison to a standard technique in Manuscript II.

The role of microzooplankton and copepod grazers during the spring bloom

The role of ciliates, heterotrophic dinoflagellates and copepods in structuring spring plankton communities was investigated during the course of a spring bloom. Experiments on microzooplankton and copepod grazing as well as on food selectivity were carried out on four occasions during different phases of the phytoplankton spring bloom. Furthermore, detailed species succession of microzooplankton as well as phytoplankton was monitored during this bloom. General patterns of grazing and selectivity in dinoflagellates and ciliates in comparison to copepods, and the role of the selective grazing of microzooplankton in shaping the phytoplankton bloom assemblage are discussed in Manuscript III.

Interactions within microzooplankton grazers

Several experiments were designed to investigate specific interactions between two different microzooplankton grazers. A model system consisting of a large ciliate predator, a small dinoflagellate predator and a phototrophic dinoflagellate prey organism co-occurring at Helgoland Roads was used. Hypothetical interactions between

the two different predators ranged from competition for the phototrophic prey to predation on the smaller predator ("intraguild predation"). Contrary to the theoretical expectations I found that the presence of the larger ciliate promoted the small dinoflagellate predator. Live observations revealed that the larger predator facilitated food uptake in the smaller predator by the pre-condition of prey cells. This commensalistic element in the interactive patterns between both predators is reported in Manuscript IV.

Discussion

Finally, a general discussion summarises the results obtained during the whole PhD research. The findings which are presented in the single chapters are discussed in a more general scientific context, thereby showing to what extent the research aims were achieved. A short outlook on the role of microzooplankton in a future global warming scenario and on future challenges completes this section.

List of manuscripts

This thesis consists of four chapters. Each chapter represents one manuscript, which has either been published (Manuscript II), been submitted (Manuscript I, III) or is about to be submitted (Manuscript IV).

Manuscript I (submitted to *Helgoland Marine Research*):

Löder, M. G. J., Kraberg, A. C., Aberle, N., Peters, S. & Wiltshire, K. H.: **Dinoflagellates and ciliates at Helgoland Roads, North Sea.**

The laboratory investigations were carried out by M. G. J. Löder. All co-authors contributed to planning and to the manuscript.

Manuscript II (published in *Marine Biodiversity Records*):

Löder, M. G. J., Aberle, N., Klaas, C., Kraberg, A. C. & Wiltshire, K. H., 2010: **Conserving original *in situ* diversity in microzooplankton grazing set-ups.** *Marine Biodiversity Records*, 3, e28. doi: 10.1017/S1755267210000254. http://journals.cambridge.org/action/displayJournal?jid=mbd.

Experiments and analyses were carried out by M. G. J. Löder. All co-authors contributed to the manuscript. C. Klaas provided a special training on standard techniques and advice on results.

Manuscript III (submitted to *Marine Biology*):

Löder, M. G. J., Meunier, C., Wiltshire, K. H., Boersma, M. & Aberle, N.:

The role of ciliates, heterotrophic dinoflagellates and copepods in structuring spring plankton communities at Helgoland Roads, North Sea.

Planning of the experiments was carried out in consultation with all co-authors. Analyses of the samples and results were carried out by M. G. J. Löder and C. Meunier following the advice of the co-authors. All authors contributed to the manuscript.

Manuscript IV (to be submitted):

Löder, M. G. J., Boersma, M., Kraberg, A. C., Aberle, N. & Wiltshire, K. H.:

They can promote their competitors - commensalism between microzooplankton predators.

Experiments and analyses were carried out by M. G. J. Löder with advisory help of the other authors. Writing the manuscript was done in close cooperation of all authors.

CHAPTER I

Dinoflagellates and ciliates at Helgoland Roads, North Sea

Dinoflagellates and ciliates at Helgoland Roads, North Sea

(submitted to *Helgoland Marine Research*)

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ABSTRACT

A monitoring program for microzooplankton was started at the long-term sampling station “Kabeltonne” at Helgoland Roads (54°11.3’N; 7°54.0’E) in January 2007 in order to provide more detailed knowledge on microzooplankton occurrence, composition and seasonality patterns at this site. Ciliate and dinoflagellate cell concentration and biomass were recorded on a weekly basis. Heterotrophic dinoflagellates were considerably more important in terms of biomass than ciliates, especially during the summer months. However, in early spring ciliates were the major grazers as they responded more quickly to food availability. Mixotrophic dinoflagellates played a secondary role in terms of biomass when compared to heterotrophic species, nevertheless, they made up the intense late summer plankton bloom in 2007. The photosynthetic ciliate *Myrionecta rubra* bloomed at the end of the sampling period. Due to its high biomass when compared to crustacean plankton especially during the spring bloom, microzooplankton can be regarded as the more important phytoplankton grazer group at Helgoland Roads. Based on these results, analyses of biotic and abiotic factors driving microzooplankton composition and abundance are necessary for a full understanding of this important group.

Keywords: dinoflagellates, ciliates, North Sea, monitoring, Helgoland Roads, seasonality

INTRODUCTION

Marine research has a long tradition on Helgoland. Water temperature has been measured at the Helgoland Roads long-term station "Kabeltonne" (54°11.3'N; 7°54.0'E) since 1873 (Wiltshire & Manly, 2004) and biological, chemical and physical parameters have been recorded continuously on a work-daily basis since 1962 (Franke et al., 2004). This makes the Helgoland long-term data series one of the longest and most detailed aquatic data sets in history. Unique in this data set are the phytoplankton species numbers counted work-daily to species level wherever possible (Wiltshire & Dürselen, 2004). Augmenting this: Since 1975 the time-series also includes meso- and macrozooplankton determined to species level three times per week (Greve et al., 2004). Thus the time-series provides an excellent basis for analyses of long term trends including changes evinced in the North Sea pelagic system over the recent decades (Schlüter et al., 2008, Wiltshire et al., 2008). Furthermore, it is a very important basis for the parameterisation and validation of mathematical ecosystem models and is invaluable in biodiversity and global change considerations (Wirtz & Wiltshire, 2005). However, one important group of planktonic organisms is underrepresented in the long term series so far - the microzooplankton. Although data on heterotrophic dinoflagellates exist in the long-term series, they were recorded with varying degrees of accuracy (Wiltshire & Dürselen, 2004) and did not always mirror the diversity in species composition (Hoppenrath, 2004). Long-term data on ciliates, another crucial microzooplankton group, are completely lacking at Helgoland Roads.

The term microzooplankton refers to the size fraction of heterotrophic planktonic organisms between 20 and 200 µm. Consisting of a diverse array of protozoa and metazoa its numerically most important components are heterotrophic dinoflagellates and ciliates (Capriulo et al., 1991). Recent research demonstrated the fundamentally important role of microzooplankton as phytoplankton grazers showing that grazing by microzooplankton can be as high as 60-75% of the daily phytoplankton production (Landry & Calbet, 2004). Furthermore, results indicate that microzooplankton tends to surpass mesozooplankton as primary consumers (Sherr & Sherr, 2007). A meta-analysis of Calbet & Landry (2004) revealed that microzooplankton grazing can be responsible for 60% of phytoplankton mortality in coastal and estuarine environments comparable to Helgoland Roads in terms of chlorophyll *a* concentration. We realized that microzooplankton could also potentially be the most important grazer group in waters around Helgoland. Therefore, the aim of this study was to supplement the regular plankton monitoring series at Helgoland with an intensive monitoring of the

microzooplankton. Investigations on species composition and seasonality of this important functional grazer group (dinoflagellates and ciliates) on a more regular basis would provide vital baseline data for studies of long-term changes in the microzooplankton community and the pelagic system at Helgoland.

MATERIAL AND METHODS

A 2.5 year microzooplankton monitoring program has been carried out at Helgoland Roads to investigate the abundance of dinoflagellates and ciliates in the Southern North Sea. This monitoring hoped to establish a higher taxonomic resolution and to improve the evaluation of biomass for single taxa of microzooplankton.

From January 2007 until June 2009 samples were taken once a week at the “Kabeltonne” (54°11.3’N; 7°54.0’E) site at Helgoland. These data supplemented the routine sampling program which is carried out week-daily and for which plankton samples are fixed with a weak neutral Lugol’s solution (final concentration 0.5%) (Wiltshire et al., 2008). Although dinoflagellates are counted within the long-term program, the taxonomic focus lies on phytoplankton groups such as diatoms. Due to the time-consuming counting procedure for phytoplankton and the high frequency of samples (work-daily), rare, small or un-common dinoflagellate taxa are inevitably neglected or categorized into size classes. The present study investigated such under-represented species more intensely during the 2.5 year microzooplankton monitoring. Apart from three ciliates that have recently been included in the counting program (1999, 2007, 2008), no ciliate species have previously been recorded. The new microzooplankton monitoring thus was to provide completely new data on ciliate biomass and seasonality patterns at a hitherto unavailable taxonomic resolution.

The loss of microzooplankton species due to fixative problems has been discussed often in the literature (Stoecker et al., 1994). Thus, we diverged from the neutral fixative used for the long-term monitoring and used acidic Lugol’s solution (final concentration of 2%) as this is the standard fixative used in most studies on microzooplankton composition. The concentration we used has been proven to be the best compromise for both conserving higher concentrations of ciliates and preventing intensive shrinkage of cells (Stoecker et al., 1994). A subsample of 250 mL was fixed immediately (final concentration 2%) (Throndsen, 1978). Samples were stored in the cold and dark, then 50 mL of the sample were settled for 24 hours and counted under an inverted microscope (Zeiss Axiovert 135) using the Utermöhl method (Lund et al., 1958, Utermöhl, 1958). At least half of the surface or the whole sedimentation chamber was

counted out at 200-fold magnification, thus reducing counting biases against rare species. Identification of naked dinoflagellates and especially of ciliates in Lugol's-preserved samples is often difficult below genus level (Johansson et al., 2004), even with the modified fixation method applied here. Therefore, problematic ciliates and dinoflagellates were identified to genus level or, otherwise, pooled into size-dependent groups and "morphotypes", based on their similar shape. Mixotrophy of the ciliates was not measured, therefore, we have no exact data on the percentage of mixotrophic ciliates in the samples. However, up to date all mixotrophic ciliates have been shown to be phagotrophic (Sherr & Sherr, 2002) and consequently all ciliates except *Myrionecta rubra* were considered heterotrophic (Johansson et al., 2004). This species acts essentially as a phototroph (Montagnes et al., 2008) but recent studies have shown that it also has some phagotrophic capabilities (Park et al., 2007). The identification of dinoflagellates was primarily based on Dodge (1982), Tomas (1996) and Hoppenrath et al. (2009). Ciliates were determined based on Kahl (1932), Carey (1992) and Montagnes (2003).

Also a new feature compared to the regular long-term series, the carbon content of each taxon was estimated from pictures taken during counting. These pictures were also used for documentation of rare and prior un-registered species and subsequent taxon assignments. Pictures of individuals from each taxon were taken for exact biovolume estimations: After measuring linear dimensions of each cell the biovolume was calculated using the geometric models described by Hillebrand et al. (1999). Biovolume was converted into carbon using the conversion factor given by Putt & Stoecker (1989) for ciliates and Menden-Deuer & Lessard (2000) for dinoflagellates.

In vivo fluorescence as proxy for phytoplankton biomass is measured on a week-daily basis (Algae Analyser, BBE Moldaenke, Kiel, Germany) as part of the routine monitoring at Helgoland Roads. These data were used for the purpose of illustration of phytoplankton food availability and are shown in the results.

For the evaluation of the microzooplankton monitoring data we compared them with the available data of the Helgoland Roads long-term data-set on plankton. After evaluation of the literature on the quality of this data-set (Wiltshire & Dürselen, 2004) and the results of an unpublished revision by S. Peters and M. Scharfe, two species were identified for the comparison: The dinoflagellate *Noctiluca scintillans* and the ciliate *Myrionecta rubra*.

RESULTS AND DISCUSSION

2.5 year microzooplankton monitoring

During the 2.5 year monitoring program 125 different taxa of dinoflagellates and ciliates were recorded. Each group contributed roughly 50 percent to the total number of taxa.

62 dinoflagellate taxa were recorded and 39 of them could be regarded as truly heterotrophic because they lacked chloroplasts. As most chloroplast-bearing dinoflagellates are capable of mixotrophic nutrition via phagotrophy (Du Yoo et al., 2009), the remainder were also considered to be potential grazers with more or less marked phagotrophic capabilities. Heterotrophic dinoflagellates were always present in carbon concentrations between 0.5 and 620 µgC L^{-1}. The most important group of dinoflagellates in terms of biomass were the Noctilucales, followed by mixotrophic and heterotrophic Gymnodiniales and Peridiniales (Figure 1, left panel). Prorocentrales and Dinophysiales played only a minor role from a biomass perspective.

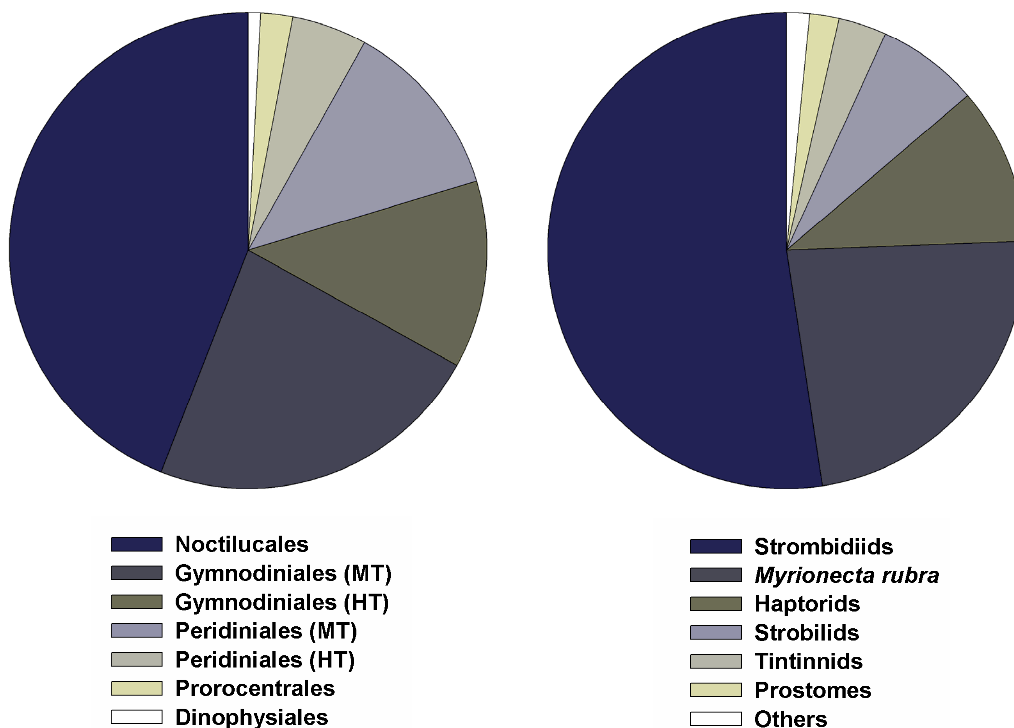

Figure 1: Shares of different dinoflagellate (left panel) and ciliate groups (right panel) during the 2.5 year monitoring based on their biomass contribution. MT = mixotrophic, HT = heterotrophic.

Dinoflagellates closely followed the chlorophyll *a* development in spring and biomass started to increase from March onwards (Figure 2).

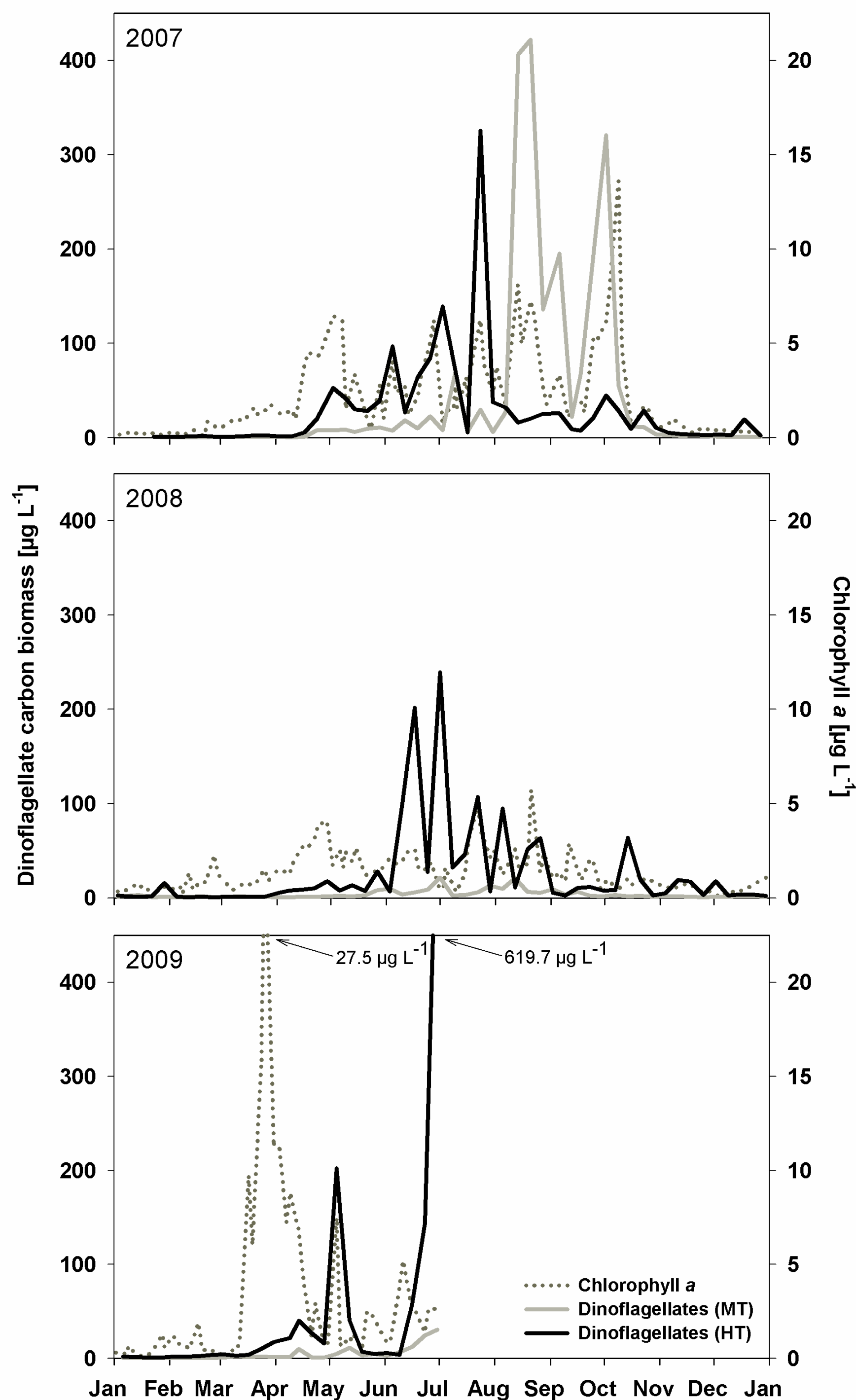

Figure 2: Biomass [µgC L^{-1}] of mixotrophic (MT) and heterotrophic (HT) dinoflagellates during the time of a weekly monitoring at Helgoland Roads in comparison to chlorophyll *a* concentration [µg L^{-1}] measured on a work daily basis via *in situ* fluorescence as a regular parameter of the long-term series.

Maximum values always occurred during the summer months (June-August) when *Noctiluca scintillans, Gyrodinium* spp. and *Protoperidinium* spp. occurred together. Towards winter in tandem with decreasing chlorophyll *a* concentrations, heterotrophic dinoflagellate biomass reached its minimum suggesting close coupling with its phytoplankton food. Outliers in biomass of heterotrophic dinoflagellates in December 2007 and January 2008 stem from the presence of single cells of *N. scintillans*. During the investigation period mixotrophic dinoflagellates (Figure 2) usually played a minor role compared to heterotrophic species (0.3–30 µgC L^{-1}). Only in summer 2007 did they form an intense bloom from the end of July to mid of October thereby exceeding the biomass of heterotrophic dinoflagellates by far (Figure 2). The bloom was first composed mainly of *Lepidodinium chlorophorum* as well as *Scrippsiella* sp. and *Prorocentrum triestinum* in lower densities. From mid September onwards the bloom comprised mainly *Akashiwo sanguinea*. During the rest of the sampling period mixotrophic dinoflagellates were usually present in much lower concentrations than heterotrophic ones.

The ciliates found comprised 63 taxa. As mentioned above ciliates were considered heterotrophic, with the exception of *Myrionecta rubra*, and were grouped together for illustration (Figure 3). Ciliated protozoa were present throughout the time of monitoring with concentrations varying between 0.2 and 106 µgC L^{-1}. In terms of biomass, strombidiids played the most important role during the monitoring program, followed by *M. rubra* and then haptorid and strobilid ciliates (Figure 1, right panel). Tintinnids and prostomatid ciliates as well as other ciliates were only of minor importance. Ciliates showed a different succession pattern when compared with dinoflagellates. Although they also followed the development of chlorophyll *a* in spring they responded with an earlier and steeper increase to enhanced food availability (Figure 3). Maxima were again found earlier in the year (March-early June) compared to dinoflagellates and mainly comprised *Strombidium* spp. and *Cyclotrichium* spp.. During the summer months ciliate biomass fluctuated synchronized with chlorophyll *a* concentration. Towards winter it also decreased parallel with declining chlorophyll *a* concentrations. The species *M. rubra* gained in importance during late spring and summer where it sometimes surpassed the biomass of the residual ciliates. Maximum concentrations of this ciliate (97 µgC L^{-1}) were found in June 2009.

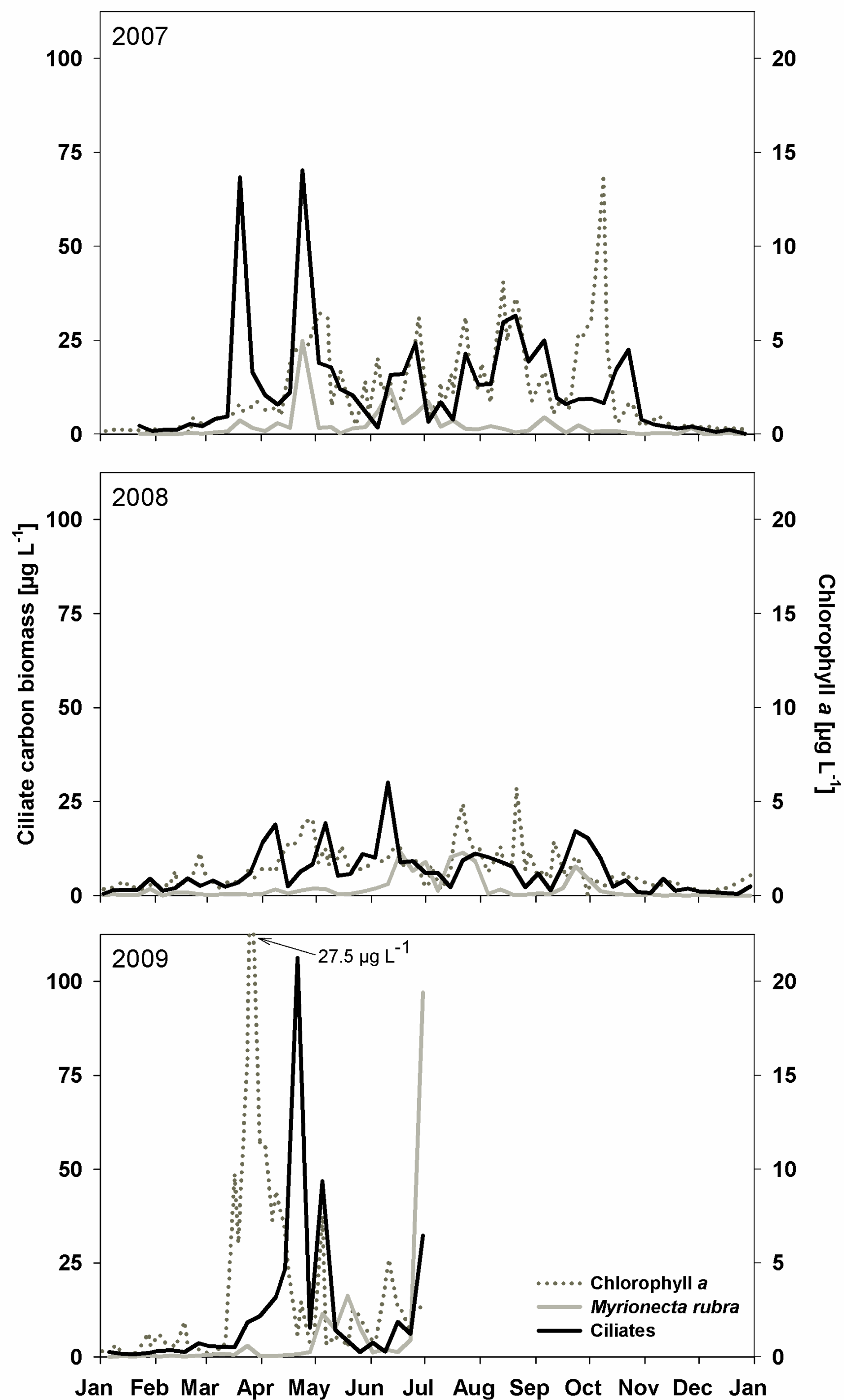

Figure 3: Biomass [µgC L^{-1}] of the ciliate *Myrionecta rubra* and other ciliates during the time of a weekly monitoring program at Helgoland Roads in comparison to chlorophyll *a* concentration [µg L^{-1}] measured on a work daily basis via *in situ* fluorescence as a regular parameter of the long-term series.

Status quo of long-term monitoring of dinoflagellates and ciliates at Helgoland Roads

The revision and quality analysis of the of the long–term data set of plankton by Witshire & Dürselen (2004) showed that quality control was very arduous and is an ongoing process: Reasons which hampered the evaluation were on the one hand methodological in nature (e.g. fixation procedures or new microscope optics) and on the other hand due to the frequent change in counting persons. The personal element involved in the recognition of microplankton species can never be eliminated completely and especially for the dinoflagellates it became evident that there was a large difference in the taxonomic knowledge between the ten different analysts. The revision also revealed that several taxa which have been recorded continuously since 1962 can be used without any restriction (12 diatom and 6 dinoflagellate taxa) and that others can be used with only minor restrictions (7 diatom and 2 dinoflagellate taxa) (Wiltshire & Dürselen, 2004).

Here we focus on the long-term data of dinoflagellates and ciliates as these two groups were the major interest of this study. A new revision of the long term data in 2008 showed that 9 dinoflagellate taxa were recorded continuously since the start of long–term monitoring and that these can be used without limitation (M. Scharfe & S. Peters, unpublished). These comprised different *Ceratium* species (*C. furca*, *C. fusus*, *C. horridum, C. lineatum*, *C. tripos*), *Prorocentrum micans*, the groups *Gyrodinium* spp. and *Protoperidinium* spp. as well as the species *Noctiluca scintillans*. No ciliate species was recorded before 1999 when the plankton monitoring started to include *Myrionecta rubra*. In the year 2007 *Laboea strobila* and in the year 2008 *Mesodinium pulex* were additionally counted in the samples.

Comparison of the two monitoring programs

Due to their important contribution to planktonic biomass when concerning our data (Figure 1) and due to the availability of long-term quality-checked cell concentration data we chose the dinoflagellate *Noctiluca scintillans* and the ciliate *Myrionecta rubra* for comparison of the 2.5 year data set with the data of the long-term series. As the long-term series provided only rough carbon biomass values for those two species (Wiltshire & Dürselen, 2004) we used cell concentration [n L^{-1}] for comparisons.

Noctiluca scintillans (Figure 4a) has continuously been recorded in the long-term data since 1962. It is the largest heterotrophic dinoflagellate species (usually > 500 µm) at Helgoland Roads. This species cannot be overlooked and its characteristic appearance

prevents confusion with other dinoflagellate species. Therefore, this species can be regarded as absolutely quality-proof in terms of counting mistakes. *N. scintillans* usually occurred in higher densities from May to September with only rare observations in the other months of the year. One exception was the year 1965 where it was recorded only on two days at very low densities. Maxima were found in summer (June-August) reaching concentrations of up to 22.500 cells L^{-1}.

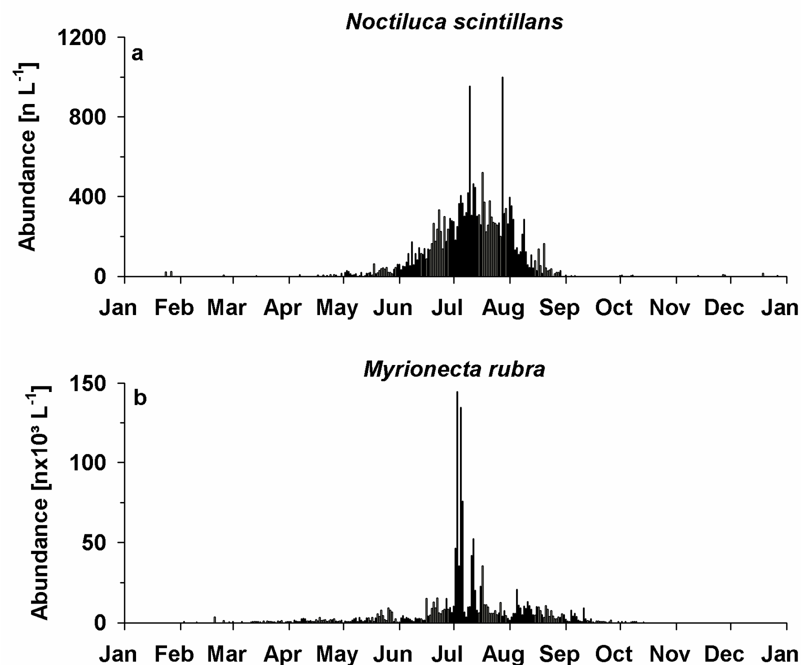

Figure 4: Mean daily concentration of (a) the dinoflagellate *Noctiluca scintillans* [n L^{-1}] during the years 1962-2009 and (b) the ciliate *Myrionecta rubra* [n x 10^3 L^{-1}] during the years 1999-2009 of long-term monitoring at Helgoland Roads.

Myrionecta rubra (Figure 4b) has been recorded since 1999. This bloom-forming ciliate can be found in different size classes (Montagnes et al., 2008) and at Helgoland Roads the size classes ~15 µm and ~35 µm were recorded during the microzooplankton monitoring, whereas no differentiation in size classes was made in the long term monitoring. It showed a year round occurrence at Helgoland Roads with minimal cell concentrations in wintertime. Frequently two distinct maxima were found within the year: A lower spring maximum and a pronounced summer maximum where cell concentration partly rose up to over 1.1 x 10^6 cells L^{-1}. In the recent years (2007 – 2009) *M. rubra* concentration was generally lower than in previous years. Interestingly, when looking at the data of the first two years in which this species has been counted, it

became obvious that *M. rubra* cells were only recorded during a narrow window in the summer months, while in the following years it occurred year-round. This pattern is due to the two size classes of *M. rubra*. The smaller size class is more abundant in winter times than the bigger one, thus it can easily be overlooked especially by an inexperienced analyst that has just started to count *M. rubra*. This was the case in the year 1999.

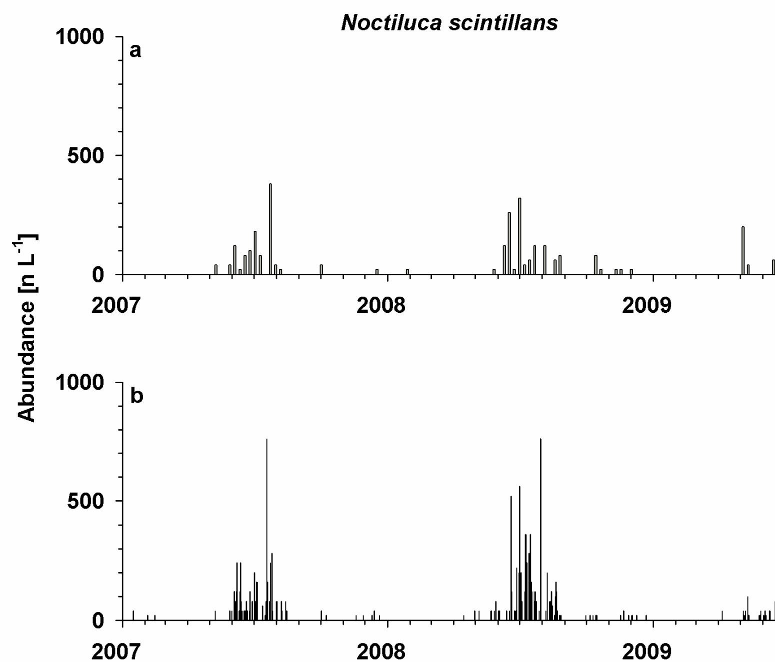

Figure 5: Comparison of cell concentration data on *Noctiluca scintillans* [n L^{-1}] between the 2.5 year microzooplankton monitoring (a) and the long-term monitoring (b).

The comparison of the data of the weekly microzooplankton monitoring with the data of the work-daily counts (Figure 5+6) revealed that despite small differences the lower resolution in the microzooplankton monitoring could nevertheless describe the seasonal patterns of distribution in both species. Discrepancies between both monitoring programs were more pronounced in *N. scintillans* (Figure 5a+b) where especially the maximal values of the years 2007 and 2009 were not reflected in the weekly samples. *M. rubra* (Figure 6a+b) concentrations from the microzooplankton monitoring mirrored the long-term data quite well with some exceptions. The most obvious outlier was in April 2007 where the microzooplankton monitoring recorded much higher concentrations which was due to counting method differences.

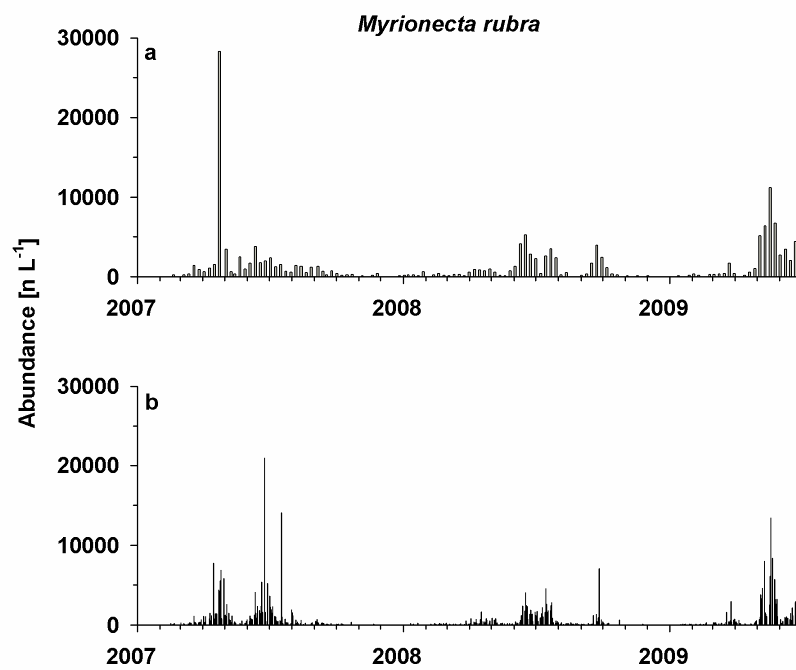

Figure 6: Comparison of cell concentration data on *Myrionecta rubra* [n L^{-1}] between the 2.5 year microzooplankton monitoring (a) and the long-term monitoring (b).

Besides the different counting frequencies deviations in the recordings of both species resulted most probably from differences in counting methodology. While in the long-term monitoring lower volumes are settled during blooms due to cell densities (usually 25 mL) and often stripes are counted for the smaller species, at least half of the sedimentation chamber was counted during the microzooplankton monitoring and 50 mL were always used for sedimentation. Despite these minor differences data on *N. scintillans* and *M. rubra* of both monitoring programs matched quite well.

Ecological implications of the microzooplankton monitoring data

Our results for ciliates are similar to results from monitoring programs in the Baltic Sea and the Gulf of Maine (Montagnes et al., 1988) where they also form distinct spring peaks (Smetacek, 1981, Johansson et al., 2004). Heterotrophic dinoflagellates are generally directly related to the availability of larger phytoplankton prey (Hansen, 1991) and often occur at high concentrations during diatom blooms (Sherr & Sherr, 2007) especially in spring (Stelfox-Widdicombe et al., 2004). Hansen (1991) reported a close relationship between dinoflagellate concentration and prey availability as also shown by our results.

Microzooplankton can be both prey and competitor for mesozooplankton. At Helgoland Roads small calanoid copepods can be regarded as direct competitors of ciliates and dinoflagellates for phytoplankton food. Their concentration ranges between 2 and 10 individuals L^{-1} throughout the year with highest values during the summer period (Greve et al., 2004).

The mean carbon content (annual mean 2007, n = 45) of the abundant small calanoid copepod *Temora longicornis* (Greve et al., 2004) was 9.5 µg carbon $female^{-1}$ (K. L. Schoo, unpublished) at Helgoland Roads. Assuming a maximum carbon content of 10 µg per copepod combined with the maximum concentrations given by Greve et al. (2004) would therefore result in maximum copepod carbon biomass of 100 µg L^{-1} (June/July). These values were surpassed by microzooplankton biomass, especially during the spring bloom. At this time the combined effects of a faster metabolism and higher productivity (Fenchel & Finlay, 1983, Montagnes & Lessard, 1999) allowed microzooplankton an undelayed direct response to an increase in prey availability (Johansson et al., 2004, Aberle et al., 2007) compared to its copepod competitors. Therefore, it is hardly surprising that recent studies have shown that microzooplankton competes not only for the same resources with copepods (Aberle et al., 2007) but may exert a stronger grazing pressure on phytoplankton than copepods (Sherr & Sherr, 2007) especially during bloom events. Our results confirm such a pivotal role of microzooplankton as phytoplankton grazers at Helgoland Roads.

We found that during the summer months ciliate biomass was generally lower when compared with dinoflagellate biomass and only with their decreasing concentrations at the end of summer ciliate biomass gained the same importance as dinoflagellate biomass again. Ciliates are, however, the first microzooplankton grazers which react to enhanced food availability in spring when the concentration of small flagellated prey increases at Helgoland. Such an earlier onset of ciliate blooms can be directly linked to their higher metabolic rates and growth rates when compared to dinoflagellates (Hansen, 1992, Strom & Morello, 1998). On the other hand they are generally more restricted to the availability of particular prey types (Tillmann, 2004), especially flagellates, than dinoflagellates are (Jeong, 1999). Thus, ciliates can respond more rapidly than dinoflagellate to enhanced food concentrations but their potential for surviving starvation periods is low (Jackson & Berger, 1985) compared to dinoflagellates (Hansen, 1992, Menden-Deuer et al., 2005). This implies rapid responses to increasing food concentrations but also quick declines of ciliate concentrations as a direct response to decreasing prey concentrations. Ciliate maxima

should therefore occur only when their appearance is coupled with the sufficient availability of adequate prey.

Another factor potentially influencing abundances of both ciliates and dinoflagellates is predation, e.g., by copepods. Microzooplankton contributes substantially to copepod diets and is often positively selected by them (Nejstgaard et al., 1997, Fileman et al., 2007). The capacity of microzooplankton to synthesize highly unsaturated fatty acids and sterols makes them good quality food for copepods (Klein Breteler et al., 1999, Tang & Taal, 2005). Especially when phytoplankton prey is nutrient limited, rendering it a low quality food, microzooplankton predators are able to dampen stoichiometric constraints of their prey to a certain extent (Malzahn et al., 2010) and are therefore of better nutritional value for copepods compared to phytoplankton.

We showed that microzooplankton is an important component of the food web at Helgoland Roads. Due to its temporarily high biomass concentration and occurrence at times throughout the year it can probably be regarded as the most important phytoplankton grazer group here. Microzooplankton is additionally an important food source for higher trophic levels such as copepods. As the routine plankton monitoring has a different focus it cannot resolve the diversity of microzooplankton sufficiently. Given its key role in the food web we recommend the long-term implementation of microzooplankton, especially dinoflagellates and ciliates, into the Helgoland Roads long-term sampling program. Further multivariate statistical analyses are necessary to evaluate the biotic and abiotic factors that drive microzooplankton composition and abundance patterns. The Helgoland Roads long-term series provides these important parameters and the extensive data on microzooplankton will provide an excellent background for such analyses.

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CHAPTER II

Conserving original *in situ* diversity in microzooplankton grazing set-ups

Conserving original *in situ* diversity in microzooplankton grazing set-ups

(published in *Marine Biodiversity Records*)

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ABSTRACT

Grazing experiments targeting the determination of *in situ* grazing rates are standard. In two separate experiments the effect of the frequently used siphon filling technique on the abundance of microzooplankton during the set-up of grazing experiments was investigated and compared with results from an alternative filling method. Hereby, water containing natural communities from Helgoland Roads, Germany (54°11.3’N; 7°54.0’E), was transferred into incubation bottles using a funnel system (“Funnel Transfer Technique = FTT”). The impact of pre-screening with a 200 µm net for excluding larger mesozooplankton grazers from the incubations was evaluated. Results show that the ciliate community was strongly affected by siphoning and pre-screening, leading to significant differences in abundance and Margalef diversity. The most affected ciliates were *Lohmanniella oviformis* and *Myrionecta rubra*, both important species in the North Sea. Dinoflagellates did not show any significant response to neither siphoning nor pre-screening with the exception of one athecate species. Such artificial bias in ciliate assemblages is very problematic for biodiversity consideration and grazing investigations. Simply changing the method of filling during the experimental set-up can ensure the measurement of accurate grazing rates of field abundances of microzooplankton. We thus recommend using conservative filling approaches like the FTT in experiments, especially when sensitive species are present, in order to avoid shifts in the overall microzooplankton community. Furthermore, we recommend introducing a control to evaluate the degree of changes in the target community due to the experimental set-up.

Keywords: ciliates, dilution experiments, dinoflagellates, FTT, microzooplankton, siphoning

INTRODUCTION

As the importance of microzooplankton as fundamental grazers in planktonic food webs became recognised, diverse techniques for determining the grazing impact of microzooplankters were developed (Kivi & Setälä, 1995). The most widely used method to estimate *in situ* grazing rates is Landry & Hassett's dilution technique (Landry & Hassett, 1982, Calbet & Landry, 2004) which facilitates the estimation of grazing rates in barely manipulated grazer communities. Consequently, this method is now standard for assessments of *in situ* grazing rates of smaller microzooplankton (< 200 μm).

As part of these experiments, mesozooplankton is removed and samples are checked for screening effectiveness with regard to the mesozooplankton (Fonda Umani et al., 2005) or losses in the phytoplankton fraction. However, to our knowledge no published study has so far considered potential losses of microzooplankton during the set-up of these experiments (Suzuki et al., 2002, Paterson et al., 2008, Pearce et al., 2008). This is especially problematic if the *in situ* abundance and biodiversity of microzooplankton grazers are the main targets of an experiment and results are transferred to the field.

Microzooplankton mainly consists of very delicate organisms (in particular ciliates and dinoflagellates: Gifford, 1985, Suzuki et al., 2002, Broglio et al., 2003), thus manipulation of water samples while setting up grazing experiments could significantly alter the grazer community through the loss of sensitive taxa, affecting estimates of grazing rates. This would defeat the goal of a grazing experiment aimed at the determination of the *in situ* grazing rate.

To avoid the loss of microzooplankters during the experimental set-up a widely used technique involves the siphoning off of water (Figure 1B) using silicone tubing and leaving the end of the tubing submerged in the water (Stelfox-Widdicombe et al., 2004, Strom et al., 2007a, Paterson et al., 2008). This technique prevents destructive air bubbles that can occur in pouring processes (Figure 1A) and is thus believed to conserve fragile species.

In preliminary experiments we found lower abundances of sensitive microzooplankton in siphoned samples when compared to the field, a pattern which was especially true for ciliates. Thus, we hypothesized that the set-up technique (here siphoning) might cause (1) diminished microzooplankton abundances, (2) pronounced effects on ciliates compared to dinoflagellates and (3) a selection in species composition in favour of non-sensitive species and thus an artificially modified grazer community. Consequently we compared two gentle filling techniques, siphoning (Figure 1B) versus a modified

application, the alternative funnel-transfer technique (FTT) (Figure 1C) while setting up dilution experiments. Here, we report the effects of both filling techniques on microzooplankton abundance and Margalef diversity (dinoflagellates, ciliates) of North Sea samples. We concentrate on *in situ* grazing experiments, but the results are equally applicable to any situation where zooplankton communities containing physically fragile species are to be manipulated in the laboratory.

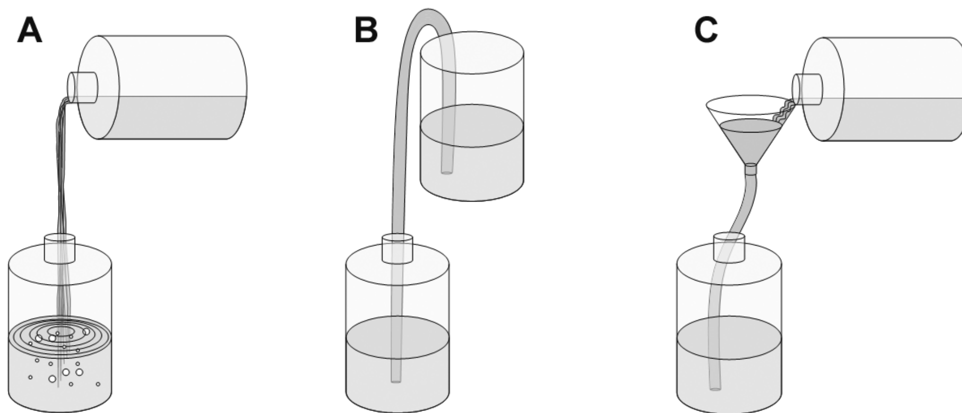

Figure 1: Illustration of the different filling techniques: (A) A simple, more destructive pouring process producing a lot of air bubbles. (B) Siphoning the water via a tube into a container without air bubbles. (C) Our new, more gentle approach to fill incubation bottles: Combination of a funnel and tube (FTT).

MATERIAL AND METHODS

Two different methods of filling experimental bottles for grazing experiments were tested in two separate experiments using water taken from the North Sea.

Sampling site

Helgoland is located in the German Bight (Southern North Sea) approximately 50 km off the German coast. It is subject to both coastal influences from the shallow Wadden Sea as well as marine influences from the open North Sea. Since 1962 bucket water samples are taken as part of a long term monitoring program at the "Kabeltonne" site at Helgoland Roads (54°11.3'N; 7°54.0'E) (Wiltshire et al., 2008). Water samples for the experiments were taken here.

Filling techniques

We compared two filling techniques: Siphoning and FTT. Siphoning is believed to be conservative for sensitive species as it avoids bubbling when the flow-end of the tube is kept under the water surface (Stelfox-Widdicombe et al., 2004, Strom et al., 2007, Paterson et al., 2008). Water is transferred only via hydrostatic pressure through a tube from one container to another when both containers have different water levels relative to geodetic height (Figure 1B). The FTT consists of a funnel mounted on a tube. For the transfer to a container, water is filled gently into the funnel keeping the tube compressed completely until the funnel is half-full. Thereafter water flow to the container is adjusted via the internal diameter by compressing the flexible tube compartment (Figure 1C). This technique also avoids air bubbles that could harm sensitive species.

Experiment 1

Experiment 1 (July 2007) was conducted to compare the two filling techniques using the experimental set-up of dilution grazing experiments. During the experiment both techniques were tested with a 200 µm net for pre-screening. Pre-screening is routine in dilution experiments and excludes mesozooplankton grazers ensuring that only the microzooplankton grazing is measured (Liu & Dagg, 2003, Fonda Umani et al., 2005, Sakka Hlaili et al., 2007).

Containers, bottles, tubing and other material used for the experiments were acid washed (10% HCL) and rinsed with deionised water. Approximately 50 L of surface seawater were sampled at Helgoland Roads using a bucket and poured without bubbling into a wide-necked carboy. The samples were brought to the laboratory immediately. The homogeneous distribution of the plankton in the initial seawater was ensured by gentle mixing. After homogenisation, 10 L were gently transferred into a 10 L carboy (as usually used for the set-up of a dilution series) with the FTT (Figure 1C). Special care was taken during the filling process that no air bubbles were produced.

When the carboy was filled, the flow was terminated by compressing the tube. In parallel to the FTT method 10 L of seawater were siphoned off with a silicone tube into a separate carboy (Figure 1B). 2.3 L narrow-necked polycarbonate incubation bottles were filled with water from each corresponding 10 L carboy using both methods in three replicates. Before each filling step gentle mixing was carried out. In both approaches a 200 µm mesh was fixed at the end of the tube. All incubation bottles were filled to the top, closed and stored cool in the dark until sampling for microzooplankton and chlorophyll *a*.

Experiment 2

Experiment 2 (April 2008) was designed to investigate both filling techniques with and without the 200 µm pre-screening to record additional effects of the pre-screening process on the monitored parameters.

The experimental set-up in Experiment 2 was as described for Experiment 1 above. Siphoning and the FTT were also applied without pre-screening, resulting in a total of four treatments. Furthermore, a control with pure seawater served as a reference value: Water for the determination of chlorophyll *a* content and microzooplankton species composition was scooped out with a 1 L beaker from the initial seawater at the beginning of the experiment. During the set-up of both experiments special care was taken not to produce bubbles.

Determination of microzooplankton species

A 250 mL aliquot of each incubation bottle was subsampled into amber bottles and immediately fixed at a final concentration of 2% acid Lugol's iodine solution (Throndsen, 1978). Samples were stored cool and dark until further analysis. For species determination 50 mL sample were settled in Utermöhl sedimentation chambers (HYDRO-BIOS) for 24 hours (Utermöhl, 1958). To reduce a possible counting bias caused by patchy settlement, the whole surface of the sedimentation chamber was counted at 200-fold magnification under a Zeiss Axiovert 135 inverted microscope. The microzooplankton fractions were considered as two major groups: Dinoflagellates and ciliates. Each group was identified to genus or species level or when this was impossible pooled into size-dependent groups or morphotypes. The identification of dinoflagellates was primarily based on Drebes (1974), Dodge (1982) and Tomas (1996). Ciliates were determined based on Kahl (1932) and Montagnes (2003).

Chlorophyll *a* analysis

Filtration of a subsample was carried out in a laboratory under dim light (< 5 µmol PAR) to avoid the loss of pigments during the filtration procedure. We used the method of extraction and analysis as described by Wiltshire et al. (1998). Pigments were separated via high-performance liquid chromatography (HPLC) (Waters 2695 Separation Module), and detected with a Waters 996 Photodiode Array Detector (Wiltshire et al., 2000).

Data analysis

The Margalef index 'd' was calculated for the most abundant ciliate and dinoflagellate species according to Equation (1).

$$d = (S-1)/(\ln N) \qquad (1)$$

This index was chosen as a simple measure for the relationship between total number of species (S) and total abundance of individuals (N) in a sample (Washington, 1984). Therefore it is a useful tool for the analysis of samples in the present study: If in two samples the total number of species is the same (as in our analysis), then the Margalef index gives direct information of the differences in the total abundance of individuals between the two samples. The index will be lower in the sample with the higher total abundance and therefore reflects changes due to the different filling methods.

Margalef indices, chlorophyll *a* contents and abundances of dinoflagellates or ciliates between the treatments in Experiment 1 were tested for significant differences using t-tests. Differences between the four treatments and the control sample in Experiment 2 were in a first step analysed using t-tests. This was necessary because the control was not influenced by any treatment and could therefore not be included in an ANOVA. The effects of the two filling techniques and the pre-screening process on the above-mentioned parameters as well as the interaction between both were tested using a 2x2 factorial ANOVA.

Due to the normal count uncertainties of microzooplankton samples and, connected to that, a high variability in abundance of rare species, only dinoflagellate and ciliate species or groups that contained more than 200 cells L^{-1} in one of the two treatments (Experiment 1) or the control (Experiment 2) are shown here. It has been shown that many chloroplast-bearing dinoflagellate species are capable of mixotrophic nutrition via phagotrophy (Tillmann, 2004). Therefore, all dinoflagellate species were considered as potential grazers and were included in the analysis. Statistical analyses were conducted with the software Statistica 7.1 (Stat Soft).

RESULTS

Experiment 1

Microzooplankton

Microzooplankton was classified into 12 categories of ciliates and 15 categories of dinoflagellates. The microzooplankton community was numerically dominated by dinoflagellates (61%, FTT).

Ciliates

The three most abundant ciliate groups were: *Myrionecta rubra* 'intermediate' (35-50 µm length), Scuticociliates and *Myrionecta rubra* 'small' (15-25 µm length). These three groups accounted for 84% of the total abundance of the FTT treatment and 77% of the siphoned treatment (mean values). They therefore dominated the ciliate community. We found a significant ciliate cell loss of 41% due to siphoning (t = 3.162, df = 4, p = 0.034, two-sided t-test) (Figure 2A, Table 1) for pooled values of the three most abundant groups leading to significant changes in diversity. The Margalef index 'd' (Figure 2B, Table 1) for the three most abundant ciliate groups was significantly lower for the FTT (t = -2.569, df = 4, p = 0.031, single-sided t-test) compared to siphoning. No difference in the Margalef index was observed between both treatments for the whole ciliate community (data not shown).

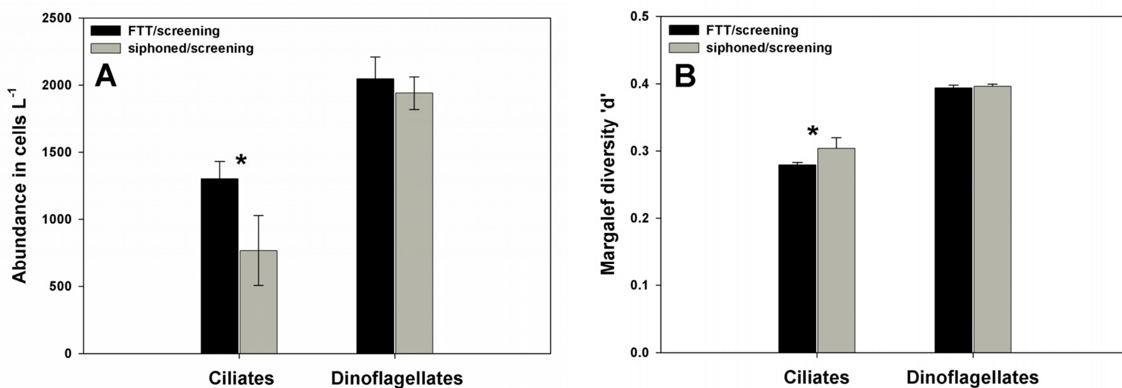

Figure 2: Experiment 1. (A) Mean abundance and (B) Margalef diversity 'd' of the most abundant ciliate and dinoflagellate species. Both treatments were done using a 200 µm pre-screened natural seawater sample. Significant difference marked with asterisk. Error bars correspond to one standard deviation.

At species/group level the loss of cells was most pronounced in the category *M. rubra* 'intermediate'. This size class of *M. rubra* accounted for 54% of total ciliate cells (FTT) and displayed a significant loss of 56% in cell numbers in the siphoned treatments (t = 3.483, df = 4, p = 0.025, two-sided t-test). The category *M. rubra* 'small' showed a similar pattern to *M. rubra* 'intermediate' with a 34% lower mean cell number when siphoned, however this effect was not statistically significant given the large count uncertainties. For the category "Scuticociliates" no significant difference between both treatments could be detected.

Although chlorophyll *a* concentrations during our experiment were relatively low (< 0.2 µg/L), we detected significant differences between both methods, with lower chlorophyll *a* contents in the siphoned treatments ($t = 5.366$, $df = 4$, $p = 0.006$, two-sided t-test) (Table 1), possibly linked to the loss of *M. rubra* cells.

Dinoflagellates

Four groups, accounting for 85% of the total dinoflagellate abundance in both treatments, were used for statistical analysis: Thecate dinoflagellates 'small' (20-25 µm length), *Scrippsiella sp.*, *Gyrodinium sp.* 'intermediate' (30-50 µm length) and *Torodinium sp*. (Table 1). In contrast to observations for the most abundant ciliate categories, we found no differences between the two treatments for the four most abundant dinoflagellate groups pooled together or at species level (Figure 2A, Table 1). Margalef indices 'd' calculated for the four most abundant and all dinoflagellate categories also revealed no differences in abundance for both treatments (Figure 2B, Table 1).

Comparison of Treatments	**Siphoned - 200 µm screening**		
	Mean values - Abundance in comparison to FTT	**Loss in comparison to FTT**	**p**
Ciliates			
***Myrionecta rubra* 'intermediate'** [a]	44.0%	56.0%	0.025
Scuticociliates [a]	102.6%	-2.6%	n.s.
***Myrionecta rubra* 'small'** [a]	65.6%	34.4%	n.s.
Sum 3 most abundant ciliates	59.0%	41.0%	0.034
Dinoflagellates			
Thecate dinoflagellates 'small' [a]	96.9%	3.1%	n.s.
Scrippsiella sp. [a]	77.9%	22.1%	n.s.
***Gyrodinium sp.* 'intermediate'** [a]	103.4%	-3.4%	n.s.
Torodinium sp. [a]	126.9%	-26.9%	n.s.
Sum 4 most abundant dinoflagellates	94.8%	5.2%	n.s.
Chlorophyll *a*	75.3%	24.7%	0.006
Margalef diversity (d)			
Sum 3 most abundant ciliates	108.6%	-8.6%	0.031*
Sum 4 most abundant dinoflagellates	100.5%	-0.5%	n.s.

Table 1: Percentage of loss based on abundances of the most common ciliate and dinoflagellate groups, chlorophyll *a* contents and Margalef index 'd' during siphoning compared to the FTT for Experiment 1 (4. July 2007). P-values derived from double-sided t-tests ($n = 3$). n.s. = not significant ($p > 0.05$). * P-value derived from single-sided t-test.

Experiment 2

Microzooplankton

In the second experiment 21 categories were established for ciliates and dinoflagellates corresponding to different species, group or size classes. Ciliates numerically dominated the microzooplankton community (60%, control).

Ciliates

Ciliate categories that presented abundances above 200 cells L^{-1} were: *Lohmanniella oviformis*, *Strombidium* cf. *tressum*, *Strombidium* cf. *epidemum*, *Balanion comatum* and *Myrionecta rubra* ‘small’ (15-25 µm length). The five ciliate categories grouped together made up over 80% of the total ciliates’ community in all treatments.

In a first step we pooled together the top five abundant ciliates and carried out a single-sided t-test (Table 2). It revealed a significant, 13% decline in abundance ($t = 2,390$, $df = 4$, $p = 0.038$) when siphoned without pre-screening compared to the control, while the FTT without pre-screening showed no difference. Both treatments with additional pre-screening exhibited significant losses in cell numbers compared to the control (siphon technique: mean loss of 26%, $t = 4,820$, $df = 4$, $p = 0.004$, FTT: mean loss 19%, $t = 3,927$, $df = 4$, $p = 0.009$, single-sided t-tests).

Comparison of Treatments	Siphoned		FTT	
	loss in comparison to control	p	loss in comparison to control	p
Sum 5 most abundant ciliates	13.3%	0.038	-0.7%	n.s.
Sum 4 most abundant dinoflagellates	0.0%	n.s.	-13.8%	n.s.

Treatment	Siphoned - 200 µm screening		FTT - 200 µm screening	
	loss in comparison to control	p	loss in comparison to control	p
Sum 5 most abundant ciliates	25.9%	0.004	19.2%	0.009
Sum 4 most abundant dinoflagellates	-1.3%	n.s.	-12.5%	n.s.

Table 2: Percentage of loss in abundance of the most abundant ciliate and dinoflagellate groups due to the different treatments and treatment combinations compared to the control values during Experiment 2 (1. April 2008). P-values derived from single-sided t-tests ($n = 3$). n.s. = not significant ($p > 0.05$).

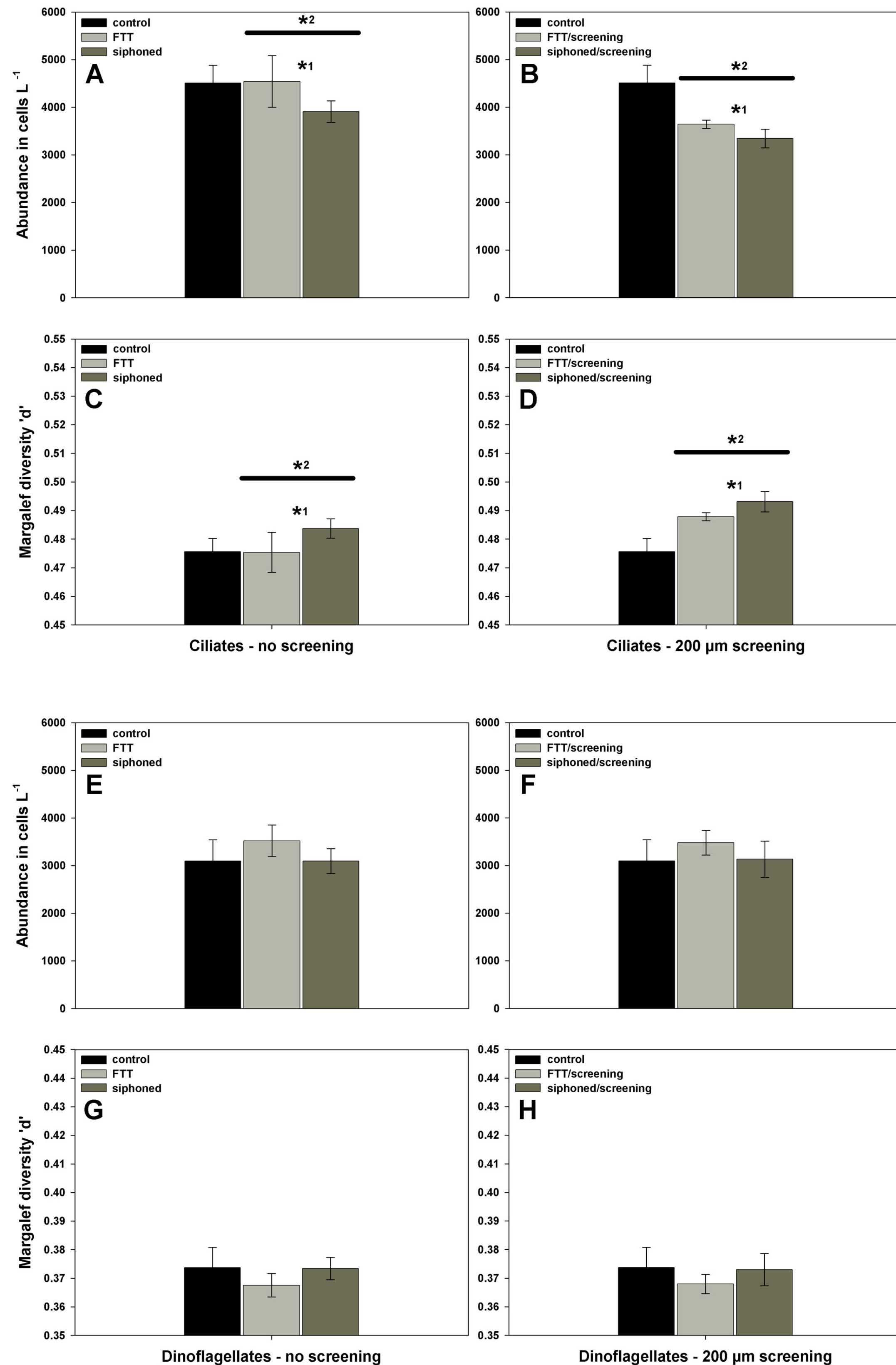

Figure 3: Experiment 2. (A, B) Mean abundance of the most abundant ciliate species, (E, F) mean abundance of the most abundant dinoflagellate species and (C, D, G, H) corresponding Margalef diversity ‘d’ in the control and using the FTT or the siphoning technique in experiments without (A, C, E, G) or with pre-screening with a 200 µm net (B, D, F, H). Control treatments were obtained by scooping samples from the initial seawater without pre-screening. Significant differences are marked with asterisks.

Asterisk *[1] symbolizes significant differences between siphoning and the FTT. Asterisk *[2] on the top of the horizontal bar symbolizes differences between 'pre-screening' and 'no pre-screening' independently from the filling technique used (therefore compare bars under the horizontal bar for both filling treatments in A and B, C and D). Error bars correspond to one standard deviation.

In the two-factorial ANOVA pooled abundances of the five most abundant ciliates showed significantly lower cell concentrations after siphoning ($F_{1,8} = 6.685$, $p = 0.03$) and pre-screening ($F_{1,8} = 16.508$, $p = 0.004$) as compared to the FTT and no screening, respectively (Figure 3A+B, Table 3), leading in both cases to significant changes in diversity. The Margalef index 'd' (Table 3, Figure 3 C+D) showed no difference at the community level (data not shown) but was significantly lower for the five most abundant ciliate species when 'no pre-screening' took place ($F_{1,8} = 19.0$, $p = 0.002$). Similar to Experiment 1, the Margalef index 'd' was also lower for the FTT ($F_{1,8} = 7.4$, $p = 0.026$, Table 3). No significant treatment interactions were observed for either abundances or Margalef index.
At the species level our results could be ascribed to the two dominant ciliates. *Lohmanniella oviformis,* showed a significant cell loss (17%, $F_{1,8} = 14.173$, $p = 0.006$) after siphoning as well as after pre-screening (loss: 13%, $F_{1,8} = 7.471$, $p = 0.026$). For *Strombidium* cf. *tressum* no significant difference between both filling treatments was found but pre-screening resulted in a significant loss of cells (up to 51% cell loss, $F_{1,8} = 56.3325$, $p = 0.00007$). The interaction of both filling methods and pre-screening showed a significant negative effect ($F_{1,8} = 6.4228$, $p = 0.035$) on *S.* cf. *tressum* with a stronger reduction in cell numbers when the combination of the FTT and pre-screening was used. The other three important ciliate species (*Strombidium* cf. *epidemum*, *Balanion comatum* and *Myrionecta rubra* 'small') were not significantly affected by any treatment. With a mean concentration of ~1.3 µg L^{-1} the chlorophyll *a* content was approximately 10-fold higher in Experiment 2 compared to Experiment 1. However, the differences between the treatments were not as clear as in Experiment 1.

Dinoflagellates

The four most abundant dinoflagellate categories were considered for analyses: Athecate dinoflagellates 'intermediate' (25-40 µm length), *Gyrodinium sp.* 'intermediate' (30-50 µm length), *Protoperidinium bipes* and *Torodinium* sp.. These four species contributed 82% to the dinoflagellate community. As in Experiment 1, no differences in abundance were found when the four dinoflagellate groups pooled were

compared to the control (Table 2). For the pooled group an ANOVA proved insignificant (Figure 3E+F, Table 3) for the treatments as well as their interaction. At species level the athecate dinoflagellates 'intermediate' revealed significantly lower cell numbers in the siphoned treatment ($F_{1,8} = 8.2718$, $p = 0.02$, Table 3) as compared to the FTT. No impact of the pre-screening process or the combination of treatment and pre-screening on the other dinoflagellates could be found. As in Experiment 1, no differences were found for the Margalef index 'd' calculated for the whole community (data not shown) or when considering only the five most abundant groups (Table 3, Figure 3 G+H).

Comparison of Treatments	Siphoned		Screening		Screening x Treatment	
	Mean values - Loss in comparison to FTT	p	Mean values - Loss in comparison to No Screening	p	-	p
Ciliates						
Lohmanniella oviformis [a]	16.9%	0.006	12.6%	0.026		n.s.
Strombidium* cf. *tressum [a]	11.6%	n.s.	50.7%	0.00007		0.035[a]
Strombidium* cf. *epidemum [a]	3.1%	n.s.	9.7%	n.s.		n.s.
Balanion comatum [a]	10.3%	n.s.	2.8%	n.s.		n.s.
***Myrionecta rubra* 'small'** [a]	9.2%	n.s.	-11.8%	n.s.		n.s.
Sum 5 most abundant ciliates	11.4%	0.032	17.4%	0.0036		n.s.
Dinoflagellates						
Athecate dinoflagellates 'intermediate' [a]	17.6%	0.021	-2.7%	n.s.		n.s.
***Gyrodinium sp.* 'intermediate'** [a]	14.6%	n.s.	19.7%	n.s.		n.s.
Protoperidinium bipes [a]	-17.9%	n.s.	-17.9%	n.s.		n.s.
Torodinium sp. [a]	-15.2%	n.s.	2.8%	n.s.		n.s.
Sum 4 most abundant dinoflagellates	11.0%	n.s.	0.0%	n.s.		n.s.
Chlorophyll *a*	7.2%	0.0025[b]	2.6%	n.s.		0.027[b]
Margalef diversity (d)						
Sum 5 most abundant ciliates	-1.4%	0.026	-2.3%	0.002		n.s.
Sum 4 most abundant dinoflagellates	-1.5%	n.s.	0.002%	n.s.		n.s.

Table 3: Percentage of loss in abundance of the most abundant ciliate and dinoflagellate species, chlorophyll *a* contents and Margalef index 'd' due to siphoning in comparison to the FTT and pre-screening in comparison to no pre-screening and effects of the combination between pre-screening and treatment during Experiment 2 (1. April 2008). P-values derived from ANOVA comparing all treatments (n = 6). n.s. = not significant (p > 0.05). [a] significantly lower abundance in the FTT with pre-screening treatments. [b] significantly higher content in the FTT with pre-screening treatments.

DISCUSSION

Transfer technique

The main aim of this study was to determine whether filling and screening techniques can influence abundance and diversity of microzooplankton during the set-up of grazing experiments. Although the FTT superficially seems to represent only a marginal modification of siphoning the change has a significant effect on microzooplankton diversity. Overall, siphoning significantly reduced the abundance of ciliates in North Sea water, while the dinoflagellate community seemed to be unaffected. The lower impact on the ciliate community using the FTT was reflected in the lower Margalef index ‘d’ for the most abundant ciliates in both experiments. The abundance and Margalef index ‘d’ of both microzooplankton groups were found to be similar to the control (Experiment 2) when the FTT was used, indicating that in contrast to siphoning no significant effect occurred using the FTT.

While the overall ciliate numbers appeared to be lower when siphoning was used, this was statistically significant only for two of the dominant ciliate species: *Myrionecta rubra* and *Lohmanniella oviformis*. The taxa *Lohmanniella* and *M. rubra* are widely distributed in coastal and open ocean environments (Smetacek, 1984, Kivi & Setälä, 1995, Myung et al., 2006, Park et al., 2007) and are also important members of the microzooplankton community in the North Sea around Helgoland (Figure 4). Both are known to feed on nanoplankton (Jonsson, 1986, Christaki, 1998, Aberle et al., 2007, Park et al., 2007) and bacteria (Myung et al., 2006).

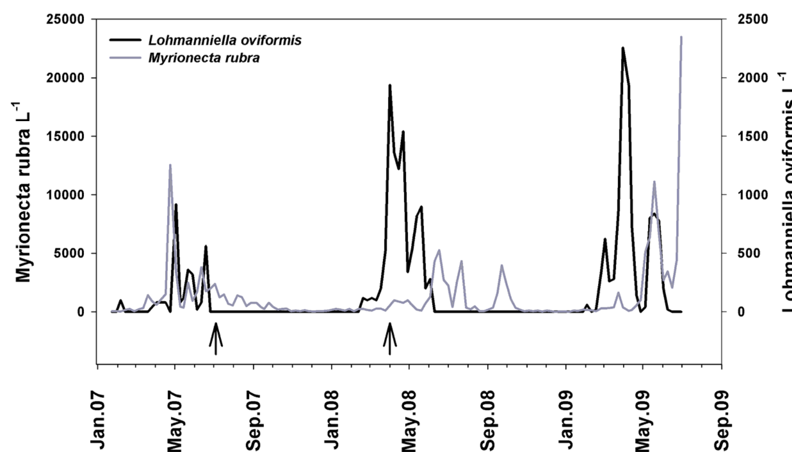

Figure 4: Abundance of *Myrionecta rubra* and *Lohmanniella oviformis* during a 2.5 year period. Values from a weekly monitoring program (n = 128). Arrows mark dates of the two experiments.

Both ciliates can be considered important grazers in temperate waters in general and dominated the communities during our experiments. Therefore, retaining natural abundances is important for accurate estimations of microzooplankton grazing.

In contrast to ciliates the sensitivity of dinoflagellates to the transfer method was more variable and species-dependent. Our results show that only the most abundant dinoflagellate group (athecate dinoflagellates 'intermediate') in Experiment 2 was significantly affected by siphoning. No effect was detected for all other investigated species. Heterotrophic dinoflagellates, including both thecate and athecate species are microzooplankton grazers of global importance (Tillmann, 2004) frequently contributing more than 50% to the microzooplankton biomass (Sherr & Sherr, 2007). As they often occur at high abundances during diatom blooms and are known to be more efficient grazers of bloom-forming diatoms than copepods and other mesozooplankters (Sherr & Sherr, 2007) it is vital not to loose dinoflagellates during grazing experiment set-ups.

Our results also show that siphoning can affect chlorophyll *a* concentration when setting up experiments. It has been reported that mixotrophic ciliates like *Myrionecta rubra* dominate plankton assemblages at certain times of the year (Stoecker et al., 1987). This was also the case during Experiment 1 (Figure 4). Our results show that the loss of these mixotrophic cells due to siphoning may induce an additional bias into dilution experiments through the concomitant loss of chlorophyll *a*, a commonly used estimate for overall phytoplankton biomass.

The effect of siphoning on microzooplankton might be explained by hydro-mechanical disturbance of water when using this method. Mechanoreceptors are common in planktonic organisms (Singarajah, 1969, Titelman, 2003, Robinson et al., 2007), including ciliates (Buskey & Stoecker, 1989, Jakobsen, 2001, 2002, Jakobsen et al. 2006) and dinoflagellates (Jakobsen et al., 2006). Different ciliates are reported to respond with long jumps to siphon-simulated feeding currents (Jakobsen, 2001, Fenchel & Hansen, 2006) and to orientate themselves against the current in a siphon flow (Fenchel & Hansen, 2006). They also respond with escape during the isolation processes with a pipette (tip: 1 mm diameter) during culture attempts (Löder pers. obs.). Although the siphon-tube diameter (7 mm) we used was bigger than that used by Jakobsen (2001) (0.25- 0.48 mm), the flow velocity and therefore also the shear stress around the tube tip was in the same order of magnitude as that of Jakobsen (2001). The ability of ciliates to detect suction at the top of a tube and respond with escape is reported by Jakobsen (2002) and could explain their lower numbers in the incubation

bottles when siphoning was used. Interestingly along with *Lohmanniella oviformis* only the larger *Myrionecta rubra* 'intermediate' cells were significantly affected by the siphon technique, whereas no significant difference was found for the category *M. rubra* 'small'. This could be linked to the ability of the larger *M. rubra* to perform longer escape jumps than the smaller, which probably enables them to escape the siphon suction more successfully.

Similar to ciliates, mechanoreceptors that enable predator detection have also been described for dinoflagellates (Maldonado, 2007). Jakobsen et al. (2006) reported highly effective escape behaviour for two different dinoflagellates when being attacked by a predator. Although the differences were less obvious for dinoflagellates, siphoning had an effect on one naked dinoflagellate species in Experiment 2. These differences could also be due to the same predator detection and escape behaviour as described above for ciliates.

The high fragility of microzooplankton could also be an explanation for the effect of siphoning on some ciliate species (Gifford, 1985, Suzuki et al., 2002, Broglio et al., 2003). During the passage of the organism through the tube physical stress is likely to act on the organism and the period of time the organism is exposed to that should increase with the length of the tubing. Necessarily, siphoning requires a longer tube compared to FTT and could therefore also cause greater damage in fragile species.

Importantly, the error introduced by the siphoning technique in our experiments was not uniform across taxa. If experiments are replicated at different times of the year this could potentially lead to a different source of error in every single experiment depending on the community structure present leading to difficulties in comparison.

Pre-screening

Several authors recommend not using pre-screening for dilution experiments to avoid retention of phytoplankton chains or breakage of the cells. Our results also show that pre-screening had an effect on ciliates. This effect was stronger than the effect of siphoning as it led to a greater loss of ciliates at species level. Importantly, different ciliate species did not respond uniformly to pre-screening. A negative effect of pre-screening on fragile ciliates due to mechanical shear forces has been reported previously (Gifford, 1985) and could be shown here for *Strombidium* cf. *tressum* and more moderately for the strobilid *Lohmanniella oviformis*. The reduced abundance of ciliates resulted in a higher Margalef index (Table 3) compared to the unscreened treatments. The difference in response to the pre-screening process could indicate different degrees

of fragility in ciliates, even in the same genus such as *Strombidium* cf. *tressum* and *Strombidium* cf. *epidemum*. Both ciliates are almost in the same size range (25-40 µm maximal length) but the impact on *S.* cf. *tressum* was large while *S.* cf. *epidemum* was not significantly affected. The impact on *S.* cf. *tressum* (51% loss) was very strong taking into account that the mesh size was 200 µm and therefore at least five times the size than the biggest individuals of this species. Compared to the loss rates of oligotrichs due to 202 µm screening (23-37%) published by Gifford (1985) our results for the pool of the most abundant ciliates are almost in the same size range (17%). In contrast to the ciliates, no effect of the pre-screening process was detectable on dinoflagellates. To our knowledge no work has been published on whether dinoflagellates are sensitive to pre-screening. Despite the observed effect of pre-screening due to a high loss of particular ciliate species, the 200 µm pre-screening process remains unavoidable whenever mesozooplankton grazers are present in the water column in high numbers in order to prevent trophic cascade effects and to ensure correct estimates of microzooplankton grazing impact alone.

Conclusions

We have demonstrated that care is required when setting up grazing experiments particularly when sensitive species are present. Commonly used techniques such as siphoning can reduce microzooplankton abundance and diversity. Siphoning had a negative effect on ciliates compared to dinoflagellates resulting in shifts in species composition and producing an "artificial" community that did not realistically reflect the *in situ* situation. Therefore, extrapolations to the field are difficult.

The FTT presented here was shown to be a good alternative to siphoning conserving the natural microzooplankton abundance and diversity. Negative effects of pre-screening on natural microzooplankton communities were determined. With the methods currently available this cannot be avoided if mesozooplankters are to be excluded. We therefore highly recommend the use of a control sample taken from the initial seawater during experiments whenever a loss of species is expected. This enables the evaluation of possible biases in abundances and diversity, especially of ciliates, introduced by pre-screening or filling technique. We recommend the use of conservative techniques like FTT during *in situ* grazing experiments when sensitive microzooplankton is present. This technique maintains the natural grazer community more closely and thus enables an accurate estimation of the grazing rate.

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CHAPTER III

The role of ciliates, heterotrophic dinoflagellates and copepods in structuring spring plankton communities at Helgoland Roads, North Sea

The role of ciliates, heterotrophic dinoflagellates and copepods in structuring spring plankton communities at Helgoland Roads, North Sea

(submitted to *Marine Biology*)

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ABSTRACT

Mesocosm experiments coupled with dilution grazing experiments were carried out during the phytoplankton spring bloom 2009. The interactions between phytoplankton, microzooplankton and copepods were investigated using natural plankton communities obtained from Helgoland Roads (54°11.3'N; 7°54.0'E), North Sea.

In the absence of mesozooplankton grazers the microzooplankton rapidly responded to different prey availabilities; this was most pronounced for ciliates such as strombidiids and strobilids. The occurrence of ciliates was strongly dependent on specific prey. Abrupt losses in their relative importance with the disappearance of their prey were observed. Thecate and athecate dinoflagellates had a broader food spectrum and slower reaction times compared to ciliates. In general, high microzooplankton grazing impacts with an average consumption of 120% of the phytoplankton production (P_p) were measured. Thus, the decline in phytoplankton biomass could be attributed to an intensive grazing by microzooplankton. Copepods were less important phytoplankton grazers consuming on average only 47% of P_p. Microzooplankton in turn contributed a substantial part to the copepods' diets especially with decreasing quality of phytoplankton food due to nutrient limitation during the course of the bloom. Copepod grazing rates on microzooplankton exceeded microzooplankton growth. As a result of selective grazing by microzooplankton less preferred diatom species bloomed both in our mesocosms and in the field with specific species (*Thalassiosira* spp., *Rhizosolenia* spp. and *Chaetoceros* spp.) dominating the bloom. This study demonstrates the importance of microzooplankton grazers for structuring and controlling phytoplankton spring blooms in temperate waters and the important role of copepods as top-down regulators of the microzooplankton.

Keywords: microzooplankton, selective grazing, spring bloom, North Sea, mesocosm, *Temora longicornis*

INTRODUCTION

Since Azam et al. (1983) introduced the term "microbial loop", microzooplankton has received ever increasing attention as an important structural and functional group in planktonic ecosystems. Indeed, microzooplankton is one of the major functional groups in microbial food webs (Landry & Calbet, 2004) and links the smaller planktonic unicellular organisms with higher metazoan trophic levels (Johansson et al., 2004). It contributes substantially to mesozooplankton diets (Kleppel, 1993). Furthermore, microzooplankton facilitates the rapid recycling of nutrients back to primary producers (Calbet & Saiz, 2005, Irigoien et al., 2005). Microzooplankton is both prey and competitor for mesozooplankton. A literature synthesis by Landry & Calbet (2004) revealed that microzooplankton grazing accounts for 60-75% of the mortality of phytoplankton production across a spectrum of open-ocean and coastal systems, and indeed recent studies showed that microzooplankton may exert a stronger grazing pressure on phytoplankton than copepods (Sherr & Sherr, 2007). During bloom events, unicellular microzooplankton can respond quickly to increasing phytoplankton availability (Johansson et al., 2004, Aberle et al., 2007) with cell division rates in the same range as those of its prey. The combination of its faster metabolism and higher production compared to mesozooplankton (Fenchel & Finlay, 1983, Müller & Geller, 1993, Montagnes & Lessard, 1999) and no egg and larval stages allows microzooplankton a rapid direct response to prey availability when compared to mesozooplankton competitors.

Irigoien et al. (2005) suggested that phytoplankton blooms can only occur, when the bloom-forming species are released from the grazing pressure by microzooplankton. According to Irigoien's hypothesis such "loopholes" for phytoplankton growth are opened by the combined efforts of (1) mesozooplankton predation on microzooplankton and thus, grazing reduction on phytoplankton (trophic cascade effect) and (2) defence mechanisms of the algae (e.g. size, colony–formation, toxicity, spines) and consequently avoidance by predators. The latter presupposes that predators can actively choose their prey and show preference or avoidance tactics for specific prey items.

Although foraging strategies are fundamental to trophic ecological considerations, e.g. trophic cascade effects, they are poorly understood for marine microzooplankton. Knowledge on the ability of microzooplankton to select specific food items is scarce in contrast to knowledge on selective feeding of mesozooplankton. Copepods, for example, are known to choose specific food actively related to taxonomical differences of the prey (Gentsch et al., 2009), prey size (Paffenhöfer, 1988), nutrient composition of

the prey (Cowles et al., 1988) and according to their own life stages (Mauchline, 1998). Microzooplankton species are in contrast often difficult to culture and the rare laboratory investigations on feeding behaviour have focussed on easily cultivable species (Flynn et al., 1996, Hamels et al., 2004).

Given the diverse feeding modes within the microzooplankton community, also food preference and selectivity are likely to be highly diverse in this group of grazers. As there is still a lot of debate on the feeding habits and the selectivity of microzooplankton, investigations on microzooplankton grazing under conditions as close to nature as possible while considering inter-specific interactions in the plankton as well as the cumulative effect of the differences in selectivity of the present grazers are imperative. Our investigations focused on North Sea plankton communities at Helgoland Roads. Although this station has been sampled for plankton since 1962, the microzooplankton has hardly been investigated so far and, in contrast to other plankton components, their role in the pelagic food web at Helgoland is unclear. In this study, we hypothesize that: **(1)** the microzooplankton with its various feeding modes can control phytoplankton spring blooms, **(2)** selective grazing by microzooplankton leads to blooms of less-favoured phytoplankton species and **(3)** microzooplankton succession in spring can be directly linked to the availability of different prey.

We conducted a mesocosm experiment and simulated a natural spring bloom using *in situ* plankton communities from Helgoland Roads. Copepod grazing on microzooplankton can be severe, especially in a restricted mesocosm environment, and can cause strong trophic cascade effects (Sommer et al., 2003, Sommer & Sommer, 2006, Zöllner et al., 2009) thus hampering investigations on the effects of microzooplankton grazing on phytoplankton. By excluding mesozooplankton grazers from the incubations, microzooplankton was relaxed from the grazing of mesozooplankton and we could explicitly examine the role of microzooplankton grazing on phytoplankton communities during the bloom. Grazing experiments for detailed investigations on microzooplankton grazing and selectivity were conducted at four defined points of the phytoplankton spring bloom: Pre-bloom (exponential growth phase = Experiment 1), bloom peak (biomass maximum = Experiment 2), early post-bloom (one week after biomass maximum = Experiment 3) and later post-bloom (two weeks after biomass maximum = Experiment 4). The role of copepods in structuring the spring phyto- and microzooplankton community was also examined via measuring copepod grazing and selectivity during these distinct bloom phases.

MATERIAL AND METHODS

Sampling site

Helgoland is located in the German Bight (Southern North Sea). It is subject to both coastal influences from the shallow Wadden Sea as well as marine influences from the open North Sea. Since 1962 water samples are taken as part of a long term monitoring for plankton and nutrients at the "Kabeltonne" site at Helgoland Roads (54°11.3'N; 7°54.0'E) (Wiltshire et al., 2008). Water for the mesocosm experiment was taken at this site.

Set up

The aim of the mesocosm experiment was to follow a typical spring plankton succession under near-natural conditions. The mesocosm experiment took place from the middle of March until mid-April 2009 in a constant temperature room with a starting temperature of 4.2°C and a quick rise towards the end temperature of ~6.8°C within a few days. Start and end temperature were similar to *in situ* conditions (4.2°C/6.7°C), but the rise in temperature in the mesocosms was somewhat faster than in the field.

Three cylindrical mesocosms with a volume of 750 L each were filled by gravity feed with natural seawater from Helgoland Roads. Pumps were not used to ensure the survival of the whole plankton community and particularly delicate organisms (Löder et al., 2010).

Water was first repeatedly scooped from the water surface using an open 850 L container suspended from the crane of the research vessel Uthörn and three 1000 L containers were subsequently filled by hose via gravitational power. In order to remove mesozooplankton but to allow for the passage of chain-forming diatoms and microzooplankton the water was screened over the feed using a 200 µm gauze bag connected to the end of the hose which floated in the container during filling. Back on land, this water was transferred to the mesocosms via gravitational feed. The even distribution of the water from each container to the three mesocosms was ensured by an interconnected triple-split hose distributor mounted on the main hose. Thus after the filling each mesocosm contained identical over-wintering/spring populations of bacteria, phytoplankton and zooplankton smaller than 200 µm (microzooplankton).

The mesocosms were stirred by a propeller (107.5 rpm, 15 minutes on, then 15 minutes off) to ensure the continuous mixing of the water column and to avoid sedimentation of the plankton. Light was provided by computer-controlled light units (Profilux II, GHL Groß Hard- and Software Logistics, Kaiserslautern, Germany) operated via an external control computer (Programme 'Prometheus', GHL, modified version 'Copacabana'). The light units were equipped with two different fluorescent tubes to obtain full light spectra ('Solar Tropic' and 'Solar Nature', JBL, Neuhofen, Germany), enabling the simulation of a daily triangular light curve (see Sommer et al. (2007) for details). The light cycle and intensity was adjusted daily to account for changes in the photoperiod during the experimental run according to the geographical position of Helgoland following the model by Brock (1981).

In order to initiate the phytoplankton spring bloom after filling a light intensity of 60% of surface irradiance was chosen, simulating the intensity of light at 1.50 m water depth with a light attenuation coefficient of 0.34 (5 m Secchi depth) under *in situ* conditions. Calculation of the light intensity was done via equations given by Tyler (1968) and Poole and Atkins (1929).

Stocking with natural inocula

During early seasonal succession many plankton organisms hatch from cysts, resting eggs or other resting stages. To ensure the same successive patterns of the plankton in the enclosed mesocosms like in the field, including those organisms hatching from cysts, resting stages etc., we introduced a small inoculum of natural seawater from Helgoland Roads on a weekly basis. Five litres of 200 µm screened seawater were added to each mesocosm. An additional 15 L of filtered seawater (0.2 µm) were added to the mesocosms to compensate for evaporation and water removal due to the sampling for monitoring and experiments.

Sampling the mesocosms

Daily measurements

Daily measurements of temperature, pH and *in vivo* fluorescence (chlorophyll *a*) (Algae Analyser, BBE Moldaenke, Kiel, Germany) were conducted between 8 and 9 am.

Weekly monitoring

In addition to the daily measurements, three litres of each mesocosm were sampled and analysed every Monday, Wednesday and Friday as well as on days with grazing experiments.

Nutrients

Silicate, phosphate, DIN (nitrite, nitrate and ammonia) were determined colorimetrically after filtration of at least 0.3 L of sample through 0.45 µm Nylon filters (Falcon) following the method of Grasshoff et al. (1999).

Phytoplankton and microzooplankton composition

For the determination of phytoplankton species 100 mL of the sample were subsampled into amber bottles and immediately fixed with neutral Lugol’s iodine solution (final conc. 0.5%) (Throndsen, 1978). For the determination of the microzooplankton 250 mL were fixed with acid Lugol’s iodine solution immediately (final conc. 2%) (Throndsen, 1978). Samples were stored cool and dark. For phytoplankton species determination 25 mL and for microzooplankton 50 mL of the sample were settled in sedimentation chambers (HYDRO-BIOS) for 24 hours and counted under a Zeiss Axiovert 135 inverted microscope using the Utermöhl method (Utermöhl, 1958). Phytoplankton (diatoms, phytoflagellates except dinoflagellates) and microzooplankton (ciliates, dinoflagellates and others) were identified where possible to genus or species level or, otherwise, pooled into size-dependent groups or “morphotypes”. It is known that most chloroplast-bearing dinoflagellates are capable of mixotrophic nutrition via phagotrophy (Du Yoo et al., 2009). Therefore, all dinoflagellate species were considered potentially heterotrophic and were assigned to the microzooplankton. For microzooplankton the whole surface of the sedimentation chamber was counted at 200-fold magnification, thus reducing counting biases against rare species. The identification of phytoplankton and dinoflagellates was primarily based on Dodge (1982), Tomas (1996), Hoppenrath et al. (2009). Ciliates were determined based on Kahl (1932), Carey (1992) and Montagnes (2003).

Net growth of plankton in the mesocosms

Phytoplankton and microzooplankton net growth rates in the mesocosms were calculated using the exponential growth model and the abundance values from the monitoring (see section 'Growth and grazing calculations').

Grazing experiments

Dilution experiments on microzooplankton grazing (Landry & Hassett, 1982, Landry, 1993) and bottle incubations with the copepod *Temora longicornis* were carried out simultaneously at four different times (see introduction) of the phytoplankton bloom (Figure 1).

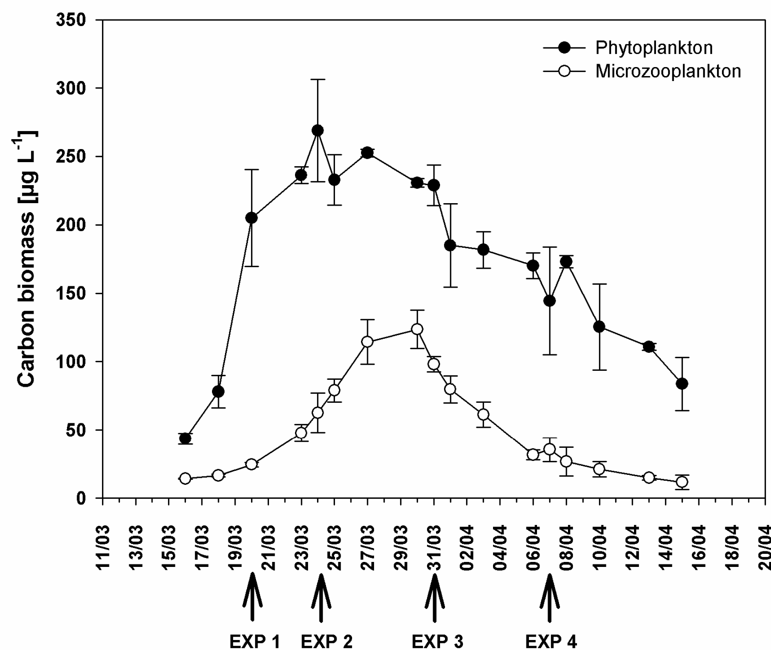

Figure 1: Development of phytoplankton and microzooplankton biomass during the mesocosm experiment. Dates at which the grazing experiments were performed are marked with arrow. Error bars correspond to one standard deviation (n = 3).

Microzooplankton grazing set up

A pool of water for the purpose of dilution was established at the same time as the mesocosms were filled. Water was filtered at low pressure through a pre-washed 0.45 + 0.2 µm sterile in-line membrane filter capsule (Sartobran® 300, 300 cm²) after pre-

filtering with a combination of 3 µm GFF + 0.2 µm membrane filter. The water was stored sterile and dark in the thermo-constant room together with the mesocosms. Particle-freeness was proved via flow-cytometry (FACSCalibur, Becton & Dickinson) before each experiment started. Four exact dilutions of 10, 25, 50 and 100% of undiluted seawater from each mesocosm were prepared. For the incubation three 2.3 L polycarbonate bottles were gently filled with water from each dilution. The Funnel-Transfer-Technique appropriate for ciliates (Löder et al., 2010) was used for filling purposes as these organisms are very sensitive to destruction by vigorous filling and mixing procedures (Landry, 1993).

To prevent nutrient limitation biases during the phytoplankton bloom sterile filtered nutrient solutions (F/2 medium, Guillard & Ryther (1962)) were added to the dilution series (8 x 10^{-4} mNO_3, 1.3 x 10^{-5} mPO_4 and 2.4 x 10^{-5} $mSiO_2$, Experiment 1+2 no SiO_2). One control bottle per mesocosm was incubated without the addition of nutrients.

Copepod grazing set up

The most reliable method to quantify feeding rates of mesozooplankton on both phytoplankton and non-pigmented microzooplankton, is the analysis of particle removal in bottle incubations (Båmstedt et al., 2000). Because of interferences with microzooplankton grazing activity, especially when both micro- and mesozooplankton prey upon the same species, it is necessary to simultaneously estimate the microzooplankton grazing rates in separate dilution experiments (Nejstgaard et al., 1997, Nejstgaard et al., 2001). Thus, for copepod grazing experiments three 2.3 L bottles per mesocosm (100% undiluted water with added nutrients) were prepared along with the dilutions and 25 female copepods of the species *Temora longicornis* were added to each bottle (~11 copepod L^{-1}). This copepod concentration was at the upper limit of *in situ* densities in the period March-April (Greve et al., 2004). *T. longicornis* is known to be a selective and omnivorous grazer feeding on phytoplankton and microzooplankton in size classes > 20 µm (Tackx et al., 1990, Maar et al., 2004, Gentsch et al., 2009). Its role in the planktonic food web makes *T. longicornis* a key species and therefore it was selected as copepod grazer in our experiment. These copepods were caught by vertical net hauls at Helgoland Roads and transferred to the laboratory immediately. Only actively swimming females of *T. longicornis* were sorted out under a dissecting microscope and acclimated to mesocosm conditions for 24 hours

prior to the experiments. The 100% undiluted bottles in the dilution series served as a control for the *T. longicornis* grazing experiments.

The whole set of incubation bottles (dilutions series + *T. longicornis* bottles = 48 bottles) was incubated for 24 hours on two plankton wheels (0.8 rpm) at the same light and temperature conditions as the mesocosms. Sampling for plankton took place at the beginning of the experiments and after 24 hours.

Biovolume and carbon calculation

Biovolume of each plankton species was calculated from the measurement of cell dimensions using geometrical formula according to Hillebrand et al. (1999). The cell volume was converted into carbon (C) according to the equations given by Menden-Deuer and Lessard (2000) for diatoms (pgC $cell^{-1} = 0.288 \times V^{0.811}$), dinoflagellates (pgC $cell^{-1} = 0.760 \times V^{0.819}$) and all other protist plankton except ciliates (pgC $cell^{-1} = 0.216 \times V^{0.939}$), whereby V refers to cell volume in μm^3. Ciliate carbon was calculated using a conversion factor of 0.19 pgC μm^{-3} (Putt & Stoecker, 1989). Rotifer carbon was estimated according to McCauley (1984) and Park and Marshall (2000): After a calculation of the biovolume by means of geometric formulas this biovolume was converted to wet weight assuming a specific gravity of 1. Wet weight was then converted to dry weight by a factor of 0.1 and 50% of dry weight was assumed to be carbon. Carbon values for the copepod species *T. longicornis* derived from measurements with an elemental analyser (EA 1110 CHNS-O, Thermo-Finnigan). The mean carbon content (annual mean 2007, n = 45) of this copepod was 9.5 µg carbon $female^{-1}$ (K. L. Schoo, unpublished).

Growth and grazing calculation – Microzooplankton

Growth rates of phytoplankton species and grazing rates of the microzooplankton community were calculated using linear regressions of apparent phytoplankton growth (calculated for the total phytoplankton community, at a species level as well as functional phytoplankton groups) against the dilution factor (Landry & Hassett, 1982, Landry, 1993). Start values for the diluted samples were calculated from the 100% undiluted samples according to their dilution factor. The growth of phytoplankton (d^{-1}) was described by the exponential growth model in equation (1):

$$C_{t_{24}} = C_{t_0} \times e^{(k-g)\times\Delta t} \qquad (1)$$

Whereby C_{t_0} is the concentration of phytoplankton biomass at the beginning of the experiment, $C_{t_{24}}$ after 24 hours, k is the phytoplankton growth coefficient, g the microzooplankton grazing coefficient and Δt the incubation time in days.

Where in our experiments non-linearity induced by saturated feeding of microzooplankton (Gallegos, 1989) was seen, especially in Experiment 1 and 4 where predator abundance was low, only the three most diluted samples (10, 25, 50%) were used for regression analysis (Paterson et al., 2008). The obtained value of apparent phytoplankton growth was used to calculate the grazing coefficient at 100% undiluted seawater level. For comparisons between microzooplankton and mesozooplankton grazing we normalized grazing parameters according to predator carbon concentration:

Daily carbon specific grazing rates g_c, filtration rates F_c and ingestion rates I_c of the microzooplankton community were calculated for average (during the time interval t_0-t_{24}) prey carbon concentrations $[C_{prey}]$ after Frost (1972) with g and k obtained from the dilution experiments.

F_c and I_c was adjusted for the growth of predators using mean predator carbon concentration $[C_{predator}]$ according to Heinbokel (1978a) with equations (2) – (5):

$$F_c = g \times [C_{predator}]^{-1} \qquad (2)$$

$$I_c = F_c \times [C_{prey}] \qquad (3)$$

$$[C_{prey}] = \frac{C_{t_0} \times (e^{(k-g)\times\Delta t} - 1)}{(k-g)\times\Delta t} \qquad (4)$$

$$[C_{predator}] = \left(\frac{C_{predator,t_{24}} - C_{predator,t_0}}{\ln C_{predator,t_{24}} - \ln C_{predator,t_0}} \right) \qquad (5)$$

The instantaneous (natural) growth rate of phytoplankton μ_0 was calculated by adding grazing mortality to values of apparent phytoplankton growth obtained from the

incubation bottles without added nutrients (Landry, 1993, Caron, 2000); negative values of g were set to zero for calculation. Based on the coefficients obtained for μ_0 and g applied on the initial phytoplankton biomass C_{t_0}, the loss of phytoplankton standing crop per day P_i and the percentage loss of potential phytoplankton production P_p of each species were calculated according to equation (6) and (7) (Quinlan et al., 2009).

$$P_i = \frac{C_{t_0} \times (e^{g} - 1)}{C_{t_0}} \times 100 \qquad (6)$$

$$P_p = \frac{C_{t_0} \times [(e^{\mu_0} - 1) - (e^{(\mu_0 - g)} - 1)]}{C_{t_0} \times (e^{\mu_0} - 1)} \times 100 \qquad (7)$$

Copepod grazing - correcting for trophic cascade effects

The uncorrected grazing coefficient $g_{cop,p}$ of *T. longicornis* was calculated for each prey type p after Frost (1972) at average prey concentrations, whereby the undiluted seawater incubation bottles of the dilution experiment served as control. The corrected copepod grazing coefficient ($g_{corr,p}$, equation (8)) was calculated after the general method of Nejstgaard (2001) by adding a correction factor k_p for reduced microzooplankton grazing rates due to predation by *T. longicornis* to $g_{cop,p}$:

$$g_{corr,p} = g_{cop,p} + k_p \qquad (8)$$

$$k_p = g_{micro,p} \times \left(\frac{[C_{predator}] - [C_{predator}]^*}{[C_{predator}]} \right) \qquad (9)$$

whereby $[C_{predator}]$ in equation (9) is the mean microzooplankton carbon concentration in the undiluted seawater from the dilution series and $[C_{predator}]^*$ is the mean microzooplankton carbon concentration in the *T. longicornis* bottles. Only significant microzooplankton grazing rates were used for the correction, negative grazing rates were set to zero. Carbon specific grazing (g_c) and filtration rates (F_c) and carbon specific ingestion rates (I_c) (phytoplankton and microzooplankton prey) of the added *T. longicornis* were calculated as described above for the microzooplankton. Microzooplankton growth was assumed not to be influenced by nutrient addition and

therefore, instead of μ_0, values of microzooplankton growth rate k obtained from the 100% undiluted seawater incubation bottles were taken to calculate P_p (equation 7).

Sometimes negative P_i and P_p values were found in our grazing experiments. These resulted from either negative g (for P_i) or μ_0/k (mortality in the control without grazer) (for P_p) and were set to zero. The same was done for positive P_p values resulting from negative g and μ_0.

Selectivity and Electivity

Prey selectivity α of the microzooplankton community and *T. longicornis* was calculated for each prey type according to Chesson (1978, 1983):

$$\alpha_i = \frac{r_i / n_i}{\sum_{j=1}^{m} r_j / n_j} \qquad (10)$$

Whereby r_i is the frequency of prey i in the diet and n_i is the frequency of prey in the environment, divided by the sum of all relationships between the frequency of prey in the diet and in the environment. Negative *T. longicornis* ingestion rates were set to zero for the calculation of the frequency of prey in the diet according to Nejstgaard (2001).

We chose Chesson's case 1 equation (n_i assumed to be constant) (Chesson, 1983) because our values of ingestion and percentage of prey in the environment were obtained by averaged prey concentrations and phytoplankton initial stocks were high, so that a strong depletion of food was unlikely.

Values of α were used to calculate the electivity index E^* according to Vanderploeg and Scavia (1979a, 1979b).

$$E^* = \frac{\alpha_i - \frac{1}{n}}{\alpha_i + \frac{1}{n}} \qquad (11)$$

(n = total number of prey types)

Values of E^* cover a range from -1 to 1. E^* values of 0 indicate non selective feeding, values > 0 indicate preference, values < 0 indicate discrimination against a prey type.

Data analysis

To monitor possible negative effects of our set-up technique on abundances we statistically compared microzooplankton communities at the start of the experiments in the mesocosms with the communities in the experimental bottles (t-tests) for differences. Insignificance was a requirement to apply the results of the experiments to the mesocosms. Regression analyses for the determination of *k* and *g* were conducted using “SigmaPlot 9.0” (SYSTAT Software); further statistical analyses were conducted using the software “Statistica 7.1” (StatSoft), both at significance levels of 0.05. Values of *g* and *k*, *F*, *I* and *E** obtained in the *T. longicornis* grazing experiments were tested against zero using two-tailed t-tests (Köhler et al., 1995).

RESULTS

Bloom dynamics in the laboratory and in the field

The mesocosms were filled with water from the open North Sea at Helgoland Roads. On the 20.03.09 an inflow of nutrient-rich coastal water was detected in the field as seen by a decrease in salinity and increased SiO_2 and DIN (Figure 2a+b). Chlorophyll *a* concentration is measured via *in situ* fluorescence on a work-daily basis at the “Kabeltonne” station in the field (Wiltshire et al., 2008). When comparing field values to those in the mesocosms (Figure 2c), both blooms showed a similar development particularly at the beginning and during the last two weeks of the experiment when salinity reached the highest values. During the detection of a nutrient-rich waterbody of lower salinity (20.-27.03.09) phytoplankton biomass in the field (“Kabeltonne”) showed values twice as high as in the mesocosms.

Developments in the mesocosms

Nutrients

Within the first four days of bloom development phosphate (start 0.36 µmol L^{-1}) and silicate (start 4.75 µmol L^{-1}) decreased rapidly to values below detection limit (< 0.01 µmol L^{-1}) and no remarkable relaxation from nutrient limitation could be observed during the course of the experiment. Dissolved inorganic nitrogen (DIN) dropped from around 14 µmol L^{-1} to around 7 µmol L^{-1} after the first week and remained at a level between 5 and 7 µmol L^{-1} during the rest of the experiment.

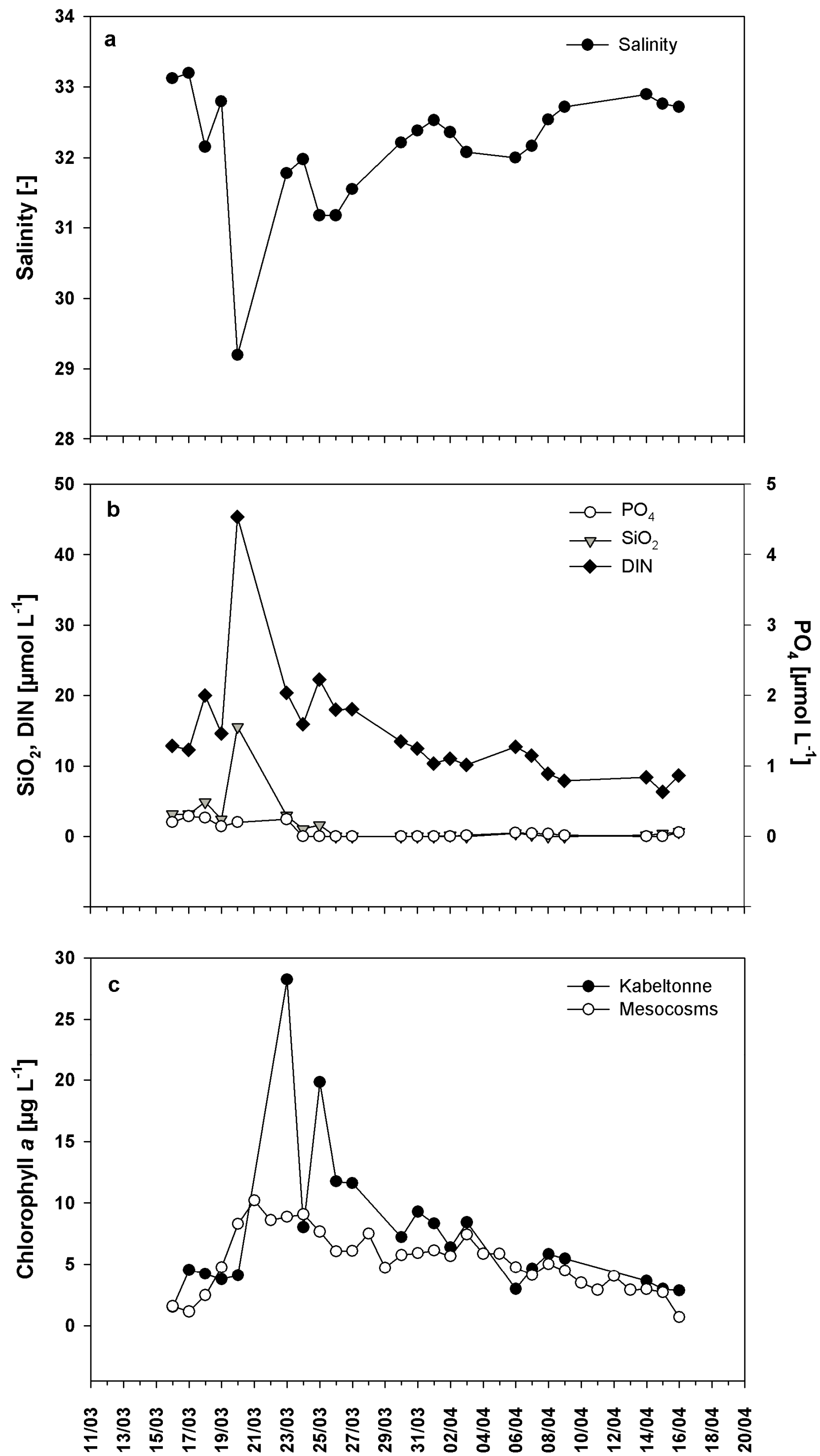

Figure 2: Field parameters (2a: Salinity, 2b: Nutrients) during the mesocosm experiments measured at "Kabeltonne" site. 2c: Mean *in situ* fluorescence (chlorophyll *a*) in the mesocosms as compared to the field.

General development of the spring bloom

Starting with 44 µgC L^{-1} phytoplankton biomass, the spring bloom evolved immediately and reached its maximum at 269 µgC L^{-1} within the first eight days of the experiment (24.03.09) (Figure 1). At this exponential phase phytoplankton had a maximal net growth rate of 0.48 d^{-1} (18.-20.03.09) and a mean net growth rate of 0.23 d^{-1} (16.-24.03.09). During the following three weeks the biomass decreased at a mean rate of -0.05 d^{-1} to a final value of 84 µgC L^{-1}.

Microzooplankton, starting with 14 µgC L^{-1}, followed the phytoplankton bloom with a delay of roughly a week and peaked on the 30.03.09 with 124 µgC L^{-1} (Figure 1). Microzooplankton reached growth rates of up to 0.27 d^{-1} (23.-24.03.09) but grew at a mean rate of 0.16 d^{-1}. Until the end of the experiment microzooplankton biomass decreased at a mean rate of -0.15 d^{-1} to 12 µgC L^{-1}, i.e. close to the starting value.

Phytoplankton composition

The spring bloom was dominated by diatoms and small flagellates (five size classes of 5 – 25 µm length) see Figure 3a+b. While flagellates contributed 34% to phytoplankton biomass at the start they played only a minor role during the bloom phase. The bloom itself was dominated by diatoms (96%) of the genera *Chaetoceros (C. danicus* and other *Chaetoceros* spp. of different size classes), *Thalassiosira* (*T. rotula* and *T. nordernskjoeldii*) and *Rhizosolenia* (*R. stylisformis/hebetata* group and *R. pungens*), each genus contributing roughly one third. In the later bloom phase *Thalassiosira* became more dominant and represented up to 49% of the phytoplankton carbon. The category 'other diatoms' (*Pseudonitzschia* spp., *Navicula* spp., *Asterionellopsis glacialis* and others) contributed only 1-4% to the phytoplankton biomass. Flagellate biomass peaked four days earlier than the diatoms and showed a steeper decline. Along with decreasing diatom shares, it increased again to around 20% of the total phytoplankton carbon in the last week of the experiment.

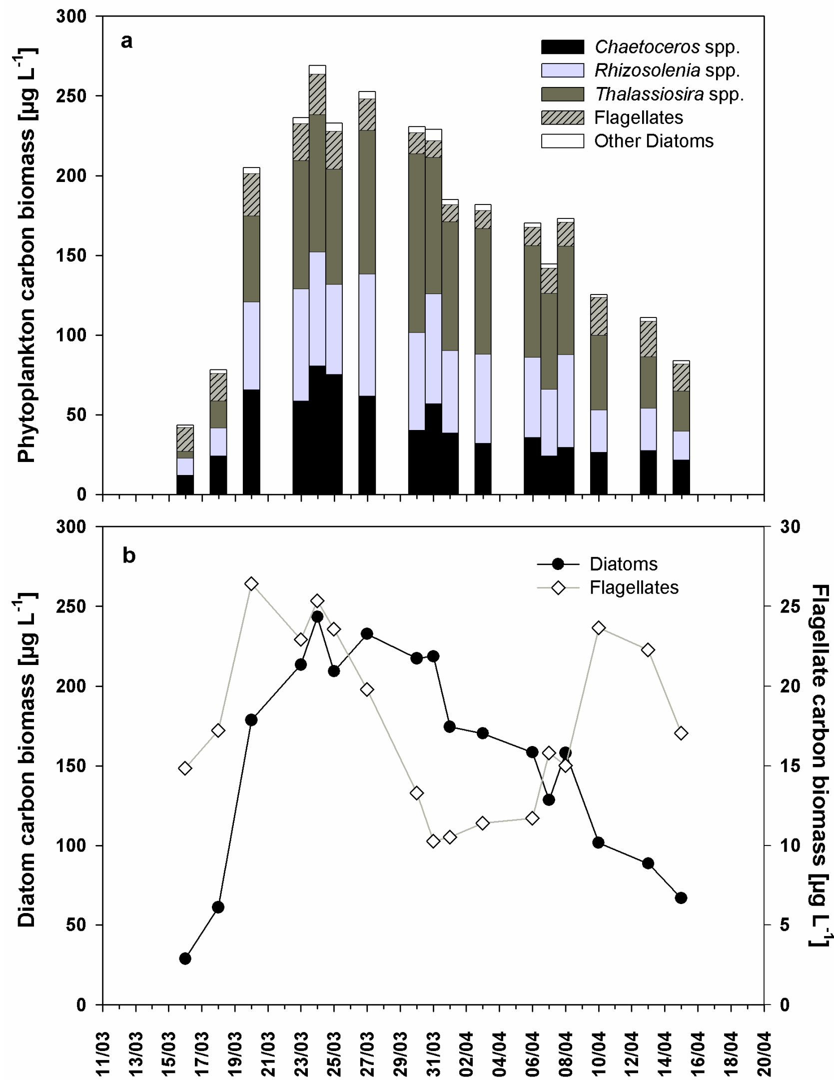

Figure 3: Phytoplankton species succession (3a) and development of diatom and flagellate carbon biomass (3b) during the mesocosm experiment. Mean values of the three mesocosms.

Microzooplankton composition

Microzooplankton (Figure 4) comprised four groups: Dinoflagellates, ciliates, rotifers and thecate amoebae, whereby ciliates and dinoflagellates dominated during the first three weeks of the experiment. Due to their very low abundances other metazoans like nauplii or polychaete larvae were detected only sporadically and were neglected in our analyses.

Ciliate community

After a week of stability (~6 µgC L^{-1}) ciliate biomass increased at rates of around 0.36 d^{-1} from 23.03.09 onwards reaching a peak on 30.03.09 (96 µgC L^{-1}) (Figure 4a). Afterwards it decreased rapidly to a final 5 µgC L^{-1}. Ciliates contributed 27-46% to the total microzooplankton biomass and dominated the bloom (up to 78%). A clear succession was found in the community. Until the end of March *Strombidium* spp. (*S. capitatum*, *S.* cf. *emergens*, *S.* cf. *epidemum*, *Laboea strobila*, *Tontonia gracillima* and others) dominated. The most important species was *S. capitatum* contributing 92% to the strombidiids and 64% to the total ciliate biomass at the ciliate peak. Co-occurring strobilids (*Rimostrombidium* sp., *Lohmanniella oviformis*, *Leegaardiella* sp., *Strombidinopsis* sp. and others) contributed 6-21% to the ciliate biomass until the 31.03.09. After the maximum both genera declined to values below 5% of total ciliate biomass and strombidiids finally disappeared. Simultaneously the big haptorid *Cyclotrichium* sp. started to dominate until the end up till 40-67% of the total biomass. Only initially cyclotrichids (*Myrionecta rubra*, *Mesodinium* sp. and *Askenasia* sp.) played a major role. During the last 10 days the category 'other ciliates' (mainly *Acineta* sp. and *Euplotes* spp.) gained importance (up to 55% of ciliate biomass).

Dinoflagellate community

Dinoflagellate biomass increased directly after the start (~7 µgC L^{-1}) at rates lower than those of ciliates (mean 0.15 d^{-1}) but peaked already five days earlier (28 µgC L^{-1}, 25.03.09) (Figure 4b). During the following 8 days it fluctuated on a high level (20-25 µgC L^{-1}) and declined afterwards to a final 4 µgC L^{-1}. Dinoflagellates contributed 21-62% to the total microzooplankton biomass with a more pronounced role before and after the ciliate peak. *Gyrodinium* spp. dominated the community at 38-52% in the first 10 days and thereafter increased to 66-87% reaching a maximum of 21 µgC L^{-1} on the 03.04.09. Different *Protoperidinium* species (*P. ovatum*, *P. thorianum*, *P. pellucidum*, *P.* cf. *leonis*, *P. bipes*, *P. brevipes*. *P.* cf *pyriforme* and others) contributed 2-23% to the total dinoflagellate biomass. The group 'athecate dinoflagellates' (*Warnowia* sp., *Torodinium* sp., *Katodinium* sp. and small athecate dinoflagellates < 15 µm) contributed 2-19% to the total biomass until 27.03.09 and thereafter declined below 1%. 'Thecate dinoflagellates' (*Diplopsalis* sp., *Dinophysis* sp., small thecate dinoflagellates < 15 µm and others) contributed 27-48% to total biomass until the 25.03.09, but declined

afterwards to only 6%. The decline in the last two groups was caused by the loss of the smallest dinoflagellates (< 15 µm).

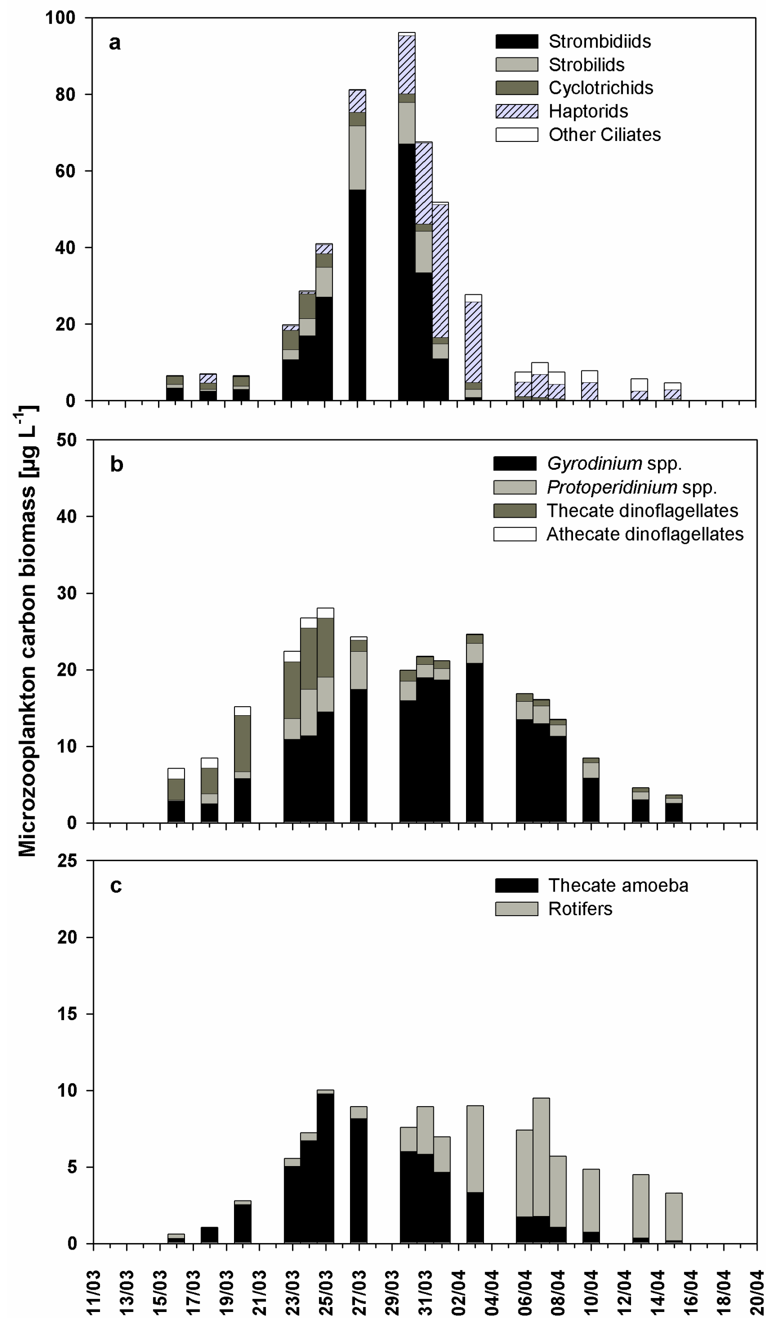

Figure 4: Microzooplankton species succession during the mesocosm experiment (4a: Ciliates, 4b: Dinoflagellates and 4c: Other microzooplankton). Mean values of the three mesocosms.

Other microzooplankton

Beside ciliates and dinoflagellates, a thecate amoeba and a rotifer (*Synchaeta* sp.) occurred in the microzooplankton community (Figure 4c). Both species together contributed 4-30% to the total microzooplankton biomass with values over 13% during the last 10 days of the experiment when *Synchaeta* sp. became more abundant, whereas the parasitic thecate amoeba (mainly attached to *Chaetoceros* spp.) dominated this group until the end of March.

Microzooplankton grazing and selectivity

The microzooplankton community showed carbon specific grazing rates g_c between 0.006 and 0.014 (µgC predator)$^{-1}$ d^{-1} during the grazing experiments (Figure 5). All groups of phytoplankton where grazed while we detected different selectivity patterns for different genera/species. Detailed information is given in Table 1+2 and on prey taxon level in the appendix.

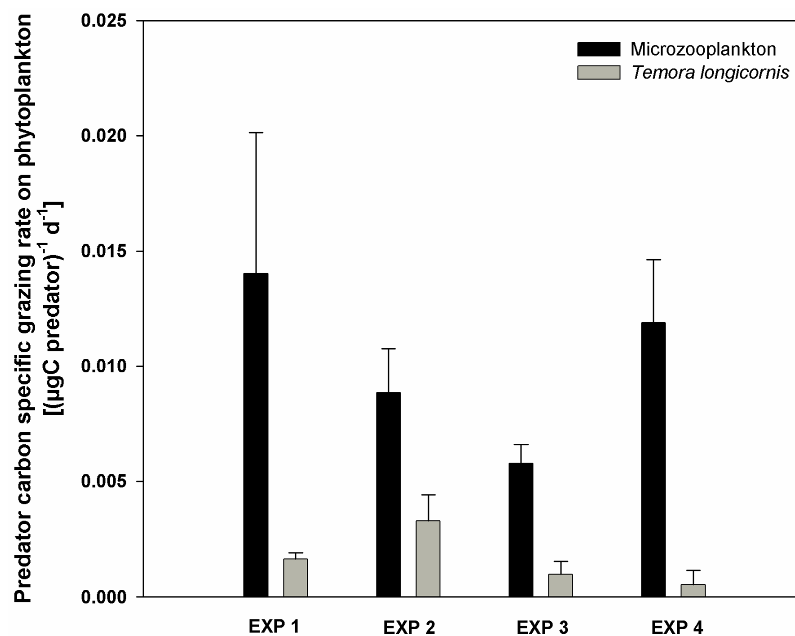

Figure 5: Predator carbon specific grazing rates g_c of microzooplankton and *Temora longicornis* grazing on phytoplankton during the four experiments. Error bars correspond to one standard error (n = 36 for microzooplankton, n = 9 for *T. longicornis*).

	Microzooplankton													
	Experiment 1 - (MMC 30.33 µg L^{-1})							Experiment 2 - (MMC 74.47 µg L^{-1})						
Phytoplankton	k	p	g	p	μ_0	P_i	P_p	k	p	g	p	μ_0	P_i	P_p
***Chaetoceros* spp.**	0.46		0.60	*	0.40	83	139	1.15	****	1.27	****	1.50	256	92
***Rhizosolenia* spp.**	0.25		0.28	**	-0.01	32	0	0.70	****	0.76	****	0.78	114	98
***Thalassiosira* spp.**	0.53	****	0.56	**	0.42	75	124	0.60	****	0.63	****	0.85	88	82
Flagellates	0.21		0.19	**	-0.47	21	0	0.58	***	0.84	*	0.74	132	109
other Diatoms	0.02		-0.10	*	-0.47	0	0	0.35	****	0.41	**	0.27	51	141
TOTAL PHYTOPLANKTON	**0.41**	*	**0.43**	*	**0.17**	**53**	**223**	**0.80**	****	**0.66**	***	**0.77**	**93**	**90**

	Experiment 3 - (MMC 93.82 µg L^{-1})							Experiment 4 - (MMC 33.12 µg L^{-1})						
Phytoplankton	k	p	g	p	μ_0	P_i	P_p	k	p	g	p	μ_0	P_i	P_p
***Chaetoceros* spp.**	0.49	***	0.52	*	0.41	68	121	0.80	****	0.70	****	1.27	102	70
***Rhizosolenia* spp.**	0.43	****	0.42	****	0.65	52	72	0.51	****	0.49	****	0.76	64	73
***Thalassiosira* spp.**	0.14	***	0.15	*	0.19	16	78	0.68	****	0.69	****	0.89	100	85
Flagellates	0.58	****	0.53	***	0.72	70	81	-0.20		0.10		-0.47	10	0
other Diatoms	0.38	***	0.37	*	0.32	45	114	0.72	****	0.71	****	0.98	103	81
TOTAL PHYTOPLANKTON	**0.39**	****	**0.54**	****	**0.65**	**72**	**87**	**0.41**	****	**0.39**	***	**0.52**	**48**	**80**

Table 1: Microzooplankton grazing g [d^{-1}] and phytoplankton growth rates k [d^{-1}] determined in four dilution experiments for different phytoplankton groups. Food saturation marked with gray background. Instantaneous growth rate values μ_0 [d^{-1}] from bottles without added nutrients. Percentage of initial stock P_i [%] and potential production grazed P_p [%]. Negative P_i and P_p values resulting from negative g (P_i) or μ_0 (P_p) and were set to zero. The same was done for positive P_p values resulting from negative g and μ_0. MMC = mean microzooplankton carbon biomass. P-values from linear regression analysis of apparent phytoplankton growth against dilution factor (n = 36). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Before the bloom (Experiment 1) dinoflagellates dominated the grazer biomass (62%) followed by ciliates (27%) and displayed maximal growth rates of ~0.3 d^{-1}. Due to the overall low grazer biomass (30 µgC L^{-1}) saturated feeding was detected in 12 out of 20 phytoplankton prey species (Appendix, Table 1). However, microzooplankton showed a total grazing rate g of 0.43 d^{-1} (Table 1), a total filtration rate F of 0.43 L d^{-1} (Table 2) and the highest carbon specific ingestion rate I_c of 1.57 µgC prey µgC predator^{-1} d^{-1} among the four experiments, leading to a total daily ingestion of 47.65 µgC L^{-1} d^{-1} (Table 2) on community level. Microzooplankton grazed 53% of the phytoplankton initial stock (P_i) and 223% of the total potential production (P_p) (Table 1) due to the lowest instantaneous growth μ_0 (0.17 d^{-1}) of prey of our four experiments. Based on the index E^* microzooplankton clearly selected the groups *Chaetoceros* spp. and *Thalassiosira* spp. (Table 2).

	Microzooplankton									
	Experiment 1 - (MMC 30.33 µg L^{-1})					Experiment 2 - (MMC 74.47 µg L^{-1})				
Phytoplankton	F_c	p	I_c	p	E^*	F_c	p	I_c	p	E^*
***Chaetoceros* spp.**	1.99E-08	*	0.30	*	0.30	1.70E-08	****	0.16	****	0.24
***Rhizosolenia* spp.**	9.11E-09	**	0.33	**	-0.08	1.02E-08	****	0.41	****	-0.01
***Thalassiosira* spp.**	1.85E-08	**	0.73	**	0.26	8.46E-09	****	0.35	****	-0.11
Flagellates	6.35E-09	**	0.10	**	-0.26	1.13E-08	*	0.11	*	0.04
other Diatoms	-3.46E-09	*	-0.02	*	-1.00	5.49E-09	**	0.03	**	-0.31
TOTAL PHYTOPLANKTON	**1.40E-08**	*	**1.57**	*		**8.85E-09**	***	**1.00**	***	
RELATED TO TOTAL MICROZOOPLANKTON CARBON	***F*** **0.425**		***I*** **47.65**			***F*** **0.659**		***I*** **74.58**		
	Experiment 3 - (MMC 93.82 µg L^{-1})					Experiment 4 - (MMC 33.12 µg L^{-1})				
Phytoplankton	F_c	p	I_c	p	E^*	F_c	p	I_c	p	E^*
***Chaetoceros* spp.**	5.53E-09	*	0.08	*	0.13	2.12E-08	****	0.17	****	0.13
***Rhizosolenia* spp.**	4.48E-09	****	0.16	****	0.03	1.49E-08	****	0.39	****	-0.04
***Thalassiosira* spp.**	1.59E-09	*	0.08	*	-0.46	2.09E-08	****	0.49	****	0.13
Flagellates	5.68E-09	***	0.09	***	0.14	2.92E-09		0.06		-0.70
other Diatoms	3.98E-09	*	0.01	*	-0.03	2.14E-08	****	0.02	****	0.14
TOTAL PHYTOPLANKTON	**5.77E-09**	****	**0.62**	****		**1.19E-08**	***	**0.97**	***	
RELATED TO TOTAL MICROZOOPLANKTON CARBON	***F*** **0.542**		***I*** **58.02**			***F*** **0.394**		***I*** **32.13**		

Table 2: Microzooplankton carbon specific filtration rates F_c [L µgC predator^{-1} d^{-1}] and carbon specific ingestion rates I_c [µgC prey µgC predator^{-1} d^{-1}], total filtration rates F [L d^{-1}], total ingestion rates I [µgC prey L^{-1} d^{-1}] and electivity E^* [-] for different phytoplankton groups. Positive selection marked with gray background. MMC = mean microzooplankton carbon biomass. P-values are the same as for the grazing rates of microzooplankton. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

At the phytoplankton peak (Experiment 2) total grazer biomass (74 µgC L^{-1}) was more than twice as high as during the pre-bloom experiment and shares of dinoflagellate and ciliate biomass were almost equal. Ciliates displayed the highest growth rates of 0.37 d^{-1} in this phase. The community showed an I_c of 1.00 µgC prey µgC predator^{-1} d^{-1} at a total filtration rate F of 0.66 L d^{-1} (Table 2) and a total grazing rate g of 0.66 d^{-1} (Table 1). As μ_0 (0.77 d^{-1}) was higher than g 93% of P_i and 90% of P_p (Table 1) were grazed. Food selectivity reflected the high grazer diversity and was spread over all categories of phytoplankton resulting in a total daily ingestion of 74.58 µgC L^{-1} d^{-1} (Table 2). On group level microzooplankton selected flagellates and *Thalassiosira* spp. (Table 2).

The early post bloom phase (Experiment 3) was characterized by the highest grazer biomass of all experiments (94 µgC L^{-1}) and ciliates clearly dominated the community (69%). Microzooplankton grazed 72% of P_i and 87% of P_p at a rate of 0.54 d^{-1} (g) and filtered 0.54 L d^{-1} (F) (Table 1+2). Phytoplankton displayed a higher instantaneous growth rate μ_0 (0.65 d^{-1}) than in the fertilized incubation bottles (k = 0.39 d^{-1}). I_c (0.62

µgC prey µgC predator^{-1} d^{-1}) was the lowest detected in our experiments leading to a total daily ingestion I of 58.02 µgC L^{-1} d^{-1} (Table 2). Microzooplankton selected for flagellates (except flagellates 5 µm), *Rhizosolenia* spp. and *Chaetoceros* spp. (Table 2).

In the late post-bloom phase (Experiment 4), grazer biomass (33 µgC L^{-1}) was as low as before the bloom and again saturated feeding was detected in 5 phytoplankton species (Appendix Table 1). Beside the now dominating dinoflagellates, rotifers became as important as ciliates. The community grazed 48% of P_i and 80% of P_p at a rate of 0.39 d^{-1} (g) and filtered 0.39 L d^{-1} (F) (Table 1+2). I_c (0.97 µgC prey µgC predator^{-1} d^{-1}) increased to a value similar to Experiment 2 resulting in a total daily ingestion I of 32.13 µgC L^{-1} d^{-1}. Again instantaneous growth μ_0 (0.52 d^{-1}) of the phytoplankton exceeded the growth in fertilized bottles (0.41 d^{-1}) (Table 1). Selectivity was similar to Experiment 1 whereas also the category 'other diatoms' was selected.

Microzooplankton predator-prey relationships

A direct coupling between ciliate and flagellate biomass was observed. This resulted in a strong suppression of flagellate biomass from 26 to 10 µgC coincident with the ciliate peak (Figure 6a). This was most pronounced for thecate and athecate dinoflagellates < 15 µm which disappeared totally during the S*trombidium capitatum* bloom (Figure 6b). Simultaneously with the disappearance of both dinoflagellate groups *S. capitatum* started to form cysts and its population collapsed totally within one week. With the break-down of strombidiid and strobilid biomass at the end of March a relaxation from grazing pressure enabled the flagellates to regenerate again.

The majority of dinoflagellate species we found in the mesocosms are reported to prefer diatom prey. As diatoms did hardly change in composition, dinoflagellate succession in the mesocosms was not as pronounced as for the ciliates. While two groups disappeared during the first half of our experiment due to predation by ciliates as described above, the remaining dinoflagellate community composition remained relatively constant until the end of the experiment.

We also found a strong predator-prey relationship between a thecate amoeba and *Chaetoceros* spp.. The apparently parasitic amoeba was found to be attached only to cells of this genus. The abundance of the amoeba followed the development of *Chaetoceros* spp. and showed a similar decline towards the end of the experiment.

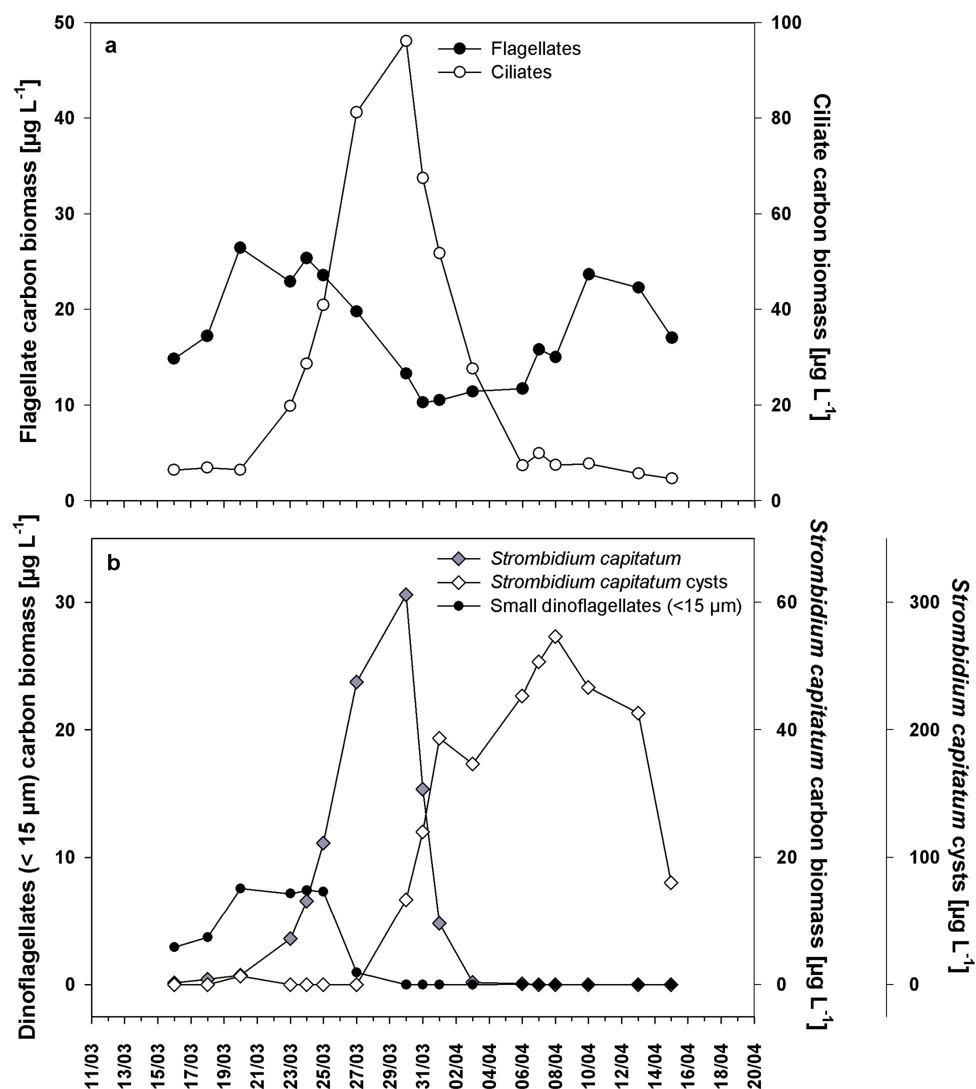

Figure 6: (6a) General development of ciliate and flagellate biomass in the mesocosms. (6b) Close predator-prey relationship between members of both groups: Development of small dinoflagellates and their predator *Strombidium capitatum* and *Strombidium capitatum* cysts. Mean values of the three mesocosms.

Temora longicornis grazing and selectivity

The *T. longicornis* (103 µgC L^{-1}) biomass we added was always higher than the microzooplankton biomass in our experiments. Nevertheless, the copepod species had a much lower grazing impact on the phytoplankton community (Figure 5) than the microzooplankton (g_c: 0.001-0.003 (µgC predator)$^{-1}$ d^{-1}). During the course of the experiments *T. longicornis* switched its diet along a gradient from a phytoplankton-dominated towards a microzooplankton-dominated diet (Figure 7a, Table 4). This was

also reflected in E* values and in general a positive selection for microzooplankton when compared with phytoplankton prey was observed, with the exception of the phytoplankton peak experiment (Figure 7b, Table 4). However, on taxon level some phytoplankters were also positively selected (see appendix for details). Detailed information on grazing parameters is given in Table 3+4.

Temora longicornis (TC 103.26 µg L^{-1})														
	Experiment 1							Experiment 2						
Phytoplankton	*k*	p	*g*	p	μ_0	P_i	P_p	*k*	p	*g*	p	μ_0	P_i	P_p
***Chaetoceros* spp.**	-0.13		0.34	***	0.40	40	88	0.24	*	0.47	*	1.50	60	48
***Rhizosolenia* spp.**	-0.02		0.13	*	-0.01	14	0	0.01		0.20		0.78	23	34
***Thalassiosira* spp.**	0.00		0.21	**	0.42	23	54	0.31	**	0.56	*	0.85	74	74
Flagellates	0.01		0.05		-0.47	5	0	-0.26	*	0.08		0.74	8	14
other Diatoms	0.11		-0.05		-0.47	0	0	-0.16		0.02		0.27	2	9
TOTAL PHYTOPLANKTON	**-0.01**		**0.17**	***	**0.17**	**18**	**100**	**0.13**	*	**0.34**	*	**0.77**	**40**	**54**
Microzooplankton														
***Gyrodinium* spp.**	-0.02		1.11	**		203	0	0.32	****	0.27	***		31	86
***Protoperidinium* spp.**	0.20		1.32	***		275	406	0.21		0.33	**		39	150
other athecate dinoflagellates	0.03		0.18			20	497	-0.01		0.27	*		30	0
other thecate dinoflagellates	0.39	****	-0.03			0	0	-0.43	****	0.20	*		23	0
***Strombidium* spp.**	0.30	*	1.18	***		224	267	0.46	****	0.79	****		121	148
***Strobilidium* spp.**	0.26		0.92	*		150	262	0.66	***	0.36	**		43	62
Cyclotrichids	0.18		0.95	**		158	374	-0.32		0.16			18	0
Haptorids	-0.28		0.93	*		154	0	0.39		0.35			42	91
other Ciliates	-0.03		0.04			4	0	0.17		0.58	*		79	289
thecate amoeba sp.	0.16		0.32	***		38	191	0.25	**	0.23	*		26	93
Rotifers	0.00		-			-	-	-		-			-	-
TOTAL MICROZOOPLANKTON	**0.21**	****	**0.40**	****		**49**	**174**	**0.27**	****	**0.43**	****		**53**	**147**
	Experiment 3							Experiment 4						
Phytoplankton	*k*	p	*g*	p	μ_0	P_i	P_p	*k*	p	*g*	p	μ_0	P_i	P_p
***Chaetoceros* spp.**	-0.02		0.23	*	0.41	25	61	0.28	*	0.21		1.27	23	26
***Rhizosolenia* spp.**	-0.09		0.03		0.65	3	5	0.13		0.11		0.76	12	20
***Thalassiosira* spp.**	-0.35		-0.03		0.19	0	0	0.08		0.21	*	0.89	24	32
Flagellates	-0.06		0.23	**	0.72	26	40	-0.28	**	-0.24	***	-0.47	0	0
other Diatoms	-0.14		0.40	*	0.32	49	120	0.23	**	0.21	*	0.98	24	31
TOTAL PHYTOPLANKTON	**-0.18**		**0.10**		**0.65**	**11**	**20**	**0.04**		**0.05**		**0.52**	**6**	**13**
Microzooplankton														
***Gyrodinium* spp.**	0.05		0.21	***		24	425	-0.11		0.66	**		93	0
***Protoperidinium* spp.**	0.12		0.76	**		114	487	-0.06		0.53	**		70	0
other athecate dinoflagellates	0.05		0.22			25	418	-		-			-	-
other thecate dinoflagellates	-0.14		0.14			15	0	-0.35		0.07			7	0
***Strombidium* spp.**	-1.61	****	0.15			16	0	1.30		0.32			37	37
***Strobilidium* spp.**	-0.61	****	0.22			25	0	-0.21		0.40			50	0
Cyclotrichids	0.01		0.59	*		80	3066	-0.46		0.49			63	0
Haptorids	0.87	**	0.54	**		72	72	-0.52		2.48			1097	0
other Ciliates	0.54		0.44			56	85	-0.11		0.10			11	0
thecate amoeba sp.	-0.11		0.13	*		13	0	-0.05		0.25	***		29	0
Rotifers	0.02		0.33			39	1183	0.03		1.84	***		529	3349
TOTAL MICROZOOPLANKTON	**0.07**		**0.38**	***		**46**	**478**	**-0.04**		**0.78**	****		**118**	**0**

Table 3: *Temora longicornis* grazing g [d^{-1}], phytoplankton and microzooplankton growth rates k [d^{-1}] determined in four grazing experiments for different prey groups. Instantaneous growth rate values μ_0 [d^{-1}] from dilution experiment bottles without added nutrients. Percentage of initial stock P_i [%] and potential production grazed P_p [%]. Negative P_i and P_p values resulting from negative g (P_i) or μ_0/k (P_p) were set to zero. The same was done for positive P_p values resulting from negative g and μ_0/k. TC = Total *T. longicornis* carbon biomass. P-values derived from t-tests against zero. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Before the bloom (Experiment 1) *T. longicornis* filtered 0.17 L d^{-1} (F) and ingested 17.87 µgC L^{-1} d^{-1} of the phytoplankton community at a carbon specific ingestion rate I_c of 0.17 µgC prey µgC predator^{-1} d^{-1} (Table 4) leading to 18% reduction of P_i and 100% of P_p (Table 3). Due to the lower biomass of microzooplankton prey, *T. longicornis* reached a higher F (0.40 L d^{-1}) and it ingested 9.58 µgC L^{-1} d^{-1} (I) of the microzooplankton community at an I_c of 0.09 µgC prey µgC predator^{-1} d^{-1}, leading to a 49% decrease of P_i and 174% of P_p (Table 3).

Only at the bloom peak (Experiment 2), electivity for microzooplankton prey was insignificant. *T. longicornis* ingested the highest amount of biomass during our experiments (57.50 µgC L^{-1}, phytoplankton + microzooplankton) and filtered 0.34 L d^{-1} for phytoplankton and 0.43 L d^{-1} for microzooplankton, at carbon specific ingestion rates I_c of 0.31 and 0.24 µgC prey µgC predator^{-1} d^{-1}, respectively (Table 4). Copepods grazed 40% of phytoplankton P_i (highest value for *T. longicornis*) and 54% of its P_p and 53% of the microzooplankton P_i and 147% of its P_p.

In the early post bloom phase (Experiment 3) the total amount of I dropped again to 38.22 µgC L^{-1} d^{-1} (Table 4 phytoplankton + microzooplankton). *T. longicornis* cleared 0.10 L d^{-1} of phytoplankton and 0.38 L d^{-1} (F) of microzooplankton at an I_c of 0.09 and 0.28 µgC prey µgC predator^{-1} d^{-1}, respectively (Table 4). The impact on the phytoplankton community was once again lower with 11% of P_i and 20% of P_p grazed. On the other hand *T. longicornis* grazed 46% of microzooplankton P_i and 478% of its P_p.

During the late post-bloom (Experiment 4) I of *T. longicornis* further dropped to 21.8 µgC L^{-1} d^{-1} (Table 4 phytoplankton + microzooplankton). While the copepods filtered only 0.05 L d^{-1} (F) of phytoplankton at a carbon specific ingestion rate I_c of 0.04 µgC prey µgC predator^{-1} d^{-1}, they cleared 0.78 L d^{-1} (F) of microzooplankton at an I_c of 0.17 µgC prey µgC predator^{-1} d^{-1} (Table 4). This led in turn to a reduced impact on the

phytoplankton community (P_i: 6%, P_p: 13%) and an even more pronounced impact on the microzooplankton biomass (P_i: 118%, P_p: not defined).

Temora longicornis **(TC 103.26 µg L^{-1})**

	Experiment 1						**Experiment 2**					
Phytoplankton	F_c	p	I_c	p	E^*	p	F_c	p	I_c	p	E^*	p
***Chaetoceros* spp.**	3.28E-09	***	0.0424	**	-0.21		4.56E-09	*	0.0351	*	-0.03	
***Rhizosolenia* spp.**	1.26E-09	*	0.0448	*	-0.57	**	1.97E-09		0.0725		-0.30	
***Thalassiosira* spp.**	1.99E-09	**	0.0725	**	-0.40	**	5.38E-09	*	0.1879	*	-0.01	
Flagellates	4.46E-10		0.0034		-0.65	**	7.46E-10		0.0065		-0.61	**
other Diatoms	-5.23E-10		-0.0024		-0.80	***	2.02E-10		0.0029		-0.62	**
TOTAL PHYTOPLANKTON	**1.63E-09**	***	**0.1730**	***	**-0.28**	**	**3.29E-09**	*	**0.3130**	*	**-0.28**	
RELATED TO TOTAL *TEMORA* CARBON	***F*** **0.17**		***I*** **17.87**				***F*** **0.34**		***I*** **32.32**			
Microzooplankton												
***Gyrodinium* spp.**	1.07E-08	**	0.0277	**	0.18		2.61E-09	***	0.0348	***	-0.19	
***Protoperidinium* spp.**	1.28E-08	***	0.0084	****	0.38	**	3.17E-09	**	0.0097	*	-0.19	
other athecate dinoflagellates	1.74E-09		0.0022		-0.48	**	2.57E-09	*	0.0028	*	-0.23	
other thecate dinoflagellates	-2.48E-10		-0.0022		-0.89	****	1.97E-09	*	0.0144	*	-0.39	*
***Strombidium* spp.**	1.14E-08	***	0.0264	****	0.36	**	7.66E-09	****	0.1159	****	0.38	****
***Strobilidium* spp.**	8.87E-09	*	0.0044	*	0.05		3.45E-09	**	0.0201	**	-0.10	
Cyclotrichids	9.17E-09	**	0.0189	****	0.25	**	1.57E-09		0.0054		-0.31	
Haptorids	9.03E-09	*	0.0007	*	-0.06		3.38E-09		0.0002		-0.12	
other Ciliates	3.42E-10		-0.0002		-0.36		5.63E-09	*	0.0010	*	0.02	
thecate amoeba sp.	3.13E-09	***	0.0064	***	-0.26		2.27E-09	*	0.0150	*	-0.27	
Rotifers	-		-		-		-		-		-	
TOTAL MICROZOOPLANKTON	**3.87E-09**	****	**0.0927**	****	**0.15**	**	**4.14E-09**	****	**0.2438**	****	**0.04**	
RELATED TO TOTAL *TEMORA* CARBON	***F*** **0.40**		***I*** **9.58**				***F*** **0.43**		***I*** **25.18**			

	Experiment 3						**Experiment 4**					
Phytoplankton	F_c	p	I_c	p	E^*	p	F_c	p	I_c	p	E^*	p
***Chaetoceros* spp.**	2.20E-09	*	0.0268	*	-0.34		1.99E-09		0.0139		-0.45	**
***Rhizosolenia* spp.**	2.44E-10		0.0062		-0.68	***	1.10E-09		0.0245		-0.57	*
***Thalassiosira* spp.**	-2.97E-10		-0.0273		-0.71	**	2.06E-09	*	0.0450	*	-0.32	
Flagellates	2.25E-09	**	0.0320	**	-0.26		-2.36E-09	***	-0.0465	***	-1.00	
other Diatoms	3.84E-09	*	0.0046	*	-0.27		2.05E-09	*	0.0020	*	-0.36	
TOTAL PHYTOPLANKTON	**9.83E-10**		**0.0912**		**-0.45**	*	**5.23E-10**		**0.0390**		**-0.74**	***
RELATED TO TOTAL *TEMORA* CARBON	***F*** **0.10**		***I*** **9.42**				***F*** **0.05**		***I*** **4.02**			
Microzooplankton												
***Gyrodinium* spp.**	2.07E-09	***	0.0337	***	-0.23	*	6.35E-09	**	0.0456	****	0.15	
***Protoperidinium* spp.**	7.38E-09	**	0.0108	**	0.23		5.11E-09	**	0.0071	*	0.07	
other athecate dinoflagellates	2.15E-09		0.0002		-0.55		-		-		-	
other thecate dinoflagellates	1.37E-09		0.0007		-0.29		6.45E-10		0.0002		-0.58	*
***Strombidium* spp.**	1.44E-09		-0.0179		-0.52		3.08E-09		-		-	
***Strobilidium* spp.**	2.13E-09		0.0090		-0.33		3.90E-09		0.0001		-0.17	
Cyclotrichids	5.70E-09	*	0.0056		0.07		4.74E-09		-0.0007		-0.08	
Haptorids	5.23E-09	**	0.1175		-0.08		2.40E-08		0.0209		0.23	
other Ciliates	4.31E-09		0.0004		0.01		9.88E-10		0.0031		-0.58	**
thecate amoeba sp.	1.22E-09	*	0.0049	*	-0.44	*	2.44E-09	***	0.0041	***	-0.29	
Rotifers	3.22E-09		0.0050		-0.16		1.78E-08	***	0.0744	***	0.55	**
TOTAL MICROZOOPLANKTON	**3.69E-09**	***	**0.2789**	**	**0.18**	*	**7.54E-09**	****	**0.1722**	****	**0.28**	****
RELATED TO TOTAL *TEMORA* CARBON	***F*** **0.38**		***I*** **28.80**				***F*** **0.78**		***I*** **17.78**			

Table 4: *Temora longicornis* carbon specific filtration rates F_c [L µgC predator^{-1} d^{-1}] and carbon specific ingestion rates I_c [µgC prey µgC predator^{-1} d^{-1}], total filtration rates F [L d^{-1}], total ingestion rates I [µgC prey L^{-1} d^{-1}] and electivity E^* [-] for different prey groups. Positive selection marked with gray background. TC = Total *T. longicornis* carbon biomass. P-values derived from t-tests against zero.* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

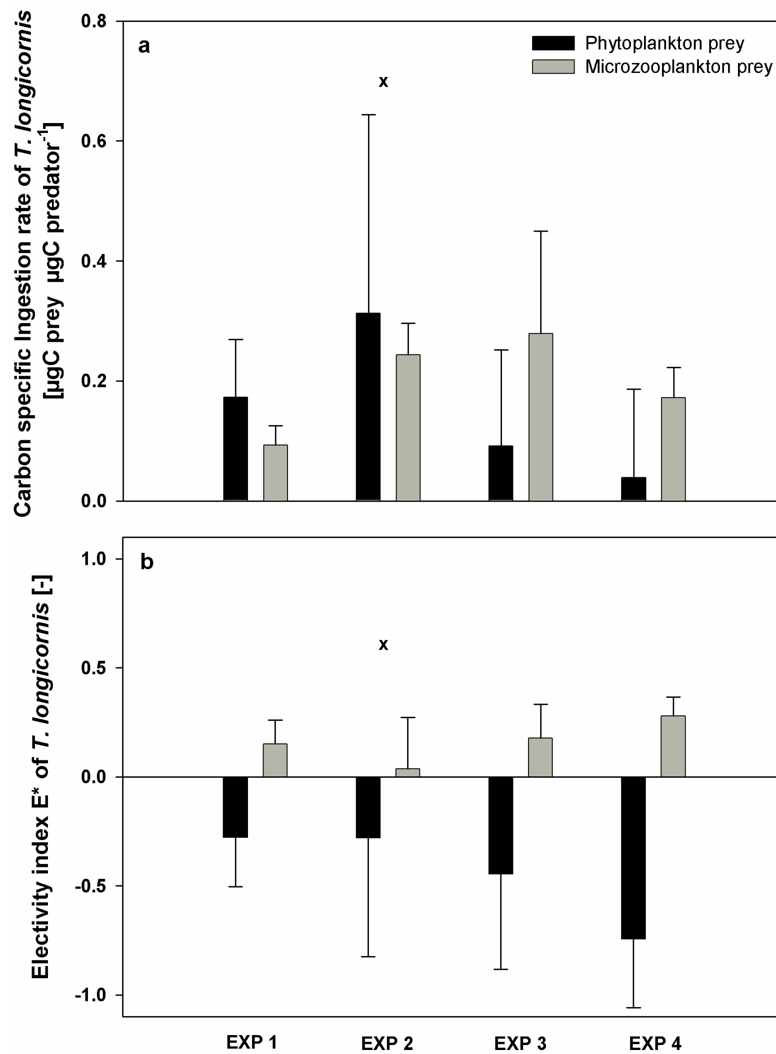

Figure 7: (7a) Carbon specific ingestion rate I_c and (7b) electivity index E^* of *Temora longicornis* for phytoplankton and microzooplankton prey. x marks experiment with insignificant differences of I_c and E^* between both prey groups. Error bars correspond to one standard deviation (n = 9).

DISCUSSION

The main finding of this study is that microzooplankton was the main grazer throughout the whole period of the phytoplankton spring bloom 2009 in the North Sea at Helgoland while copepods played only a minor role, especially as the densities we used were rather high. Using a mesocosm set up and excluding mesozooplankton grazers allowed us to follow plankton spring succession focussing on top-down control mechanisms by microzooplankton solely. In fact, the close resemblance of the bloom in the mesocosms to the natural situations (where mesograzers were present) further suggested that the microzooplankton drives the spring dynamics of the phytoplankton community around Helgoland. Furthermore, the combined approach of dilution grazing experiments and *T. longicornis* bottle incubations allowed us to analyse microzooplankton and copepod grazing and feeding preferences in the same plankton community.

Microzooplankton and *T. longicornis* impact on the phytoplankton bloom

While microzooplankton grazed on average 120% of the potential phytoplankton production (P_p) in our experiments, average grazing impact of *T. longicornis* was 47%. Microzooplankton showed an almost sevenfold higher specific ingestion rate (I_c) when preying on phytoplankton in contrast to copepods. Whereas the removal of phytoplankton by *T. longicornis* in our experiments was slightly higher than the 10-40% given by Calbet (2001) for copepods on a global scale, the grazing impact of the microzooplankton was around twofold higher than results reported by Landry & Calbet (2004). They found an average grazing impact of 59-75% of P_p by microzooplankton across a spectrum of open-ocean and coastal systems, whereas the lower border (60%) was found for estuarine systems with chlorophyll *a* values similar to those of our experiment. During our study the high availability of food during the bloom situation combined with a release from grazing pressure by metazoans enabled the development of a high microzooplankton grazer biomass in the mesocosms. Our results therefore should represent the maximum in microzooplankton grazing impact on phytoplankton in coastal regions. On the other hand the copepod biomass we used in our grazing experiments was at the upper level of field abundances at this time of the year (Greve et al., 2004) and therefore represents the maximal expectable grazing impact of copepods. Nevertheless, we found a much higher grazing impact by microzooplankton than by copepods.

The comparison of mesocosm chlorophyll *a* development with that in field revealed that they differed only during the inflow of coastal waters, even though copepods as potential predators were present potentially leading to a higher grazing impact on phytoplankton. As we did not investigate phytoplankton grazing of microzooplankton and copepods in the field we can only speculate about their relative importance. However, the similarity of the chlorophyll *a* development suggests that the same patterns of grazing as in the mesocosms were responsible for the development of phytoplankton biomass in the field. Combined with the fact that copepods avoided phytoplankton prey in the mesocosms this finding strengthens our result that spring microzooplankton can be regarded as the key phytoplankton grazer during the phytoplankton spring bloom and copepods apparently playing only a minor role at this time of the year.

Optimal bloom exploitation through different feeding strategies of microzooplankton

Different feeding strategies are recorded among heterotrophic dinoflagellates including direct engulfment, pallium-feeding and peduncle- or tube-feeding (Jacobson & Anderson, 1986, Gaines & Elbrächter, 1987). Ciliates are categorized as suspension, raptorial, deposit and diffusion feeders (Müller & Weisse, 1994). Depending on the feeding mode of the predators different prey is selected. Therefore, depending on the zooplankton community present at specific times of the year, feeding habits are directly mirrored by food selectivity patterns. Grazing selectivity itself also structures the phytoplankton composition (Irigoien et al., 2005). During the course of our experiments the microzooplankton community comprised a large variety of food preferences and preferred size spectra according to grazer species, their own size and feeding mode.
Generally, dinoflagellates can feed on a wide range of prey (Jeong, 1999) and are likely to be more quantitatively significant consumers of bloom-forming diatoms than copepods (Sherr & Sherr, 2007). Species that dominated in our study (*Gyrodinium* spp. and *Protoperidinium* spp.) are mainly associated with diatom blooms (Sherr & Sherr, 2007). Athecate *Gyrodinium* spp. (20-120 µm length) and thecate *Protoperidinium* spp. (15-75 µm diameter) dominated the grazer assemblage. Dinoflagellates can feed and grow on variable predator to prey size ratios between 5.2:1 and 0.15:1 (Naustvoll, 2000a, Naustvoll, 2000b). The upper limit of prey size reported by Naustvoll (2000a, 2000b) is probably not reached by naked phagotrophs such as *Gyrodinium* sp. as they

prefer food of their own size (Hansen, 1992), but rather by thecate, pallium-feeding dinoflagellates like *Protoperidinium* spp.. Concerning their feeding abilities and size, dinoflagellates were able to feed on the biggest diatoms in the mesocosm.

Ciliates feed mainly on nanoplankton in an optimal size corresponding to ca. 1/10 of their own size (Spittler, 1973, Heinbokel, 1978a, Jonsson, 1986). However, it is reported that they can feed on prey items sometimes larger than themselves (Kahl, 1932, Smetacek, 1981, Gifford, 1985, Johansson et al., 2004). Ciliates are thus believed to be in direct feeding competition with copepods (Aberle et al., 2007) and dinoflagellates (Hansen, 1992, Sherr & Sherr, 2007). *Strombidium capitatum,* the dominating strombidiid is known to feed on small flagellates of different groups (Stoecker & Silver, 1990, Crawford & Stoecker, 1996). Other *Strombidium* and *Strobilidium* species present in our experiment are considered to consume phytoplankton fractions ranging from 2 to 15 µm (Christaki, 1998, Sime-Ngando et al., 1999, Aberle et al., 2007). Xu and Hu (2005) found a big *Cyclotrichium* species similar to the species in the second half of the bloom feeding on different algae including diatoms. Except the latter the main prey of ciliates in the mesocosm should have been flagellates and smaller diatoms.

We found a highly diverse microzooplankton community during the spring bloom. Species of different size classes with different feeding modes were always present. It is therefore not surprising that microzooplankton grazed on all possible components of the phytoplankton ranging from smallest flagellates to large-sized diatoms. Microzooplankton was even able to graze on huge bloom-forming diatom species like *Rhizosolenia* spp. in significant numbers. We did not investigate other factors that can reduce a phytoplankton bloom (e.g. cell death, cyst forming, sedimentation, parasitism or viral lysis). Nevertheless, the measured consumption of all available phytoplankton species should have been by far the most important factor since it led to a strong suppression of phytoplankton and an almost complete decline within three weeks after the bloom peak.

Bloom of less-favoured species due to selective grazing by microzooplankton

Irigoien et al. (2005) pointed out that among other factors, defence mechanisms (e.g. large cell sizes, colonies or spine-formation) and selective predation of microzooplankton opens a “loophole” for phytoplankton blooms of less edible, unfavoured species. As food selectivity is a constant process, we have to stress that a pre-selection of phytoplankton species must have been already taken place in the field prior to our mesocosm experiment.

Flagellates contributed to one third to the phytoplankton community at the start of the experiment. As they lost importance towards the end of March we assume that the growth of flagellates was controlled by selective predation of microzooplankton and flagellates were therefore not able to form a bloom. By contrast, shortly after the start the bloom in the experiment was dominated by three diatom genera: *Rhizosolenia*, *Thalassiosira* and *Chaetoceros*. *Rhizosolenia* represented the biggest diatoms (mean length 288 µm) occurring in the mesocosms. Although *Rhizosolenia* was grazed to some extent, electivity values showed that it was less preferred compared to other phytoplankton. It gained thereby an advantage resulting in a relatively constant biomass of ~27% of the total phytoplankton biomass throughout the experiment. A good example for the opening of "loopholes" via selective grazing is the genus *Thalassiosira*. Two species occurred during the experiments, *T. rotula* and *T. nordenskjoeldii*, whereas the latter one dominated the total *Thalassiosira spp.* biomass with up to 92% and dominance increased during the bloom. Both *Thalassiosira* species are able to form long chains but, in addition, *T. nordenskjoeldii* possesses spines. During our experiment *T. nordenskjoeldii* was always less preferred as prey. This resulted in an increase in *Thallassiosira* spp. from 10 to 49% of total phytoplankton biomass. In contrast, the spine-possessing and chain-forming genus *Chaetoceros* showed an ambivalent picture. It consisted of species of different size classes from 10 to 40 µm diameter per cell. While small *Chaetoceros* spp. (10 µm) totally disappeared due to grazing, others, especially the bigger ones with long spines were grazed less and remained as a constant fraction of the phytoplankton. Overall, even if certain bloom dynamics have been observed, the consumption and selective grazing by microzooplankton shaped the phytoplankton assemblage and left a bloom of three less-favoured diatom genera over.

Microzooplankton species succession - a direct response to different food availability

Ciliates showed a direct response and distinct succession patterns in relation to food availability which was most pronounced in the genera *Strombidium* and *Strobilidium*. Their abundance was directly coupled with the availability of flagellate prey and resulted in a strong suppression of flagellates right at the ciliate peak. With the disappearance of their predators due to food shortage the relaxation from grazing pressure in turn enabled a positive net growth of flagellates again. The fact that they did not disappear completely due to grazing is most probably due to selective predation on specific flagellate species. This predator-prey relationship was most obvious in

Strombidium capitatum, which is known to be directly dependent on flagellate prey (Stoecker & Silver, 1990) and forms cysts as soon as unfavourable conditions occur (Kim et al., 2008). Simultaneously with the disappearance of its potential prey (dinoflagellates < 15µm) *S. capitatum* started to form cysts and abundance of the ciliate population collapsed within one week. After the decrease of strombidiids and strobilids, *Cyclotrichium* sp. a ciliate which also feeds on diatoms (Xu et al., 2005) started to dominate and towards the end of the bloom, bacterivorous ciliates (*Acineta* sp., *Euplotes* sp.) gained importance.

In contrast, dinoflagellates showed unclear succession patterns compared to ciliates. While two groups disappeared due to predation by ciliates (thecate and athecate dinoflagellates < 15 µm), the remaining dinoflagellate community remained relatively stable until the end of the experiment. This was most likely related to the fact that diatoms, the preferred prey of dinoflagellates (Sherr & Sherr, 2007), were always present. The opposing patterns found for dinoflagellates and ciliates might also be related to a contrasting ecological strategy of these groups. Dinoflagellates are considered to have lower growth rates than ciliates (Hansen, 1992) and therefore their ability to react rapidly to enhanced food availability is limited. On the other hand, dinoflagellates can prey on almost every organic particle present in the oceans (Jeong, 1999, Tillmann, 2004). They also have a higher starving potential (Hansen, 1992, Menden-Deuer et al., 2005), and thus can survive periods of food shortage (Sherr & Sherr, 2007). In contrast, ciliates can respond rapidly to enhanced food availability showing growth rates higher than those of dinoflagellates (Strom & Morello, 1998) but their potential to survive starvation periods is low (Jackson & Berger, 1985, Hansen, 1992) and they are more restricted to certain prey items (Tillmann, 2004). Thus, the succession of microzooplankton observed in the present study is mainly triggered by the availability of food and contrasting survival strategies. Ciliates can be considered as specialists and dinoflagellates more as generalists.

Factors determining the microzooplankton bloom in the mesocosms

Interestingly, microzooplankton biomass declined to values close to start values at the end, even though a considerable amount of phytoplankton food was still available. Besides predator-prey relationships that negatively influence the predator when its prey is absent, this pattern could be due to changes in the food quality of the preferred food. With the duration of the bloom phytoplankton got increasingly nutrient-depleted (Schoo, 2010) and therefore did not meet the nutritional needs of the microzooplankton.

The effort to capture, handle, digest the prey and egest the excess carbon might have been more energy demanding than the energy benefit the prey offered. Negative effects due to poor food have been reported (Jensen & Hessen, 2007) and if predators have the choice between good and bad food they naturally choose the good one. Other microzooplankters, which feed on nutrient-limited phytoplankton represent the better food when compared to the phytoplankton itself (Malzahn et al., 2010). Thus, an extra effect introduced by "bad quality phytoplankton" may have been predation within microzooplankton. Pronounced carnivory towards the end of phytoplankton blooms has been described by Irigoien (2005) and in our experiment microzooplankton might also have switched its feeding strategy. Towards the end of the bloom rotifers gained in importance (up to 28% of biomass). About 10-40% of rotifer food can consist of heterotrophic organisms of the microbial food web as rotifers are efficient predators on protozoans (Arndt, 1993). It is therefore most likely that the combined effects of both, predation within the microzooplankton especially by rotifers and the bad nutritional quality of the food sources, resulted in an overall decline in microzooplankton abundance.

The microzooplankton fate in a real bloom

Microzooplankton is able to compete with copepods for the same food sources and to exploit food stocks more efficiently due to their fast metabolic abilities and growth rates. They in turn are preferred food for higher trophic levels, e.g. mesozooplankton, even if phytoplankton is available at high numbers but at low food quality (Hansen et al., 1993). Microzooplankton contributes as a substantial part to copepods' diets and it is often positively selected (Nejstgaard et al., 1997, Fileman et al., 2007). Even in predominately herbivorous species such as *Acartia tonsa* microzooplankton can make up to 41% of the diet even when present in low abundances (Gifford & Dagg, 1988). Grazing on microzooplankton by copepods can have severe trophic cascade effects. The release of microzooplankton grazing pressure can promote nanoflagellates, an important prey of ciliates, and thus affect bacterial abundance positively as bacteria are the main food source of nanoflagellates (Zöllner et al., 2009). Even more pronounced effects were reported on chlorophyll *a* concentration: Enrichment in copepod grazers reduced microzooplankton biomass and led to overall higher chlorophyll *a* concentrations due to the release of small sized flagellates from microzooplankton grazing (Sommer et al., 2003, Sommer et al., 2005).

With decreasing food quality of the phytoplankton in our experiments *T. longicornis* changed its diet along a gradient from phytoplankton-dominated to microzooplankton-dominated during the course of the bloom and we observed high positive selection for microzooplankton. We thus assume that microzooplankton is top-down controlled by copepod grazing during bloom situations. Trophic upgrading of food by heterotrophic protists (Martin-Creuzburg et al., 2005, Tang & Taal, 2005, Bec et al., 2006) has been demonstrated and data show the ability of protozoan grazers to dampen stoichiometric imbalances to a certain extent when they feed on low quality food (Malzahn et al., 2010). This fact as well as their capacity to synthesize highly unsaturated fatty acids and sterols makes them good quality food from a copepod perspective (Klein Breteler et al., 1999, Tang & Taal, 2005). Therefore, microzooplankton can not only be regarded as the major phytoplankton grazer but has also an important role in channelling the energy of primary production as food source for higher trophic levels and furthermore dampens potential nutritional shortfalls of herbivory.

Conclusions

(1) Microzooplankton reacted quickly to enhanced availability of prey and its high grazing led to a decrease to pre-bloom values within three weeks after the bloom peak.
(2) Microzooplankton was the more efficient grazer when compared to copepods.
(3) Selective grazing by microzooplankton led to a bloom of less-favoured phytoplankton species and to constant shares of bloom-forming species during the course of the bloom.
(4) Ciliates responded with rapid growth and mortality to differences in prey availability, leading to a short but high peak during the bloom. Dinoflagellates had a broader food spectrum and lower growth and mortality rates, which led to a longer duration of the dinoflagellate bloom.
(5) As a substantial part of the copepod diet microzooplankton gained in importance with decreasing food quality of the phytoplankton during the course of the bloom.

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APPENDIX

Table 1: Microzooplankton grazing g [d^{-1}] and phytoplankton growth rates k [d^{-1}] determined in four dilution experiments for each prey category. Food saturation marked with gray background. Instantaneous growth rate values μ_0 [d^{-1}] from bottles without added nutrients. Percentage of initial stock P_i [%] and potential production grazed P_p [%]. Negative P_i and P_p values resulting from negative g (P_i) or μ_0 (P_p) and were set to zero. The same was done for positive P_p values resulting from negative g and μ_0. MMC = mean microzooplankton carbon biomass. P-values from linear regression analysis of apparent phytoplankton growth against dilution factor (n = 36). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Table 2: Microzooplankton carbon specific filtration rates F_c [L µgC predator^{-1} d^{-1}] and carbon specific ingestion rates I_c [µgC prey µgC predator^{-1} d^{-1}], total filtration rates F [L d^{-1}], total ingestion rates I [µgC prey L^{-1} d^{-1}] and electivity E^* [-] for each prey category. Positive selection marked with gray background. MMC = mean microzooplankton carbon biomass. P-values are the same as for the grazing rates of microzooplankton. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Table 3 a+b: *Temora longicornis* grazing g [d^{-1}], phytoplankton and microzooplankton growth rates k [d^{-1}] determined in four grazing experiments for each prey category. Instantaneous growth rate values μ_0 [d^{-1}] from dilution experiment bottles without added nutrients. Percentage of initial stock P_i [%] and potential production grazed P_p [%]. Negative P_i and P_p values resulting from negative g (P_i) or μ_0/k (P_p) were set to zero. The same was done for positive P_p values resulting from negative g and μ_0/k. TC = Total *T. longicornis* carbon biomass. P-values derived from t-tests against zero.* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Table 4 a+b: *Temora longicornis* carbon specific filtration rates F_c [L µgC predator^{-1} d^{-1}] and carbon specific ingestion rates I_c [µgC prey µgC predator^{-1} d^{-1}], total filtration rates F [L d^{-1}], total ingestion rates I [µgC prey L^{-1} d^{-1}] and electivity E^* [-] for each prey category. Positive selection marked with gray background. TC = Total *T. longicornis* carbon biomass. P-values derived from t-tests against zero.* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Table 1

	Microzooplankton														
	Experiment 1 - (MMC 30.33 μg L^{-1})							Experiment 2 - (MMC 74.47 μg L^{-1})						
Phytoplankton	k	p	g	p	μ_0	P_i	P_p	k	p	g	p	μ_0	P_i	P_p
***Pseudonitzschia* spp. (120 μm)**	0.43	***	0.62	**	0.31	86	172	0.87	****	1.04	****	1.52	183	83
***Pseudonitzschia* spp. (80 μm)**	0.14		0.29		-0.46	34	0	0.57	***	0.66	*	0.85	93	84
***Pseudonitzschia* spp. (60 μm)**	0.44		1.08		0.51	195	164	0.99	****	0.88	*	0.50	142	148
***Navicula* spp. (40 μm)**	0.66	*	0.39	*	0.16	48	220	0.98	****	1.35	****	1.62	285	92
***Navicula* spp. (20 μm)**	0.08		-0.21		-0.28	0	74	0.20		0.52	*	0.23	68	199
***Navicula* spp. (10 μm)**	-0.32	*	-0.50		-0.39	0	135	0.24	*	0.44	*	0.16	56	237
Chaetoceros danicus	1.14	**	1.11	****	0.65	203	141	0.40	**	0.16		0.30	18	58
***Chaetoceros* spp. (40 μm)**	0.72	**	1.16	**	0.62	220	149	1.07	****	0.91	**	1.25	150	84
***Chaetoceros* spp. (30 μm)**	0.48	**	0.77	**	0.86	116	93	0.59	***	0.54	*	0.93	71	69
***Chaetoceros* spp. (20 μm)**	0.41		0.55	*	0.40	72	129	1.60	****	1.69	****	1.70	443	100
***Chaetoceros* spp. (10 μm)**	0.71	*	1.19	*	0.73	230	135	1.34	***	0.75		0.66	113	109
Rhizosolenia styliformis/hebetata	0.32		0.35	**	0.03	42	1063	0.53	****	0.61	****	0.62	85	99
Rhizosolenia pungens/setigera	-0.58	*	-0.73	*	0.05	0	0	1.09	****	1.02	****	1.03	177	99
Thalassiosira nordenskioeldii	0.50	***	0.50	*	0.33	65	141	0.66	****	0.39	*	0.63	47	69
Thalassiosira rotula	0.56	****	0.68	**	0.64	96	104	-0.14		-0.29		0.16	0	0
Flagellates (25 μm)	0.54		0.68	*	0.83	98	88	0.49	*	0.74	*	0.78	110	97
Flagellates (20 μm)	0.11		0.02		-0.12	2	0	0.07		0.47	*	-0.06	60	0
Flagellates (15 μm)	-0.52	**	-0.36		-0.47	0	0	0.53	***	0.91	***	0.69	149	119
Flagellates (10 μm)	0.49	*	0.31	**	-0.23	36	0	0.46	**	0.66	**	0.58	94	109
Flagellates (5 μm)	0.24		0.28	**	-0.61	32	0	0.79	****	1.21	***	1.42	236	93
TOTAL PHYTOPLANKTON	**0.41**	*****	**0.43**	*****	**0.17**	**53**	**223**	**0.80**	********	**0.66**	*******	**0.77**	**93**	**90**

	Experiment 3 - (MMC 93.82 μg L^{-1})							Experiment 4 - (MMC 33.12 μg L^{-1})						
Phytoplankton	k	p	g	p	μ_0	P_i	P_p	k	p	g	p	μ_0	P_i	P_p
***Pseudonitzschia* spp. (120 μm)**	0.38	***	0.41	*	0.46	51	91	0.78	****	0.44	****	0.75	55	67
***Pseudonitzschia* spp. (80 μm)**	0.91	****	0.94	***	1.26	156	85	1.08	****	1.14	***	1.82	213	81
***Pseudonitzschia* spp. (60 μm)**	1.63	****	2.04	****	2.02	666	100	1.02	***	1.22	**	1.46	239	92
***Navicula* spp. (40 μm)**	1.35	****	1.62	****	0.16	404	541	0.20		0.50	*	-0.20	66	0
***Navicula* spp. (20 μm)**	0.15		0.52		0.45	68	111	-0.18		0.18		0.18	19	96
***Navicula* spp. (10 μm)**	0.30		0.62	*	0.23	85	223	1.32	**	1.43	*	2.18	316	86
Chaetoceros danicus	-0.03		-0.07		-0.02	0	0	-0.28		-0.11		0.21	0	0
***Chaetoceros* spp. (40 μm)**	1.09	****	1.42	***	1.72	313	92	1.18	****	1.00	**	1.67	173	78
***Chaetoceros* spp. (30 μm)**	0.45	*	0.34		0.50	40	73	1.17	****	0.84	****	1.66	131	70
***Chaetoceros* spp. (20 μm)**	0.27	*	0.51	*	0.08	66	515	0.64	****	0.16		0.68	18	31
***Chaetoceros* spp. (10 μm)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhizosolenia styliformis/hebetata	0.41	****	0.47	****	0.73	61	73	0.50	****	0.36	**	0.65	43	63
Rhizosolenia pungens/setigera	0.64	**	0.66	*	0.74	93	92	0.33		0.28		0.06	32	409
Thalassiosira nordenskioeldii	0.17	**	0.49	****	0.49	63	99	0.62	****	0.58	****	0.73	79	85
Thalassiosira rotula	0.51	***	0.87	**	1.14	138	85	0.86	****	0.70	***	1.20	102	72
Flagellates (25 μm)	1.34	*	1.92	*	2.36	584	94	1.33		1.33		3.04	278	77
Flagellates (20 μm)	0.43		1.03	*	0.76	180	121	0.66	*	0.98	*	0.73	165	121
Flagellates (15 μm)	0.84	****	1.38	****	0.72	299	146	0.28		0.50		0.68	65	79
Flagellates (10 μm)	0.82	****	0.99	****	0.95	169	102	-0.49		-0.12		-0.59	0	0
Flagellates (5 μm)	0.57	****	0.54	**	0.76	72	79	0.007		0.26	*	-0.33	30	0
TOTAL PHYTOPLANKTON	**0.39**	********	**0.54**	********	**0.65**	**72**	**87**	**0.41**	********	**0.39**	*******	**0.52**	**48**	**80**

Table 2

	Microzooplankton									
	Experiment 1 - (MMC 30.33 µg L^{-1})					Experiment 2 - (MMC 74.47 µg L^{-1})				
Phytoplankton	F_c	p	I_c	p	E^*	F_c	p	I_c	p	E^*
***Pseudonitzschia* spp. (120 µm)**	2.05E-08	**	0.0179	**	0.11	1.40E-08	****	0.0070	****	0.16
***Pseudonitzschia* spp. (80 µm)**	9.58E-09		0.0015		-0.26	8.83E-09	*	0.0009	*	-0.06
***Pseudonitzschia* spp. (60 µm)**	3.57E-08		0.0003		0.37	1.19E-08	*	0.0002	*	0.08
***Navicula* spp. (40 µm)**	1.29E-08	*	0.0008	*	-0.12	1.81E-08	****	0.0018	****	0.29
***Navicula* spp. (20 µm)**	-6.98E-09		-0.0034		-1.00	7.00E-09	*	0.0032	*	-0.18
***Navicula* spp. (10 µm)**	-1.64E-08		-0.0542		-1.00	5.97E-09	*	0.0244	*	-0.26
Chaetoceros danicus	3.65E-08	****	0.0914	****	0.38	2.20E-09		0.0046		-0.64
***Chaetoceros* spp. (40 µm)**	3.83E-08	**	0.0301	**	0.40	1.23E-08	**	0.0096	**	0.10
***Chaetoceros* spp. (30 µm)**	2.53E-08	**	0.0500	**	0.21	7.23E-09	*	0.0292	*	-0.16
***Chaetoceros* spp. (20 µm)**	1.80E-08	*	0.1322	*	0.04	2.27E-08	****	0.0673	****	0.39
***Chaetoceros* spp. (10 µm)**	3.93E-08	*	0.0826	*	0.41	1.01E-08		0.0046		0.00
Rhizosolenia styliformis/hebetata	1.17E-08	**	0.4002	**	-0.17	8.23E-09	****	0.2548	****	-0.10
Rhizosolenia pungens/setigera	-2.41E-08	*	-0.0588	*	-1.00	1.37E-08	****	0.1286	****	0.15
Thalassiosira nordenskioeldii	1.65E-08	*	0.5005	*	0.00	5.21E-09	*	0.1975	*	-0.32
Thalassiosira rotula	2.23E-08	**	0.2031	**	0.15	-3.95E-09		-0.0396		-1
Flagellates (25 µm)	2.26E-08	*	0.0073	*	0.16	9.99E-09	*	0.0035	*	0.00
Flagellates (20 µm)	7.35E-10		0.0006		-0.91	6.32E-09	*	0.0092	*	-0.23
Flagellates (15 µm)	-1.17E-08		-0.0262		-1.00	1.22E-08	***	0.0263	***	0.10
Flagellates (10 µm)	1.02E-08	**	0.0524	**	-0.24	8.89E-09	**	0.0271	**	-0.06
Flagellates (5 µm)	9.17E-09	**	0.0698	**	-0.28	1.63E-08	***	0.0366	***	0.24
TOTAL PHYTOPLANKTON	**1.40E-08**	*	**1.57**	*		**8.85E-09**	***	**1.00**	***	
RELATED TO TOTAL MICROZOOPLANKTON CARBON	***F*** **0.43**		***I*** **47.65**			***F*** **0.66**		***I*** **74.58**		

	Experiment 3 - (MMC 93.82 µg L^{-1})					Experiment 4 - (MMC 33.12 µg L^{-1})				
Phytoplankton	F_c	p	I_c	p	E^*	F_c	p	I_c	p	E^*
***Pseudonitzschia* spp. (120 µm)**	4.41E-09	*	0.0043	*	-0.36	1.32E-08	****	0.0105	****	-0.18
***Pseudonitzschia* spp. (80 µm)**	1.00E-08	***	0.0008	***	0.03	3.45E-08	***	0.0021	***	0.29
***Pseudonitzschia* spp. (60 µm)**	2.17E-08	****	0.0002	****	0.40	3.69E-08	**	0.0002	**	0.32
***Navicula* spp. (40 µm)**	1.72E-08	****	0.0008	****	0.29	1.52E-08	*	0.0007	*	-0.11
***Navicula* spp. (20 µm)**	5.52E-09		0.0012		-0.26	5.36E-09		0.0007		-0.56
***Navicula* spp. (10 µm)**	6.56E-09	*	0.0026	*	-0.18	4.31E-08	*	0.0019	*	0.39
Chaetoceros danicus	-7.81E-10		-0.0016		-1	-3.34E-09		-0.0041		-1
***Chaetoceros* spp. (40 µm)**	1.51E-08	***	0.0117	***	0.23	3.03E-08	**	0.0146	**	0.23
***Chaetoceros* spp. (30 µm)**	3.59E-09		0.0185		-0.45	2.53E-08	****	0.0511	****	0.14
***Chaetoceros* spp. (20 µm)**	5.41E-09	*	0.0336	*	-0.27	4.94E-09		0.0275		-0.59
***Chaetoceros* spp. (10 µm)**	-	-	-	-	-	-	-	-	-	-
Rhizosolenia styliformis/hebetata	5.05E-09	****	0.1467	****	-0.30	1.08E-08	**	0.2776	**	-0.28
Rhizosolenia pungens/setigera	7.02E-09	*	0.0321	*	-0.14	8.46E-09		0.0192		-0.38
Thalassiosira nordenskioeldii	5.19E-09	****	0.1874	****	-0.29	1.77E-08	****	0.3726	****	-0.03
Thalassiosira rotula	9.24E-09	**	0.0501	**	-0.01	2.12E-08	***	0.0669	***	0.06
Flagellates (25 µm)	2.05E-08	*	0.0013	*	0.37	4.02E-08		0.0017		0.36
Flagellates (20 µm)	1.10E-08	*	0.0017	*	0.08	2.95E-08	*	0.0049	*	0.22
Flagellates (15 µm)	1.47E-08	****	0.0020	****	0.22	1.51E-08		0.0027		-0.11
Flagellates (10 µm)	1.05E-08	****	0.0151	****	0.06	-3.70E-09		-0.0284		-1
Flagellates (5 µm)	5.80E-09	**	0.0834	**	-0.24	7.96E-09	*	0.0857	*	-0.41
TOTAL PHYTOPLANKTON	**5.77E-09**	****	**0.62**	****		**1.19E-08**	***	**0.97**	***	
RELATED TO TOTAL MICROZOOPLANKTON CARBON	***F*** **0.54**		***I*** **58.02**			***F*** **0.39**		***I*** **32.13**		

Table 3a

	Temora longicornis (TC 103.26 µg L^{-1})													
	Experiment 1							Experiment 2						
Phytoplankton	k	p	g	p	μ_0	P_i	P_p	k	p	g	p	μ_0	P_i	P_p
Pseudonitzschia spp. (120 µm)	-0.11		-0.02		0.31	0	0	-0.15	*	0.02		1.52	2	2
Pseudonitzschia spp. (80 µm)	-0.25		0.07		-0.46	8	0	-0.08		-0.04		0.85	0	0
Pseudonitzschia spp. (60 µm)	-0.29		0.51		0.51	67	99	0.26		-0.03		0.50	0	0
Navicula spp. (40 µm)	0.25		0.35		0.16	42	202	-0.27		0.21		1.62	24	24
Navicula spp. (20 µm)	0.30	*	0.01		-0.28	1	0	-0.25		0.02		0.23	2	11
Navicula spp. (10 µm)	0.15		-0.06		-0.39	0	0	-0.16		0.04		0.16	4	27
Chaetoceros danicus	0.00		1.01	**	0.65	174	134	0.36		0.08		0.30	8	29
Chaetoceros spp. (40 µm)	-0.30		0.17		0.62	19	34	0.33		0.38		1.25	46	44
Chaetoceros spp. (30 µm)	-0.15		0.29		0.86	33	43	0.31		0.43		0.93	54	58
Chaetoceros spp. (20 µm)	-0.13		0.41	**	0.40	50	102	0.08		0.43	*	1.70	53	42
Chaetoceros spp. (10 µm)	-0.31		-0.10		0.73	0	0	1.29		2.06		0.66	686	180
Rhizosolenia styliformis/hebetata	-0.04		0.13		0.03	14	432	-0.06		0.11		0.62	12	23
Rhizosolenia pungens/setigera	0.17		0.29		0.05	34	500	0.20		0.42	**	1.03	52	53
Thalassiosira nordenskioeldii	0.02		0.17	*	0.33	18	55	0.34	**	0.51	*	0.63	67	86
Thalassiosira rotula	-0.07		0.30	**	0.64	35	55	0.15		0.37	*	0.16	45	212
Flagellates (25 µm)	0.03		0.99		0.83	170	112	-0.20		0.08		0.78	8	14
Flagellates (20 µm)	-0.05		0.32		-0.12	38	0	-0.36		0.37		-0.06	45	0
Flagellates (15 µm)	-0.24		0.02		-0.47	2	0	-0.38	**	0.22	**	0.69	25	40
Flagellates (10 µm)	0.14		0.31		-0.23	37	0	-0.09		0.20		0.58	22	41
Flagellates (5 µm)	-0.04		-0.14		-0.61	0	0	-0.44		-0.43		1.42	0	0
TOTAL PHYTOPLANKTON	**-0.01**		**0.17**	***	**0.17**	**18**	**100**	**0.13**	*	**0.34**	*	**0.77**	**40**	**54**
Microzooplankton														
Gyrodinium spp. (30-75 µm)	-0.05		1.12	**		207	0	0.33	****	0.24	**		27	76
Gyrodinium spp. (75-120 µm)	0.30	*	0.95	**		158	236	0.17		1.00	****		172	407
Protoperidinium spp. (20-40 µm)	0.41		-0.14			0	0	0.11		0.37	*		45	287
Protoperidinium spp. (50-80 µm)	0.17		1.15	**		217	435	0.23		0.32	*		38	136
Ceratium spp.	-0.21		1.51			354	0	0.06		0.19			21	325
Torodinium spp.	0.02		0.23			26	1047	0.15		-0.03			0	0
other athecate dinoflagellates	0.02		0.18			19	729	-0.02		0.31	*		37	0
other thecate dinoflagellates	0.40	****	-0.03			0	0	-0.44	****	0.21	*		23	0
Strombidium spp. (25-40 µm)	-0.12		0.39			47	0	0.34	***	0.75	**		111	181
Strombidium spp. (40-110 µm)	0.32	*	1.24	***		245	257	0.46	****	0.80	****		122	148
Strobilidium spp.	0.26		0.92	*		150	262	0.66	***	0.36	**		43	62
Myrionecta rubra	0.18		0.95	**		158	373	-0.32		0.17			18	0
other Cyclotrichids	0.00		0.00			0	-	-0.67		-			-	-
Haptorids	-0.28		0.93	*		154	0	0.39		0.35			42	91
Tintinnids	0.11		-0.08			0	0	0.09		0.52	*		69	452
other Ciliates	-		-			-	-	-		-			-	-
thecate amoeba sp.	0.16		0.32	***		38	191	0.25	**	0.23	*		26	93
Rotifers	0.00		-			-	-	-		-			-	-
TOTAL MICROZOOPLANKTON	**0.21**	****	**0.40**	****		**49**	**174**	**0.27**	****	**0.43**	****		**53**	**147**

Table 3b

	Temora longicornis (TC 103.26 µg L^{-1})													
	Experiment 3							Experiment 4						
Phytoplankton	*k*	p	*g*	p	μ_0	P_i	P_p	*k*	p	*g*	p	μ_0	P_i	P_p
***Pseudonitzschia* spp. (120 µm)**	-0.04		0.34		0.46	41	78	0.38	***	0.13		0.75	14	23
***Pseudonitzschia* spp. (80 µm)**	-0.05		0.14		1.26	15	18	0.06		0.88	**	1.82	140	70
***Pseudonitzschia* spp. (60 µm)**	-0.37		0.09		2.02	9	10	-0.24		0.57		1.46	78	57
***Navicula* spp. (40 µm)**	-0.28		0.74	***	0.16	109	353	-0.47		0.23		-0.20	26	0
***Navicula* spp. (20 µm)**	-0.46		-0.01		0.45	0	0	-0.36	*	-0.11		0.18	0	0
***Navicula* spp. (10 µm)**	-0.39	*	0.45	**	0.23	56	175	-0.08		0.20		2.18	22	20
Chaetoceros danicus	0.02		0.18		-0.02	19	0	-0.19		0.43		0.21	53	182
***Chaetoceros* spp. (40 µm)**	-0.23		0.35		1.72	42	36	0.23		0.48		1.67	61	47
***Chaetoceros* spp. (30 µm)**	0.19		-0.10		0.50	0	0	0.32	*	0.24		1.66	28	27
***Chaetoceros* spp. (20 µm)**	-0.16		0.56	***	0.08	75	555	0.38		-0.10		0.68	0	0
***Chaetoceros* spp. (10 µm)**	-		-		-	-	-	-		-		-	-	-
Rhizosolenia styliformis/hebetata	-0.09		0.02		0.73	2	4	0.14		0.04		0.65	4	7
Rhizosolenia pungens/setigera	0.06		0.15		0.74	16	27	0.03		0.42	**	0.06	52	569
Thalassiosira nordenskioeldii	-0.36	*	0.04		0.49	5	11	0.06		0.17	*	0.73	19	30
Thalassiosira rotula	-0.34		-0.02		1.14	0	0	0.21		0.24		1.20	27	30
Flagellates (25 µm)	-0.28		1.40		2.36	305	83	0.00		-1.05		3.04	0	0
Flagellates (20 µm)	-0.51	*	0.07		0.76	7	12	-0.21		0.24		0.73	28	42
Flagellates (15 µm)	-0.39	*	0.42		0.72	53	67	-0.10		-0.11		0.68	0	0
Flagellates (10 µm)	-0.15		-0.07		0.95	0	0	-0.36	****	-0.16		-0.59	0	0
Flagellates (5 µm)	-0.04		0.27	**	0.76	32	45	-0.20		-0.18	*	-0.33	0	0
TOTAL PHYTOPLANKTON	**-0.18**		**0.10**		**0.65**	**11**	**20**	**0.04**		**0.05**		**0.52**	**6**	**13**
Microzooplankton														
***Gyrodinium* spp. (30-75 µm)**	0.02		0.15	**		16	593	-0.22	*	0.42	**		52	0
***Gyrodinium* spp. (75-120 µm)**	0.20	*	0.68	****		98	276	0.18		1.23	***		242	439
***Protoperidinium* spp. (20-40 µm)**	0.03		0.24			27	802	-0.25		0.15			16	0
***Protoperidinium* spp. (50-80 µm)**	0.14		0.91	**		149	467	-0.05		0.68	*		98	0
***Ceratium* spp.**	0.26		0.50			65	170	-0.30		-0.39			0	0
***Torodinium* spp.**	0.27		0.22			25	85	-		-			-	-
other athecate dinoflagellates	-		-			-	-	-		-			-	-
other thecate dinoflagellates	-0.26		-0.02			0	0	-0.47	*	0.06			6	0
***Strombidium* spp. (25-40 µm)**	-0.15		1.34			284	0	-		-			-	-
***Strombidium* spp. (40-110 µm)**	-1.61	****	0.15			16	0	0.61		0.19			20	37
***Strobilidium* spp.**	-0.61	****	0.22			25	0	-0.21		0.40			50	0
Myrionecta rubra	-1.09	***	0.34	*		40	0	-0.72		0.49			63	0
other Cyclotrichids	0.96	*	0.83			129	91	-		-			-	-
Haptorids	0.87	**	0.54	**		72	72	-0.52		2.48			1097	0
Tintinnids	0.26		0.69			100	216	-		-			-	-
other Ciliates	0.32		0.27			31	87	-0.11		0.10			11	0
thecate amoeba sp.	-0.11		0.13	*		13	0	-0.05		0.25	***		29	0
Rotifers	0.02		0.33			39	1183	0.03		1.84	***		529	3349
TOTAL MICROZOOPLANKTON	**0.07**		**0.38**	***		**46**	**478**	**-0.04**		**0.78**	****		**118**	**0**

Table 4a

	Temora longicornis (TC 103.26 µg L^{-1})											
	Experiment 1						Experiment 2					
Phytoplankton	F_c	p	I_c	p	E^*	p	F_c	p	I_c	p	E^*	p
***Pseudonitzschia* spp. (120 µm)**	-1.56E-10		-0.0002		-0.80	***	1.84E-10		0.0001		-0.71	***
***Pseudonitzschia* spp. (80 µm)**	7.15E-10		-0.0001		-0.56	*	-3.41E-10		0.0000		-0.52	*
***Pseudonitzschia* spp. (60 µm)**	4.94E-09		0.0000		-0.52		-2.61E-10		0.0000		-0.48	
***Navicula* spp. (40 µm)**	3.43E-09		0.0001		-0.22		2.07E-09		0.0001		-0.49	*
***Navicula* spp. (20 µm)**	1.07E-10		0.0001		-0.58	**	2.21E-10		0.0004		-0.60	***
***Navicula* spp. (10 µm)**	-5.59E-10		-0.0019		-0.82	***	4.07E-10		0.0027		-0.59	*
Chaetoceros danicus	9.77E-09	**	0.0129	**	0.21		7.68E-10		0.0005		-0.65	**
***Chaetoceros* spp. (40 µm)**	1.67E-09		0.0005		-0.40		3.68E-09		0.0019		-0.18	
***Chaetoceros* spp. (30 µm)**	2.79E-09		0.0050		-0.34		4.21E-09		0.0034		-0.01	
***Chaetoceros* spp. (20 µm)**	3.92E-09	**	0.0232	**	-0.21		4.13E-09	*	0.0106		-0.13	
***Chaetoceros* spp. (10 µm)**	-9.87E-10		-0.0026		-0.86	****	2.00E-08		0.0069		0.51	
Rhizosolenia styliformis/hebetata	1.25E-09		0.0420		-0.60	**	1.07E-09		0.0333		-0.46	*
Rhizosolenia pungens/setigera	2.83E-09		0.0075		-0.01		4.04E-09	**	0.0306	**	-0.16	
Thalassiosira nordenskioeldii	1.63E-09	*	0.0463	*	-0.52	***	4.98E-09	*	0.1381	*	-0.07	
Thalassiosira rotula	2.91E-09	**	0.0235	**	-0.32	*	3.57E-09	*	0.0268	*	-0.15	
Flagellates (25 µm)	9.63E-09		0.0030		0.12		7.57E-10		-0.0015		-0.42	
Flagellates (20 µm)	3.11E-09		0.0012		-0.12		3.59E-09		0.0038		-0.31	
Flagellates (15 µm)	2.37E-10		0.0009		-0.44	**	2.17E-09	**	0.0041	*	-0.34	*
Flagellates (10 µm)	3.02E-09		0.0087		-0.29		1.94E-09		0.0048		-0.43	
Flagellates (5 µm)	-1.40E-09		-0.0141		-0.82	****	-4.13E-09		-0.0083		-0.87	****
TOTAL PHYTOPLANKTON	**1.63E-09**	***	**0.1730**	***	**-0.28**	**	**3.29E-09**	*	**0.3130**	*	**-0.28**	
RELATED TO TOTAL *TEMORA* CARBON	***F*** **0.17**		***I*** **17.87**				***F*** **0.34**		***I*** **32.32**			
Microzooplankton												
***Gyrodinium* spp. (30-75 µm)**	1.09E-08	**	0.0249	**	0.10		2.33E-09	**	0.030	**	-0.27	
***Gyrodinium* spp. (75-120 µm)**	9.17E-09	**	0.0029	**	0.34		9.68E-09	****	0.0055	***	0.41	***
***Protoperidinium* spp. (20-40 µm)**	-1.40E-09		-0.0001		-0.67	**	3.61E-09	*	0.0010		-0.23	
***Protoperidinium* spp. (50-80 µm)**	1.12E-08	**	0.0086	**	0.31		3.12E-09	*	0.0087	*	-0.20	
***Ceratium* spp.**	1.47E-08		0.0011		0.56		1.86E-09		0.0003		-0.43	
***Torodinium* spp.**	2.25E-09		0.0000		-0.33		-3.06E-10		0.0000		-0.70	**
other athecate dinoflagellates	1.72E-09		0.0020		-0.50	**	3.03E-09	*	0.0029	*	-0.16	
other thecate dinoflagellates	-2.72E-10		-0.0025		-0.88	****	2.02E-09	*	0.0147	*	-0.40	*
***Strombidium* spp. (25-40 µm)**	3.75E-09		0.0004		-0.25		7.22E-09	**	0.0010	**	0.19	
***Strombidium* spp. (40-110 µm)**	1.20E-08	***	0.0255	****	0.37	**	7.71E-09	****	0.1149	****	0.34	***
***Strobilidium* spp.**	8.87E-09	*	0.0044	*	0.04		3.45E-09	**	0.0201	**	-0.15	
Myrionecta rubra	9.16E-09	**	0.0189	****	0.23	*	1.63E-09		0.0055		-0.33	
other Cyclotrichids	0.00E+00		-		-		-		-		-	
Haptorids	9.03E-09	*	0.0007	*	-0.08		3.38E-09		0.0002		-0.16	
Tintinnids	-7.46E-10		0.0000		-0.40		5.07E-09	*	0.0012	**	0.08	
other Ciliates	-		-		-		-		-		-	
thecate amoeba sp.	3.13E-09	***	0.0064	***	-0.26	*	2.27E-09	*	0.0150	*	-0.30	
Rotifers	-		-		-		-		-		-	
TOTAL MICROZOOPLANKTON	**3.87E-09**	****	**0.0927**	****	**0.15**	**	**4.14E-09**	****	**0.2438**	****	**0.04**	
RELATED TO TOTAL *TEMORA* CARBON	***F*** **0.40**		***I*** **9.58**				***F*** **0.43**		***I*** **25.18**			

Table 4b

	Temora longicornis (TC 103.26 µg L^{-1})											
	Experiment 3						Experiment 4					
Phytoplankton	F_c	p	I_c	p	E*	p	F_c	p	I_c	p	E*	p
***Pseudonitzschia* spp. (120 µm)**	3.30E-09		0.0019		-0.31		1.24E-09		0.0009		-0.54	**
***Pseudonitzschia* spp. (80 µm)**	1.34E-09		0.0001		-0.49	*	8.48E-09	**	0.0003	**	0.16	
***Pseudonitzschia* spp. (60 µm)**	8.59E-10		0.0000		-0.60		5.56E-09		0.0000		0.00	
***Navicula* spp. (40 µm)**	7.16E-09	***	0.0002	**	0.22		2.22E-09		0.0001		-0.37	
***Navicula* spp. (20 µm)**	-5.06E-11		-0.0003		-0.65	**	-1.06E-09		-0.0002		-0.68	**
***Navicula* spp. (10 µm)**	4.33E-09	**	0.0014	*	-0.10		1.94E-09		0.0000		-0.54	
Chaetoceros danicus	1.72E-09		0.0025		-0.42		4.12E-09		0.0037		-0.16	
***Chaetoceros* spp. (40 µm)**	3.37E-09		0.0015		-0.35		4.61E-09		0.0011		-0.27	
***Chaetoceros* spp. (30 µm)**	-9.56E-10		-0.0098		-0.76	****	2.37E-09		0.0028		-0.41	
***Chaetoceros* spp. (20 µm)**	5.43E-09	***	0.0273	**	0.09		-9.27E-10		-0.0097		-0.72	***
***Chaetoceros* spp. (10 µm)**	-		-		-		-		-		-	
Rhizosolenia styliformis/hebetata	2.02E-10		0.0051		-0.73	***	3.44E-10		0.0022		-0.72	**
Rhizosolenia pungens/setigera	1.46E-09		0.0043		-0.45	*	4.03E-09	**	0.0057	*	-0.14	
Thalassiosira nordenskioeldii	4.33E-10		0.0011		-0.66	**	1.66E-09	*	0.0320	*	-0.42	*
Thalassiosira rotula	-1.60E-10		-0.0026		-0.60	*	2.31E-09		0.0057		-0.48	*
Flagellates (25 µm)	1.36E-08		0.0020		0.56		-1.02E-08		-0.0014		-1.00	
Flagellates (20 µm)	6.37E-10		0.0001		-0.41		2.36E-09		0.0005		-0.19	
Flagellates (15 µm)	4.11E-09		0.0006		-0.15		-1.11E-09		-0.0005		-0.70	**
Flagellates (10 µm)	-6.98E-10		-0.0006		-0.82	***	-1.57E-09		-0.0119		-0.86	****
Flagellates (5 µm)	2.66E-09	**	0.0326	**	-0.28		-1.70E-09	*	-0.0235	*	-0.95	****
TOTAL PHYTOPLANKTON	**9.83E-10**		**0.0912**		**-0.45**	*	**5.23E-10**		**0.0390**		**-0.74**	***
RELATED TO TOTAL *TEMORA* CARBON	***F*** **0.10**		***I*** **9.42**				***F*** **0.05**		***I*** **4.02**			
Microzooplankton												
***Gyrodinium* spp. (30-75 µm)**	1.46E-09	**	0.0217	**	-0.47	***	4.07E-09	**	0.0241	***	-0.15	
***Gyrodinium* spp. (75-120 µm)**	6.61E-09	****	0.0112	****	0.24	**	1.19E-08	***	0.0185	****	0.44	***
***Protoperidinium* spp. (20-40 µm)**	2.33E-09		0.0002		-0.46		1.42E-09		0.0001		-0.52	*
***Protoperidinium* spp. (50-80 µm)**	8.82E-09	**	0.0104	**	0.25		6.63E-09	*	0.0065	*	0.02	
***Ceratium* spp.**	4.86E-09		0.0005		-0.06		-3.82E-09		-0.0014		-1.00	
***Torodinium* spp.**	2.15E-09		0.0002		-0.54		-		-		-	
other athecate dinoflagellates	-		-		-		-		-		-	
other thecate dinoflagellates	-1.99E-10		-0.0008		-0.34		5.67E-10		0.0000		-0.63	*
***Strombidium* spp. (25-40 µm)**	1.30E-08		0.0005		0.42		-		-		-	
***Strombidium* spp. (40-110 µm)**	1.41E-09		-0.0182		-0.53		1.79E-09		-		-	
***Strobilidium* spp.**	2.13E-09		0.0090		-0.38		3.90E-09		0.0001		-0.18	
Myrionecta rubra	3.26E-09	*	0.0017		-0.20		4.74E-09		-0.0007		-0.14	
other Cyclotrichids	8.04E-09		0.0019		0.00		-		-		-	
Haptorids	5.23E-09	**	0.1175		-0.10		2.40E-08		0.0209		0.19	
Tintinnids	6.71E-09		0.0006		0.24		-		-		-	
other Ciliates	2.62E-09		-0.0002		-0.05		9.88E-10		0.0031		-0.58	**
thecate amoeba sp.	1.22E-09	*	0.0049	*	-0.52	**	2.44E-09	***	0.0041	***	-0.33	*
Rotifers	3.22E-09		0.0050		-0.22		1.78E-08	***	0.0744	***	0.53	***
TOTAL MICROZOOPLANKTON	**3.69E-09**	***	**0.2789**	**	**0.18**	*	**7.54E-09**	****	**0.1722**	****	**0.28**	****
RELATED TO TOTAL *TEMORA* CARBON	***F*** **0.38**		***I*** **28.80**				***F*** **0.78**		***I*** **17.78**			

CHAPTER IV

They can promote their competitors - commensalism between microzooplankton predators

They can promote their competitors - commensalism between microzooplankton predators

(to be submitted)

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ABSTRACT

The intra-specific interactions between two competing microzooplankton predators in a three species model system were investigated experimentally. The large tintinnid *Favella ehrenbergii* and the small heterotrophic dinoflagellate *Gyrodinium dominans* both prey on the phototrophic dinoflagellate *Scrippsiella trochoidea*. The experimental system included the possibility of "intraguild" predation since the smaller predator was also a potential prey item for *F. ehrenbergii*. We followed the development of the three species in treatments containing either one of the two predators or both together with the prey. As the only predator on *S. trochoidea*, *F. ehrenbergii* grew at a mean rate of 0.77 d^{-1} and *G. dominans* grew at a mean rate of 0.32 d^{-1}. *F. ehrenbergii* growth rate did not differ between single predator treatments and treatments with both predators (0.77 d^{-1}). In treatments containing only the two predators without the autotrophic prey, high *F. ehrenbergii* grazing on *G. dominans* was detected. However, in the treatments with all three species, *G. dominans* displayed significantly higher growth rates although the second predator was present (0.42 d^{-1}). To test the mechanisms responsible for this increase in growth rate, we investigated whether chemical communication processes played a role. Exposing *G. dominans* to exudates of *F. ehrenbergii*, showed that this was not the case as neither swimming speed of the small predator nor of the prey changed in the presence of *F. ehrenbergii* or its exudates. Observation of *F. ehrenbergii* cultures revealed that the tintinnid egests a significant proportion of the catch after initial uptake again. In this way *S. trochoidea* cells are immobilized by *F. ehrenbergii* at a rate of 1.4 immobile cells predator^{-1} h^{-1}. This corresponds to an immobilization of around 26% of the cells caught by the tintinnid. Results from experiments with artificially immobilized *S. trochoidea* showed that *G. dominans* benefits from these immobilized prey resulting in higher growth rates. The dinoflagellate was shown to positively select for immobile prey cells. As both predators co-occur in the same environment from a spatial and temporal point of view the feeding relationship between the two competing predators should increase exploitation efficiency of common mobile prey items. Their commensalistic interaction potentially opens a loophole for the stable coexistence of both predators.

Keywords: intraguild predation, competition, ciliates, dinoflagellates, commensalism, interaction

INTRODUCTION

One basic element of interest in ecology is the relationships of organisms and their interactions (Begon et al., 2006). The most obvious interaction between two predators preying on the same limiting resource is competition whereby both can be negatively affected by each other and the more competitive predator theoretically outcompetes the less competitive one (Gause's law) (Gause, 1934). However, such competitive exclusion is actually rarely observed in natural ecosystems, and many biological communities appear to violate Gause's law, which led to Hutchinson's term "paradox of the plankton" (Hutchinson, 1961). Meant was the fact that in the seemingly homogenous environment of the world's oceans an incredibly high number of phytoplankton species coexist, despite the fact that they all compete for the same limiting resources (e.g. CO_2, light and nutrients). One solution to this paradox is externally imposed variability in the surrounding environment, such that the systems are never in equilibrium. Another important factor may be found in the trophic interaction between phytoplankton and zooplankton, with typical oscillatory (Scheffer et al., 2003) or even chaotic population cycles (Beninca et al., 2008). Although considerable work has been carried out on predation in single predator-prey relationships of planktonic species, less is known about inter-specific interactions between planktonic predators. Whereas mesozooplankton refers to larger metazoan grazers, microzooplankton is the size fraction of heterotrophic planktonic organisms between 20 and 200 µm. Microzooplankton has recently gained attention as the main grazer in the oceans, capable of grazing up to 60-75% of the daily phytoplankton production (Landry & Calbet, 2004). Interestingly, almost nothing is known about the inter-specific interactions of predators within the microzooplankton. Although it includes a large variety of taxonomic groups, the most important ones in terms of abundance are heterotrophic dinoflagellates and ciliates (Capriulo et al., 1991).

Generally, the majority of marine planktonic ciliates feed on prey which is about one tenth of their own size (Spittler, 1973, Heinbokel, 1978a, Jonsson, 1986) and mainly nanoflagellates of different taxonomic groups. In contrast, heterotrophic dinoflagellates seem to prefer food of their own size (Hansen, 1992) and they also can feed on prey that is actually larger than the predator (Naustvoll, 2000a, Naustvoll, 2000b). Large heterotrophic dinoflagellates thus tend to feed on larger planktonic organisms (e.g. chain-forming diatoms), while small heterotrophic dinoflagellates feed mainly on nanoflagellates. As a result of their preferred prey size, small heterotrophic dinoflagellates potentially compete with larger planktonic ciliates for prey (Jakobsen &

Hansen, 1997). In turn, small dinoflagellates are themselves within the optimal size range of prey preferred by larger ciliates. Thus, small dinoflagellates may not only compete for food with large ciliates but are also potential prey for them.

Such "intraguild" predation (Polis & Holt, 1992), here using the term in a broader sense for all taxa in a community that compete for similar resources regardless of different tactics in resource exploitation (Polis et al., 1989), is the combination of two basic inter-specific interactions: Predation and competition. The immediate energetic benefit for the killing predator distinguishes intraguild predation from "classic" competition. Furthermore, it differs from classic predation by directly reducing potential competitors. Therefore, killing and eating of species that use the same limiting food resources is more complex than either competition or predation alone (Polis et al., 1989). Predation on smaller predators which are competitors for the same food source, so called "closed loop omnivory" (Sprules & Bowerman, 1988) is ubiquitous in the world's food webs (Polis et al., 1989). However, such investigations in the microzooplankton community of the marine food web are still scarce.

The large ciliate *Favella ehrenbergii* co-occurs with the smaller heterotrophic dinoflagellate *Gyrodinium dominans* in the microzooplankton community of the North Sea, both spatially and temporally, despite the fact that *Favella ehrenbergii* is a predator on *Gyrodinium dominans.* Furthermore, both predators prey on the same type of prey. In this study, we set out to investigate the mechanisms that can explain this coexistence, using the small autotrophic dinoflagellate (*Scrippsiella trochoidea*) as prey. We addressed the following questions: (1) Is there any kind of measureable interaction between both predators, if so, (2) how can this interaction be categorized, (3) what are the mechanisms that drive the interaction?

MATERIAL AND METHODS

Cultures

Cultures of the heterotrophic dinoflagellate *Gyrodinium dominans* (~30 µm length), the tintinnid *Favella ehrenbergii* (~160 µm length) and the phototrophic dinoflagellate *Scrippsiella trochoidea* (~20 µm length) were obtained from isolates of 20 µm net samples from Helgoland Roads, Germany (54°11.3'N; 7°54.0'E). *S. trochoidea* was isolated in May 2007 and grown in F/2 medium (Guillard & Ryther, 1962) without silicate using double sterile-filtered seawater in sterile six well plates at 14.5°C and an illumination of ~40 µmol photosynthetically active radiation (PAR) at constant light conditions. Isolates were checked for contamination and transferred into 73.5 mL tissue

culture bottles. The culture of *G. dominans* was established in July 2007 by adding *S. trochoidea* as prey to a 20 µm net sample and incubating it on a plankton wheel. After *G. dominans* started to grow in this raw sample, cells were isolated and transferred into 73.5 mL tissue culture bottles containing F/2 medium and their prey organism *S. trochoidea*. The culture of *F. ehrenbergii* was established in October 2007 by adding isolated *F. ehrenbergii* cells to culture bottles containing F/2 medium and the prey *S. trochoidea*. Cultures without contaminations were realised by repeated re-isolation. Both predator species were maintained on a plankton wheel (1.1 rpm) at 14.5°C and constant illumination of ~30 µmol PAR in 73.5 mL tissue culture bottles without air bubbles. Culture bottles, food and media were renewed weekly. As the first *F. ehrenbergii* culture died after the first experiment a new culture was established in August 2009. This culture was used in the later experiments.

Biovolume estimation

Cell volumes of the three species were estimated from the linear dimensions of acid Lugol's fixed cells (2% final conc., n = 43-50) assuming geometric shapes according to Hillebrand et al. (1999). Cell volume was converted to wet weight assuming a specific gravity of 1 and is thereafter referred to as "biomass".

General patterns of interaction

This experiment aimed at determining population growth patterns of both predators as well as general effects of the presence of a second competitive predator within the microzooplankton predator system. Growth and grazing parameters of both predators were determined in single predator treatments and in a combined two predator treatment. In the single predator treatments the same biomass of the predators *F. ehrenbergii* (1.2 cells mL^{-1}) and *G. dominans* (270 cells mL^{-1}) was added to a starting prey concentration of *S. trochoidea* of ~590 cells mL^{-1} which is roughly equivalent to a bloom of this species (Qi et al., 2004). The predator biomass in the two predator treatment was the same as in the single predator treatments comprising equal proportions of *F. ehrenbergii* (0.6 cells mL^{-1}) and *G. dominans* (130 cells mL^{-1}). Predator cultures used for the experiment had reduced prey cells to a minimum but were not starved for more than one day. Predation of *F. ehrenbergii* on the smaller predator was investigated in an additional approach where only *G. dominans* (840 cells mL^{-1}) was offered to *F. ehrenbergii* in the same biomass as the prey organism *S. trochoidea*. Treatments comprising single species served as controls. The experiments were carried

out in F/2 medium over 72 hours in 73.5 mL tissue culture bottles as batch cultures with four replicates. Incubation conditions were the same as described for the predator cultures before. The total volume of each incubation bottle was fixed immediately with acid Lugol's solution at a final concentration of 2% for the determination of cell concentrations. Samples were taken at the start and after 24, 48 and 72 hours of incubation. To avoid density-dependent differences and allow comparability between the experiments we maintained start concentrations, incubation conditions and replication of this first experiment in the following experiments if not explicitly otherwise stated.

Specific interactions

As we observed that *G. dominans* responded to the presence of the larger tintinnid predator *F. ehrenbergii* we focused on this species interaction with the other predator when designing the following experiments.

Chemical stimulation and swimming behaviour

To test whether chemical compounds excreted by *F. ehrenbergii* influenced the growth of *G. dominans*, a well fed exponentially growing *F. ehrenbergii* culture (15 cells mL^{-1}) was filtered over 0.2 µm nylon filters (Falcon). 10 mL of filtrate, equalling a final *F. ehrenbergii* concentration of 2 cells mL^{-1}, was added to incubation bottles containing *G. dominans* and *S. trochoidea*. Controls received 10 mL of F/2 filtrate. As the enhancement of swimming speed can increase predator-prey encounter rates (Gerritsen & Strickler, 1977) and thus potentially promotes higher grazing rates, we also investigated the swimming behaviour and velocity of the prey organism *S. trochoidea* and the predator *G. dominans* in the presence and absence of compounds released by *F. ehrenbergii* in this experiment. After exposure to the filtrate for 24 hours, *S. trochoidea* and *G. dominans* cells were filmed under a stereo microscope (SZX16, Olympus) at 50-fold magnification for 10 seconds at a rate of 15 frames per second. Samples for cell concentrations were fixed as described above at the start and immediately after filming.

Experiments on the pre-condition of the prey

From the detailed observation of a freshly fed *F. ehrenbergii* culture we found that not every *S. trochoidea* cell captured by the predator was actually ingested. A certain number of cells was egested again – becoming immobile after this "manipulation" by the predator. This has led to the hypothesis that *F. ehrenbergii* promotes growth in *G.*

dominans by immobilizing the potential prey for the smaller species, and thus making it easier to catch.

Prey immobilisation rate of Favella ehrenbergii

The rate of *S. trochoidea* prey cells not eaten but immobilized by *F. ehrenbergii* was experimentally determined after 24 and 48 hours of incubation. Differentiation between mobile and immobile *S. trochoidea* in fixed samples is not possible, thus after the incubation immobile cells were allowed to settle in the experimental bottles for 15 minutes. This timeframe was found to be sufficient for the complete sedimentation of the immobile fraction of *S. trochoidea* cells. To discriminate between mobile and immobile cells, a grid was defined and ten sites of the bottom surface of each experimental bottle were filmed for 5 seconds under conditions as described above. Cells that did not change their position during the film were counted as immobile ones. The concentration of immobile cells was calculated via the extrapolation of immobile cells of the filmed area to the whole bottom plate and thereafter to bottle volume. By dividing the mean immobile *S. trochoidea* concentration by the mean *F. ehrenbergii* concentration during the time of incubation (Frost, 1972, Heinbokel, 1978a) we calculated the immobilisation rate of *F. ehrenbergii* per day [cells immobilized predator^{-1} day^{-1}]. The percentage of immobilisation of cells caught by the tintinnid was also calculated. Samples for the determination of total cell concentrations were fixed as described above immediately after filming.

Growth and grazing response of Gyrodinium dominans *on immobilized prey*

To test if *G. dominans* benefits from immobile prey we investigated its growth when fed with artificially immobilized *S. trochoidea*. The immobilisation of prey took place in an ultrasound bath via sonication of *S. trochoidea* in six cycles, each cycle lasting three minutes. The ratio of immobile cells was determined via films as described above. We measured growth and grazing of *G. dominans* as well as selectivity for mobile or immobile prey after incubation for 24 hours with sonicated *S. trochoidea*. Untreated prey cultures served as controls. Samples for cell counts were fixed with acid Lugol's solution immediately before and after the time of incubation.

Commensalism experiment

A final experiment was designed to evaluate the results of the previous experiments. We investigated if the presence of a different newly isolated *F. ehrenbergii* culture led to

the same patterns in *G. dominans* in a three-species treatment as was found in the first experiment. Bottles containing only *S. trochoidea* and *G. dominans* served as controls. We also monitored the effect of the direct presence of the larger predator on swimming behaviour and speed of *S. trochoidea* and *G. dominans* after exposure to *F. ehrenbergii* for 24 hours. Fixation for cell counts with acid Lugol's solution took place before and immediately after the time of incubation and filming.

Counting

Samples were enumerated under a Zeiss Axiovert 135 inverted microscope using the Utermöhl method (Utermöhl, 1958). The samples were settled overnight and 4-6 circular transects of each settling chamber were counted at 200 or 400-fold magnification depending on cell concentration of *S. trochoidea* and *G. dominan*s. For *F. ehrenbergii* the whole chamber was enumerated. Apart from the first experiment *G. dominans* cells containing fresh food vacuoles (indicated by the dark colour of the vacuoles) were recorded separately and food vacuoles were used as a proxy for ingestion.

Calculations

Growth and grazing parameters

Growth rates k [d^{-1}] of *F. ehrenbergii*, *G. dominans* and *S. trochoidea* in the experiments were calculated assuming exponential growth. Grazing rates g [d^{-1}], specific filtration rates F [mL µg predator^{-1} d^{-1}] and specific ingestion rates I [prey cells predator^{-1} d^{-1}] of the predators were calculated according to Frost (1972) with the modification of Heinbokel (1978a) for the growth of predators. Predator biomass specific ingestion rates I_b [µg prey µg predator^{-1} d^{-1}] were calculated to allow for comparisons between the treatments by normalization of the specific ingestion rates to mean predator and prey biomass. Growth of phytoplankton is known to be density dependent. The *S. trochoidea* growth control in the general interaction experiment significantly exceeded the initial start concentrations after 24 and 48 hours of incubation. In contrast, the initial concentrations of *S. trochoidea* in the grazing treatments at these times were close to starting concentrations of the first day. We therefore always used the growth rate of *S. trochoidea* determined after the first day for our calculations in the general interaction experiment.

Selectivity and Electivity of G. dominans *for mobile and immobile prey*

The prey selectivity index α of *G. dominans* preying on a population of *S. trochoidea* containing both mobile and immobile prey was calculated for each prey type according to Chesson (1978, 1983). We chose Chesson's case 1 equation (prey population assumed to be constant) (Chesson, 1983) because our values of ingestion and percentage of prey in the environment were obtained by averaged prey concentrations and a strong depletion of food was not observed during our experiments. Values of α were then used to calculate the electivity index E* according to Vanderploeg and Scavia (1979a, 1979b).Values of E* cover a range from -1 to 1. E* values of 0 indicate non selective feeding, values > 0 indicate preference, values < 0 indicate discrimination against a prey type.

Swimming behaviour and velocity

The films of swimming behaviour were converted to single frame pictures using the freeware program "Avi4Bmp" (Bottomap Software). The first 30 pictures (equalling two seconds of each original film) were stacked with the function "overlay frames" of the freeware program "Trace" (© Heribert Cypionka, 2000-2010). These stacked images showed the path of a cell during the two-second period (Fischer & Cypionka, 2006). The length of the path of 60 cells per treatment was measured with the open source software "ImageJ". Swimming speed [$\mu m\ s^{-1}$] of the cells was then calculated by dividing path length by the timeframe needed for it. Changes in swimming behaviour were analysed by comparing the patterns of the swimming paths in the stacked pictures.

Data analysis

Statistical analyses were conducted with the software "Statistica 7.1" (StatSoft) using two-tailed t-tests and one way ANOVAs followed by Newman-Keuls post hoc tests both at significance levels of 0.05.

RESULTS

General patterns of interaction

In the first experiment we investigated the differences in successive patterns between treatments with one predator species and treatments with two predator species preying on the same organism.

Both predator species preying on *Scrippsiella trochoidea* displayed positive growth rates in the single predator treatments throughout the whole experiment (Figure 1a+b).

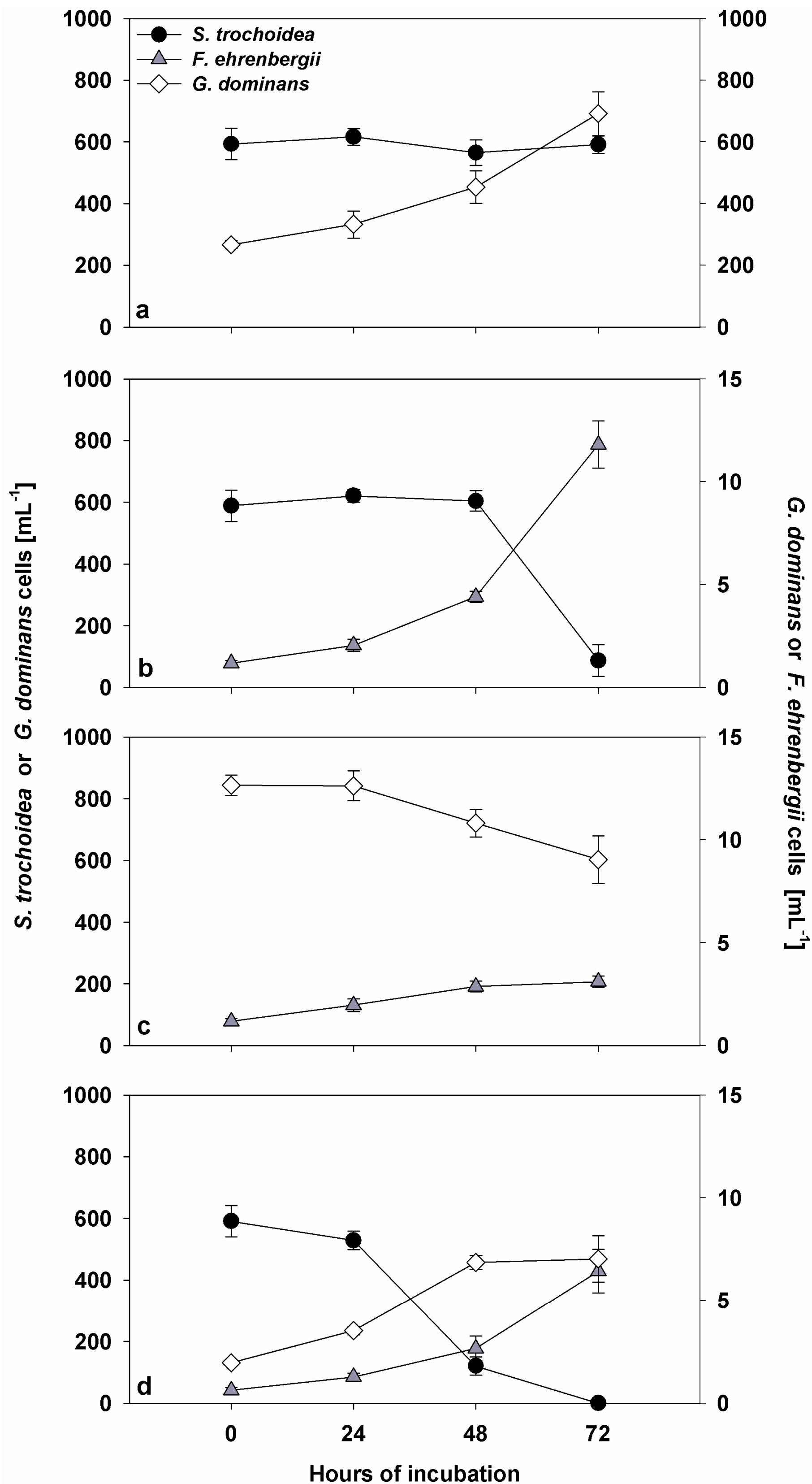

Figure 1: General development in abundance [cells mL^{-1}] of the predators *Gyrodinium dominans* and *Favella ehrenbergii* and their prey *Scrippsiella trochoidea* in single predator (1a+b+c) and two predator treatments (1d) during the time of incubation. Error bars correspond to one standard deviation, n = 4.

Gyrodinium dominans (Figure 1a) grew at a mean rate of 0.32 d^{-1} during the three days in the single predator treatment and continuously increased its growth rate from 0.21 to 0.42 d^{-1} (Figure 2a). The specific ingestion of the dinoflagellates decreased from 0.9 to 0.5 cells predator^{-1} d^{-1} (mean 0.7 cells predator^{-1} d^{-1}) during the experiment. With a mean grazing rate of 0.48 d^{-1} (0.44-0.57 d^{-1}) *G. dominan's* consumption averaged the production of *S. trochoidea* (mean growth rate 0.45 d^{-1}) and the predator was not able to graze down its prey substantially during the experiment. Mean predator specific filtration rates recorded for *G. dominans* ranged between 0.31 and 0.59 mL µg predator^{-1} d^{-1} (mean value 0.49 mL µg predator^{-1} d^{-1}).

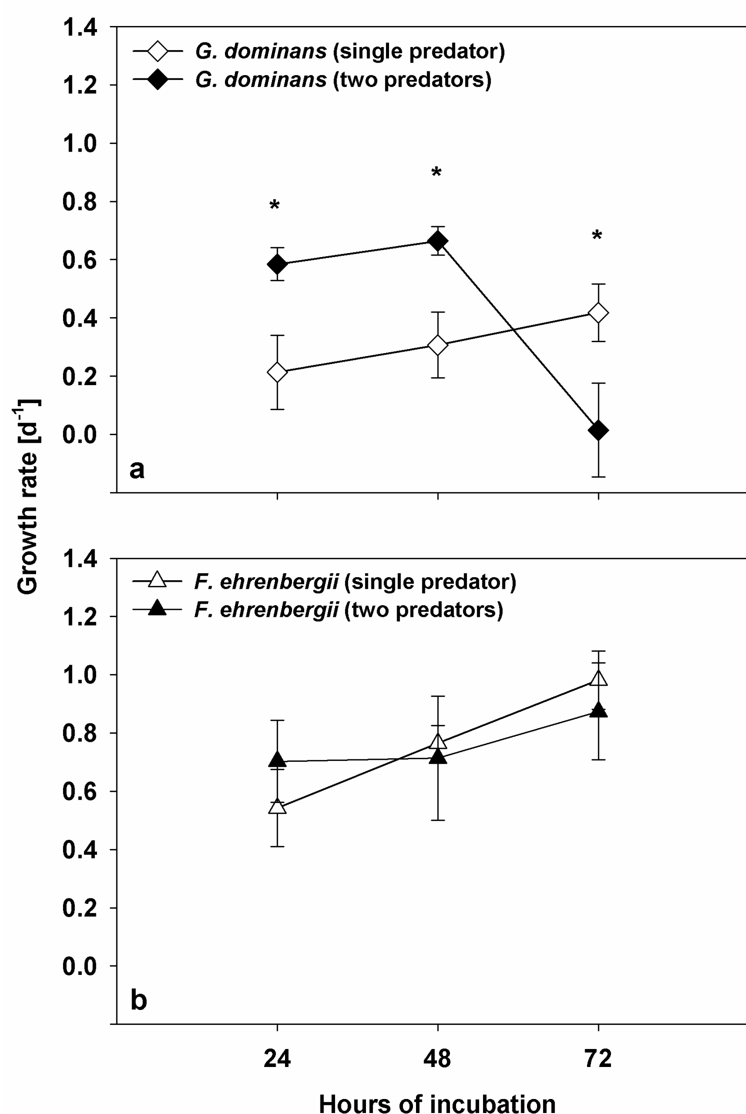

Figure 2: Growth rates [d^{-1}] of both predators (2a: *Gyrodinium dominans*, 2b: *Favella ehrenbergii*) preying on *Scrippsiella trochoidea* in single predator and two predator treatments after 24, 48 and 72 hours of incubation; calculated for 24 hour intervals. Error bars correspond to one standard deviation, n = 4. Significant differences are marked by asterisk (two-tailed t-tests).

Favella ehrenbergii (Figure 1b) generally grew faster ($t = 6.27$, $df = 22$, $p < 0.001$, two-tailed t-test) in the single predator treatment when compared to *G. dominans* (Figure 2b). Mean growth rates of the tintinnid increased constantly from 0.54 to 0.98 d^{-1} and were on average 0.77 d^{-1}. Specific ingestion rates dropped from 165 to 86 cells predator^{-1} d^{-1} and averaged 64 cells predator^{-1} d^{-1} during the course of the experiment. As found for *G. dominans* the grazing rates of *F. ehrenbergii* hardly exceeded the growth rates of *S. trochoidea* for the first two days (0.43 and 0.51 d^{-1}). However, during the last day of the experiment as a result of the increasing predator population the grazing rate amounted 2.55 d^{-1} leading to a sharp decline in the prey population. Predator specific filtration rates recorded for *F. ehrenbergii* were not different ($t = 0.05$, $df = 22$, $p = 0.96$, two-tailed t-test) from those recorded for *G. dominans* and ranged between 0.31 and 0.64 mL µg predator^{-1} d^{-1} (mean value 0.49 mL µg predator^{-1} d^{-1}).
In treatments with *G. dominans* as sole prey for *F. ehrenbergii* (Figure 1c), the larger predator displayed a mean grazing rate of 0.20 d^{-1}. Although prey was always available at high concentrations, grazing rates decreased from higher values (0.21 and 0.34 d^{-1}) to a value of 0.04 d^{-1} on the last day of the experiment. This was also reflected by the specific ingestion: The ciliate initially ingested 115 and 111 cells predator^{-1} d^{-1} on the second day but then ingestion dropped to a value of 6 cells predator^{-1} d^{-1} (mean 70 cells predator^{-1} d^{-1}). Even if *F. ehrenbergii* ingested high numbers of prey cells it was not able to increase growth rates as with the prey *S. trochoidea.* Growth rates amounted to 0.32 d^{-1} on average and dropped from 0.50 to 0.08 d^{-1} during the experiment.
While both predators in the single predator treatments roughly only consumed the daily production of *S. trochoidea* until day three of the experiment, a completely different picture emerged in the two predator treatments (Figure 1d, 3). Although the initial predator and prey biomass were the same as in the single predator treatments, *S. trochoidea* biomass already decreased after 24 hours and was completely grazed down at the end of the experiment. Consequently, grazing rates in the two predator treatments were consistently higher (0.60 and 1.89 d^{-1}) than those measured in the single predator treatments on the first two days of the experiment (after 24 hours: $t = 5.91$, $df = 10$, $p < 0.001$, after 48 hours: $t = 16.50$, $df = 10$, $p < 0.001$, two-tailed t-tests: pooled g of single predator treatments against g in the two predator treatment). After 72 hours this effect was only measurable in one replicate due to the extinction of the prey (Figure 3).

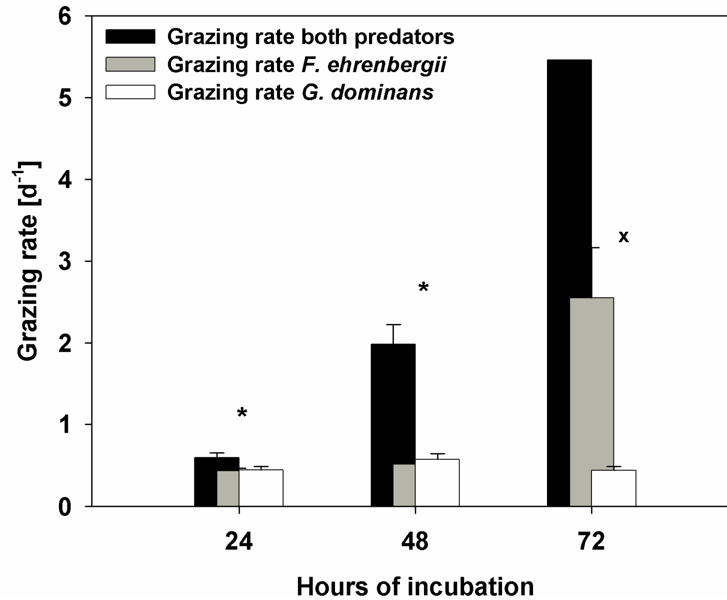

Figure 3: Grazing rates [d^{-1}] of *Gyrodinium dominans* and *Favella ehrenbergii* on *Scrippsiella trochoidea* in single predator treatments and two predator treatments after 24, 48 and 72 hours of incubation; calculated for 24 hour intervals. Error bars correspond to one standard deviation, n = 4. Significant differences between single predator treatments and two predator treatment are marked by asterisk (two-tailed t-tests). x: No comparison possible as grazing was measurable only in one replicate of the two predator treatment while the prey population was grazed down totally in the others.

Despite higher grazing rates, biomass specific ingestion rates I_b were not different between the single predators nor between single predator treatments and two predator treatment (*G. dominans*: mean 0.98 µg $µg^{-1}$ d^{-1}, *F. ehrenbergii*: mean 0.85 µg $µg^{-1}$ d^{-1}, two-predator treatment: mean 0.97 µg $µg^{-1}$ d^{-1}, $F_{2,9} = 1.18$, $p = 0.35$, ANOVA) after the first 24 hours of the experiment. Keeping in mind that the start concentrations of cells were the same, this indicates that the higher grazing rates in the two predator treatments were due to the faster growth of predator biomass only. After 48 hours I_b differed between all treatments (*G. dominans*: mean 0.92 µg $µg^{-1}$ d^{-1}, *F. ehrenbergii*: mean 0.52 µg $µg^{-1}$ d^{-1}, two-predator treatment: mean 0.80 µg $µg^{-1}$ d^{-1}, $F_{2,9} = 100.95$, $p < 0.001$, ANOVA). No difference was found between both predators at the end of the experiment (*G. dominans*: mean 0.48 µg $µg^{-1}$ d^{-1}, *F. ehrenbergii*: mean 0.44 µg $µg^{-1}$ d^{-1}, t = 1.45, df = 6, p = 0.20, two-tailed t-test) whereas a comparison of I_b with the two predator treatment was not possible due to the extinction of prey.

We observed no difference in the growth rates of *F. ehrenbergii* between the single and two predator treatments when looking at the single sampling days (Figure 2b)

(24, 48, 72 hours of incubation: df = 6, t = 1.66, 0.46, 1.11, p = 0.15, 0.66, 0.31, two-tailed t-tests). This was also reflected in the mean growth rate of 0.77 d^{-1} over 72 hours of incubation in both cases. A completely different picture was observed in the smaller predator *G. dominans* as it always showed significantly different growth rates at each sampling day (24, 48, 72 hours of incubation: df = 6, t = 5.32, 5.81, 4.28, p = 0.002, 0.001, 0.005, two-tailed t-tests) in the two predator treatments compared to the single predator treatments (Figure 2a). Growth rates were twice as high as in the single predator treatments (mean 0.58 and 0.66 d^{-1}) for the first two days. This was surprising as predation on *G. dominans* by the larger predator *F. ehrenbergii* has been shown. During the last 24 hours of the experiment growth of *G. dominans* dropped to a mean value of 0.01 d^{-1} along with the complete disappearance of the prey *S. trochoidea.* As our starving control of *G. dominans* displayed positive growth rates after the first two days of starvation (mean value 0.20 d^{-1}) we conclude that this drop was due to pronounced feeding of *F. ehrenbergii* on *G. dominans* when *S. trochoidea* disappeared as potential prey during day three of the experiment.

Chemical stimulation of *G. dominans* by *F. ehrenbergii*

We exposed *G. dominans* for 24 hours to a filtrate of *F. ehrenbergii*. Measured growth rates in treatments with filtrate (mean: 0.04 d^{-1}) and in the control (mean: 0.1 d^{-1}) were lower than those observed in the first experiment but were statistically not different from each other (Figure 4a) (t = 1.85, df = 6, p = 0.11, two-tailed t-test). The same pattern was found for ingestion rates (t = 0.52, df = 6, p = 0.62, two-tailed t-test). *G. dominans* only showed a weak ingestion in treatments that received filtrate (mean 0.06 prey cells $predator^{-1}$ d^{-1}) as well as in the control (mean 0.02 prey cells $predator^{-1}$ d^{-1}) (Figure 4a), which was probably due to differences in predator condition compared to the first experiment. We additionally investigated swimming patterns and velocity during this experiment for both, the predator and the prey. There were no visible differences in the swimming patterns of both species, when looking at the paths of the cells. Swimming velocity was not different between treatments with and without filtrate of *F. ehrenbergii* (*G. dominans*: t = 0.45, df = 118, p = 0.66, *S. trochoidea*: t = 0.25, df = 118, p = 0.80, two-tailed t-tests). It differed significantly between both species (*G. dominans*: 177 µm s^{-1}, *S. trochoidea* 414 µm s^{-1}, t = 26.14, df = 238, p < 0.0001, two-tailed t-test).

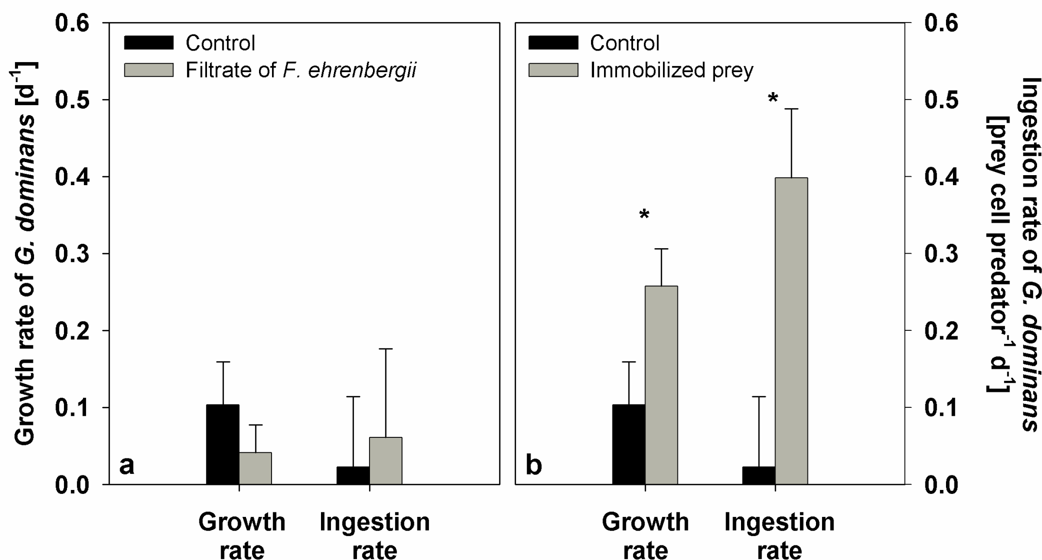

Figure 4: Growth rates [d^{-1}] and ingestion rates [prey cell predator^{-1} d^{-1}] of *Gyrodinium dominans* preying on *Scrippsiella trochoidea* when exposed for 24 hours to (4a) a filtrate of *Favella ehrenbergii* or to artificially immobilised prey (4b). Error bars correspond to one standard deviation, n = 4. Significant differences between treatments and the control are marked by asterisk (two-tailed t-tests).

Prey immobilisation by *Favella ehrenbergii*

During the time of incubation the amount of immobile cells in the control treatment was 3% after 24 hours and 8% after 48 hours and therefore always below 10% of the population. However, this proportion in the presence of *F. ehrenbergii* was 24 and 26% respectively, resulting in an additional 21 and 18% of immobilized prey by the predator. The amount of immobile prey cells in the presence of the tintinnid was always significantly higher both with regard to the total number of immobile cells mL^{-1} (24 hours: $t = 11.34$, $df = 6$, $p < 0.0001$, 48 hours: $t = 5.39$, $df = 6$, $p = 0.002$, two-tailed t-tests) and relative shares to the total number of cells mL^{-1} when compared to the control (24 hours: $t = 29.83$, $df = 6$, $p < 0.0001$, 48 hours: $t = 11.34$, $df = 6$, $p < 0.0001$, two-tailed t-tests). We determined a mean immobilisation rate of 1.4 cells predator^{-1} h^{-1} for 24 and 48 hours, respectively. Using a different *F. ehrenbergii* culture, ingestion rates of the predator were similar to the first experiment and only decreased slightly from 102 to 90 cells predator^{-1} d^{-1}. For that experiment 26 ± 3% of the cells caught by the tintinnid were egested and immobilized.

Growth response of *G. dominans* to immobilised prey

Artificial immobilisation was achieved using sonication. This resulted in 58% immobile cells of the total prey cells at the beginning of the experiment (7% in control) of which after 24 hours 21% were still immobile due to sonication and only 3% in the control. While we measured low growth rates in the control treatment (mean: 0.10 d^{-1}) we detected significantly higher growth rates of *G. dominans* preying on immobilised prey (mean: 0.26 d^{-1}, $t = 4.17$, $df = 6$, $p = 0.006$, two-tailed t-test) (Figure 4b). This difference was even stronger for the ingestion rates ($t = 5.85$, $df = 6$, $p = 0.001$, two-tailed t-test) (Figure 4b). Those differed by a factor of 20 in the mean values and accounted for 0.4 prey cells $predator^{-1}$ d^{-1} in the immobilised prey treatment and only 0.02 prey cells $predator^{-1}$ d^{-1} in the control. This pattern was also reflected by the percentage of predators containing food vacuoles. Whereas only 15% of the predator community contained visible food vacuoles in the control almost every second predator cell (42%) contained visible food when offered prey with immobile cells in higher amounts. The finding of increased growth and higher ingestion when immobile prey was offered to *G. dominans* was also confirmed by prey selectivity. Selectivity patterns for mobile and immobile prey were significantly different ($t = 7.96$, $df = 6$, $p < 0.001$, two-tailed t-test). *G. dominans* selected for immobile *S. trochoidea* (E^*: 0.18-0.28) and it strongly avoided mobile prey cells (E^*: -0.67 - -0.29).

Commensalism experiment

This experiment served to confirm the findings of the previous experiments. As it was difficult to disentangle predation shares of each predator in our model system we focused on the food vacuole content of *G. dominans* as a proxy for ingested prey (Figure 5). We also investigated swimming behaviour and velocity of *S. trochoidea* and *G. dominans* when exposed directly to *F. ehrenbergii*. We found no differences between the control and treatments with *F. ehrenbergii* (*G. dominans*: $t = 0.97$, $df = 118$, $p = 0.34$, *S. trochoidea*: $t = 0.44$, $df = 118$, $p = 0.67$, two-tailed t-tests). No visible changes in swimming patterns were detected and swimming velocity again differed significantly between species (*G. dominans*: 167 $\mu m\ s^{-1}$, S. trochoidea 355 $\mu m\ s^{-1}$, $t = 23.34$, $df = 238$, $p < 0.0001$, two-tailed t-test).

The presence of *F. ehrenbergii* had a strongly significant effect on the food ingested by *G. dominans* shown by the percentage of individuals containing food vacuoles ($t = 6.42$, $df = 6$, $p < 0.001$, two-tailed t-test). While the percentage of individuals containing food vacuoles increased only slightly from 12 to 18% in the absence of *F. ehrenbergii* during

the time of incubation, the proportion increased from 13 to 46% when the second predator was present. This was similar to the results found in experiments with artificially immobilised prey. As we detected a mortality rate of around $-0.22\ d^{-1}$ in the presence of *F. ehrenbergii*, indicating predation on the smaller *G. dominans*, differences in food vacuole content could possibly have been resulting from selective predation of *F. ehrenbergii* on *G. dominans* without vacuole contents. This would have increased artificially the percentage of vacuole containing cells in the population. We therefore also looked at the total amount of *G. dominans* containing food vacuoles in the treatments. However, this hypothesis could then be rejected as there were also significantly higher total numbers of cells containing food vacuoles per mL in treatments where the larger predator was present when compared to the control ($t = 9.89$, $df = 6$, $p < 0.0001$, two-tailed t-test).

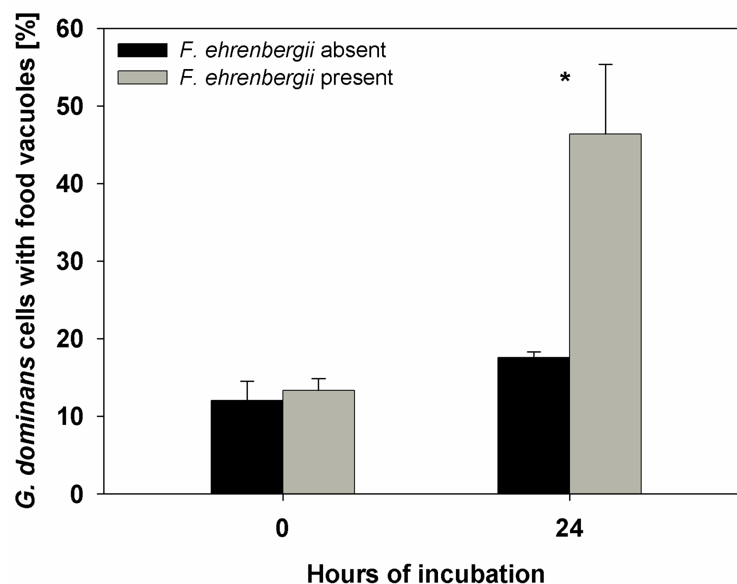

Figure 5: Percentage of the *Gyrodinium dominans* population containing food vacuoles in presence or absence of *Favella ehrenbergii* before and after incubation for 24 hours. Error bars correspond to one standard deviation, n = 4. Significant differences between treatments are marked by asterisk (two-tailed t-tests).

DISCUSSION

We investigated the interactions between two naturally competing microzooplankton predator species including the possibility of "intraguild" predation. Our results clearly show that the presence of the larger competitive predator *F. ehrenbergii* enhances ingestion in the smaller predator *G. dominans.* We showed conclusively that this was

due to pre-conditioning of the prey for *G. dominans* via immobilisation of the prey items by the tintinnid.

Enhanced "intraguild" predation?

From a theoretical perspective "intraguild" predation would be profitable for the top-predator in a double sense (Polis et al., 1989). First, the predatory competitor would benefit directly by ingesting the other predator as food and second, eating a competitor would indirectly release the predator from competition pressure. In our case *G. dominans* was also a potential prey organism for its larger competitor *F. ehrenbergii*. Food selectivity of *F. ehrenbergii* was not directly measurable in our two-predator treatment because we could not discriminate between the shares of each predator in the observed grazing rates. However, given the highest growth rates (first experiment) of *G. dominans* measured throughout our experiments when *F. ehrenbergii* was present it seems reasonable that *F. ehrenbergii* did not feed exclusively on *G. dominans*. Although not directly measured we can at least assess the likelyhood of predation of *F. ehrenbergii* on *G. dominans*. The growth rate of *G. dominans* was promoted by a factor of 2.6 when fed artificially immobilised prey. Applying this factor to its growth in treatments of the first experiment containing only *G. dominans* as a single predator of *S. trochoidea* provides an estimate on how *G. dominans* growth would have resulted, taking into account the positive effect of immobilized prey in the absence of the predation by *F. ehrenbergii*. Using this estimate we could predict the observed growth rates of *G. dominans* in the two predator treatment after 24 hours quite well (i.e. real growth mean: 0.58 d^{-1}, estimate mean: 0.55 d^{-1}). The estimate indicated predation on *G. dominans* after 48 and 72 hours as predicted growth was much higher than the observed one. However, as food density effects were not included, this has to be seen as a fairly rough result. Nevertheless, predation has been shown in the treatments with *G. dominans* as the only prey organism for the tintinnid. Whereas it first ingested *G. dominans* in numbers comparable to the other prey organism *S. trochoidea*, *F. ehrenbergii* ingestion and also growth declined to a minimum even when prey was still available. One possible explanation could be that predation pressure induced predator avoidance mechanisms in *G. dominans*. Increased escape velocity as reported for other dinoflagellates (Jakobsen et al., 2006) seems unlikely because we did not detect any change in swimming speed or behaviour in the presence of the tintinnid. Toxicity is also not reported for *G. dominans* and other ciliate species are able to feed on this dinoflagellate without negative effects for the predator (Jeong et al., 2004). In the past

feeding rates of *F. ehrenbergii* have been shown to be inhibited by a number of dissolved free amino acids (Strom et al., 2007b) which could theoretically also be released by *G. dominans*. However, in our work growth rates of the tintinnid in the presence of the smaller predator were the same as when preying on *S. trochoidea* alone and thus such a chemical influence could be rejected.

A different picture was observed in the last experiment with a new *F. ehrenbergii* culture. Here we detected a mortality rate of around -0.22 d^{-1} in the presence of *F. ehrenbergii* indicating predation on the smaller *G. dominans*. However, a pronounced selective predation on *G. dominans* that would also promote the autotrophic prey *S. trochoidea* due to the partial release of grazing pressure (Stoecker & Evans, 1985) was not observed in our experiments.

Competitive predator relationship with a commensalistic element

Our findings are in contrast to results of another study where no difference in growth rates was found for a dinoflagellate or its potential ciliate predator competing for the same prey when compared to the single predator treatments (Jakobsen & Hansen, 1997) and another study where intraguild predation between the predators favoured the prey (Stoecker & Evans, 1985). Even if both predators competed for the same prey organism in our experiments *G. dominans* was directly supported by the presence, especially by the feeding, of the other predator leading to a higher efficiency in resource exploitation. This observed paradox could only be solved when looking at the feeding behaviour of *F. ehrenbergii*. The dinoflagellate directly benefited from immobilised but not ingested prey cells of the tintinnid. Benefits from "pre-conditioned prey" have been reported for dinoflagellates before, e.g. when feeding on faecal pellets of copepods (Poulsen & Iversen, 2008).

Although *G. dominans* can feed on different planktonic prey in the laboratory (Nakamura et al., 1995a) it is often highly abundant during red tides of mobile dinoflagellate prey (Nakamura et al., 1995b, Kim & Jeong, 2004). Interestingly, *G. dominans* selected strongly for immobilised dinoflagellates in our experiments even if mobile prey was available in the same concentration. This is most probably related to the feeding habit of *G. dominans*. *Gyrodinium* species display a smooth pre-capture swimming behaviour around the prey before it is captured and ingested (Hansen, 1992). Taking this habit and the swimming speed of the prey organism *S. trochoidea* into account it is clear that immobile prey cells are easier captured by *G. dominans* even if there were higher encounter rates with swimming prey (Gerritsen & Strickler, 1977).

This was also confirmed by our own personal observations. *G. dominans* cultures always grew best on old *S. trochoidea* batch cultures that started to form immobile cysts. The immobilisation of prey by *F. ehrenbergii* facilitates prey ingestion of the dinoflagellate without directly observable negative effects for the larger predator and therefore their interaction can be categorized as a competitive predator relationship with a commensalistic element**.**

Implications of the findings

Both predators occur at the same time in the field, especially when dinoflagellate prey is abundant (Buskey & Stoecker, 1989, Nakamura et al., 1995b). This has also been observed at Helgoland Roads during a bloom of *S. trochoidea* (pers. observation). Extrapolating our results to the field during a bloom of *S. trochoidea* when prey is available in high abundance, the predator relationship between *F. ehrenbergii* and *G. dominans* directly supports the fact that *G. dominans* can ingest more prey. This promotion consequently causes a faster growth of *G. dominans* and thus an increase in grazer biomass. Finally more grazers lead to higher grazing rates and potentially a faster decline of the prey population. At times during a bloom when nutrients become limiting, *S. trochoidea* starts to form immobile cysts (Gottschling et al., 2005, Wang et al., 2007). These encysting immobile cells represent an "easier" food for *G. dominans*, when compared to fast swimming mobile *S. trochoidea* cells, and this fact has been personally observed during culturing of *G. dominans*. The additive availability of "easy" prey at the end of a bloom would enhance an already high *G. dominans* concentration even more.

Copepods are known to selectively feed on dinoflagellates (Gentsch et al., 2009), thus also heterotrophic dinoflagellates are an important food source for them. This is due to the "trophic upgrading" capability of heterotrophic dinoflagellates when feeding on nutritional "poorer" phytoplankton food (Klein Breteler et al., 1999). Consequently the availability of heterotrophic dinoflagellates can enhance the reproductive success in copepods (Tang & Taal, 2005). In turn, copepods are themselves an important part in the diet of fish larvae and are also crucial for their survival (Castonguay et al., 2008). A high availability of heterotrophic biomass due to the promotion of *G. dominans* could thus promote higher copepod densities and enhance the export of energy to higher trophic levels such as fish.

Finally: Can the interaction we observed here lead to a stable coexistence between the three species? There are several assumptions which need to be considered when

discussing the coexistence of "intraguild predator" and "intraguild prey" (Diehl & Feissel, 2000) with their communal prey in a three species system. The most crucial one is that the intraguild prey must be the superior competitor for the resource (Diehl & Feissel, 2001). We did not measure parameters that are necessary for the determination of superior resource competition in our system, but from our results for *G. dominans* as single predator it seems doubtful that this species is necessarily the superior competitor for the prey resource when compared with *F. ehrenbergii*, especially in terms of its low growth rates. The picture changes in the presence of the intraguild predator. Even though intraguild predation was possible the promotion of *G. dominans* by pre-conditioned prey was of such magnitude that the apparent growth rate of the dinoflagellate was not different from the growth rate of the intraguild predator any longer. This observation allows the prediction that *G. dominans* could be the superior competitor for the resource, but only in the presence of the other predator. Thus, the presence of the intraguild predator causes the intraguild prey to do better, which could lead to the coexistence of both predators in this system (Diehl & Feissel, 2001).

Outlook

Our results show that interactions within the microzooplankton community can be more complex than previously thought. To our knowledge, the results we presented here are unique for the reported microzooplankton predator interactions in marine systems. Further research is necessary to elucidate the diverse interactive patterns that can occur between various members of the microzooplankton and also to clarify their implications for food web interactions, particularly in looking at multiple predator-prey systems. Mathematical modelling is an important tool here, which could underpin the understanding of the impact of such inter-specific interactions. Especially the commensalistic aspect we observed, which could lead to the coexistence between intraguild predator and intraguild prey, deserves further experimental attention in marine systems.

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DISCUSSION

This thesis aimed at contributing deeper insights into the general role of microzooplankton in marine food webs, with an emphasis on heterotrophic dinoflagellates and ciliates, at the base of the food web in the North Sea at Helgoland. The most important findings which emerged from my research can be summarized as follows.

1. The monitoring showed that heterotrophic dinoflagellates and ciliates were the most important members among the microzooplankton community in the North Sea around Helgoland throughout the whole year and they showed a clear seasonality with maxima during summer and minima during winter.

2. The most appropriate technique for investigations on the grazing impact of microzooplankton, the dilution technique, was improved by an alternative filling technique that conserves the original diversity of the investigated microzooplankton community better than the standard method. This modified method is applicable for any experiment that requires microzooplankton sample collection.

3. Microzooplankton was a more important grazer than copepods during a phytoplankton spring bloom. Its selective grazing shaped the bloom assemblage towards three diatom genera and its grazing impact determined the end of the bloom. Copepods, in turn, selectively fed on microzooplankton and could therefore be regarded as the main "top down" controlling factor of microzooplankton.

4. Heterotrophic dinoflagellates and ciliates showed different patterns in succession during the spring bloom experiment which can be ascribed to their contrasting survival strategies. Their wide food spectrum and higher starvation tolerance facilitated a long lasting dinoflagellate bloom with a relatively uniform species composition, whereas ciliates had higher growth rates but a more restricted food spectrum as well as starvation tolerance. This fact led to a high but short ciliate bloom peak and a clear succession of different taxa.

5. Experimental examinations of the interactions between two microzooplankton predators in a three species "intraguild predation" system revealed that the inter-specific interactions between microzooplankton predators can be complex and cannot be

described by competition or predation alone. I detected a commensalistic relationship that potentially enables a coexistence of both competing predators. These results demonstrated the great need for further research in the field of inter-specific interactions between members of the microzooplankton in the planktonic food web.

On the following pages these major findings will be discussed in a more general context.

The significance of heterotrophic dinoflagellates and ciliates

Microzooplankton consists of a diverse array of zooplankton grazers smaller than 200 µm that play an important role in the marine food web. Although new insights into the pathways and rates comprising trophic interactions are published regularly, a full understanding of microbial food webs is hampered by the small sizes of the players (Moline et al., 2008). Futhermore, investigations on microzooplankton are aggravated by their varied and variable trophic strategies, including autotrophy, heterotrophy, and mixotrophy and not least by the difficulties in culturing microzooplankton (Sherr & Sherr, 2002).

The principal goal of this thesis was to gain fundamental knowledge about the most important microzooplankton groups, dinoflagellates and ciliates (Capriulo et al., 1991), in the North Sea. Although phytoplankton dynamics in the North Sea were studied quite frequently and for longer time periods (Cadee & Hegeman, 2002, Wiltshire & Dürselen, 2004, Gowen & Stewart, 2005, Muylaert et al., 2006, Bresnan et al., 2009, Peperzak, 2010), combined data sets on heterotrophic dinoflagellates and ciliates at a high taxonomic resolution are not available. Thus, a monitoring was started on a weekly basis to elucidate species composition, abundance and seasonal succession of both groups at Helgoland Roads (Chapter I) and to supplement the regular plankton monitoring series with new data (ciliates). It was furthermore aimed at revealing "key" model species that are of functional importance in the North Sea. Cultures of these species served as the basis for detailed laboratory experiments on grazing and interaction of predators (compare Chapter IV).

My results from a 2.5-year period show the importance of dinoflagellates and ciliates as the major microzooplankton groups in the North Sea around Helgoland (Chapter I). Other microzooplankters (nauplii, rotifers, larvae, amoebae, silicoflagellates and ebriaceae) were only observed occasionally during the monitoring. Dinoflagellates and ciliates played a key role at Helgoland in terms of their biomass contribution (Chapter I)

and showed a clear seasonality with minima during winter and maxima during summer. The heterotrophic part of both groups contributed to the planktonic biomass with carbon concentrations ranging from 2 to 652 µg L^{-1}. Including also mixotrophic species yielded maxima of 779 µg L^{-1}. These concentrations are in a typical range for the North Sea (Riegman et al., 1993, Brussaard et al., 1995).

The community I observed was highly diverse, with 62 dinoflagellate taxa and 63 ciliate taxa typical for the North Sea (Riegman et al., 1993, Brussaard et al., 1995, Hoppenrath, 2004, Stelfox-Widdicombe et al., 2004). The numerically most important heterotrophic dinoflagellates were species of the genera *Gyrodinium* and *Protoperidinium*, while *Noctiluca scintillans* contributed large proportions to the biomass. Mixotrophic dinoflagellates of several genera partly also reached a high planktonic biomass during blooms. The most important ciliates, in terms of abundance, were species of the genera *Strombidium* and *Strobilidium*, the functionally phototrophic *Myrionecta rubra* and tintinnids. Large *Cyclotrichium* spp. contributed substantially to ciliate biomass. The data on ciliates presented in Chapter 1 are completely new for Helgoland Roads, whereas dinoflagellates have been counted since 1962 (Wiltshire & Dürselen, 2004). However, these records do not mirror the natural diversity (Hoppenrath, 2004). My monitoring aimed at a higher taxonomic resolution in dinoflagellates and ciliates compared to the Helgoland time series on plankton. This could partially be achieved (Chapter I) (*Protoperidinium* spp., ciliates). The longer monitoring period compared to other studies (Riegman et al., 1993, Brussaard et al., 1995, Stelfox-Widdicombe et al., 2004) also revealed general patterns in the seasonality of both groups.

However, there is still a great need for further work on this topic at Helgoland Roads and a microzooplankton monitoring aiming at a higher taxonomic resolution should be included into the long-term observation for detailed insights into the species composition. Due to the methodology used (Lugol fixation and light microscopy) many cells could be identified to genus level only or were assigned to morphologically similar groups (Johansson et al., 2004). Especially naked ciliates and dinoflagellates should receive further attention. Elucidating the taxonomy of these groups by applying live observation, epifluorescence (Elbrächter, 1994, Kraberg et al., 2010) and scanning microscopy techniques (Hernandez-Becerril et al., 2010), as well as staining techniques (Agatha & Tsai, 2008) is feasible. Applying these methods in combination with molecular methods (Sherr & Sherr, 2002) provides further insights into the taxonomy of dinoflagellates (Gottschling et al., 2005) and ciliates (Agatha, 2004). For gaining fundamental knowledge on the role of microzooplankton in the food web we first have

to know who they are. Therefore, investigations on species composition and seasonality as presented in this study are imperative for a basic understanding of processes within the planktonic food web and should be extended especially on locations providing excellent background data as the Helgoland time series.

Methods for grazing experiments with microzooplankton grazers

Grazing plays a major role in the fate of biomass in the oceans (Moline et al., 2008). The measurement of grazing rates is thus crucial for the understanding of the flow of matter in the marine pelagic food web. Since microzooplankton was recognized to be one driving factor in the consumption of phytoplankton diverse techniques to measure the grazing impact of microzooplankton have been developed and a multitude of techniques for the investigation of protozoan feeding is available (Kivi & Setälä, 1995). Direct methods measure food uptake in the predators, indirect methods measure the disappearance of food from the environment (see introduction for a more detailed description). Both types of methods mostly involve an artificial alteration of natural food web relationships. One aim of this thesis was to find the most appropriate method for the measurement of the grazing under such "natural" conditions.

Laboratory grazing experiments are restricted to simple model systems containing only a few different organisms and are biased towards culturable species (Heinbokel, 1978a, Jonsson, 1986, Hansen, 1992, Jeong et al., 2004). Alternative techniques dealing with natural microzooplankton assemblages mostly make use of artificial food particles (Heinbokel, 1978b, Kivi & Setälä, 1995) or extrapolate from laboratory-determined feeding relationships to field situations (Heinbokel & Beers, 1979). Although important for the understanding of basic mechanisms, e.g. functional and numerical responses of predator species, all these different techniques manipulate natural conditions or neglect food web interactions.

Microzooplankton dilution experiments provide us with an alternative to determine grazing rates of microzooplankton by indirect, labour-intensive, and taxonomically selective techniques (Landry & Hassett, 1982, Calbet & Landry, 2004). The dilution technique also includes the simultaneous measurement of specific growth rates of the phytoplankton along with the specific grazing rate of the microzooplankton. There are, however, methodological restrictions which have to be taken into account (Gallegos, 1989, Landry et al., 1995, Dolan, 2000, Moigis, 2006, Teixeira & Figueiras, 2009). The most crucial restrictions are related to the theoretical assumptions the dilution method is based on (see introduction for detailed information). To overcome for, e.g., nutrient

limiting conditions, nutrients are added in excess to the dilution series and unfertilized bottles serve as control for the calculation of the natural phytoplankton growth rate (Landry, 1993). Sometimes the feeding response of the microzooplankton is not linear due to food saturation at lower dilution levels. In these cases, additional results from higher dilution levels at which feeding is still linear can be applied to estimate the grazing rate (Gallegos, 1989, Gallegos et al., 1996).

While recognizing the restrictions of the dilution technique, it still has the fundamental advantage of barely altering natural prey and grazer communities and only excluding larger zooplankton. Thus, natural interactions within the plankton community are included in dilution experiments. This technique is now standard for assessment of *in situ* grazing rates of microzooplankton and was also used during this study (see Chapter III).

One principal restriction of dilution experiments was addressed during this thesis: Several microzooplankton species are highly fragile and sensitive to handling. Filling and mixing procedures during experiments (Gifford, 1985, Landry, 1993, Broglio et al., 2003) can cause considerable losses in those species. Based on this fragility non-destructive methods have been developed to prevent the loss of sensitive species. However, these methods which were meant to handle microzooplankton with greater care have never truly been evaluated in an experimental set-up.

Results presented in Chapter II show that even techniques previously considered conservative for microzooplankton species can have significant negative impacts on their abundance and diversity. Handling procedures are always necessary during laboratory experiments and especially dilution experiments require several treatment steps (e.g. screening, preparation of the dilution series, filling of incubation bottles). I showed that the consequences of such manipulation of water samples while setting up grazing experiments can significantly alter the grazer community through the loss of sensitive taxa. This defeats the goal of a grazing experiment aimed at the determination of the *in situ* grazing rate, which can indeed only be measured when the natural *in situ* grazer community is present in an experiment. The consequence of such alteration is even worse when the degree of bias in the community is unknown. My results show that it is imperative to monitor potential effects of handling procedures on the community under study. Such evaluation is crucial not only for the extrapolation of experimental results to the field, but also for the understanding of processes within the investigated system. It futhermore leads to the advancement of present techniques and further innovations.

Microzooplankton grazers at the base of the North Sea food web

"Classic" food chain theories stress the role of primary production of photosynthetic phytoplankton grazed by crustacean mesozooplankters (> 200 µm) and those consumed by larger predators such as fish. Towards the end of the last century scientific findings emphasized the role of the microbial loop (Azam et al., 1983) as a major biological force in the ocean (Azam, 1998) and the significant importance of microzooplankters (< 200 µm) for the consumption of primary production. The aim of this thesis was to contribute in elucidating their role as phytoplankton grazers in the planktonic food web of the North Sea (Figure 1).

Heterotrophic dinoflagellates and ciliates are the most important groups within the microzooplankton (Capriulo et al., 1991) especially in water of higher productivity (Calbet, 2008) and from a biomass perspective this was also confirmed in this study. The monitoring program conducted as an important basis for this thesis (Chapter I) verified the importance of heterotrophic dinoflagellates and ciliates. Summarized they showed carbon concentrations (652 µgC L^{-1}) comparable to results from other studies (Riegman et al., 1993, Brussaard et al., 1995). During a phytoplankton spring bloom situation (Chapter III) the biomass of heterotrophic dinoflagellates and ciliates made up around 70-96% of the biomass of the microzooplankton and other studies confirm such an importance of both groups in the North Sea (Brussaard et al., 1995, Stelfox-Widdicombe et al., 2004). Whereas in my experiment (Chapter III) the phytoplankton showed a maximum carbon biomass concentration of 269 µgC L^{-1}, the microzooplankton reached concentrations of up to 126 µgC L^{-1}. At its maximum, microzooplankton contributed more than 50% to the available carbon of the plankton size fraction > 5µm.

Especially during bloom situations, unicellular microzooplankton can respond quickly to increased phytoplankton food availability (Johansson et al., 2004, Aberle et al., 2007). This pattern has also been observed during a mesocosm spring bloom situation (Chapter III) where the phytoplankton peak (24.03.09) was followed by a microzooplankton maximum with less than one week delay (30.03.09).

A meta-analysis by Calbet & Landry (2004) showed that microzooplankton grazing accounts on average for 60% of the mortality of the daily phytoplankton production in estuarine and coastal environments with chlorophyll *a* concentrations (3-13 µg L^{-1}) comparable to those at Helgoland Roads (0.05-28 µg L^{-1}, Chapter I). The results for the grazing impact of microzooplankton obtained during a typical North Sea spring bloom

(average 120%, Chapter III) even surpassed this range and showed the big potential and importance of this grazer group in waters around Helgoland.

Copepods have long been considered the main herbivorous force in the plankton. However, taking into account the grazing impact of copepods during the spring bloom experiment (average 47%, Chapter IV) I showed that throughout the bloom phase the microzooplankton was the more important phytoplankton grazer group, even though it was always present in lower biomass concentrations (30-94 µg L^{-1}) compared to copepods (103 µg L^{-1}). In addition, our findings (Chapter I) suggest that in the field copepods biomass only plays a minor role when compared to microzooplankton. The combination of those two facts stresses the importance of microzooplankton grazers in the North Sea. Furthermore, my findings support results reported in other studies (Calbet, 2001, Calbet & Landry, 2004, Putland & Iverson, 2007, Sherr & Sherr, 2007).

Irigoien (2005) proposed that microzooplankton grazing is of such importance that only phytoplankton species which can escape control by microzooplankton are able to bloom. Supporting this view, I showed in Chapter III that microzooplankton selective grazing can also have a stabilizing function on the blooming phytoplankton assemblage, leading to constant shares of the bloom-forming taxa. In my study the high grazing impact of ciliates prevented small flagellates from blooming, whereas the selective feeding of dinoflagellates led to a diatom bloom consisting of only three genera (*Rhizosolenia*, *Thalassiosira*, *Chaetoceros*).

Microzooplankton is not only in direct competition with herbivorous mesozooplankters, such as copepods (Hansen, 1992, Aberle et al., 2007, Sherr & Sherr, 2007), but it is also an important food source for higher trophic levels (Kleppel, 1993). Thus, microzooplankters play a fundamental role as trophic intermediaries. They link small planktonic size fractions, unavailable to most metazoan consumers (Gifford, 1991), to mesozooplankton (Klein Breteler et al., 1999). Grazing experiments with copepods during the spring bloom experiments conducted in this thesis (Chapter III) showed that microzooplankton was always an important food source for them. Several other studies support this finding (Nejstgaard et al., 1997, Calbet & Saiz, 2005, Fileman et al., 2007, Figueiredo et al., 2009, De Laender et al., 2010).

Nutritionally “poor” food can have negative effects on copepods (Schoo, 2010) and if they have the choice they obviously actively choose food according to their nutritional needs (Cowles et al., 1988, Kleppel, 1993).

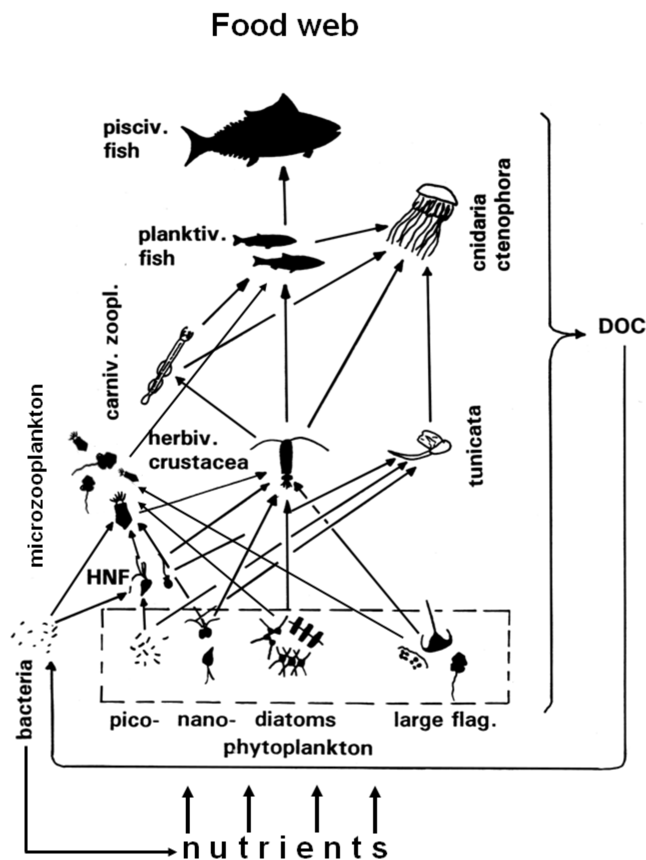

Figure 1: A rather simplified food web of the North Sea (after Sommer, 2005).

A microzooplankton diet transfers not only energy but also minerals, vitamins, amino acids, fatty acids and sterols from lower trophic levels, thus balancing any nutritional shortfalls of herbivory by "trophic upgrading" (Klein Breteler et al., 1999, Tang & Taal, 2005). Such shortfalls occur frequently during nutrient-limiting conditions for phytoplankton in an ongoing bloom (Malzahn et al., 2010). These facts support my finding that during the course of the phytoplankton bloom microzooplankton became even more dominant in the diet of the copepods than phytoplankton, although autotrophic prey was available in much higher concentrations. The reproductive success in copepods can be directly enhanced by the availability of nutritionally "better" microzooplankton prey (Tang & Taal, 2005). Copepods in turn are themselves an important diet of fish larvae and the availability of this food source is crucial for their survival (Castonguay et al., 2008). The availability of microzooplankton prey can thus potentially influence fish abundances. More recent studies suggest that microzooplankton is also important prey for fish larvae, thereby directly influencing fish abundance (Montagnes et al., 2010).

From a food web perspective microzooplankton can thus be regarded as keystone group (Calbet, 2008) (Figure 1). On the one hand it is the most important group of grazers which accumulates the energy of primary production. On the other hand microzooplankton is a key link to higher trophic levels serving as an important, "trophic upgrading" (Klein Breteler et al., 1999) food source. Concerning the results of this study, the keystone role of microzooplankton is also true for the North Sea food web.

Contrasting strategies in heterotrophic dinoflagellates and ciliates

Apart from their role as predators and prey organisms I also observed different strategies in dinoflagellates and ciliates during the spring bloom (Chapter III). Ciliates showed distinct predator-prey relationships resulting in a clear succession of ciliate taxa from strombidiid/strobilid-dominated to haptorid-dominated. The abundance of the bloom-forming species (*Strombidium* spp.) was closely coupled to availability of flagellate prey. This resulted in a short but pronounced peak, the disappearance of the bloom-forming species together with their prey followed by a substitution with other ciliate species. Dinoflagellate biomass did not increase as steeply as ciliate biomass but remained on a high level forming a broad peak over a longer period. Dinoflagellates also displayed no clear succession and were dominated by *Gyrodinium* spp. and *Protoperidinium* spp. This pattern was mainly triggered by the availability of food and contrasting survival strategies in ciliates and dinoflagellates.

Ciliates are highly effective grazers on their preferred food, mainly flagellates, and can respond rapidly to increasing food availability with higher growth rates than dinoflagellates (Strom & Morello, 1998), but their ability to survive starvation periods is low (Jackson & Berger, 1985, Hansen, 1992) and they are more specialized on a certain prey (Tillmann, 2004). When food is scarce they consequently die off or form cysts (Kim et al., 2008). In contrast, dinoflagellates can prey on almost any particle of organic origin present in the water column (Jeong, 1999, Tillmann, 2004). They have a greater ability to survive starvation (Hansen, 1992, Menden-Deuer et al., 2005), but lower growth rates than ciliates (Hansen, 1992). The broader food spectrum of dinoflagellates is also linked to a higher variety of feeding strategies when compared to ciliates (Tillmann, 2004). These feeding strategies enable dinoflagellates to feed on bacteria-sized food at the same time as on prey that is much larger than themselves (Jacobson & Anderson, 1986, Schnepf & Elbrächter, 1992, Hansen & Calado, 1999). Many dinoflagellates prefer diatom food (Sherr & Sherr, 2007). Ciliates, with few exceptions (Smetacek, 1981, Aberle et al., 2007), are restricted to smaller prey (Jonsson,

1986, Tillmann, 2004) consisting mainly of flagellates (Kivi & Setälä, 1995). Apart from obvious exceptions, ciliates can thus be classified as rapid reaction food specialists and dinoflagellates more as generalists with longer response times but greater persistence. These ecological strategies were confirmed by succession patterns visible during my spring bloom experiments (Chapter III). The observed seasonality of both groups in the monitoring data (Chapter I) also supported this to a certain extent. Ciliates played a key role during spring (Riegman et al., 1993) as they responded more quickly to increasing phytoplankton concentrations (flagellates) and formed an earlier peak than dinoflagellates. These in turn displayed longer lasting biomass maxima especially during the summer months.

Let us apply these findings to a simplified situation in the field shortly before a bloom of different phytoplankton species, e.g., small flagellates and different diatoms. Furthermore let us neglect top down control of dinoflagellates and ciliates and ask the question: Concerning the different features of dinoflagellates and ciliates, what would be the effect of both grazer groups on bloom formation? The resulting scenario could be as follows:

The phytoplankton constituting the preferred food of the ciliates, e.g. flagellates, would be eaten before being able to form a bloom due to the effective grazing of their fast-growing ciliate predators. Ciliates therefore would prevent their preferred prey from blooming. The other phytoplankters would not be controlled by ciliates. They would be able to grow faster as they would be grazed by their more slowly growing dinoflagellate predators. These phytoplankton species, e.g. different diatoms, would be the bloom-forming species. Although this depiction is an oversimplification of the real mechanisms that drive phytoplankton blooms a similar scenario has been observed during the spring bloom experiments reported in this study (Chapter III). Such size-differential grazing control promoting diatom spring blooms has also been reported from other studies (Riegman et al., 1993, Brussaard et al., 1995) in the North Sea.

Interactions between microzooplankton predators

The relationships and interactions between species are of fundamental interest in ecology (Begon et al., 2006). Although the interactions of microzooplankton with other members of the marine food web, especially phytoplankton and mesozooplankton (Calbet, 2008), have been investigated in some detail, studies on the interactions between members of the microzooplankton are rare (Stoecker & Evans, 1985, Jakobsen & Hansen, 1997). Due to the wide range of members of different taxonomic groups and

a high diversity of nutritional strategies in the microzooplankton (Sherr & Sherr, 2002), interactions between microzooplankters can be as variable as the players themselves comprising different nuances of competition as well as predation or neutralism. Investigations on possible interactions are made difficult by problems with culturing microzooplankton, particularly ciliates (Gifford, 1985).

During one part of this thesis I focused on the interactions between small heterotrophic dinoflagellates and large ciliates and succeeded in taking them into culture. The experimental organisms, according to the monitoring data “key” model species at Helgoland Roads, were isolated from North Sea samples and cultures were established.

As a result of their preferred prey size, small heterotrophic dinoflagellates potentially compete with bigger planktonic ciliates for prey (Jakobsen & Hansen, 1997). The system I investigated included the possibility of intraguild predation realized when the ciliate preys on the smaller dinoflagellate. Other studies with the same interactive conditions of the predators have dealt with two competing ciliates (Stoecker & Evans, 1985) or with a ciliate and a dinoflagellate species (Jakobsen & Hansen, 1997). Unlike such studies, results presented in Chapter IV of this study showed that interactions between intraguild prey and predator are more complex and not just a combination of competition and predation between the predators.

Diehl & Feissel (2000) developed a theoretical framework for three-level food chains including omnivory. One fundamental condition in this is that the intraguild prey must be the superior resource competitor, otherwise it will be outcompeted by the intraguild predator. My experiments with both predators as single grazers suggested that the intraguild predator was the superior competitor. Against all expectations and theoretical suggestions (Diehl & Feissel, 2000, Diehl & Feissel, 2001) no negative effect on the intraguild prey was detected in the three-species treatments conducted during my thesis. The small heterotrophic dinoflagellate grew even faster in the presence of its competing intraguild predator. Looking closer into the interactive patterns I found a kind of commensalistic relationship preceding other possibilities of interaction. The small dinoflagellate was promoted by pre-conditioned prey items produced by the ciliate and showed the same growth rates as its competitor. Due to a faster growing predator biomass this led to a more efficient use of the resource in the system. Theoretical assumptions suggest that the promotion of the intraguild prey is of such magnitude that it can potentially favor a stable coexistence of both predators which is of further relevance as both predators co-occur in the same environment.

To my knowledge similar results have not been published for the marine system up to now. These novel results show that extrapolating laboratory results of single species experiments to the field can be extremely difficult, as this strategy strongly neglects food web interactions. My findings show furthermore that we are just starting to understand marine food web interactions. Especially the impact of such interactive relationships between members of the microzooplankton needs further research. Mathematical models can provide deeper insights into the effect of such inter-specific interactions within the microzooplankton and will be applied in future studies.

Microzooplankton in a "climate change" environment

We know now that microzooplankton can play a crucial role in the marine food web, especially as phytoplankton grazer. Understanding its role in a changing environment caused by the anthropogenic increase of CO_2 will be one of our future challenges.
Current climate scenarios predict a further rise in air and water temperature (IPCC, 2007). Indeed a strong warming trend has already been observed for the North Sea, where the mean annual temperature rose by 1.7°C since 1962 (Wiltshire et al., 2010). As growth and grazing rates of heterotrophic organisms like microzooplankton species are linked to temperature (Müller & Geller, 1993, Montagnes & Lessard, 1999) it can be assumed that these rates will also increase with increasing ambient temperatures. Similarly, a study on maximal growth rates of algal grazers showed that these decrease much more rapidly with decreasing temperature than those of their algal prey. This fact partly explains the phenomenon of distinct blooms in temperate and arctic oceans at times when coldest temperatures co-occur with conditions that favor increased phytoplankton growth and also the absence of such blooms in warmer regions (Rose & Caron, 2007).
In the temperate North Sea the seasonal succession of plankton is initiated by the spring bloom of phytoplankton which is predominantly triggered by the combined effects of increasing light and nutrient availability (Sommer, 1996). The spring bloom is almost a start from zero, because only few phytoplankters will have survived the winter (Sommer et al., 2007). Warmer water temperatures especially during the colder period of the year might be expected to induce a higher metabolic rate in microzooplankton and consequently higher grazing rates. These higher grazing rates potentially lead to shifts in seasonal patterns of phytoplankton density: Even less phytoplankton survives winter time due to grazing by the more active microzooplankton species. This has already been

reported for the North Sea around Helgoland where an increase in water temperature has led to a later onset of the spring bloom (Wiltshire & Manly, 2004).
Furthermore, higher water temperatures also led to an increase in average cell size of the phytoplankton (Wiltshire et al., 2008), which could also be a result of selective grazing of microzooplankton on smaller phytoplankton fractions (compare Chapter III). A temperature-dependent shift in the size spectrum of phytoplankton has also been reported for the Baltic Sea (Sommer et al., 2007) where an increase in temperature also resulted in altered microzooplankton community structures and enhanced grazing rates (Aberle et al., 2007).
With growth rates in the same range as those of its prey (Müller & Geller, 1993, Montagnes & Lessard, 1999), unicellular microzooplankton can respond faster to increasing phytoplankton availability (Johansson et al., 2004, Aberle et al., 2007), when compared to mesozooplankton, i.e. copepods. Copepods are at a disadvantage in that they need to develop from egg and larval stages and have overall lower growth rates (Rose & Caron, 2007). Although increased water temperatures favor all zooplankton to some extent (Rose & Caron, 2007) microzooplankton grazers will be favored even more by higher temperature than their mesozooplankton competitors. This could lead to a more pronounced recycling and retention of energy in the microzooplankton fraction and less energy being transferred directly from phytoplankton to the classic herbivorous food chain with copepods as important grazers.
In addition, the recruitment success of higher trophic levels such as copepods is highly dependent on synchronization with phytoplankton blooms (Edwards & Richardson, 2004) especially in temperate environments like the North Sea. Shifts in bloom timing, as mentioned above, can therefore lead to classic mismatch situations.
Consequences are not assessable yet but have potentially severe implications for trophic interactions, food web structures and also for higher trophic levels of human interest such as fish stocks. On the other hand there is evidence that the reproductive success of copepods can be enhanced directly by a microzooplankton diet (Tang & Taal, 2005) and higher copepod densities could consequently support higher fish stocks (Castonguay et al., 2008).
Another aspect that has also to be taken into account when dealing with climate change are alterations in plankton biodiversity (Beaugrand et al., 2009, Goberville et al., 2010) which can also concern the microzooplankton. Warmer waters could promote warm-adapted species (Wiltshire et al., 2010) and thus also support the invasion of new species from warmer regions. With ships' ballast water planktonic species are

transported around the world and released in environments where the new species can have severe impacts on the original biocoenosis, and therefore indirectly or directly on the human society (Shiganova et al., 2003, Gollasch, 2006, Gregg & Hallegraeff, 2007). Higher temperatures broaden the basis for such species invasions. In turn, cold-adapted species at the southernmost border of their geographic range can be suppressed by rising temperatures (Beaugrand et al., 2003), their occurrence can be restricted to colder seasons or they can disappear totally. As plankton forms the basis of the marine food web the effects of climate-induced biodiversity changes in the plankton are not yet assessable at the food web level but are possibly profound (Beaugrand & Kirby, 2010). Continuous plankton monitoring programs can reveal changes in the plankton community on an early stage and are therefore of utmost importance. Only in observing the roots of change an estimation of the consequences of change is possible.
This short glimpse into potential impacts of climate change shows that assessing the role of microzooplankton in future climate scenarios is extremely difficult and further research is necessary to reveal the effects on microzooplankton and its implication for food web functioning.

Outlook

It is obvious that we are just beginning to understand the food web processes in the marine system. The results of the work presented here contribute to the elucidation of the role of dinoflagellates and ciliates in a small part of the marine food web of the North Sea. It is now clear that both groups are indeed fundamentally important intermediaries in terms of grazing as well as for transporting energy to higher trophic levels. In addition to these deeper insights, a lot of new questions have been raised from this study. Fundamental for a better understanding of its ecological role in the North Sea, we need to increase our knowledge on the species composition of microzooplankton. This can only be achieved by more detailed and continuous taxonomical observations. In combination with long-term data such as the Helgoland time series, such observations can provide a powerful tool to reveal the biotic and abiotic factors that drive microzooplankton abundance and composition. Having demonstrated the important role of microzooplankton during the course of a spring bloom, further investigations are now necessary for elucidating the role of microzooplankters as grazers and also as prey items during other specific times of the year. Clarifying interactive patterns between microzooplankton members has also been neglected up to date. However, as such interactions can have considerable effects on

other trophic levels in the food web it is thus of the utmost importance to broaden our knowledge in this field by further laboratory experiments and mathematical modelling.
Investigations on the role of the diverse community of microzooplankton in the food web will continue to be a challenge in the future especially against the backdrop of a changing climate. However, despite new insights into microzooplankton ecology arise continually, microzooplankton provides a great potential for future research.

SUMMARY

This thesis investigates the role of microzooplankton in the food web of the North Sea. For a basic understanding of the importance and seasonal distributions of dinoflagellates and ciliates in the North Sea a monitoring of both groups was established on a weekly basis and continued for 2.5 years. The results show that dinoflagellates and ciliates are key organisms in terms of planktonic biomass in the North Sea around Helgoland and constitute a highly diverse and abundant community with a clear seasonal trend. Dinoflagellate biomass surpassed that of ciliates, especially during summer months, whereas ciliates played an important role at the onset of the phytoplankton spring bloom.

The evaluation of methodological sources of error in standard grazing experiments resulted in a new set-up technique, which was tested and shown to represent an improvement of the state-of-the-art method, conserving fragile microzooplankton species and grazer biodiversity.

Applying the new technique, experiments on the grazing impact and food selectivity of microzooplankton and copepods were carried out during key phases of a typical North Sea spring bloom. The grazing experiments showed the overall important role of ciliates and dinoflagellates as phytoplankton grazers when compared to copepods. Their grazing impact terminated the bloom after roughly three weeks. Microzooplankton food selectivity shaped the bloom and at the same time led to a stabilized assemblage of the bloom-forming diatoms. Copepods, in turn, selectively fed on microzooplankton and could therefore be regarded as the main top down controlling factor of this group. As the positive selection for microzooplankton food gained importance during the course of the bloom, when nutrients became limiting for the phytoplankton, microzooplankton can be regarded as an important dietary component for copepods, particularly in cases of nutritional shortfall in phytoplankton.

Microzooplankton does not only interact with other functional groups of the food web but also with other members of the microzooplankton. Such interactions were investigated within a three species model system consisting of two different Helgoland microzooplankton predators and their common prey. The system included the possibility of “intraguild” predation as the smaller dinoflagellate predator could be a prey for the larger ciliate predator. In spite of theoretical assumptions that predicted the extinction of the smaller predator a commensalistic relationship between both predators was shown. The smaller predator was promoted by prey items that were pre-conditioned

by its larger competitor. The advantage for the smaller predator was of such magnitude that it potentially enables a stable coexistence of both predators.
This study contributed new insights on: (1) the species composition and seasonality of dinoflagellates and ciliates in the North Sea, (2) methodological improvements concerning microzooplankton grazing experiments, (3) the important role of dinoflagellates and ciliates as grazers and also as prey for higher trophic levels and (4) interactive relationships between microzooplankton predators.

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DECLARATION

I herewith declare that this thesis is my own work and effort and has been written independently. Where other sources of information have been used, these have been cited and are listed in the references. Furthermore, I declare that this work has not been submitted to any other university for the conferral of a degree.

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