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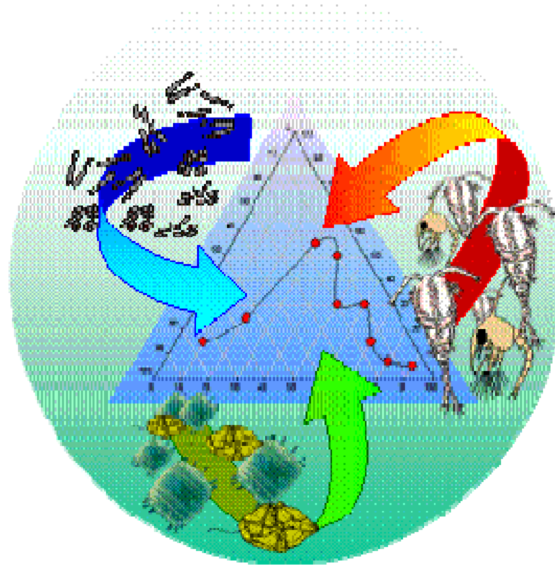
Phytoplankton-microzooplankton interactions



The role of food quality and selective feeding of microzooplankters in the Helgoland food web

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Summary

Since microzooplankton is not well studied so far in contrast to other components of the plankton, their role in the food web remains still unclear and further investigations are needed. The final aim of this study was to measure the grazing impact of microzooplankton on the spring bloom, to investigate microzooplankters feeding preferences and to elucidate the role of food quality in their diets. This was investigated by dilution experiments within a one month mesocosm experiment. This work led to several conclusions (1) Microzooplankters are able to suppress phytoplankton spring blooms and to graze down phytoplankton biomass substantially, (2) They are able boost the regenerated production by contributing to a fast nutrient remineralisation, (3) The selective grazing by microzooplankters led to a bloom of inedible phytoplankton species (e.g. *Rhizolenia styliformis* and *Pseudonitzschia sp.* known to produce toxic components, even if this seems not to be the case in our experiments because they were grazed) while edible components of the phytoplankton (*Chaetoceros sp.* and flagellates) were grazed down substantially, (4) When microzooplankton was released from grazing pressure by copepods, the phytoplankton community was biased by selectivity grazing patterns of microzooplankters. Furthermore, food quality differences between *Pentapharsodinium sp.* and *Scrippsiella sp.*, two abundant and similar dinoflagellate species at Helgoland Roads, were investigated and the potential of an active choice of these two dinoflagellates by grazers, their C:N ratio and fatty acids content were analysed. We proved that no clear difference exists between *Pentapharsodinium sp.* and *Scrippsiella sp.* in term on food quality when considering C:N (fatty acids being analysed only for one species) and concluded that under limited conditions these dinoflagellates increase their energy stock until a threshold after which they form cyst to ensure their survival.

Résumé

Puisque moins étudié, en comparaison à d'autres composants du plancton, le rôle du microzooplancton dans le réseau trophique planctonique reste flou et de nouvelles analyses sont nécessaires. Le but de cette étude était de mesurer l'impact du broutage du microzooplancton sur le bloom de printemps, d'investiguer les préférences trophiques de ce groupe et d'éclaircir le rôle de la qualité nutritive de leurs proies au sein de leur régime alimentaire. Pour se faire nous avons réalisé des expériences de dilution pendant une expérience en mésocosme d'un mois. Ce travail a abouti à plusieurs conclusions (1) Le microzooplancton est capable de supprimer le bloom de printemps en diminuant largement la biomasse phytoplanctonique par broutage, (2) Il est également capable de favoriser la production régénérée en contribuant à une rapide reminéralisation des nutriments, (3) Le broutage préférentiel du microzooplancton a conduit à un bloom d'espèces non comestibles (ex. *Rhizolenia styliformis* et *Pseudonitzschia sp.* connue pour produire des toxines, bien que, puisque brouté dans nos expériences, il ne semble pas que ce soit le cas ici) alors que les espèces comestibles (*Chaetoceros sp.* et flagellés) étaient peu abondantes en raison d'une forte pression de prédation, (4) Lorsque le microzooplankton ne subit pas de prédation des copépodes, la communauté phytoplanctonique se trouve biaisée par son broutage préférentiel. De plus, nous avons étudié les différences, en termes de qualité nutritive, de deux dinoflagellés abondants à Helgoland Roads, *Pentapharsodinium sp.* et *Scrippsiella sp.*. Afin d'expliquer une éventuelle préférence des consommateurs, leur C:N ratio et teneur en acides gras ont été analysés. Nous avons prouvés qu'aucune différence claire n'existe entre les deux espèces en terme de qualité nutritive, considérant leur C:N (les acides gras n'ont pu être analysés que pour une seule espèce) et avons conclu qu'en conditions de stress, ces dinoflagellés augmentent leur stock d'énergie jusqu'à un seuil à partir duquel ils forment des cistes afin d'assurer leur survie.

1. Introduction

Acting as an important structural and functional group, microzooplankton is an essential element in planktonic ecosystems. Indeed, it can be seen as one of the major predator groups in microbial food webs (Sherr & Sherr 2002, Landry & Calbet 2004) and, in addition, microzooplankters are known to form a trophic link between pico-, nano-, and microplankton as well as higher trophic levels (Johansson et al. 2004, Sommer et al. 2005). This pathway through microzooplankton thus allows a rapid recycling of nutrients (Irigoien et al. 2005; Calbet & Saiz 2005). Therefore, this group can be considered as major consumer of phytoplankton competing for food with mesozooplankton by grazing up to 60-70% of the potential primary production (Landry & Calbet 2004). Traditionally food-web models used to consider a direct transfer of carbon from phytoplankton to mesozooplankton (Cushing 1989) and it has been highlighted, only relatively recently, that microzooplankton can contribute to an important part of mesozooplankters diets (Kleppel 1993). In temperate regions, the seasonal succession of plankton is initiated by a spring bloom of phytoplankton. These blooms are initiated predominantly by an amplification in light and nutrient availabilities (Greve & Reiners 1995; Sommer 1996) and only indirectly by temperature, e.g., via the effects of thermal stratification and/or cloud cover (Sverdrup 1953; Wiltshire & Manly 2004). Especially in spring, microzooplankton is seen to be key component of planktonic communities because of its more rapid metabolism and production per unit weight than mesozooplankton (Fenchel & Finlay 1983; Müller & Geller 1993; Montagnes & Lessard 1999), and so allowing a direct response to increasing food availability occurring during the phytoplankton spring bloom (Johansson et al. 2004). Previous studies on feeding preferences of microzooplankters have confirmed their importance as grazers in general and also the main role of certain groups (e.g. ciliates) in depleting spring phytoplankton communities, especially in the Baltic and the North Sea (Smetacek 1981; Kivi et al. 1993; Leppaenen & Bruun 1988; Johansson et al. 2004). It has been evidenced that microzooplankton, and especially ciliates, can compete with mesozooplankton by grazing diatom chains and large, single-celled diatoms although they also graze on bacteria and flagellates. Three different feeding modes among heterotrophic dinoflagellates are known (Jacobson & Anderson 1986, Gaines & Elbrächter 1987, Hansen 1991 a). The first group including exclusively naked genera (e.g. *Gyrodinium, Gymnodinium),* directly swallow up intact preys through the sulcus at the posterior end of the cell. A second group includes species ingesting prey with a "pallium", a pseudopodium that extends through the flagellar pore and envelopes the prey, (e.g. *Protoperidinium* spp., the

Diplopsalis group). The third group consists of species of naked and thecate genera; these organisms suck out the contents of the prey with a peduncle (e.g. *Dinophysis* spp., *Gymnodinium* spp., *Gyrodinium* sp., *Amphidinium* spp.). The ciliates are known to have three main feeding modes: filter feeders (*Strobilidium spp.*), raptorial feeders (*Balanion spp.*), and diffusion feeders (*Histiobalantium spp.*) (Müller & Weisse 1994).

Although one of the central topics in ecology has traditionally been foraging behavior, the knowledge about the capacity of microzooplankters to select their food is very weak. In its most basic form, optimal foraging theory specifies that organisms select their food in order to optimize their energy intake per unit time. They act in a way to find, capture and consume food containing the most calories while expending as less time as possible. Because it can avoid intoxication for toxic food but also provide a balanced diet when different food qualities are available, food selectivity can be considered as a key parameter for consumers. Our knowledge about prey selectivity of planktonic grazers is growing but still far from complete. For planktonic organisms food quality is an important key factor acting and determining the development, survival and reproduction rates. Two points are important to consider (1) the inter-specific and (2) the intra-specific variability of food quality. The first one is due to taxonomic differences and specific chemical composition (e.g. C:N, fatty acids) while the second one can be engendered by light and/or nutrients availability/limitation. It is now confirmed that copepods, one of the principal herbivores in the oceans, choose their prey in function of taxonomical differences (Irigoien et al. 2000, Fileman et al. 2007), prey size (Paffenhoefer 1988) and that they change their preferences depending on their body size (Mauchline 1998) and developmental stage (Mauchline 1998). For microzooplankters, few investigations have been realised. The heterotoph flagellate *Oxyrrhis marina* was shown to be able to select between 3 algal species offered as prey (Flynn et al. 1996) and similar results were reported for four ciliate species (Hamels et al. 2004). Another interesting result is that ciliates select similar prey items

Fig. 1 Helgoland, North Sea; Yellow star, Helgoland Roads (54° 11.3’ N, 7° 54’ E)

than copepods do in mesocosm experiments (Aberle et al. 2006).

Our investigations have focused on the well studied plankton community at Helgoland Roads (Fig. 1), in the North Sea (54° 11.3' North, 7° 54' East). Although this point is sampled since 1873, microzooplankton at Helgoland Roads is not well studied so far in contrast to other components of the plankton and therefore their role in the food web remains still unclear. In this study, we hypothesized that (1) Microzooplankton is able to control phytoplankton spring blooms and to graze down phytoplankton biomass substantially, (2) Selective grazing by microzooplankton leads to a bloom of inedible phytoplankton species while edible components of the phytoplankton will be grazed down substantially and (3) Microzooplankton will be released from grazing pressure by copepods and therefore the phytoplankton community will be biased by selectivity grazing patterns of microzooplankters.

Experiment 1- Feeding selectivity

In order to investigate feeding preferences of microzooplankton consumers and to elucidate the role of food quality in the diets of microzooplankters, we conducted a mesocosm experiment on the role of microzooplankton grazing and their selective potential in natural spring plankton communities. Using three parallel mesocosms we simulated natural species succession occurring during the diatom spring bloom while excluding mesozooplankton. Thus, the spring bloom succession in our mesocosms included only phytoplankton and microzooplankton and released microzooplankton from grazing pressure through mesozooplankters (e.g. copepods). Microzooplankton is a complex group of consumers known to have several feeding modes (e.g. bacterivorous, algivorous) which could explain their succession and rapid numerical response in spring, depending on available prey items. In order to confirm or decline this hypothesis, species-specific grazing of natural microzooplankton communities was investigated. During the course of the mesocosm experiment, four dilution experiments were conducted, the two first just before and after the phytoplankton biomass peak (phytoplankton biomass maximum, bloom experiments), and the two last after the bloom (post-bloom experiments). Here I present the results of the second and third experiments; due to the time-consuming analysis of the grazing experiments the two others will not be considered in the present study but will be analysed later.

Experiment 2- Food quality aspects

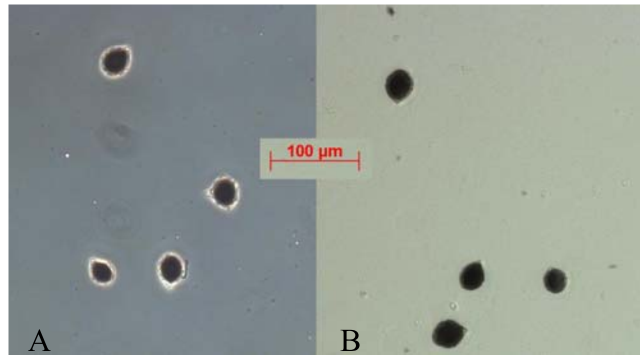


Fig. 2 A *Scrippsiella sp.* B *Pentapharsodinium sp.*

Due to their small size and their strong similarity concerning taxonomical identification, the two dinoflagellates *Scrippsiella sp.* and *Pentapharsodinium sp.* (Fig. 2) are hard to differentiate and much confusion exists about these two species. *Scrippsiella sp.* is described to have six cingular plates, five sulcal plates and a partly calcareous cyst and *Pentapharsodinium sp.* to have five cingular plates, four sulcal plates and a wholly organic cyst (Lewis 1991). At Helgoland Roads (54° 11.3' North, 7° 54' East), those two dinoflagellates are abundant components in the plankton and at specific times of the year they form considerable blooms (e.g. summer 2007, pers. comm. Loeder/Kraberg/Peters) and so act as important component in food web. In order to account for food quality differences between those two prey species and to elucidate the potential of an active choice of high quality food items by grazers, fatty acids profiles and C/N ratio of each species were analysed.

2. Materials and Methods

Experiment 1- Feeding selectivity

- General setup

This experiment, using three parallel mesocosms placed in a thermo-constant room (Fig. 3) at 6°C, was started on the 16th March 2009 and ended at the 16th April 2009. The cyclindrical mesocosms with a volume of 700 L each, were filled with natural seawater from Helgoland Roads, North Sea. Prior to the filling of the mesocosms the water was filtered gently using a 200 µm gauze, in order to remove mesozooplankton but to allow for the passage of chain-forming diatoms and microzooplankton at the same time. The natural, pre-filtered seawater

Fig. 3 Mesocosms 1 and 2 placed in the thermo-constant room.

which was filled into the mesocosms thus contained over-wintering/spring populations of bacteria, phytoplankton and microzooplankton. The water was stirred with a slow velocity to ensure a continous mixing of the water column and avoid sedimentation of the plankton. Lightening was ensured by two overhead light sources, each composed of five neon lights tubes of the type Solar Ultra Tropic (JBL ®) providing solar complete spectrum and one tube of the type Solar Ultra Natur (JBL ®) providing a spectrum enriched in blue wavelengths. Light regimes above the three mesocosms was identical simulating daily triangular light curves like in the field while the timing of sunrise and sunset and the maximum light intensity was supplied daily by a computer program (Prometeus, modified version after Sommer et al. 2007) and was adjusted daily. Each day sunrise started a little bit earlier and sunset a little bit later, to account for changes in the photoperiod during the experimental run. A light intensity of 60% was chosen to simulate the light intensity at about 1.50 m with 5 m Secchi depth at Helgoland Roads, to simulate fairly bright light conditions during spring rather than more cloudy (darker) conditions in order to mimic an early onset of the phytoplankton spring bloom under bright light conditions. The seawater which was removed from the mesocosms for sampling (appr. 10 L per week) was partially replaced by a small amount of natural seawater from the field (5 L per week), in order to add a small inoculum of natural phytoplankton and microzooplankton communities at different stages of the bloom (to account e.g. for microzooplankton hatching from cysts). This should allow the natural succession of microzooplankters leading to the occurrence of species able to graze on large-sized diatom species which usually occur during the phytoplankton spring bloom. Additional 15 L of filtered seawater (0.2µm) were added to account for water losses due to the removal of water from the mesocosms for additional grazing experiments. In order to investigate the development of the phytoplankton spring bloom daily measurements of temperature, pH and *in vivo* fluorescence as a proxy for phytoplankton biomass were conducted. In addition, nutrient measurements were conducted three times per week.

- Dilution experiment

The grazing experiments lasted for 24 hrs. and were run four times during the whole experimental period (bloom grazing experiments: 20th March 2009 and 24th March 2009; post-bloom grazing experiments: 31st March 2009 and 7th April 2009). A modified version of Landry and Hassett's (1982) dilution technique was used by replacing glass bottles by PC's culture flasks of 2 L volume filled completely to avoid air bubble which can damaged fragile species and closed by a lid. The three mesocosms were stocked, using three replicates, with 4 dilution grades (10, 25, 50, 100% of mesocosm water), the final volume was reached by adding 0.2 µm filtered seawater. In order to avoid a possible nutrient limitations, the incubations were realised with nutrients in excess (F/2 medium concentrations). The flasks were placed on a plankton wheel (Fig. 4) with ~1.1 rpm in order to avoid the settlement of phyto- and microzooplankton. The dilution technique is based upon the assumption that according to the dilution steps, the encounter rate between phytoplankton and their microzooplankton grazers is reduced stepwise and is, after logarithmic transformation, linearly related to the dilution factor.

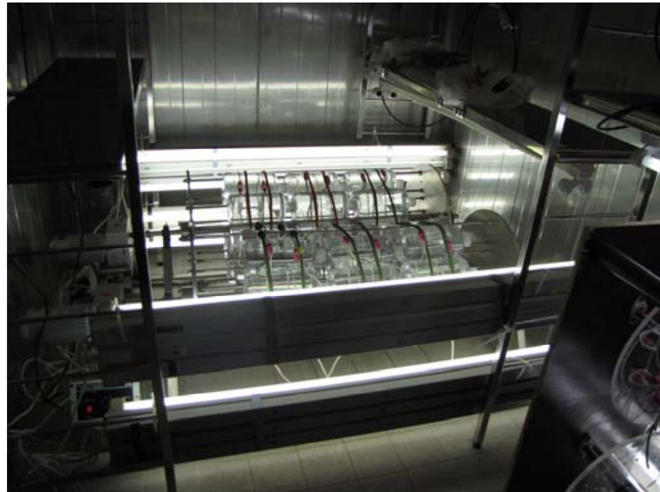
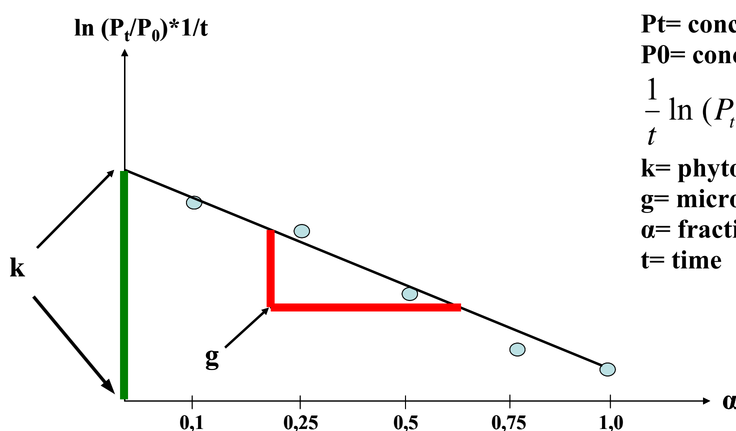


Fig. 4 PC bottles placed on the plankton wheel for the dilution experiment

Equation 1: $P_t = P_0 * e^{(k-g)t}$ Linearization: $\frac{1}{t}\ln(P_t / P_0) = k - g * \alpha$



Pt= concentration of phytoplankton biomass at time t
P0= concentration of phytoplankton biomass at time 0
$\frac{1}{t}\ln(P_t / P_0)$ **= apparent phytoplankton growth**
k= phytoplankton growth rate
g= microzooplankton grazing rate
α= fraction of natural seawater
t= time

Fig. 5 Graphical solution of microzooplankton grazing and phytoplankton growth rate in dilution experiments: the y-axis intercept of the regression line, **k**, is the apparent phytoplankton growth rate, the slope of the regression line, **g**, is the microzooplankton grazing rate.

The grazing rate of the microzooplankton community is estimated as the decrease of apparent phytoplankton growth (ratio phytoplankton biomass before/phytoplankton biomass after incubation) with dilution factor (Fig. 5). More precisely, the grazing rate is estimated as the slope of a regression of the apparent growth rate of the phytoplankton against the dilution step. Additionally, the phytoplankton growth rate could be estimated as the y-axis-intercept, when the apparent growth rate was extrapolated to 100% dilution (growth without grazers). To account for the grazing impact of mesozooplankton at the same time, a copepod treatment, using the calanoid *Temora longicornis* (25 individuals per 2.3 L bottle) was included into the dilution experiment in order to investigate species-specific grazing of *T. longicornis,* an abundant mesozooplankton grazer in the North Sea. Furthermore, in order to highlight a possible species specific nutrient limitation, a treatment without nutrient was performed.

At T24 (after 24 hrs), 250 mL of each dilution grade, for each mesocosm, were fixed with Acetic Lugol (2%) for the determination of changes in the phytoplankton and microzooplankton community. At T0, we sampled only the 100% dilution grade, assuming that differences between the different dilution grades would be only due to the dilution. For each sample (filtration and fixation) three replicates were used. In order to analyse species-specific grazing by microzooplankton, phytoplankton was counted under the inverted microscope, using four different magnifications (50x, 100x, 200x and 400x) using the Utermöhl techniques (Utermöhl 1958) after having settled the sample in 25 mL sedimentation columns. Phytoplankton was distinguished to the species or genus level. Depending on the concentration of organisms, the whole, or half of the chamber, was counted for the two first magnifications and at maximum four stripes were counted for the two last. The limit was fixed at fifty individuals, or chains, per taxonomic unit which gave 95% confidence limits of ±20%; however, this standard could not be attained for rare species. The microzooplankton composition was recorded, after settling 50 mL of each sample, via counting the whole settling chamber at 200 fold magnification and 3 stripes for small species (< 15 µm).

Experiment 2- Food quality aspects

Fatty acids profiles and C/N ratio of each phytoplankton species were analysed to account for food quality aspects of prey items as a potential reason for an active choice of food components. This analysis has been realised using batch cultures of *Pentapharsodinium sp.* and *Scrippsiella sp.* cultured in f/2 media (Guillard & Ryther, 1962) without adding

silicate. Three different treatments were chosen: (1) nutrients in access (f/2 media), (2) P-limitation (F/2 media without P addition) and (3) N-limitation (F/2 media without N addition) (Tab. 1).

Tab. 1 Media nutrient compositions, based on F/2 medium. We also add a trace metal and a vitamin solution (Guillard & Ryther 1962, Guillard 1975).

	SiO_2 µmol/L	PO_4 µmol/L	NO_2 µmol/L	NO_3 µmol/L	NO_x µmol/L	NH_4 µmol/L
Unlimited	3,62	53,1	0,55	129,02	129,57	0,92
N-limited	3,62	53,1	0,55	8,02	8,57	0,92
P-limited	3,62	0,5	0,55	129,02	129,57	0,92

Samples were taken at three different growth phases (lag phase, log phase and stationary phase). Incubations were conducted in 250 mL culture flasks closed by a lid with a filter enabling the free exchange of gas and incubated in a thermo-constant room. This experiment was conducted at 14°C under 12/12 light regime (45 µmol m^{-2} s^{-1}) (Fig. 6). For each sampling date, two filtrations (three replicates for each) of the same biovolume of each dinoflagellate were performed on GF/C Whatman® filters in order to measure the fatty acids and C/N ratio using the protocol described by Aberle & Malzahn (2007).

Fig. 6 Batch cultures in incubation bottles placed in the thermo-constant room under a 12/12 light regime.

The fatty acids of algae were measured as fatty acid methyl esters (FAMEs). Cells were disrupted by a 30 minutes ultrasounds treatment using dichloromethanol as solvent. In order to isolate the FAMEs, a 10 minutes centrifugation at 4000 rpm after having added 2 mL of KCl was realised. The bottom phase was incubated at 70°C with 3 mL methanol during 60 minutes. Finally, two 10 minutes centrifugations at 4000 rpm were performed after having added 2 mL N-Hexan. The supernatant, containing the FAMEs was analysed by gas chromatography using a CP 8400 gas chromatograph equipped with a DB-225 column (J&W Scientific, 30-m length, 0.25-mm inner diameter [ID], 0.25-mm film). The injector temperature was set to 250°C. The column oven was set to 80°C, which was heated to 150°C with an increase of 30°C min^{-1} after injection , then to 170°C at 6°C min^{-1}, and finally

to 220°C at 1.5°C min^{-1}, which was held for 21 min. The carrier gas was helium at a constant pressure of 12 PSI. The flame ionization detector was set to 300°C. Injection of the 1 µL aliquots of the samples was done in a split-less mode. FAMEs were quantified using calibrations set up for each fatty acid separately and a known amount of C 23:0 was added at the first step of the preparation as an internal standard.

For the analysis of carbon and nitrogen contents (C:N) of the algae, 50 mL of each culture were filtered on pre-combusted and acid-washed (10% HCl) Whatman® GF/F filters. The filters were dried after filtration and rolled into tin foil. The elemental analyses were done using an elementar VARIO MicroCube analyzer. The combustion tube temperature was set to 1150°C and the reduction tube to 850°C. Sulfanilamide was used as a standard.

3. Results

Experiment 1- Feeding selectivity

General observations

In order to plan the dilution experiments and also to follow the bloom development, daily measurements of *in situ* chlorophyll concentration (Fig. 7) were conducted. For all three mesocosms, the chlorophyll concentration, a proxy for phytoplankton biomass, increased from about 1.5 µg L^{-1} to 10 µg L^{-1} (biomass maximum peak reached 7 days after the beginning of the experiment. Thereafter, the biomass decreased slowly until return to the initial values, considered as the end of the bloom.

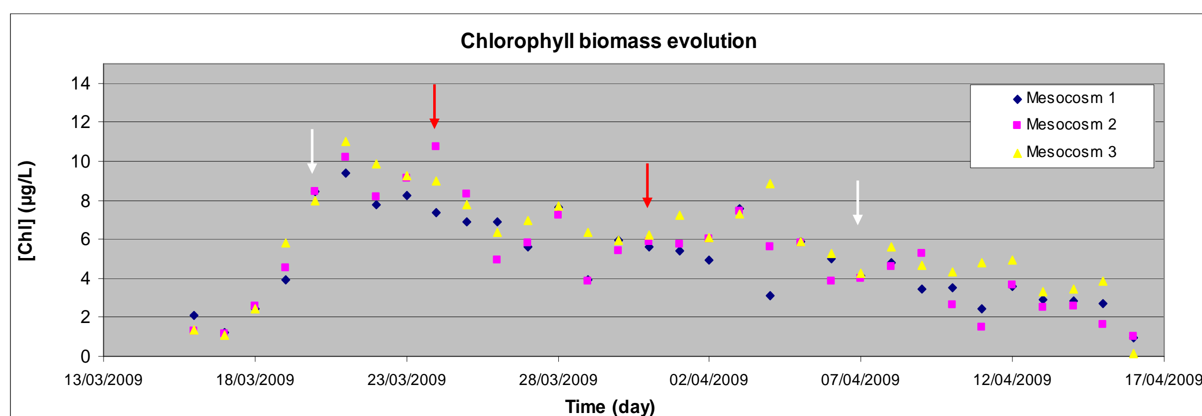


Fig. 7 BBE *in situ* fluorescence measurements: Chlorophyll biomass development; White arrows, dilution experiments not yet analyzed; Red arrows, dilution experiments analysed during the present study

Additionally, nutrient measurements were conducted three times per week. At the beginning all nutrients were available in high concentrations (4 µmol SiO_2, 0.4 µmol PO_4, 12 µmol NO_3, 0.5 µmol NH_4). With the duration of the experiment, silicate and phosphate were depleted reaching minimum values below 0.05 µmol L^{-1} on the 24th March, while nitrate and ammonium concentrations remained almost constant until the end of the experiment.

Dilution experiment

For both experiments and all mesocosms, at t_0 and t_{24}, the phytoplankton assemblages were determined. The relative abundances of each species were calculated from the biomass data in order to avoid a bias favouring the most numerous organisms. Since no visible difference was observed from the graphs between both experiments and all three mesocosms only one graph showing the data for mesocosm 1, 24th March, is presented here showing general taxonomic composition pattern of the algal assemblages (Fig. 8). The only difference in composition is the disappearance of the 10 and 20 µm *Chaetoceros sp.* size classes in the three mesocosms between the second and the third experiments. With more than 90% of the global biomass, the pennate diatom *Rhizolenia styliformis* dominates widely the phytoplankton community. The rest is divided, in the decreasing order, between *Thalassiosira nordenskioeldii*, *Thalassiosira rotula* and the 20 and 30 µm *Chateoceros sp.* size classes.

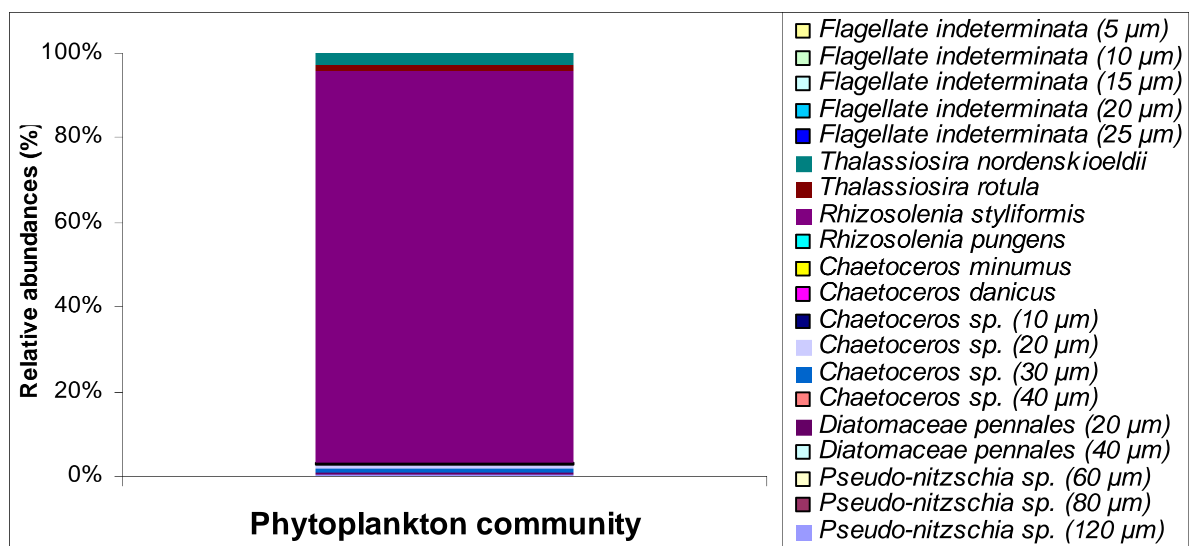


Fig. 8 Phytoplankton assemblage, relative abundances calculated with biomass data.

The relative abundances were also calculated in the same way for microzooplankton community for each mesocosm and both experiment. The microzooplankton community was divided into dinoflagellates, ciliates and an amoeba species. We consider here that all dinoflagellates present in the mesocosms were hetero- or mixotroph which means that they

are able to feed on phytoplankton. For experiment 2 (24th March), since no visible difference was observed between the three mesocosms only one graph for each sub-community (dinoflagellates and ciliates) showing the data for mesocosm 1, 24th March, is presented here showing general taxonomic composition pattern of the microzooplankton assemblages (Fig. 9). The dinoflagellate community was dominated by *Gyrodinium sp.* (75 µm) contributing to more than 50% to the overall dinoflagellate community. The rest was mostly divided between *Gyrodinium sp.* (50 µm), thecate *dinoflagellate sp.* (~10% each) and *Protoperidinium thorianum* (~ 7%). The ciliate community was mostly dominated by *Strombidium capitatum* (~50%), and *Lohmaniella oviformis* (~20%).

For experiment 3, since no difference could be detected between the three mesocosm for the dinoflagellate community, only one graph showing the data for mesocosm 1, 31st March, is presented. On the other hand, the ciliates assemblage is similar between mesocosm 1 and 2 but differs with mesocosm 3, thus two graphs are presented, for mesocosm 1 and 3 (Fig. 10).

The dinoflagellate community was dominated by *Gyrodinium sp.* (75 µm) (~70%), *Gyrodinium sp.* (100 and 50 µm) (each ~10%). In the second mesocosm, *Strombidium capitatum* (~40%), *Laboea strobilida* (~15 %) and *Rimostrombidium sp.* (~10%) dominated

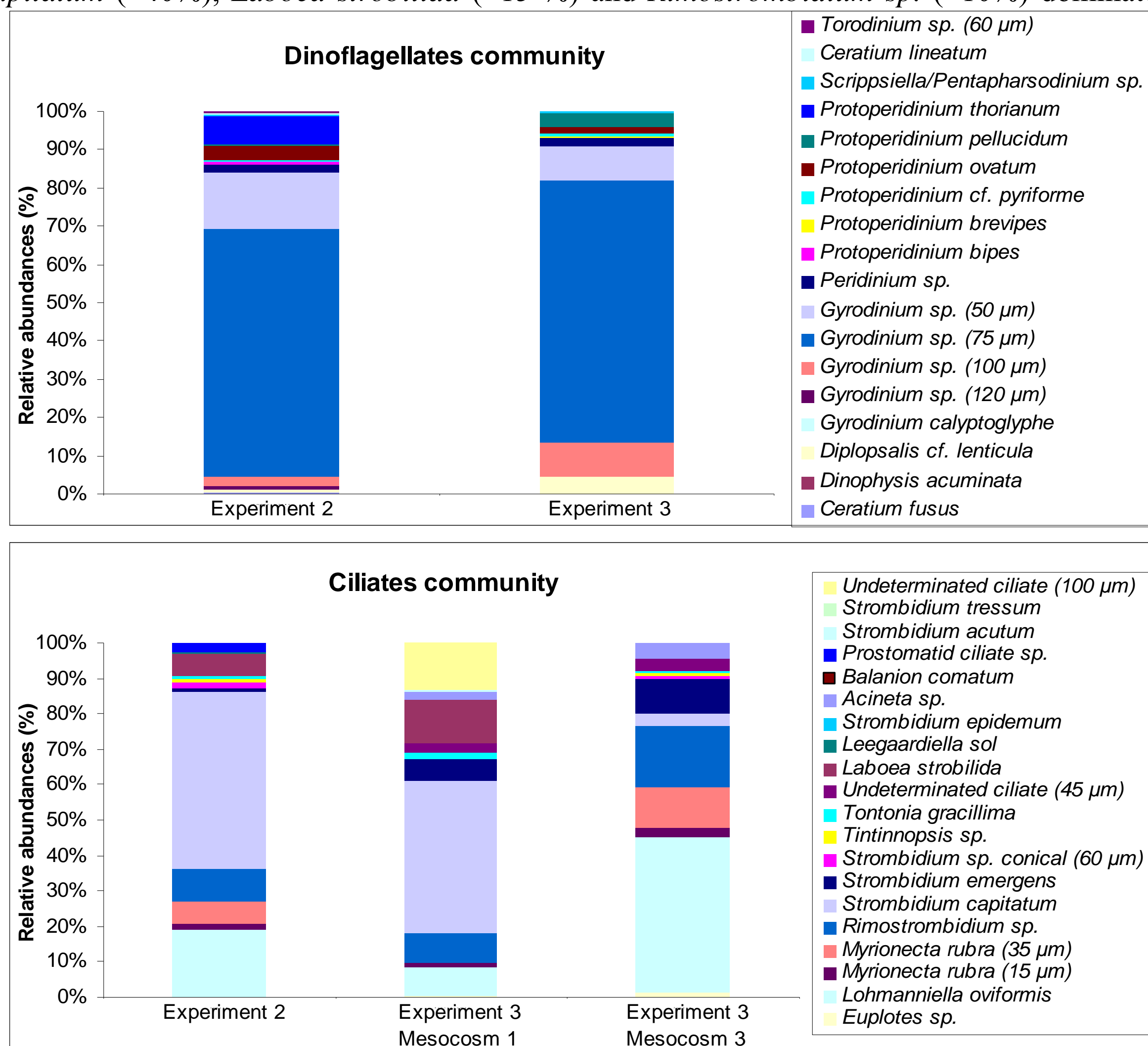


Fig. 9 Microzooplankton assemblages, relative abundances calculated from biomass data.

the ciliates community. In the third mesocosm, this community was dominated by *Lohmaniella oviformis* (~40%), *Rimostrombidium sp.* (~15%), *Myrionecta rubra* and *Strombidium emergens* (each ~10 %). Another difference between the mesocosms was detected in terms of diversity since 17 species/size classes were found in mesocosm 1 while 13 were found in mesocosm 3.

In both experiment the concentration in thecate amoeba was the same between the mesocosms and increased by a factor of two in each of the mesocosms between the second and the third experiment.

The results of both dilution experiments estimating the grazing impact of microzooplankton communities are presented in Tab. 2 and 3. Examples of graphs having allowed us to obtain the regression equations are presented in Fig.10 for the second and Fig. 11 for the third experiment.

In experiment 2 grazing coefficients of 0.007 (R^2=0.77), 0.006 (R^2=0.64) and 0.014 d^{-1} (R^2=0.98) were determined from the regression equations for the total phytoplankton community respectively for the three mesocosms. The growth coefficients for the phytoplankton communities were 0.731, 0.281 and 1.154 d^{-1} for mesocosm 1, 2 and 3 respectively and, except for mesocosm 2, less than one doubling per day occurred. However, due to an addition of nutrients to the incubation bottles, one would not expect the calculated growth coefficient from the dilution experiment to reflect accurately the growth rate of phytoplankton in the field.

In experiment 2 (24th March), in order to compare easily the three mesocosms, three clusters based on the grazing rates were chosen: (1) Level 1, >0.014 corresponds to an intensive grazing, (2) Level 2, from >0.01 to<0.014 for an intermediate grazing and (3) Level 3, <0.01 for a low grazing. Like this, we can define the most grazed species, in two or even three mesocosms: *Pseudonitzschia sp.* (60 µm), *Chaetoceros sp.* (20 and 40 µm), and flagellates (5 µm). The species that were grazed with intermediate intensity were *Rhizosolenia pungens* and flagellates (15 µm) (except in mesocosm 3, low grazing). The less grazed species were *Chaetoceros minimus*, *Rhizosolenia styliformis* (except in mesocosm 3, intermediate grazing), *Thalassiosira nordenskioeldii*, and flagellates (20 µm). Furthermore, R^2 for some species are significant for only one mesocosm. Thus, in mesocosm 2 *Chaetoceros sp*. (10 µm) and flagellates (25 µm) in mesocosm 3, were among the most grazed species; in mesocosm 3 *Chaetoceros sp.* (30 µm) was grazed at intermediate levels and, *Chaetoceros danicus* and *Thalassiosira rotula* were among the less grazed species. Finally, because of the variability inherent to such experiment, some species are not grazed in the same way between the

mesocosms. Thus, *Pseudonitzschia sp.* (120 µm) were highly grazed in mesocosm 3, fairly grazed in mesocosm 2 and grazed only to a low degree in mesocosm 1; *Pseudonitzschia sp*. (80 µm) and pennate diatoms (40 µm) were highly grazed in mesocosm 1 but respectively few and fairly grazed in mesocosm 3; pennate diatoms (20 µm) and flagellates (10 µm) were few grazed in mesocosms 1 and 2 but fairly and highly grazed in mesocosm 2.

For the phytoplankton growth rates the same kind of clusters were chosen: (1) Level 1, >1 corresponds to high growth rates, (2) Level 2, >0.5 to <1 for an intermediate growth rate and (3) Level 3, <0.5 for low growth rates. Thus, *Pseudonitzschia sp.* (120 µm) (except in mesocosm 1, intermediate growth rate), *Pseudonitzschia sp.* (60 µm), pennate diatoms (40 µm), *Chaetoceros sp.* (20 and 40 µm), *Rhizosolenia pungens* and flagellates (5 µm) showed highest growth rates. *Thalassiosira nordenskioeldii* presented a species with moderate growth rates. *Rhizosolenia styliformis* (except in mesocosm 3, high growth rate), pennate diatoms (20 µm) and flagellates (20 µm) had low growth rates. For species having significant R^2 in only one mesocosm, *Chaetoceros sp.* (10 µm) and flagellates (25 µm) (mesocosm 2 and 3) had high growth rates, *Chaetoceros danicus* and *Thalassiosira rotula* (both in mesocosm 3) presented intermediate and low growth rates. Finally, *Pseudonitzschia sp.* (80 µm) and *Chaetoceros minimus* showed intermediate growth rates in mesocosm 3 but high ones respectively in mesocosm 1 and 2; flagellates (15 and 10 µm) had low growth rates in mesocosm 1 but high ones in mesocosm 3.

In experiment 3 (31st March) grazing coefficients of 0.012 (R^2=0.85) and 0.003 (R^2=0.93) for mesocosm 2 and 3 respectively, were determined from the regression equations for the total phytoplankton community; the R^2 of mesocosm 1 was not significant. The growth coefficients for phytoplankton were 1.136 and 0.517 d^{-1} for mesocosm 2 and 3 respectively.

For this third experiment the same clusters used for experiment 2 are kept. Thus, the 80 (except mesocosm 1, low grazing) and 60 µm *Pseudonitzschia sp.* size classes as well as flagellates (15 µm) were highly grazed. *Pseudonitzschia sp.* (80 µm), *Rhizosolenia pungens* and *styliformis* and *Thalassiosira nordenskioeldii* were among the less grazed species. Species which presented significant R^2 in only one mesocosm were in mesocosm 2: *Chaetoceros minimus* and *Chaetoceros danicus*, *Chaetoceros sp.* (30 µm) were few grazed while pennate diatoms (20 µm) were fairly grazed. Finally, pennate diatoms (40 µm), *Chaetoceros sp.* (40 µm), flagellates (25, 20 and 10 µm) were highly grazed in mesocosm 2 while few or fairly grazed in the others. *Thalassiosira rotula* and flagellates (5 µm) were fairly grazed in mesocosm 2 while few grazed in mesocosm 3.

80 (except in mesocosm 1, intermediate growth rate) and 60 µm *Pseudonitzschia sp.* size classes, *Chaetoceros sp.* (40 µm) (except in mesocosm 1, intermediate growth rate), *Rhizosolenia pungens* (except in mesocosm 3, low growth rate) and flagellates (15 µm) had high growth rates while *Pseudonitzschia sp.* (120 µm) had intermediate growth rate and *Thalassiosira nordenskioeldii* had low growth rate. Species which presented significant R^2 in only one mesocosm were in mesocosm 2: *Chaetoceros minimus* and *Chaetoceros sp.* (30 µm) presented intermediate growth rates while *Chaetoceros danicus* and pennate diatoms (20 µm) low ones. Pennate diatoms (40 µm), *Thalassiosira rotula*, flagellates (25, 20 10 and 5 µm) presented high growth rates in mesocosm 2 but lower in the others. *Rhizosolenia styliformis* had intermediate growth rate in mesocosm 2 and low one in the third.

In addition, two other treatments were realised: (1) a treatment without nutrient addition in order to account for possible species-specific nutrient limitation patterns and (2) a copepod treatment accounting for the species-specific grazing of *T. longicornis* was included in the dilution experiment. The data of these treatments were added on the dilution graphs to account for possible significant differences with the dilution treatments. These differences would highlight, for the first treatment, a nutrient limitation when the value is lower compared to the 100% dot, and a grazing impact for the second treatment.

For experiment 2, pennate diatoms (20 µm) in mesocosm 1, flagellates (25µm) and *Pseudonitzschia sp.* (60 µm) in mesocosm 2 and *Chaetoceros sp.* (20µm) in mesocosm 3, were altered by nutrient limitation. For experiment 3, flagellates (15µm) in mesocosm 1, pennate diatoms (40 µm) in both mesocosms, *Pseudonitzschia sp.* (60 µm) and *Chaetoceros sp.* (20 µm) in mesocosms 2 and 3 presented a nutrient limitation.

For experiment 2, *T. longicornis* showed a species-specific grazing on flagellates (25 µm) (mesocosm 1) and on *Chaetoceros sp.* (10 µm) (mesocosms 2 and 3). For experiment 3, a species-specific grazing on pennate diatoms (20 µm) (mesocosm 2), and on flagellates (25 µm) and *Chaetoceros sp.* (20 µm) for the third mesocosm was evidenced.

In order to investigate the grazing impact of *Temora longicornis* on the microzooplankton community, tables 4 and 5 compare their growth rates in absence or presence of copepod. Only the species for which a comparison is possible are presented here.
In experiment 2, *Temora longicornis* had a strong impact on the growth of most microzooplankton species. Within the dinoflagellates community, only *Gyrodinium sp.* (50 µm), *Peridinium sp.* (35 µm), *Protoperidinium pellucidum*, *Torodinium sp.* (35 µm) were not

grazed to a considerable degree. Within the ciliate community, except in mesocosm 2, only *Laboea strobilida* and *Myrionecta rubra* (15 µm), *Strobilidium sp.* (75 µm) (except in mesocosm 1), *Strombidium sp.* (60 µm) conical (except in mesocosm 3) and *Strombidium stressum* were not affected by the copepods' presence. *Diplopsalis cf. lenticula* was the most dinoflagellate grazed in mesocosm 1 and *Protoperidinium cf. pyriforme* in mesocosms 2 and 3. *Strobilidium sp.* (75 µm) in mesocosm 1, *Strombidium epidemum* in mesocosm 2 and *Strombidium capitatum* in mesocosm 3 were the most ciliates grazed.

In experiment 3, the same strong grazing impact by *T. longicornis* was observed: only *Gyrodinium sp.* (50 µm), *Protoperidinium cf. pyriforme* (except in mesocosm 3), *Protoperidinium thorianum* and *Scrippsiella/Pentapharsodinium sp.* were not grazed among the dinoflagellate species. For the ciliates, *Acineta sp.*, *Balanion comatum*, *Euplotes sp.* (except in mesocosm 1), *Leegaardiella sol*, *Myrionecta rubra* (35 µm), *Strobilidium sp*. (75 µm), *Strombidium capitatum* (except in mesocosm 3), *Strombidium emergens* and *epidemum* were not grazed. *Protoperidinium ovatum* in mesocosm 1 and 2 and *Protoperidinium pellucidum* in mesocosm 3 were the most grazed dinoflagellates. *Tontonia gracillima* in mesocosms 1 and 2 and *Tintinnopsis sp.* in mesocosm 3 were the most grazed ciliates.

Tab. 2 Results of dilution experiment n°2 (24th March); the limit of significance for the regressions has been fixed at $R^2 > 0.5$, the other results are not presented

	Regression equation			Phytoplankton growth rate (day^{-1})			Microzooplankton grazing rate (day^{-1})			R^2		
	Mesocosm 1	Mesocosm 2	Mesocosm 3	Mesocosm 1	Mesocosm 2	Mesocosm 3	Mesocosm 1	Mesocosm 2	Mesocosm 3	Mesocosm 1	Mesocosm 2	Mesocosm 3
Pseudonitzschia sp. (120 µm)	y = -0,0081x + 0,6858	y = -0,0119x + 1,0196	y = -0,0146x + 1,2112	0,6858	1,0196	1,2112	0,0081	0,0119	0,0146	0,973	0,999	0,988
Pseudonitzschia sp. (80 µm)	y = -0,0278x + 1,7736		y = -0,0044x + 0,5226	1,7736		0,5226	0,0278		0,0044	0,844	NS	0,642
Pseudonitzschia sp. (60 µm)	y = -0,0192x + 1,8965	y = -0,016x + 1,9306		1,8965	1,9306		0,0192	0,016		0,701	0,635	NS
Pennate diatom (40 µm)	y = -0,0202x + 1,2535		y = -0,0133x + 1,2301	1,2535		1,2301	0,0202		0,0133	0,997	NS	0,923
Pennate diatom (20 µm)		y = -0,0076x + 0,3113	y = -0,0123x - 0,0893		0,3113	-0,0893		0,0076	0,0123	NS	0,644	0,765
Chaetoceros minimus		y = -0,0048x + 0,1582	y = -0,0097x + 0,5971		0,1582	0,5971		0,0048	0,0097	NS	0,880	0,742
Chaetoceros danicus			y = -0,0077x + 0,6263			0,6263			0,0077	NS	NS	0,609
Chaetoceros sp. (40 µm)	y = -0,0143x + 1,6154		y = -0,0157x + 1,5655	1,6154		1,5655	0,0143		0,0157	0,703	NS	0,889
Chaetoceros sp. (30 µm)			y = -0,0135x + 0,9106			0,9106			0,0135	NS	NS	0,990
Chaetoceros sp. (20 µm)	y = -0,0209x + 1,0774	y = -0,0169x + 1,9916	y = -0,014x + 2,108	1,0774	1,9916	2,108	0,0209	0,0169	0,014	0,922	0,662	0,735
Chaetoceros sp. (10 µm)		y = -0,0213x + 2,7527			2,7527			0,0213		NS	0,588	NS
Rhizosolenia styliformis	y = -0,0051x + 0,3732	y = -0,004x + 0,3328	y = -0,0105x + 1,0026	0,3732	0,3328	1,0026	0,0051	0,004	0,0105	0,798	0,809	0,919
Rhizosolenia pungens	y = -0,0123x + 1,1337		y = -0,0136x + 1,3957	1,1337		1,3957	0,0123		0,0136	0,848	NS	0,960
Thalassiosira nordenskioeldii		y = -0,0007x + 0,6134	y = -0,0085x + 0,9965		0,6134	0,9965		0,0007	0,0085	NS	0,320	0,820
Thalassiosira rotula			y = 0,005x - 0,4175			-0,4175			0,005	NS	NS	0,574
Flagellate (25 µm)			y = -0,021x + 1,3486			1,3486			0,021	NS	NS	0,926
Flagellate (20 µm)	y = -0,0048x - 0,0106		y = -0,0023x + 0,3112	-0,0106		0,3112	0,0048		0,0023	0,647	NS	0,705
Flagellate (15 µm)	y = -0,0043x + 0,0165	y = -0,0111x + 0,5545	y = -0,0118x + 1,0034	0,0165	0,5545	1,0034	0,0043	0,0111	0,0118	0,878	0,945	0,930
Flagellate (10 µm)	y = -0,005x + 0,3522		y = -0,0176x + 1,2081	0,3522		1,2081	0,005		0,0176	0,881	NS	0,914
Flagellate (5 µm)	y = -0,0149x + 1,4037		y = -0,016x + 1,4686	1,4037		1,4686	0,0149		0,016	0,786	NS	0,948
Total community	y = -0,0072x + 0,7306	y = -0,0056x + 0,2811	y = -0,0135x + 1,1543	0,7306	0,2811	1,1543	0,0072	0,0056	0,0135	0,769	0,644	0,981

Tab. 3 Results of dilution experiment n°3 (31th March); the limit of significance for the regressions has been fixed at $R^2 > 0.5$, the other results are not presented

	Regression equation			Phytoplankton growth rate (day^{-1})			Microzooplankton grazing rate (day^{-1})			R^2		
	Mesocosm 1	Mesocosm 2	Mesocosm 3	Mesocosm 1	Mesocosm 2	Mesocosm 3	Mesocosm 1	Mesocosm 2	Mesocosm 3	Mesocosm 1	Mesocosm 2	Mesocosm 3
Pseudonitzschia sp. (120 µm)		y = -0,0056x + 0,6697	y = -0,0047x + 0,6091		0,6697	0,6091		0,0056	0,0047	NS	0,5481	0,8298
Pseudonitzschia sp. (80 µm)	y = -0,0074x + 0,9681	y = -0,0149x + 1,3487	y = -0,0141x + 1,0555	0,9681	1,3487	1,0555	0,0074	0,0149	0,0141	0,7287	0,8139	0,8795
Pseudonitzschia sp. (60 µm)		y = -0,0211x + 1,7529	y = -0,0245x + 1,8327		1,7529	1,8327		0,0211	0,0245	NS	0,6571	0,918
Pennate diatom (40 µm)	y = -0,009x + 0,6581	y = -0,0229x + 1,0909		0,6581	1,0909		0,009	0,0229		0,7453	0,535	NS
Pennate diatom (20 µm)		y = -0,0081x + 0,3812			0,3812			0,0081		NS	0,8773	NS
Chaetoceros minimus		y = -0,0114x + 0,9064			0,9064			0,0114		NS	0,9344	NS
Chaetoceros danicus		y = 0,0039x - 0,3043			-0,3043			0,0039		NS	0,5028	NS
Chaetoceros sp. (40 µm)	y = -0,0108x + 0,8452	y = -0,0244x + 2,0149	y = -0,0113x + 1,0474	0,8452	2,0149	1,0474	-0,0108	0,0244	0,0113	0,5568	0,9365	0,6302
Chaetoceros sp. (30 µm)		y = -0,0058x + 0,5045			0,5045			0,0058		NS	0,9033	NS
Rhizosolenia styliformis		y = -0,0056x + 0,5457	y = -0,0049x + 0,3984		0,5457	0,3984		0,0056	0,0049	NS	0,7906	0,7565
Rhizosolenia pungens	y = -0,0071x + 1,1949	y = -0,0101x + 1,1664	y = -0,007x - 0,0278	1,1949	1,1664	-0,0278	0,0071	0,01	0,007	0,8625	0,6144	0,5478
Thalassiosira nordenskioeldii	y = -0,0081x + 0,2929	y = -0,0027x + 0,0389	y = -0,0035x + 0,1997	0,2929	0,0389	0,1997	0,0081	0,0027	0,0035	0,8572	0,5417	0,6462
Thalassiosira rotula		y = -0,0122x + 1,0925	y = -0,0065x + 0,594		1,0925	0,594		0,0122	0,0065	NS	0,8741	0,8462
Flagellate (25 µm)		y = -0,0236x + 2,1884	y = -0,0079x - 0,001		2,1884	-0,001		0,0236	0,0079	NS	0,8979	0,5704
Flagellate (20 µm)	y = -0,0113x + 0,0109	y = -0,0158x + 1,6006	y = -0,0026x - 0,171	0,0109	1,6006	-0,171	0,0113	0,0158	0,0026	0,9784	0,9911	0,5778
Flagellate (15 µm)	y = -0,0168x + 1,1528	y = -0,0252x + 1,602		1,1528	1,602		0,0168	0,0252		0,9119	0,8035	NS
Flagellate (10 µm)		y = -0,0167x + 1,3206	y = -0,0061x + 0,848		1,3206	0,848		0,0167	0,0061	NS	0,9151	0,8753
Flagellate (5 µm)		y = -0,0126x + 1,1854	y = -0,0027x + 0,5088		1,1854	0,5088		0,0126	0,0027	NS	0,8452	0,8954
Total community		y = -0,0122x + 1,1355	y = -0,0029x + 0,5173		1,1355	0,5173		0,0122	0,0029	NS	0,8543	0,9268

Tab. 4 Comparison of microzooplankton growth rates in absence and presence of *Temora longicornis* for experiment 2.

	Mesocosm 1		Mesocosm 2		Mesocosm 3	
	Growth rate (d^{-1})	Growth rate with *Temora* (d^{-1})	Growth rate (d^{-1})	Growth rate with *Temora* (d^{-1})	Growth rate (d^{-1})	Growth rate with *Temora* (d^{-1})
Dinoflagellates						
Diplopsalis cf. lenticula	0,000	-1,253	-1,322	-1,609	0,470	-0,223
Gyrodinium sp. (50 µm)	0,336	0,736	0,245	0,502	0,504	0,713
Gyrodinium sp. (75 µm)	0,335	-0,110	0,255	0,144	0,421	-0,099
Gyrodinium sp. (100 µm)	0,323	-0,742	0,363	-0,575	-0,140	-1,190
naked *dinoflagellate sp.*	0,227	-0,267	-0,199	-0,122	0,315	-0,140
Peridinium sp. (35 µm)	-0,405	-0,223	0,452	0,619	0,174	0,288
Protoperidinium bipes	0,109	0,109	0,071	-0,160	0,778	0,386
Protoperidinium cf. pyriforme	-0,470	-0,470	-0,080	-1,872	-0,288	-2,079
Protoperidinium leonis	0,470	0,336	0,041	-0,875	0,256	-1,232
Protoperidinium ovatum	0,916	1,099	2,485	1,946	-0,693	0,560
Protoperidinium pellucidum	0,405	0,000	-0,154	-0,154	0,000	-0,405
Protoperidinium thorianum	0,232	-0,191	0,105	-0,160	-0,105	-0,223
Scrippsiella/Pentapharsodinium sp.	1,447	0,811	0,368	-0,118	-0,134	-0,613
thecate *dinoflagellate sp.*	-0,543	-0,890	-0,664	-0,705	-0,372	-0,616
Torodinium sp. (35 µm)	-1,099	0,154	0,000	1,253	-0,288	0,560
Warnowia sp.	-0,466	-1,293	0,143	-0,956	-0,670	-0,206
Ciliates						
Laboea strobila	-0,442	-0,499	-0,300	-0,811	0,315	0,342
Lohmanniella oviformis	0,532	0,407	0,609	0,356	0,593	0,472
Myrionecta rubra (15 µm)	0,115	0,247	0,225	-0,277	0,389	0,545
Myrionecta rubra (35 µm)	-0,208	-0,488	-0,693	-1,038	-0,055	-0,285
Prostomatid ciliate sp.	-0,175	-0,130	0,061	-0,315	0,143	-0,045
Rimostrombidium sp.	0,693	0,065	0,916	0,194	0,875	0,262
Strobilidium sp. (75 µm)	0,405	-0,693	1,099		0,916	
Strombidium capitatum	0,726	-0,234	0,618	-0,134	0,555	-0,643
Strombidium emergens	0,134	-0,154	-0,773	-0,773	0,105	-0,118
Strombidium epidemum	0,405	0,742	2,197	1,386	0,693	0,386
Strombidium sp. conical (60 µm)	0,095	0,336	0,780	0,310	0,425	0,302
Strombidium sp. small	-0,091	-0,496	0,082		-0,074	
Strombidium tressum			0,693	0,693		
Tintinnopsis sp.	0,305	-0,154	-0,074	-0,624	0,191	-0,642
Tontonia gracillima	-0,606	-1,299	-1,492	-1,609	-0,182	
Amoeba						
thecate *amoeba sp.*	0,324	-0,065	0,306	0,166	0,164	-0,043

Tab. 5 Comparison of microzooplankton growth rates in absence and presence of *Temora longicornis* for experiment 3.

	Mesocosm 1		Mesocosm 2		Mesocosm 3	
	Growth rate (d^{-1})	Growth rate with *Temora* (d^{-1})	Growth rate (d^{-1})	Growth rate with *Temora* (d^{-1})	Growth rate (d^{-1})	Growth rate with *Temora* (d^{-1})
Dinoflagellates						
Ceratium fusus	1,386	0,000	0,182	-1,609	1,386	0,693
Diplopsalis cf. lenticula		-0,560	0,773	0,511	-0,310	-0,762
Gyrodinium sp. (120 µm)	0,693		0,000		0,916	-0,693
Gyrodinium sp. (50 µm)	2,031	2,477	2,693	2,909	0,453	0,489
Gyrodinium sp. (75 µm)	-0,070	-0,246	-0,177	-0,361	0,116	-0,148
Gyrodinium sp. (100 µm)	0,267	-0,154	0,087	-0,526	0,070	-0,511
Peridinium sp. (35 µm)	-0,348	-0,636	0,375	-0,201	0,251	-0,074
Protoperidinium bipes	0,105	-0,588	-0,368	-0,956	-0,470	0,272
Protoperidinium brevipes	0,288	0,288	1,609	0,693	0,693	0,000
Protoperidinium cf. pyriforme	0,000	-0,288	0,288	0,511	-0,405	-0,944
Protoperidinium ovatum	-0,105	-1,609	1,190	-1,253	0,405	-0,470
Protoperidinium pellucidum	0,288	-0,405	0,811	0,405	0,000	-1,872
Protoperidinium thorianum	-0,405	0,288	-0,773		-1,609	-1,609
Scrippsiella/Pentapharsodinium sp.	-1,253	-0,154	1,609	1,386	-0,693	0,405
Ciliates						
Acineta sp.	0,588	0,000	0,693	0,000	0,223	0,405
Balanion comatum	-1,099		1,386		0,693	1,386
Euplotes sp.	0,693	0,182	-0,693	0,693	0,118	0,223
Laboea strobila	-0,182	-0,087	-0,368	-0,486	-0,799	
Leegaardiella sol			-0,492	-0,492	-1,099	
Lohmanniella oviformis	0,336	-0,280	0,062	-0,180	-0,335	-0,327
Myrionecta rubra (15 µm)	-0,216	0,080	0,082	-0,202	-0,349	-0,443
Myrionecta rubra (35 µm)	-2,079		-1,526	-1,526	-0,989	-1,061
Rimostrombidium sp.	-1,061	-1,312	-0,709	-1,008	-1,584	-1,466
Strobilidium sp. (75 µm)	0,000		0,405	0,405		
Strombidium capitatum	-2,897	-2,175	-1,192	-1,278	-2,216	-3,314
Strombidium emergens	-2,773	-0,134	-2,398	-0,095	-0,405	0,442
Strombidium epidemum	1,386	0,693				
Tintinnopsis sp.			0,956	0,182	0,788	-0,511
Tontonia gracillima	0,636	-0,405	0,262	-0,916	-1,792	-2,485
Amoeba						
thecate *amoeba sp.*	-0,114	-0,308	-0,285	-0,356	0,043	-0,083

Experiment 2- Food quality aspects

In order to follow the growth of *Pentapharsodinium sp.* and *Scrippsiella sp.* daily *in situ* fluorescence measurements were conducted (Fig. 12).

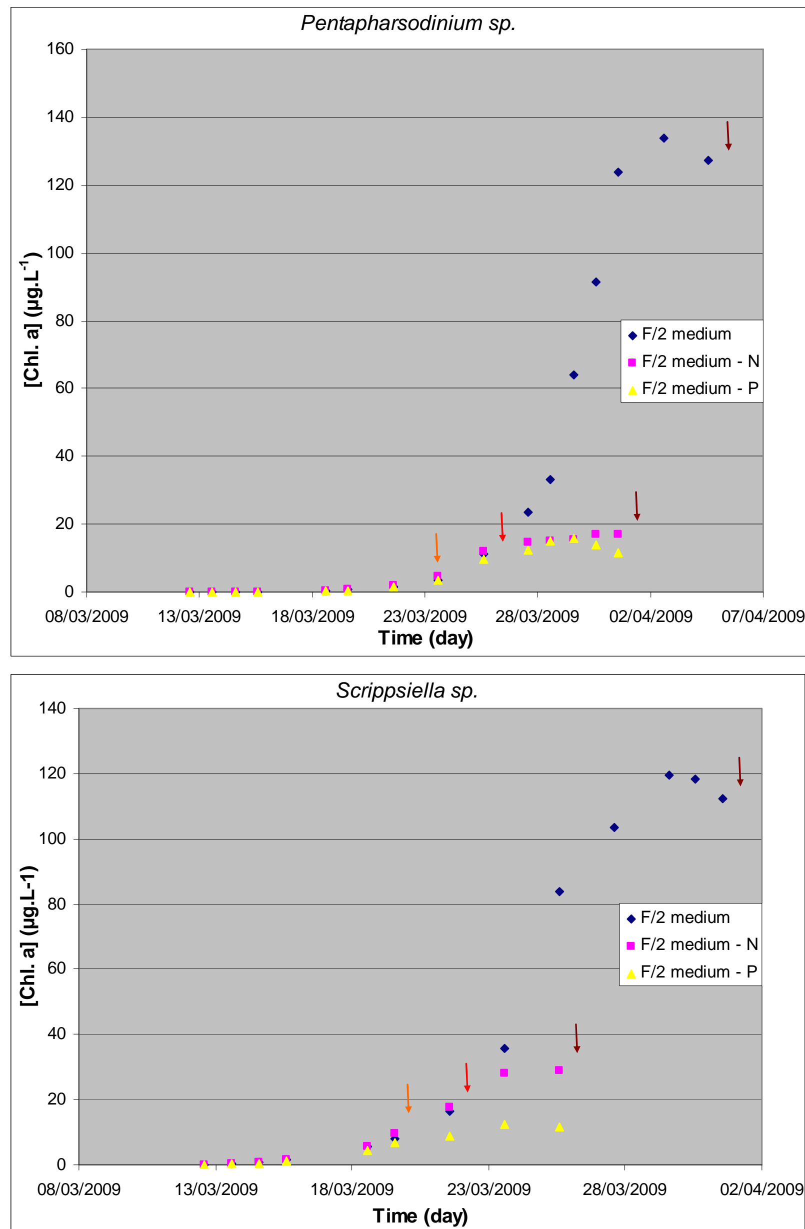


Fig. 12 Chlorophyll concentration evolution during the experiment for *Pentapharsodinium sp*. and *Scrippsiella sp.*; orange arrows, lag phase sampling; red arrows, log phase sampling; brown arrows, stationary phase sampling.

This allowed us to identify three growth phases; lag, log and stationary, during which we have investigate there food quality in terms of C:N ratio (Fig. 13) and fatty acids profile (Fig.14).

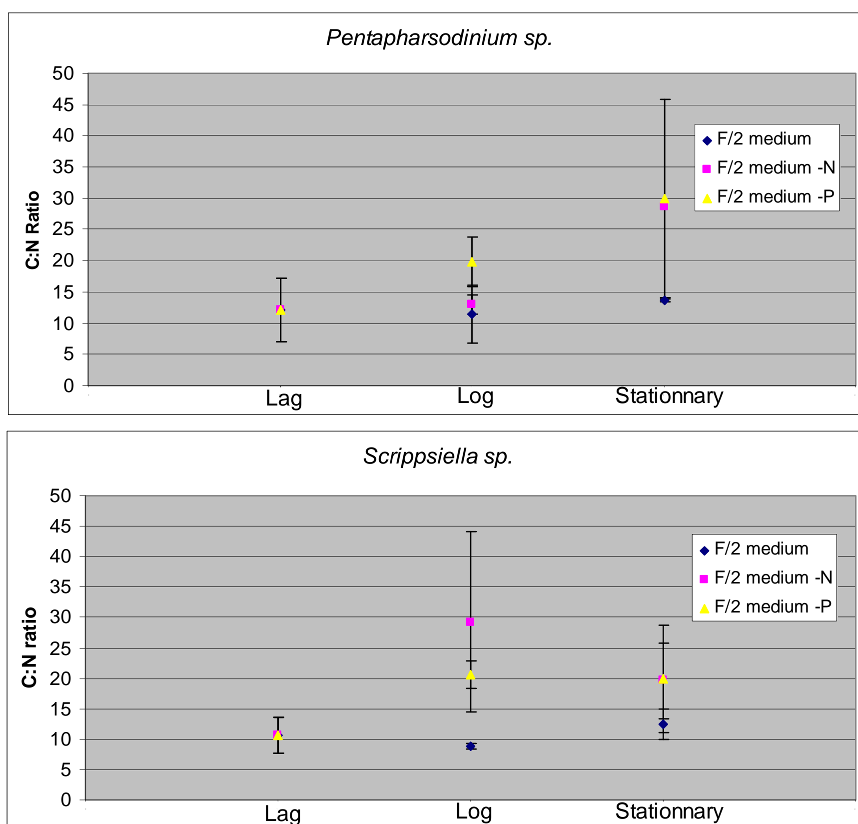


Fig.13 C:N ratios of *Pentapharsodinium sp.* and *Scrippsiella sp.*, measured during three growth phases, cultivated in three different media.

The first observation is that no significant difference can be seen between the two species. For *Pentapharsodinium sp.*, one can see a strong increase in C:N from 12 to 30 for the limited conditions, with increasing duration of the experiment. For *Scrippsiella sp.*, the highest C:N occurred during the exponential phase (around 20 and 30 for P and N limitations) and thereafter declined to 20, although this is not significant. Furthermore, C:N for both species remain constant around 12 for the unlimited condition.

Due to a problem with the gas chromatography, fatty acid profiles are only available for *Pentapharsodinium sp.*. For the unlimited condition, one can see an increase of 16_0 from 0.21 to 0.29, DPA from 0.18 to 0.34 and 18_0 and 18_1 n9 (trans) from 0.005 to 0.007. Furthermore, 22_2 n6 decreased slightly. For the N-limited medium, 16_0, DPA, 18_1 n9 (trans), 18_2 n6 increase strongly with the duration of the experiment. For the P-limited medium, 16_0, 18_1 n9 (trans), 18_3 n3, 22_2 n6 and DPA present a strong increase between the lag and log phases and a decrease or stabilisation at the stationary phase. The only fatty acid which continued to rise is 18_2 n6 (cis). The other general observation is that for the

limited media, the quantity of the fatty acids described above are almost twice as high as in the unlimited medium.

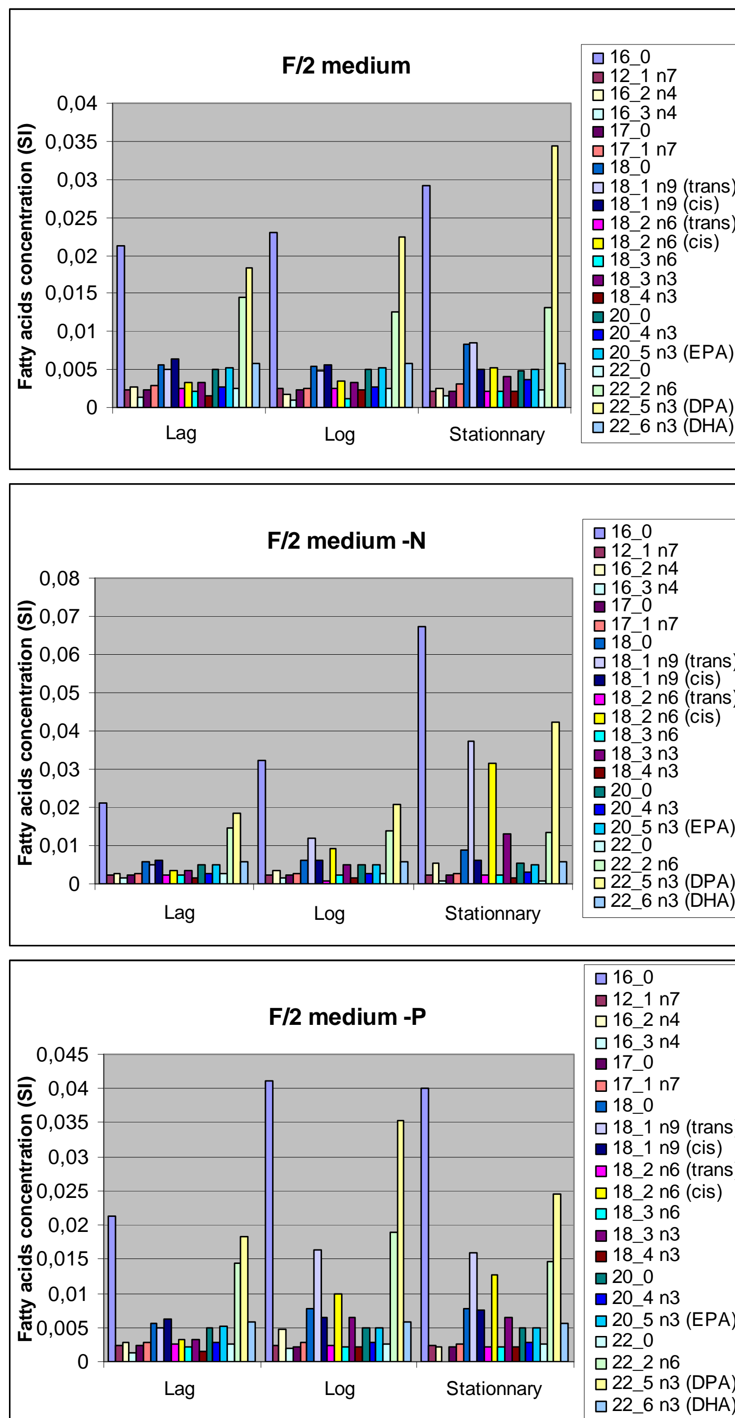


Fig.14 Fatty acid profiles of *Pentapharsodinium sp.* measured during three growth phases, cultivated in three different media.

4. Discussion

Experiment 1- Feeding selectivity

- General observations

Since photoautotrophic biomass can increase by up to three orders of magnitude within a few days, phytoplankton blooms can be considered as the biggest biological events in nature. The species composition of these blooms plays a major ecological role. Furthermore, successions occurring during blooms are temporal changes in the relative abundance and dominance of species comprising natural assemblages. These changes in natural phytoplankton assemblages can be interpreted as species/organism-specific responses to environmental variability, grazing pressure and physical factors which will lead to the formation of particular planktonic communities. R-strategists are in general considered to dominate the beginning of the bloom; while late stages are dominated by K-strategist plankton species (Poole 1979, Reynolds 2002). Environmental fluctuations cause a moving selective advantage from species to species generating typical successions during blooming events (Sommer 1988, Reynolds 1988, Sommer et al. 2007, Sommer & Lengfellner 2008).
When having a look at the development of chlorophyll concentration during the mesocosm experiment, the first observation was that the peak phytoplankton biomass was reached only within a few days. This allows us to assume that the phytoplankton in the field was already developing exponentially at the date of seawater sampling for the mesocosm experiment. This assumption was confirmed by the continuous data recording at Helgoland Roads. Furthermore, the peak in the field was reached at the same time (Peters pers. com.) as in the mesocosms which confirms the representativeness of our experiment aiming at simulating a typical spring bloom succession usually occurring in the field at that time of the year. When comparing the first value measured just after the mesocosm filling with the values after two days experimental run, the initial values were slightly higher than those recorded on day 2. It is most likely that this is related to the filling and filtration process which might have damaged cells thus leading to increased chlorophyll concentration in the mesocosms. A general pattern, in temperate marine environments, usually observed during spring bloom succession in the field is that the chlorophyll concentration decrease quickly right after a phytoplankton bloom due to nutrient limitation or/and grazing impact (Edwards & Richardson 2004, Wiltshire & Manly 2004, Aberle 2007, Wiltshire et al. 2008). In contrast, only a slow decrease in phytoplankton biomass could be observed during our mesocosm study which

could be related to the absence of mesozooplankton thus releasing phytoplankton from mesozooplankton grazing. In fact, the grazing impact of mesozooplankters is well known and in several studies it was shown that they are able to suppress a bloom (Carpenter et al. 1985, Griffin 2001, Irigoien et al. 2005). This was described by Irigoien et al. 2005, explaining that the predator–prey relation is crucial for the control of primary production; which is in part a classic match–mismatch issue (Cushing 1990). Blooms may thus be considered as events generated principally by a failure of grazers to control phytoplankton production. Despite the slower decline of chlorophyll concentrations in the mesocosms, there is evidence that microzooplankters contributed largely to the consumption of phytoplankton during the bloom even if the microzooplankton was not able to suppress the spring phytoplankton biomass as intense as in the presence of mesozooplankton (Calbet 2001, Calbet & Landry 2004). In this context the almost constant nitrate and ammonium concentrations give indirect indication for an increased nutrient recycling in the mesocosms which is most likely related to a substantial contribution of microzooplankton grazing thus leading to a higher nutrient recycling efficiency via the microbial loop. Thus, phytoplankton-microzooplankton interactions during bloom conditions can be regarded as an interplay between phytoplankton growth limited by the grazing impact of microzooplankters and, on the other hand, it is boosted by the strong nutrient remineralisation which could explain why the bloom was not depleted so quickly and remained almost constant for several days.

- Dilution experiment

Between experiment 2 and 3, in spite of their high growth rates, 10 and 20 µm *Chaetoceros sp.* disappeared which could explain the high grazing exerted on them. Furthermore, some less abundant species, like 60 µm *Pseudonitzschia sp.*, 40 *Chaetoceros sp.* and 15 and 5 µm flagellates, were also highly grazed and had high growth rates. Thus, microzooplankters seem to be able to deplete completely some species even if they are growing at high rates. Furthermore, the dominant phytoplankton species, *Rhizosolenia styliformis*, *Thalassiosira nordenskioeldii*, *Thalassiosira rotula* and 30 µm *Chateoceros sp.*, were among the less grazed and had quite high growth rates in both experiments. Consequently, microzooplankters seem to be, with its high grazing impact, perfectly able to strongly influence the phytoplankton community composition. These results, allow us to join the hypothesis emitted by Irigoien (2005), that blooming species are those able to escape control by grazers leading the bloom of inedible species (e.g. *Rhizolenia styliformis* and *Pseudonitzschia sp.*

known to produce toxic components, even if this seems not to be the case in our experiments because they were grazed) while edible components of the phytoplankton are grazed down substantially. Blooming conditions are interpreted as physical or chemical perturbations disrupting the predator–prey controls that normally operate, opening 'loopholes' into which some phytoplankton species populations can explode.
Because this was the dominant dinoflagellate species and because it is the one which increased the most between both experiments (from 50% to 70%), 75 µm *Gyrodinium sp.* can be considered as the main grazer within the dinoflagellate community. The same pattern was observed for mesocosm 1 and 2, even if it decreased in relative abundances between both experiments as this species represented 40% of the dinofalgellate biomass. These heterotrophic dinoflagellates were identified as being a strong competitor for other dinoflagellates, ciliates and copepods even if, unlike copepods, *Gyrodinium sp.* have growth rate which allow them to respond numerically (Hansen 1992, Haigh & Taylor 1991). For the third mesocosm, *Strombidium capitatum* decreased until less than 5% in aid of *Lohmaniella oviformis* and *Rimostrombidium sp.* which can so be seen as the main grazers among ciliates. These ciliates are known to be abundant in marine food webs, especially in the North Sea (Fenchel 1987, Lynn & Montagnes 1991, Montagnes 1996) and thus their significant role as phytoplankton grazers can be assumed. Furthermore, since the strongest difference in term of grazing rate between mesocosm 1 and 2, and mesocosm 3 was observed for 25 and 20 µm flagellates: they were few grazed in the third mesocosm and highly in the others, we can expect a feeding preference of *Strombidium capitatum* for these flagellates, but this hypothesis needs further consideration. This information could be important if confirmed because it provides new data concerning the species succession and the shift of grazing from species to species occuring during a bloom. These results give new information on the grazing impact of microzooplankters on the diatom spring bloom in temperate marine environments, the dominant species within the sub-communities but also on their feeding preferences on phytoplankton, thus increasing our knowledge on this planktonic food web.

The treatment without added nutrients proved that only a few species were affected by limitation. This confirms the hyptothesis emited above in the "general observation" part that the strong nutrient remineralisation by the microzooplankton in our study avoided nutrient limitation of phytoplankton growth during bloom succession in the mesocosms. Furthermore, since the species showing this limitation are almost the same between both experiments (e.g. 20 µm *Chaetoceros sp.*, 60 µm *Pseudonitzschia sp.*) these species can be assumed as the ones

with the highest nutrient demand. This is supported by other studies (Dugdale 1967, Conway & Harrison 1977, Pan et al. 1996) showing that these species are highly affected by nutrient limitation slowing down strongly their growth rate.

The treatment on the grazing impact of the copepod *Temora longicornis* highlighted that few phytoplankton species were grazed directly by *T. longicornis*. Other studies showed that e.g. *Chaetoceros sp.* is efficiently grazed by *T. longicornis* (Gasparini et al 2000, Antajan 2004) which fits well into our results as this was one of the preferred food items of *T. Longicornis* in our experiments.
Thus it can be assumed that copepods were feeding preferentially on microzooplankton, which is evinced by the comparison of the growth rate in presence and absence of copepepod predators. This result is very interesting because several studies showed that copepods used to graze mainly on phytoplankton (Mullin 1963, Richman & Rogers 1969, Paffenhöfer 1971, Smetacek et al*.,* 1997, Fransz & Gonzalez, 1997, Dubischar & Bathmann 1997), even if over the last years investigations showed that copepods feed preferentially on microzooplankton during distinct periods like e.g. *Phaeocystis* blooms, which can represent up to 50-96% of their diet at specific times of the year (Gasparini *et al.*, 2000). In our study, there was a very diverse phytoplankton assemblage, in term of size as well as taxonomic composition, allowing a large range of food choice. One reason for this preference for microzooplankton as prey could be the high abundance of phytoplankton compared to microzooplankton. In fact, to ensure a balanced diet, *Temora* would feed preferentially on the rarest prey (Gentsch et al 2008). The preferential grazing of *T. longicornis* on microzooplankton over large diatoms and *Phaeocystis* colonies was demonstrated by Antajan (2004) even if daily ingestion rates on microzooplankton would not be sufficient to sustain copepod growth (Gasparini *et al.*, 2000). The active predation of copepods on microzooplankton represents an important trophic pathway linking the microbial food web to the classical food chain (from diatoms to copepods). However this link could also be the basis of a trophic cascade where copepod grazing on microzooplankton could stimulate *Rhizolenia styliformis* blooms.

Experiment 2- Food quality aspects

Since *Pentapharsodinium sp.* and *Scrippsiella sp.* cultures were batch cultures, the high C:N is a proxy for a strong nutrient limitation which is confirmed by the comparison with the f/2 treatment in which C:N were lower. Furthermore, at the end of the log phase we observed

that both species began to form cysts and that they became very numerous during the stationary phase. The increase in C:N could be interpreted as a result of N-limitation, slowing down the N incorporation leading to an increase of this ratio, but this increase was also observed in P-limitation condition. We can emit the hypothesis that theses species, in stress conditions, begin to form cysts when they have enough energy in reserve. This is supported by the increase in C:N for *Scrippsiella sp.* between lag and log phase followed by a stabilisation at the stationary phase. Thus, we can think that the sampling for *Scrippsiella sp.* was most likely at the end of the exponential phase, when the organisms already began to form cysts, which could explain the C:N stabilisation observed. Compared to other studies on phytoplankton food quality and C:N (Ahlgren et al 1997, Kilham et al. 1997), the values found for *Pentapharsodinium sp.* and *Scrippsiella sp.* are really high, thus they can be considered as bad quality food for consumers. Actually, high C:N values do not stand for good quality: if C:N is rather high, the cells contain more or less only C and almost no N, but C is usually not a limiting element. Good algal diets have usually a C :N of 6-8 while the values found here are almost twice as high, for the unlimited condition (Ahlgren et al 1997, Kilham et al. 1997).

The hypothesis emitted above on the cyst formation can be supported for *Pentapharsodinium sp.* by the fatty acids data. Indeed, some fatty acids, like DPA, increased strongly with the duration of the experiment for the N limited medium while they reached their maximum during the log phase and decrease at the stationary phase in the P limited medium. Furthermore, *Pentapharsodinium sp.* reached the stationary phase earlier under P- than under N-limited conditions and so the sampling in the P limited treatment most likely took place at the end of the exponential phase. The high content in 16:0, DPA and 18:2 ω6 is related to the physiology of the algae and was evidenced to increase under stress conditions (Thompson et al 1990). Furthermore, low values of EPA and DHA were found in *Pentapharsodinium sp.* compare to other species (Bronk et al 1993) which seems to confirm the C:N results.

5. Conclusion

This study provides insights on the importance of food quality aspects and selective feeding of microzooplankters within the North Sea food web by clarifying their role in the plankton at Helgoland Roads. Based on the present data we were able to highlight that microzooplankters are able to suppress phytoplankton spring blooms and to graze down phytoplankton biomass substantially but also to boost the regenerated production by contributing to a fast nutrient remineralisation. We also proved that selective grazing by microzooplankters leads to a bloom of inedible phytoplankton species (e.g. *Rhizolenia styliformis* and *Pseudonitzschia sp.* known to produce toxic components, even if this seems not to be the case in our experiments because they were grazed) while edible components of the phytoplankton are grazed down substantially. Finally we evidenced that when microzooplankton is released from grazing pressure by copepods, the phytoplankton community is biased by selectivity grazing patterns of microzooplankters. This was shown by the strong grazing impact of *T. longicornis* on microzooplankton while this copepod grazed only to moderate degrees on phytoplankton cells. It was thus evinced that microzooplankters are grazed down substantially by copepods leading to the assumption that in the presence of mesozooplankton grazers the grazing impact of microzooplankters on the phytoplankton might be reduced.

Furthermore, we proved that no clear difference exists between *Pentapharsodinium sp.* and *Scrippsiella sp.* in term on food quality when considering C:N and fatty acids since measurements were done for only one species. We made the hypothesis that in limited conditions these dinoflagellates increase their energy stock until a threshold after which they form cyst to ensure their survival.

Moreover, to complete our knowledge on trophic interactions, experiments investigating stoichiometric constraints in natural food webs are needed in the future. To enhance our understanding on nutritional constraints in marine pelagic food webs, testing the consumer's capacity to choose high quality food and by investigating the propagation of different phytoplankton quality via grazers to second and third consumers should be realised. These aspects could be experimentally analysed in microcosms and mesocosms and fundamentally enhance our ability to predict the consequences of anthropogenically altered biogeochemistry in coastal waters on pelagic ecosystems.

6. Bibliography

Aberle N, Lengfellner K, Sommer U (2007) Spring bloom succession, grazing impact and herbivore selectivity of ciliate communities in response to winter warming. Oecologia 150:668-681

Aberle N, Malzahn AM (2007). Interspecific and nutrient-dependent variations in stable isotope fractionation: experimental studies simulating pelagic multitrophic systems. Oecologia, 154(2):291-303.

Ahlgren G, Goedkoop W, Markensten H, Sonesten L, Boberg M (1997) Seasonal variations in food quality for pelagic and benthic invertebrates in Lake Erken – the role of fatty acids. Fr Biol 38:555–570

Antajan E (2004). Responses of calanoid copepods to changes in phytoplankton dominance in the diatoms - *Phaeocystis globosa* dominated Belgium coastal waters. PhD Thesis. Vrije Universiteit Brussel. Laboratorium voor Ecologie en Systematiek: Brussel, Belgium. 147 pp.

Bronk DA, Glibert PM (1993b) Contrasting patterns of dissolved organic nitrogen release by two size fractions of estuarine plankton during a period of rapid NH^{4+} consumption and NO^{2-} production. Mar Ecol Prog Ser 96:291–299

Calbet A (2001) Mesozooplankton grazing effect on primary production: a global comparative analysis in marine ecosystems. Limnol. Oceanogr. 46: 1824–1830.

Calbet A, Landry MR (2004), Phytoplankton growth, microzooplankton grazing and carbon cycling in marine systems, Limnol. Oceanogr. 49:51–57.

Calbet A, Saiz E (2005) The ciliate-copepod link in marine ecosystems. Aquat Microb Ecol 38:157–167

Carpenter SR, Kitchell JF, Hodgson JF (1985) Cascading trophic interactions and lake productivity. BioSci 35:634–639

Cushing DH (1989) A difference in structure between ecosystems in strongly stratified waters and in those that are only weakly stratified. J Plankton Res 11:1–13

Cushing DH (1990) Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. Adv. Mar. Biol., 26:250–293.

Dubischar CD, Bathmann UV (1997) Grazing impact of copepods and salps on phytoplankton in the Atlantic sector of the Southern Ocean. Deep-Sea Res II 44:415–433

Dugdale RC (1967) Nutrient limitation in the sea: dynamics, identification and significance. Limnol Oceanogr. 12:685-95

Edwards M, Richardson AJ (2004) Impact of climate change on marine pelagic phenology and trophic mismatch. Nature 430:881-884

Fenchel T, Finlay BJ (1983) Respiration rates in heterotrophic, free-living protozoa. Microb Ecol 9:99–122

Fenchel T (1987) Ecology of protozoa; the biology of free-living phagotrophic protist. Science Tech Springer-Verlag. Madison,WI. Berlin. (USDE)

Flynn KJ, Davidson K, Cunningham A (1996) Prey selection and rejection by a microflagellate; implications for the study and operation of microbial food webs. J Exp Mar Biol Ecol 196:357

Fileman E, Smith T, Harris R (2007) Grazing by Calanus helgolandicus and Para-Pseudocalanus spp. on phytoplankton and protozooplankton during the spring bloom in the Celtic Sea. J Exp Mar Biol Ecol 348:70-84

Fransz HG, González SR (1997) Latitudinal metazoan plankton zones in the Antarctic Circumpolar Current along 6°W during austral spring 1992. Deep-Sea Res 44:395-414

Gaines G, Elbrächter M (1987) Heterotrophic nutrition, p. 224-268. III E J. R. Taylor [cd.], The biology of dinoflagellates. Blackwell.

Gasparini S, Daro MH, Antajan E, Tackx M, Rousseau V, Parent JY, Lancelot C (2000) Mesozooplankton grazing during the *Phaeocystis globosa* bloom in the southern bight of the North Sea. Jour Sea Res 43:345–356

Gentsch E, Kreibich T, Hagen W, Niehoff B (2008).Dietary shifts in the copepod *Temora longicornis* during spring: evidence from stable isotope signatures, fatty acid biomarkers and feeding experiments, J of Plank Res, 31(1):45-60

Greve W, Reiners F (1995) Biocoenotic process patterns in the German Bight. Olsen and Olsen, Fredensborg, pp 67–72

Griffin SL, Herzfeld M, Hamilton DP (2001) Modelling the impact of zooplankton grazing on phytoplankton biomass during a dinoflagellate bloom in the Swan River Estuary, W AustrEcol Eng 16 (3):373-394

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. Can J Microbiol 8: 229–239

Haigh R, Taylor FJR (1991) Mosaicism of microplankton communities on the northern Strait of Georgia, British Columbia. Mar Biol 110:301–314

Hamels I, Mussche H, Sabbe K, Muylaert K, Vyverman W (2004) Evidence for constant and highly specific active food selection by benthic ciliates in mixed diatoms assemblages. Limnol Oceanogr 49:58-68

Hansen PJ (1991a). Dinophysis-a planktonic dinoflagellate genus which can act both as a prey and a predator of a ciliate. Mar. Ecol. Prog. Ser 69: 201–204

Hansen PJ (1992) Prey size selection, feeding rates and growth dynamics of heterotrophic dinoflagellates with special emphasis on *Gyrodinium spirale*. Mar Biol 114:327-334

Harrison PJ, Conway HL, Holmes RW, Davis CO (1977) Marine diatoms grown in chemostats under silicate or ammonium limitation. III. Cellular chemical composition and morphology of *Chaetoceros debilis, Skeletonema costatum*, and *Thalassiosira.* Mar Biol 43:19-31

Irigoien X, Harris RP, Meyer-Harms B, Head RN, Cummings D, Harbour D (2000) Feeding selectivity and egg production of *Calanus helgolandicus* in the English channel. Limnol Oceanogr, 45:44–59.

Irigoien X, Flynn KJ, Harris RP (2005) Phytoplankton blooms: a ‘loophole’ in microzooplankton grazing impact J Plankton Res 27:313–321

Jacobsen DM, Anderson DM (1986) Thecate heterotrophic dinoflagellates: Feeding behavior and mechanisms. J. Phycol. 22: 249-258.

Johansson M, Gorokhova E, Larsson U (2004) Annual variability in ciliate community structure, potential prey and predators in the open northern Baltic Sea proper. J Plankton Res 26:67–80

Kilham SS, Kreeger DA, Goulden CE, Lynn SG (1997) Effects of algal food quality on fecundity and population growth rates of *Daphnia*. F Biol 38:639–647.

Kivi K, Kaitala S, Kuosa H, Kuparinen J, Leskinen E, Lignell R, Marcussen B, Tamminen T (1993) Nutrient limitation and grazing control of the Baltic plankton community during annual succession. Limnol Oceanogr 38:893–905

Kleppel GS (1993) On the diets of calanoid copepods. Mar Ecol Prog Ser 99:1–2

Landry MR, Calbet A (2004) Microzooplankton production in the oceans. ICES J Mar Sci 61:501–507

Landry MR, Hassett RP (1982) Estimating the grazing impact of marine micro-zooplankton. Mar Biol 67:283–288

Leppaenen JM, Bruun JE (1988) Cycling of organic matter during the vernal growth period in the open northern Baltic proper. 4. Ciliate and mesozooplankton species composition, biomass, food intake, respiration, and production. Finn Mar Res 255:55–78

Lewis J (1991) Cyst-theca relationships in *Scrippsiella* (Dinophyceae) and related orthoperidinoid genera. Bot Mar. 34: 91-106.

Lynn DH, Montagnes DJS, Dale T, Gilron GL, Strom SL (1991) A reassessment of the genus Strombidinopsis (Ciliophora, Choreotrichida) with descriptions of four new planktonic species and remarks on its taxonomy and phylogeny. J Mar Biol Assoc UK 71(3):597-612

Mauchline J (1998) The biology of calanoid copepods, Academic Press, San Diego

Montagnes DJS, Berger JD, Taylor FJR (1996) Growth rate of the marine planktonic ciliate *Strombidinopsis cheshiri* Snyder and Ohman as a function of food concentration and interclonal variability. J Exp Mar Biol Ecol 206(1-2):121-132

Montagnes DJS, Lessard EJ (1999) Population dynamics of the marine planktonic ciliate Strombidinopsis multiauris: its potential to control phytoplankton blooms. Aquat Microb Ecol 20:167–181

Müller H, Geller W (1993) Maximum growth rates of aquatic ciliated protozoa: the dependence on body size and temperature reconsidered. Arch Hydrobiol 126:315–327

Miiller H, Weisse T (1994) Laboratory and field observations on the scuticociliate *Histiobalantiurn* from the pelagic zone of Lake Constance, FRG. J Plankton Res 16:391-401

Mullin M (1963) Some factors affecting the feeding of marine copepods of the genus *Calanus*. Limnol Oceanogr 8:239–250

Paffenhöfer GA (1988) Feeding rates and behavior of zooplankton. Bull Mar Sci 43:430-445

Paffenhöfer GA (1971) Grazing and ingestion rates of nauplii, copepodids and adults of the marine planktonic copepod *Calanus helgolandicus*. Mar Biol 11:286-298

Pan Y, Subba Rao DV, Mann KH, Brown RG, Pocklington R (1996). Effects of silicate limitation on production of domoic acid, a neurotoxin, by the diatom *Pseudo-nitzschia multiseries*. I. Batch culture studies. Mar. Ecol. Prog. Ser. 131:225-243.

Reynolds CS (1988) Functional morphology and the adaptive strategies of freshwater phytoplankton. In C.D. Sandgren (ed.), Growth and reproductive strategies of freshwater phytoplankton. Cambridge University Press, New York: 388–433.

Richman S, Rogers JN (1969) Feeding of *Calanus helgolandicus* on synchronously growing populations of the marine diatom *Ditylum brightwellii*. Limnol Oceanogr 14:701–709

Sherr EB, Sherr BF (2002) Phagotrophy in aquatic microbial food webs. In: Newell S (Ed) Man Env Microb II 409–418

Smetacek V (1981) Annual cycle of protozooplankton in the Kiel Bight. Mar Biol 63:1–11

Smetacek V, De Baar HJW, Bathmann UV, Lochte K, Rutgers Van Der Loeff MM (1997) Ecology and biogeochemistry of the antarctic circumpolar current during austral spring: a summary of southern ocean JGOFS cruise ANT X/6 of R.V. *Polarstern.* Deep-Sea Research II, 44(l-2):l-21

Sommer U (1988) Phytoplankton succession in microcosm experiments under simultaneous grazing pressure and resource limitation. Limnol. Oceanogr. 33(5):1037-1054

Sommer U (1996) Plankton ecology: the past two decades of progress. Naturwissenschaften 63:293–301

Sommer U, Hansen T, Blum O, Holzner N, Vadstein O, Stibor H (2005) Copepod and microzooplankton grazing in mesocosms fertilised with different Si:N ratios: no overlap between food spectra and Si:N influence on zooplankton trophic level. Oecologia 142:274–283

Sommer U, Aberle N, Engel A, Hansen T, Lengfellner K, Sandow M, Wohlers J, Zöllner E, Riebesell U (2007) An indoor mesocosm system to study the effect of climate change on the late winter and spring succession of Baltic Sea phyto- and zooplankton. Oecologia 150:655–667

Sommer U, Langfellner K (2008) Climate change and the timing, magnitude, and composition of the phytoplankton spring bloom. Global Change Biology 14:1199–1208

Sverdrup H (1953) On conditions for the vernal blooming of phytoplankton. J Cons Explor Mer 18:287–295

Thompson PA, Harrison PJ, Whyte JNC (1990) Influence of irradiance on the fatty acid composition of phytoplankton. J Phycol 26:278–288

Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt Int Ver Limnol 9:1–38

Wiltshire KH, Manly BFJ (2004) The warming trend at Helgoland Roads, North Sea: phytoplankton response. Helgol Mar Res 58:269–273

Wiltshire KH, Malzahn AM, Wirtz K, Greve W, Janisch S, Mangelsdorf P, Manly BFJ, Boersma M (2008) Resilience of North Sea phytoplankton spring bloom dynamics: An analysis of long-term data at Helgoland Roads. Limnol. Oceanogr., 53(4):1294–1302