

BERICHTE  
aus dem  
**INSTITUT FÜR MEERESKUNDE**  
an der  
**CHRISTIAN-ALBRECHTS-UNIVERSITÄT · KIEL**

Nr. 328

Omnivory in planktonic food webs:  
a study on the impact of mixotrophic flagellates  
and microzooplankton on food web  
dynamics and productivity

by

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Kiel 2003

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Kopien dieser Arbeit können bezogen werden durch:

Institut für Meereskunde

- Bibliothek -

Düsternbrooker Weg 20

D-24105 Kiel

Germany

ISSN 0341-8561

Diese Arbeit wurde von der  
Mathematisch-Naturwissenschaftlichen Fakultät  
der Universität Kiel 2003 als  
Dissertation angenommen

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# Chapter 1

## Introduction

During the last two decades the view of the lower levels of pelagic food webs has changed considerably. Planktonic food webs have originally been viewed as more or less linear chains from phytoplankton to metazooplankton. Within the phytoplankton, larger groups as diatoms and dinoflagellates should be responsible for the bulk of primary production, and their production should be consumed mainly directly by putatively herbivorous crustacean zooplankton such as calanoid copepods, krill and filter feeding cladocerans, the latter being mainly important in freshwater systems (e.g. Ryther 1969). Meanwhile it became obvious that phytoplankton is not only directly consumed by the metazooplankton, but that various protists may be important consumers of phytoplankton, and that they may in turn be an important trophic link to the metazoan zooplankton (Kleppel 1993, Sommer et al. 2002, Sommer and Stibor 2002). Such 'omnivorous' relationships are not only found between protists and zooplankton. Rather, there is increasing evidence that omnivory is common among virtually all functional groups in planktonic ecosystems (Sommer et al. 2002, Sommer and Stibor 2002).

In general, omnivory is defined as consumption of prey on different trophic levels by one organism (Fig. 1.1). A top predator preys upon a basal resource as well as on a so-called 'intermediate consumer' (Diehl and Feissel 2000; Fig. 1.1). One reason for the ubiquity of omnivory in planktonic food webs arises from scale overlap within and between functional groups, respectively (Fig. 1.2). Phagotrophic protists are likely to prey upon other phagotrophs as well as on osmotrophic organisms. Similarly, various metazoan zooplankton may prey on smaller metazoan zooplankton as well as on larger protists, as is evident from recent results on the prey spectra of krill and calanoid copepods (Gurney et al. 2001, Zeldis et al. 2002). To sum up, omnivory is the rule rather than the exception among the lower levels of the pelagic food web, contrasting with the general ecological paradigm that considers omnivory as an exception in natural food webs

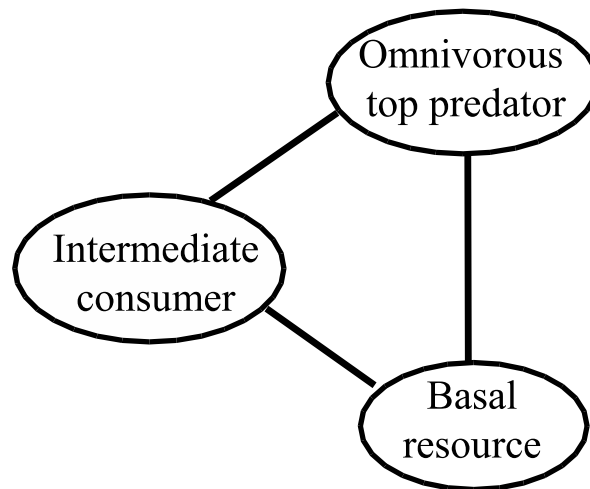


Figure 1.1: Scheme of omnivory.

(Ricklefs 1979, Pimm 1982, McCann and Hastings 1997).

Two important implications arise from omnivory: (1) The 'intermediate consumer' (Fig. 1.1) suffers from competition and predation at the same time. Theoretical analyses predict that omnivory may lead to extinction of an intermediate consumer under high resource productivity (Diehl and Feissel 2000, Mylius et al. 2001). By excluding intermediate consumers, omnivory is considered as a factor that possibly limits food chain length. (2) Aside from controlling the abundances of the intermediate consumer, feeding on the intermediate trophic level means an energetic disadvantage for the top consumer, since energy is being lost on every trophic transfer. Any heterotrophic organism respire a major portion of its ingested energy. Depending on the functional group, the net growth efficiency (achieved biomass per assimilated prey) ranges from 60 % in protozoan grazers (Fenchel 1982) to 30 - 40 % in most metazoans (Winberg 1971). Since not all ingested prey can be assimilated, the gross growth efficiency (achieved biomass per ingested prey) is considerably lower (10-15 %; Lampert and Sommer 1999) In addition to energetic losses during prey utilisation, a considerable portion of prey on any trophic level is lost by other processes than predation, like death or sinking. Therefore the ratio production of consumer level to production of producer level (ecological efficiency) is usually between 0.05 and 0.2 (Lampert and Sommer 1999). Consequently, an omnivorous consumer should do best by feeding mainly on the basal resource.

A special case of omnivory is represented by algal mixotrophy. Mixotrophy is originally defined as mixed auto- and heterotrophic mode of nutrition in one organism. In plankton ecology, the term mixotrophy is commonly used in a more restricted way for (potentially) phototrophic protists, that may additionally ingest particles (usually other protists) by phagotrophy, thereby

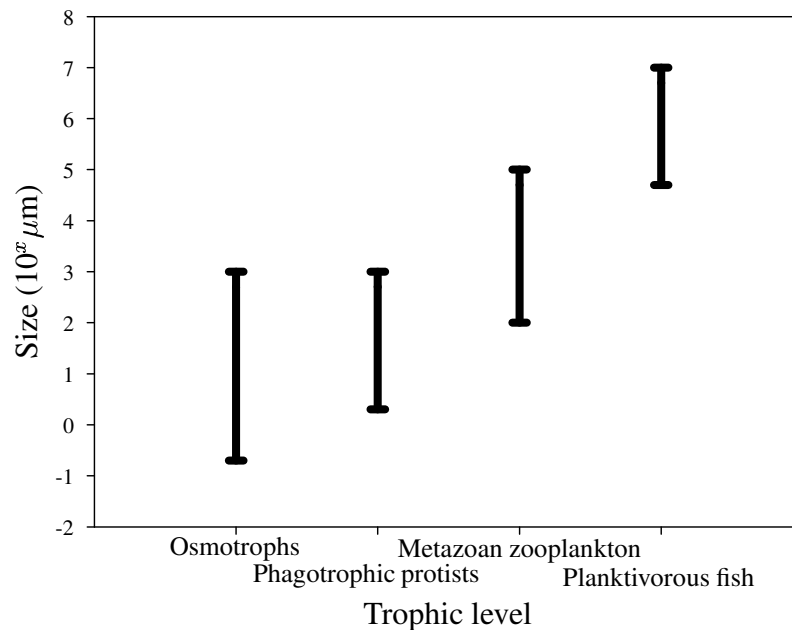


Figure 1.2: Size overlap in adjacent trophic levels in planktonic food webs.

enhancing their gain in limiting nutrients or energy (Riemann et al. 1995, Stoecker 1998). Mixotrophs compete with algae and bacteria for dissolved nutrients, and with heterotrophic protists for particulate prey as bacteria and phytoplankton. Since a mixotroph competes with osmotrophs (algae and bacteria) for a common resource and preys upon them at the same time, a mixotroph is a true omnivore (compare Fig. 1.1). Despite methodological difficulties in the identification of mixotrophs in the field (see final discussion for details), mixotrophs seem to be an inherent constituent of planktonic food webs. They are present in virtually all aquatic environments and may contribute more than 40 % of phytoplankton biomass (Havskum and Riemann 1996, Pitta et al. 2000, Sanders et al. 2000). Mixotrophs are found among virtually all flagellated taxa (*Chrysophyceae*, *Chlorophyceae*, *Cryptophyceae*, *Dinophyceae*, *Haptophyceae*) and in several ciliated protozoans (e.g. *Heterotrichia*, *Oligotrichia*, *Haptoria*). While most mixotrophic flagellates have their own chloroplasts, mixotrophic ciliates obtain chloroplasts by ingestion of autotrophs or by symbiosis with autotrophs.

While having been known for long time already, the ecological role of mixotrophs has been appreciated only recently. Any possible impact depends on their competitive abilities relative to pure autotrophic and pure heterotrophic competitors, respectively. According to general ecological wisdom, mixotrophs are generalists and should be inferior competitors compared to pure

auto- and heterotrophic protists.

The discovery of the microbial loop (Azam et al. 1983, Sherr and Sherr 1988) has shown that a considerable portion of primary and secondary production is done by autotrophic and heterotrophic picoplankton, respectively, and that this production is mainly consumed by various protozoan grazers. Regarding metazoan production, the intermediate consumers can have two roles, termed 'link' and 'sink'. By feeding on particles too small for many metazoans, heterotrophic protists make production available for larger animals ('link'). However, much of that picoplankton production can be lost by respiration of the intermediate consumers ('sink'). In this interface between picoplankton and metazooplankton, mixotrophic flagellates have recently been assumed to be an important link (Riemann et al. 1995). By combination of phagotrophy and phototrophy, they respire less energy of the ingested prey (Rothhaupt 1997) and may represent a more effective trophic link than heterotrophic protists.

In order to investigate the effects of omnivory on pelagic food webs, artificial food webs were assembled, in which the degree/presence of omnivory and mixotrophy were manipulated. In case of mixotrophy, microbial food webs were optionally extended by mixotrophic flagellates. For manipulation of omnivory on higher trophic level, the interface between phytoplankton and calanoid copepods was manipulated by addition of microzooplankton, acting as intermediate consumer between phytoplankton and copepods. Aside from the above mentioned paradigm shift in zooplankton nutrition, this interface is of particular interest because in most marine ecosystems calanoid copepods are the most important link between primary and fish production (Mann and Lazier 1996, Sommer 1998).

The performed food web experiments are sketched in Fig. 1.3: in Chapter 2 (plot a), the effects of a heterotrophic dinoflagellate on an otherwise linear diatom-copepod interaction was investigated. In Chapter 4 (plot c), an otherwise nature-like food web with calanoid copepods as top predators was manipulated by the presence of a heterotrophic dinoflagellate. Effects of mixotrophy on a microbial food web were studied in a food web as sketched in plot b (Chapter 3). More complex food webs were manipulated by the presence of mixotrophs in Chapters 4 and 5 (plot c and d). Chapter 6 deals with an application how long term cultivation of calanoid copepods may easily be achieved by a simple food web manipulation.



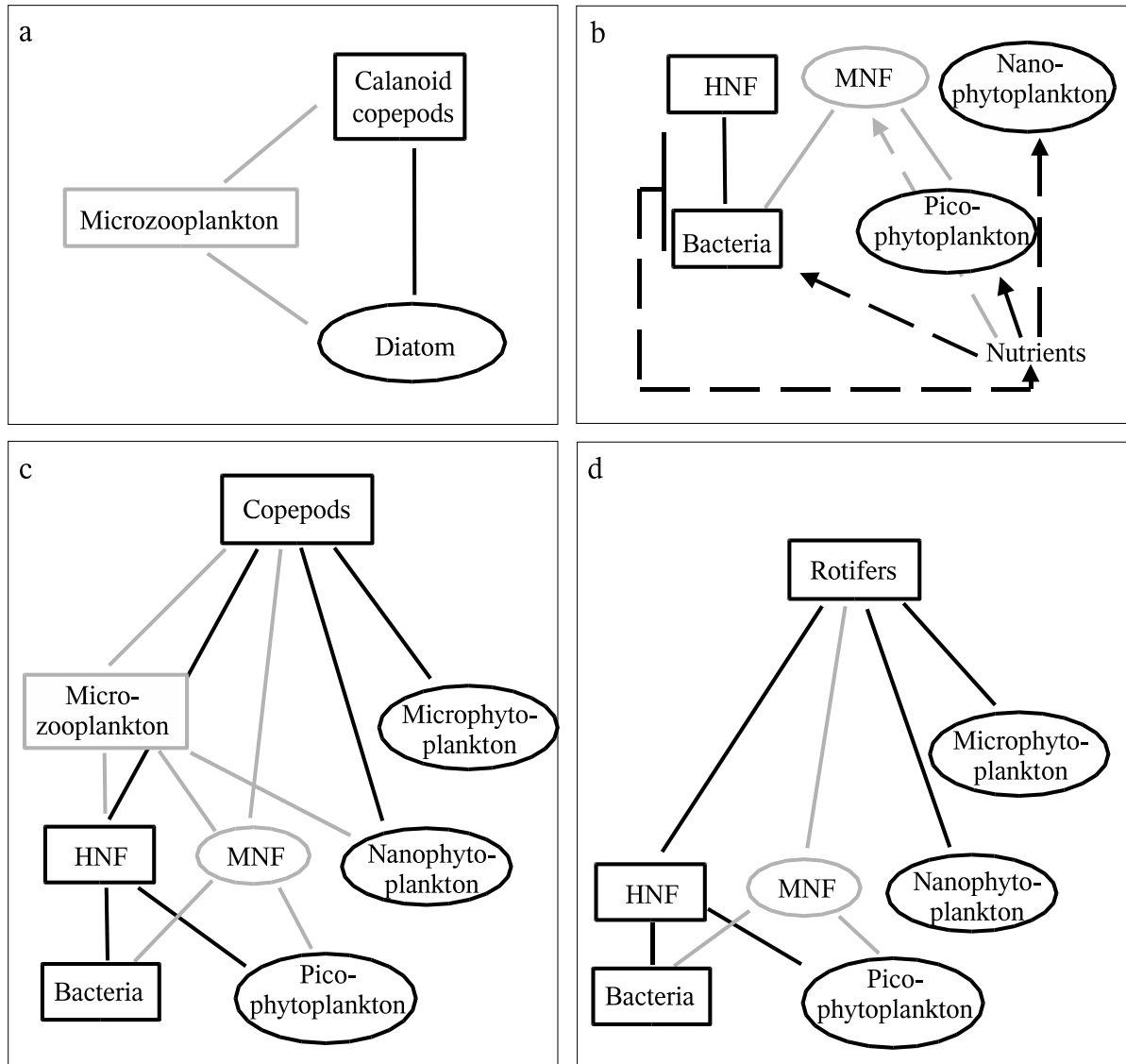


Figure 1.3: Food web configurations in the performed experiments. Plot (a) corresponds to Chapter 2, (b) to Chapter 3, (c) to Chapter 4 and (d) to Chapter 5. Heterotrophic organisms are displayed in boxes, auto- and mixotrophic in ovals. Optional components are depicted in grey. Solid lines indicate fluxes of particulate matter (grazing). Black lines represent permanent fluxes, while grey lines represent fluxes that were only present in the manipulated treatments (omnivory, mixotrophy). To illustrate nutrient competition among bacteria, auto- and mixotrophic algae, nutrient fluxes are indicated by dashed lines in plot (b).



## Chapter 2

# Calanoid copepods and diatoms: a question about the role of heterotrophic dinoflagellates

**Abstract** - In two experiments, the effects of the heterotrophic dinoflagellate *Gyrodinium dominans* acting as an intermediate consumer between the diatom *Skeletonema costatum* and the calanoid copepod *Acartia tonsa* were investigated. In a food web experiment where the copepods were incubated either with the diatom alone or with the diatom and the dinoflagellate, the presence of *Gyrodinium* enhanced egg production and hatching success of *Acartia*. In a second experiment where single females of *Acartia* were fed either with *Skeletonema* or *Gyrodinium* grown on *Skeletonema*, reproduction in *Acartia* was similar on both prey types. It is concluded that both, diatoms and heterotrophic dinoflagellates, contain essential nutrients lacking in the other type of prey and that heterotrophic dinoflagellates may have strong positive effects on copepod reproduction in diatom-dominated systems. Results are discussed in context of previous studies about interactions between phytoplankton, microzooplankton and calanoid copepods.

### 2.1 Introduction

For long time, marine calanoid copepods were viewed as so-called 'herbivorous zooplankton' (e.g. White 1979). Recent work has revealed that calanoids are omnivorous organisms and that

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ROLE OF HETEROTROPHIC DINOFLAGELLATES

Table 2.1: Studies reporting positive effects of microzooplankton acting as intermediate prey for calanoid copepods.

| Study | Phytoplankton prey          | Microzoo-plankton                      | Copepod species                             | Parameter tested                 |
|-------|-----------------------------|--|---|----------------------------------|
| 1     | natural phytoplankton       | ciliates, rotifers                     | <i>Acartia tonsa</i>                        | egg production                   |
| 2     | <i>Phaeocystis globosa</i>  | <i>Gyrodinium domi-</i><br><i>nans</i> | <i>Acartia tonsa</i>                        | egg production                   |
| 3     | <i>Amphidinium carterae</i> | <i>Oxyrrhis marina</i>                 | <i>Acartia spp.</i>                         | ingestion                        |
| 4, 5  | <i>Isochrysis galbana</i>   | <i>Oxyrrhis marina</i>                 | <i>Acartia tonsa</i>                        | egg production,<br>hatching rate |
| 6     | <i>Dunaliella sp.</i>       | <i>Oxyrrhis marina</i>                 | <i>T. longicornis</i> , <i>P. elongatus</i> | growth rate                      |

1) Stoecker and Egloff 1987; 2) Tang et al. 2001; 3) Jeong et al. 2001; 4) Kleppel and Burkart 1995; 5) Kleppel et al. 1998; 6) Klein Breteler et al. 1999.

microzooplankton can make up a major part of their diet (Kleppel 1993). Feeding experiments have shown that calanoid copepods select for larger, actively moving food items such as dinoflagellates, ciliates, nauplii, and even rotifers (Stoecker and Egloff 1987, Sell et al. 2001, Vincent and Hartmann 2001). Various feeding experiments showed that reproductive success of copepods was enhanced when the phytoplankton diet was either enriched by microzooplankton or when the phytoplankton was replaced by a microzooplankton fed by the phytoplankton species under study (see Table 2.1). In addition to such 'trophic upgrading' (Klein Breteler 1999) of phytoplankton prey, microzooplankton may even diminish toxicity of harmful algae (Jeong et al. 2001). So far, most studies investigated the effects of microzooplankton on interactions between phytoflagellates and copepods, far less effort has been devoted to the prey quality of diatoms (but see Bonnet and Carlotti 2001).

At the same time when the importance of microzooplankton in the diet of calanoid copepods became obvious, the role of diatoms in their diet became questionable. While it is still under debate whether diatoms are only nutritionally inadequate food for calanoid copepods or even toxic (review in Paffenhöfer 2002), there is no doubt that diatoms, as a single source of food, may reduce substantially their reproductive success compared to various phytoflagellates and heterotrophic flagellates and ciliates (Kleppel 1993, Ianora et al. 1996, Turner et al. 2001, Paffenhöfer 2002, Carotenuto et al. 2002).

Negative effects of diatoms on calanoids may be mitigated if other prey is admixed to their diet. Bonnet and Carlotti (2001) fed the copepod *Centropages typicus* either with the diatom

*Thalassiosira weissflogii* or with a mixed diet of the diatom and the ciliate *Strombidium sulcatum* that was grown on bacteria; both egg production and development of the offspring were enhanced in the presence of the ciliate. However, *Strombidium* did not act as an intermediate consumer in their experiment, since it was not grown on the diatom but on bacteria. Especially heterotrophic dinoflagellates may act as intermediate consumers between diatoms and copepods, since they are important consumers of diatoms, particularly in temperate and cold waters (Tiselius and Kuylenstierna 1996, Levinsen and Nielsen 2002, Suzuki et al. 2002). Contrary to ciliates and tintinnids, many heterotrophic dinoflagellates are able to feed on cells larger than themselves by external digestion (Jacobson and Anderson 1986, Buskey 1997, Graham and Wilcox 2000) and may therefore consume even large diatoms and filaments. They can be abundant during diatom blooms and should therefore be considered as a possible alternative or complementary type of prey for copepods when diatoms are abundant.

Two experiments were performed to investigate whether the heterotrophic naked dinoflagellate *Gyrodinium dominans* may enhance the food quality of the diatom *Skeletonema costatum* for the calanoid copepod *Acartia tonsa* when it acts as an intermediate consumer between the diatom and the copepod. In a food web experiment (Experiment 1), an assemblage of copepods was incubated either with a monoculture of *Skeletonema* or with a mixed community of *Skeletonema* and *Gyrodinium* for 6 days. Here survival and reproduction of the copepods have been analysed. In an egg production experiment (Experiment 2), single females of *Acartia* were fed with monocultures of either *Skeletonema* or *Gyrodinium* grown on *Skeletonema*.

## 2.2 Materials and methods

All experiments and the cultivation of the organisms were done in a walk-in environmental chamber that was set to 16 °C and a 16L-8D cycle. The light intensity (PAR) for the stock cultures of the organisms and for Experiment 1 was approximately 50  $\mu\text{E m}^2 \text{s}^{-1}$  (Licor Quantum Photometer LI-185B). Media were prepared from sterile filtered water from the western Baltic Sea (salinity appr. 15 PSU) with trace metals and vitamins added according to Rick and Dürselen (1995). Major nutrients were added to a final concentration of 30:2:15 (Exp. 1) and 40:2.5:20 (Exp. 2)  $\mu\text{mol L}^{-1}$  nitrogen:phosphorus:silica, respectively, except for the stock culture of *Skeletonema* in Experiment 2 (see there). The copepods used in the experiments originated from field catches from the Western Baltic and had been cultivated in 25 L culture vessels on a mixed diet of *Rhodomonas salina* and *Oxyrrhis marina* already for several months before the experiments were done (see Chapter 6). The diatom *Skeletonema costatum* is a strain from

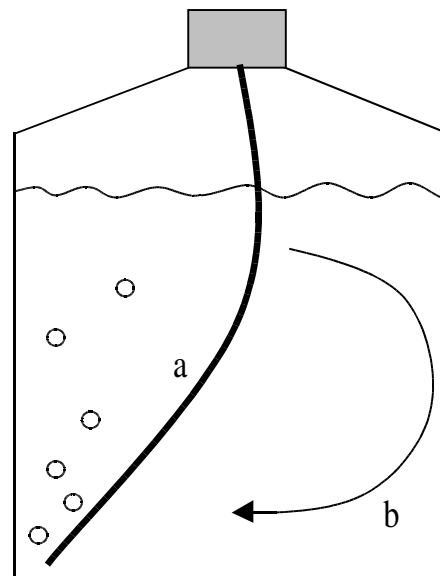


Figure 2.1: Scheme of an incubation flask used in Experiment 1. Appr. dimensions 13 x 16 x 4 cm width x height x depth. (a) Aeration tube. The rising bubbles caused a circular current of the medium as indicated by the arrow (b).

the British 'Culture Collection of Algae and Protozoa' (CCAP, strain-no. 1077/1C). The heterotrophic dinoflagellate *Gyrodinium dominans* was isolated from the Kiel Fjord in summer 2001 and determined from life observations according to Tomas (1996).

**Experiment 1** The food web experiment was performed in 750 ml polystyrene culture flasks (Fig. 2.1). Four culture flasks were filled with 500 ml of sterile medium. Two of them were inoculated with 100 ml of a culture of *Skeletonema* (control). The two remaining flasks were inoculated with 60 ml of the same *Skeletonema* culture and 40 ml of a mixed culture of *Gyrodinium* and *Skeletonema*. The same medium was used for the cultivation of *Skeletonema* and *Gyrodinium* as well as for the inoculation of the culture flasks. The culture flasks were then placed under a light bench. A small plastic tube was thrust through the lid of each flask (Fig. 2.1). Through this tube, air was gently pumped to one of the bottom corners of each flask. The ascending bubbles induced a constant circular mixing of the medium that minimized sedimentation. Two days after inoculation with the protists, 40 randomly selected copepodids of different stages (no nauplii) were added to each culture flask. Phytoplankton samples were taken at days 0 (addition of the copepods), 4, and 6 (end of experiment). Additionally, on day 6 the whole volume of each flask was filtered by a 60  $\mu\text{m}$  mesh to retain all copepods including nauplii and eggs. Phytoplankton and copepod samples were preserved with Lugol's solution and counted

under an inverted microscope (Utermöhl 1958). The copepods were classified as copepodids, nauplii and eggs. Development time from egg to 1<sup>st</sup> copepodid instar is about 10 days at 15 °C (Landry 1983). Therefore all copepodids were assumed to belong to the inoculum, while eggs and nauplii were assumed to be offspring.

**Experiment 2** In this experiment, single females of *Acartia tonsa* were incubated with a monoculture of either *Skeletonema* or *Gyrodinium*. To avoid nutrient limitation in *Skeletonema*, the diatoms were grown on a nutrient-rich medium (200:8:100  $\mu\text{mol L}^{-1}$  N:P:Si). On the day the experiment was started the atomic carbon:nitrogen (C:N) ratio of the *Skeletonema* biomass was 4.7 (carbon and nitrogen content of *Skeletonema* were measured by a FISONs NA 1500 N C:N-analyser after heat combustion of a sample filtered on WHATMAN GF/F filter). *Gyrodinium* was grown on *Skeletonema* in the light. During cultivation of *Gyrodinium*, a small coccoid cyanobacterium ( $< 2 \mu\text{m}$ ) appeared in this culture. Since *Gyrodinium* and *Acartia* are not able to ingest particles in that size range (Berggreen et al. 1988, Naustvoll 2000), the presence of the cyanobacterium may not have affected the results of this experiment. 24 hours before the start of the experiment, the copepods were incubated in two 100 ml culture flasks containing either *Skeletonema* or *Gyrodinium* at experimental concentrations. At the start of the experiment, the *Skeletonema* culture was in the exponential growth phase and was diluted with fresh medium to a final concentration of 10,200 cells  $\text{ml}^{-1}$ . The culture of *Gyrodinium* contained approximately 1,000 cells  $\text{ml}^{-1}$ , abundances of *Skeletonema* were below detection limit at this time in the *Gyrodinium* culture. In order to dilute metabolites of *Gyrodinium* contained in the culture medium, this culture was first concentrated by a 10  $\mu\text{m}$  mesh to 3,500 cells  $\text{ml}^{-1}$  and thereupon diluted it with fresh medium to a final concentration of 1,000 cells  $\text{ml}^{-1}$ . Each 12 adult females of *Acartia* were single incubated in 20 ml scintillation vials that were filled with 8 ml of the prepared *Skeletonema* and *Gyrodinium* suspensions. Additionally to treatments containing copepods, each three controls containing solely *Skeletonema* or *Gyrodinium* were prepared. All treatments were incubated under the same light-dark cycle as described above, but light intensity was reduced to minimize reproduction of *Skeletonema*. After 8 and 16 hours, all scintillation vials were closed with a lid, gently shaken, and opened again, to resuspend sedimented food particles. After 24 hours all treatments were fixed with Lugol's solution. Samples were counted under an inverted microscope (Utermöhl 1958). Additionally, samples from the two starting cultures were preserved with Lugol's solution. 30 cells of *Gyrodinium* were measured under an inverted microscope to calculate its average dimensions. Using simple geometrical bodies from Tikkanen and Willén (1992) its average cell volume was estimated. Carbon content of *Gyrodinium* was

calculated according to Menden-Deuer and Lessard (2000).

The gross growth rates of *Skeletonema* and *Gyrodinium* in the controls,  $\mu$ , were calculated as follows:

$$\mu = \frac{\ln\left(\frac{N_t}{N_0}\right)}{t_1 - t_0} \quad (2.1)$$

where  $N_0$  is the cell concentration at  $t_0$ , and  $N_t$  the cell concentrations at  $t_1$  in the control. The growth rate  $\mu_n$  in the treatments was calculated analogously by using the corresponding cell concentrations of the treatments. The loss rate  $l$  caused by the presence of the copepod is then:

$$l = \mu_n - \mu \quad (2.2)$$

Given these parameters, the absolute number of ingested cells per copepod,  $N_{ing}$ , was calculated as:

$$N_{ing} = \left(\frac{N_0 - N_t}{\mu}\right) \times l \quad (2.3)$$

These formula are simplifications of the formula given in Frost (1972).

## 2.3 Results

**Experiment 1** At the start of the experiment, average abundances of *Skeletonema* were lower in the *Gyrodinium* treatment than in the control (*Skeletonema*-only treatment) (Fig. 2.2; appr. 25,000 and 17,000 cells ml<sup>-1</sup> in control and *Gyrodinium* treatment, respectively). Thereafter abundances of the diatom declined in both treatments. On day 4, abundances in both control replicates and one *Gyrodinium* replicate were similar, but in the second *Gyrodinium* replicate (denoted with (c) in Fig. 2.2) abundances of *Skeletonema* were 10 times lower. At the end of the experiment, average abundances of *Skeletonema* were higher in the *Gyrodinium* treatment compared to the control (appr. 400 and 1,440 cells ml<sup>-1</sup> in control and *Gyrodinium* treatment, respectively). Abundances of *Gyrodinium* were of importance only on day 0 (239 and 103 cells ml<sup>-1</sup> in replicate (c) and (d)). Thereafter *Gyrodinium* declined rapidly and was below 2 cells ml<sup>-1</sup> already on day 4 in both replicates (in 5 ml samples that were completely scanned, less than 10 cells were found). On day 6, no cells at all were found in 5 ml samples. Since *Gyrodinium* proved to grow well on *Skeletonema* when cultivated without copepods, its quick disappearance can only be explained by selective grazing by the copepods (Stoecker and Egloff 1987). The low



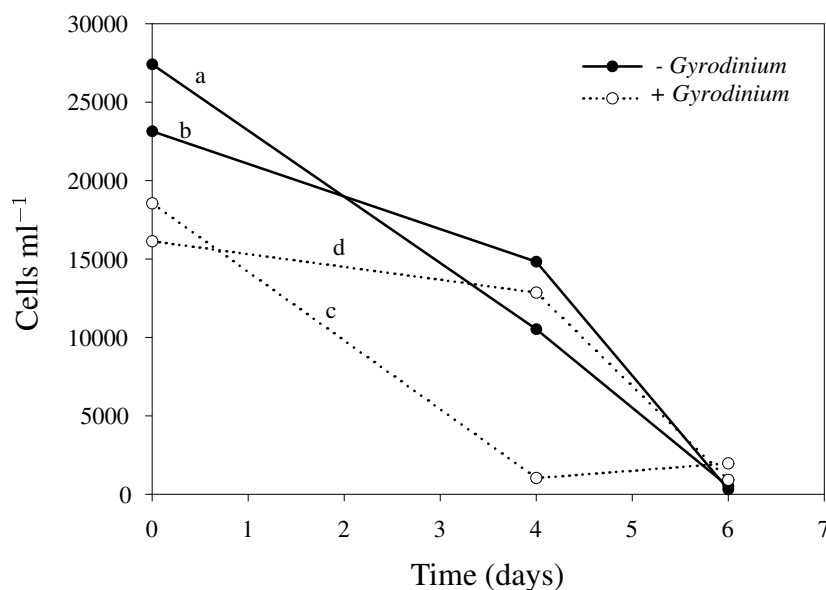


Figure 2.2: Experiment 1. Abundances of *Skeletonema costatum* over time. Small letters indicate the corresponding replicates.

abundances of *Skeletonema* in the *Gyrodinium* replicate (c) on day 4 coincided with higher abundances of *Gyrodinium* on day 0 and higher abundances of *Acartia* on day 4 and 6 in this replicate compared to replicate (d) (Fig. 2.2, 2.3). Therefore, the low abundances of *Skeletonema* in this sample may be caused by a higher grazing pressure. The copepods survived slightly better in the *Gyrodinium* treatment (abundances on day 6, Fig 2.3; 24 and 17 in the *Gyrodinium* treatment, 12 in both controls; Student's *t*-test  $p = 0.14$ ; Cochran's test on homogeneity of variances n.s.). There was a big difference in the produced offspring between both treatments. The total sum of eggs and nauplii was about five times higher in the *Gyrodinium* treatment (Fig. 2.4. 36 and 127 (control), 428 and 576 (*Gyrodinium*); Student's *t*-test  $p = 0.04$ ; Cochran's test on homogeneity of variances n.s.). Additionally, the percentage of offspring that was already hatched at the time of sampling was higher in the *Gyrodinium* treatment (11 and 5 percent in the controls, 38 and 18 percent in the *Gyrodinium* treatment; Student's *t*-test  $p = 0.14$ ; Cochran's test on homogeneity of variances n.s.). The higher percentage of hatched nauplii indicates a better hatching rate as a consequence of an improvement in food quality.

**Experiment 2** Initial food concentration expressed as carbon per volume was approximately the same in both treatments (445 and 448  $\mu\text{g C L}^{-1}$  in the *Skeletonema* (*Sk.*) and the *Gyrodinium* (*Gy.*) treatments, respectively). Within both treatments, the ingestion rates varied strongly (Fig.

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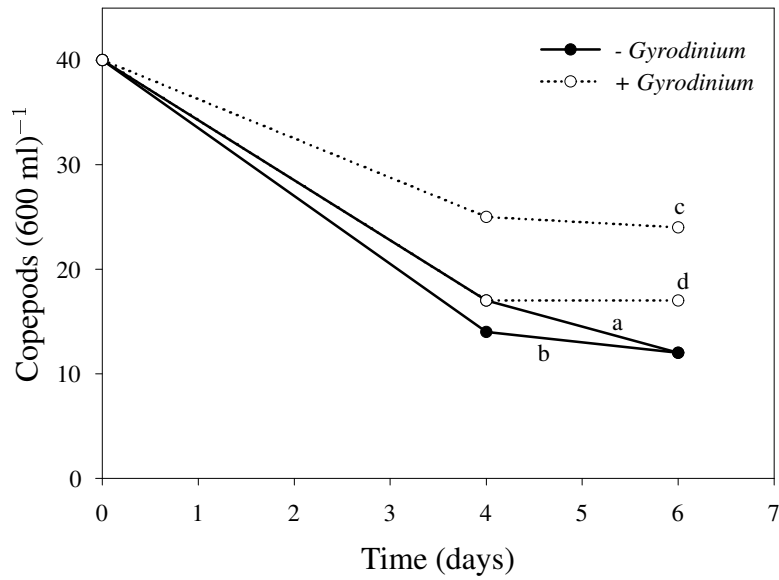


Figure 2.3: Experiment 1. Abundances of *Acartia tonsa* over time. Small letters indicate the corresponding replicates.

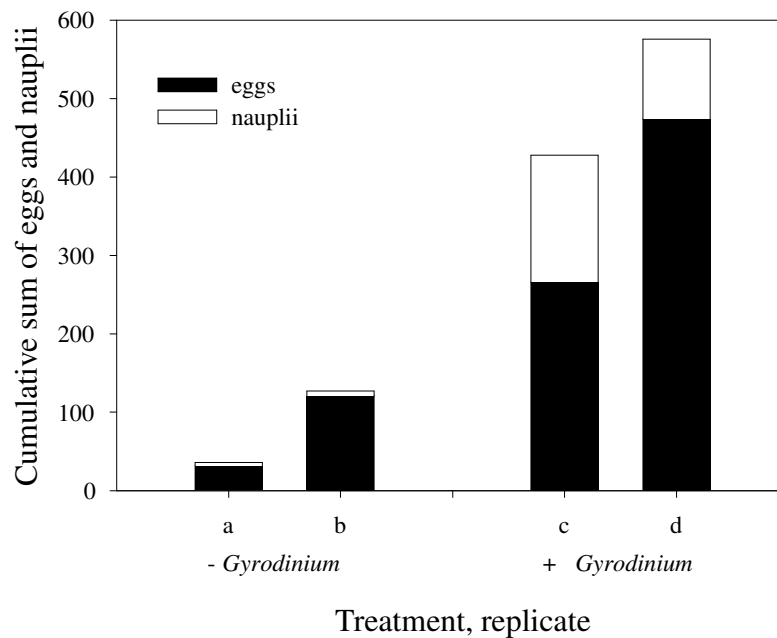


Figure 2.4: Experiment 1. Cumulative sum of eggs and nauplii at the end of the experiment. Small letters indicate the corresponding replicates.

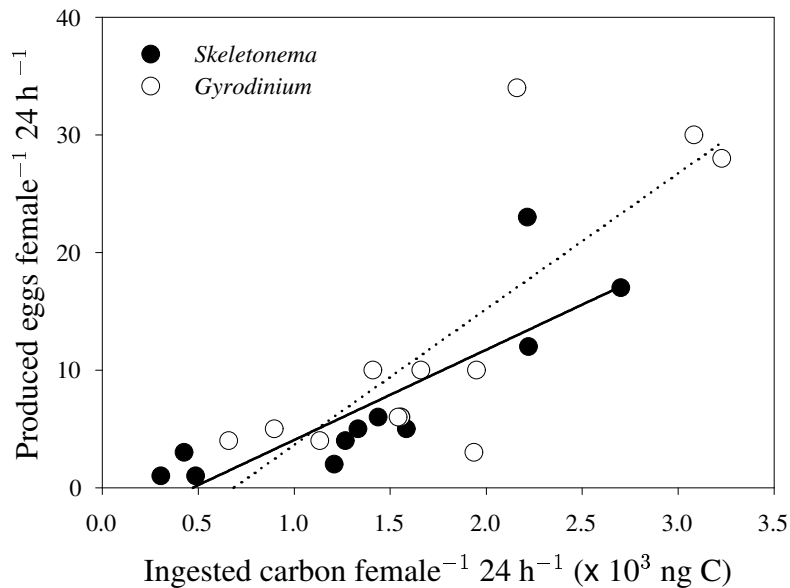


Figure 2.5: Experiment 2. Cumulative sum of eggs and nauplii at the end of the experiment. Lines represent linear regressions between ingested carbon (C) and produced eggs (solid, *Skeletonema*; dotted, *Gyrodinium*)

2.5; coefficient of variance (CV) 0.54 (*Sk.*) and 0.44 (*Gy.*). The copepods feeding on the dinoflagellate ingested insignificantly more carbon than the copepods feeding on the diatom (1.35 (*Sk.*) and 1.77 (*Gy.*) ng C day<sup>-1</sup>; Student's *t*-test  $p = 0.54$ ). The number of eggs laid per female varied even more strongly (CV 0.96 (*Sk.*), 0.90 (*Gy.*)), and were a linear function of the ingested prey volume (Fig. 2.5; linear regression between ingested prey volume and produced eggs,  $r^2 = 0.7$ ,  $p < 0.001$  (*Sk.*) and  $r^2 = 0.64$ ,  $p = 0.002$  (*Gy.*)). On average, copepods feeding on *Gyrodinium* laid more eggs than those feeding on *Skeletonema* (12.5 and 7.1 eggs, respectively), but due to the high variability in both treatments, this difference was not significant (Student's *t*-test  $p = 0.17$ ). When the ingested prey (as units carbon, C) was included into an ANCOVA, only ingested carbon, but not the prey type had a significant effect of the number of eggs laid per copepod ( $r^2 = 0.66$ ,  $p < 0.001$ ; ingested C (fixed factor)  $p < 0.001$ , prey type (covariate)  $p = 0.47$ ; Box-M test on homogeneity of variances n.s.). The high variability in the food consumption and egg production may reflect differences in the nutritional condition or age of the individual copepods. Additionally, the copepods may have suffered under the experimental treatment (small volume) to a different degree, depending on stochastic effects.

## 2.4 Discussion

The results of Experiment 1 show that the heterotrophic dinoflagellate *Gyrodinium dominans*, acting as an intermediate consumer between the calanoid copepod *Acartia tonsa* and the diatom *Skeletonema costatum*, may have a strong positive effect on copepod reproduction. The results of Experiment 2 show that this effect cannot be attributed to different ingestion rates, since *Acartia* is able to ingest both types of prey equally well. Therefore, *Gyrodinium* obviously enhanced food quality of the diatom diet. Similar results were obtained for various species of phytoflagellates when they were fed either directly or indirectly to calanoid copepods (Table 2.1). Kleppel and Burkart (1995) and Kleppel et al. (1998) investigated food quality of the haptophyte *Isochrysis galbana* and of the heterotrophic dinoflagellate *Oxyrrhis marina*, grown on *Isochrysis*. They found that *Oxyrrhis* enhanced reproduction of *Acartia tonsa* due to a higher content of polyunsaturated fatty acids compared to *Isochrysis*.

When *Gyrodinium* was offered as a single food (Experiment 2), both carbon ingestion rates and produced eggs were slightly enhanced compared to the copepods fed with *Skeletonema*; however, as a single prey type, *Gyrodinium* had a less pronounced effect than in Experiment 1. Therefore, the dinoflagellate does not seem to be a significantly better source of food than the diatom, but to complement nutrients lacking in the diatom. In the study by Kleppel and Burkart (1995), egg production in *Acartia tonsa* increased in the same order as in this study: *Isochrysis* < *Oxyrrhis* < *Isochrysis* + *Oxyrrhis*. Similar results were obtained by Bonnet and Carlotti (2001) for the copepod *Centropages typicus* when they either added a heterotrophic ciliate (grown on bacteria) to a diatom diet, or replaced the diatom by the ciliate. Roman (1984) found that detritus of a macrophyte enhanced survival and growth in the copepod *Acartia tonsa* when it was added to a diatom diet, though the copepods did not survive on a pure detrital diet.

In the studies listed in Table 2.1, the same amount of food (in units carbon per volume) was offered to the copepods in the phytoplankton and the microzooplankton treatments. However, according to the energy flow hypothesis (Oksanen et al. 1981), adding an intermediate consumer to an otherwise 2-guild food chain should reduce the productivity of the top predator (if ingestion rate and food quality are comparable for both types of prey). In Experiment 1, the positive effects of the intermediate consumer outweighed this energetic loss.

In previous studies that compared food quality of phytoplankton and microzooplankton for calanoid copepods, microzooplankton was either offered as a pure diet (Kleppel and Burkart 1995, Kleppel et al. 1998, Klein Breteler et al. 1999), or made up at least 50 % of the total food concentration (in carbon) of a mixed phytoplankton-microzooplankton diet (Kleppel and Burkart 1995, Kleppel et al. 1998, Bonnet and Carlotti 2001, Tang et al. 2001). In contrast,

in Experiment 1 the proportion of microzooplankton on overall food concentration was low, but had nevertheless a pronounced effect on copepod reproduction. In natural systems, proportions of microzooplankton on overall protist plankton are highly variable (e.g. Levinsen and Nielsen 2002). According to our results, microzooplankton should be considered as an important type of prey even at low concentrations. Furthermore, future studies investigating the impact of microzooplankton on copepod growth and reproduction should include natural ratios of phytoplankton to microzooplankton.

CHAPTER 2. CALANOID COPEPODS AND DIATOMS: A QUESTION ABOUT THE  
ROLE OF HETEROTROPHIC DINOFLAGELLATES

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## Chapter 3

# Effects of a mixotrophic flagellate in a microbial food web

**Abstract** - The ecological role of a mixotrophic chrysophyte, *Ochromonas minima*, was studied in nature-like marine microbial food webs, consisting of bacteria, heterotrophic nanoflagellates, pico- and nanophytoplankton. Bacterial productivity was manipulated by three levels of glucose addition (zero - low - high). The biomass of the mixotroph increased with increasing glucose enrichment. *Ochromonas* grazed effectively on bacteria and on picophytoplankton, and reduced their abundances to lower levels than did its heterotrophic competitor. By retaining nutrients contained in the mixotrophs' prey, nutrient remineralization was reduced, leading to a reduction of the autotrophic nanoflagellate. Effects on overall microbial biomass were context dependent: while the presence of the mixotroph caused a reduction in treatments without glucose addition, seston biomass was enhanced by the mixotroph in the enriched treatments. Maximum growth rates of the mixotroph were well below the maximum growth rates of its specialised auto- and heterotrophic competitors. However, when the resources of the auto- and the heterotrophic flagellates (nutrients and picoplankton, respectively) became low, the mixotroph was able to maintain higher growth rates than its specialised competitors.

### 3.1 Introduction

In nutrient limited surface layers of seas and lakes, phototrophs ('algae') compete with heterotrophic bacteria for limiting soluble nutrients like nitrogen, phosphorus or iron. Competitive abilities for soluble nutrients increase with decreasing cell size, and therefore the smallest organisms like bacteria and picophytoplankton are the best competitors for limiting nutrients (Sommer 1994). Larger sized algae like many phytoflagellates are weaker competitors, and they depend on nutrient regeneration by the microbial loop or external inputs such as vertical mixing or inflow from the watershed (Sommer et al. 1986, Uz et al. 2001). Under such circumstances, ingestion of particulate food by phototrophic protists ('mixotrophy') seems to be an attractive strategy to gain additional nutrients bound in prey biomass.

Though mixotrophy may serve for both, enhancing energy gain as well as essential nutrient gain, acquisition of essential nutrients should be the prevailing benefit for mixotrophy in light surface strata. Indeed, in various potentially phagotrophic phytoflagellates ingestion of small particles as bacteria can be triggered by nutrient limitation (Jones et al. 1993, Nygaard and Tobiesen 1993, Stibor and Sommer 2003). Mixotrophs can be abundant components of the phytoplankton (Sanders 1991, Pitta and Giannakourou 2000). In a number of field studies, it has been shown that mixotrophic flagellates may be equally important consumers of picoplankton as heterotrophic protists (Havskum and Riemann 1996, Baretta-Bekker et al. 1998, Sanders et al. 2000). Yet, though mixotrophic flagellates are seemingly an important component of planktonic food webs, their ecological impact on microbial food webs and on nutrient dynamics is barely known. Stickney et al. (2000) investigated the roles of mixotrophs in dynamic models. Based on the assumption that mixotrophs are feeding on phytoplankton, they predicted that mixotrophs are likely to reduce the productivity of microbial food webs. In contrast, Baretta-Bekker et al. (1998) found a pronounced positive effect of mixotrophy on primary production when modelling a nutrient-limited plankton community. In their systems, mixotrophs turned out to be important consumers of bacteria. By utilizing nutrients bound in bacteria for primary production, the mixotrophs enhanced overall primary production (Baretta-Bekker et al. 1998).

Competition for nutrients between phytoplankton and heterotrophic bacteria strongly depends on the availability of dissolved organic carbon (DOC; Grover 2002). If DOC is supplied in excess, bacteria can take advantage of their high affinity for soluble nutrients and outcompete phytoplankton (Rothhaupt 1992, Joint et al. 2002). Mixotrophy might be a particularly successful strategy when dissolved nutrients are reduced by high bacterial productivity. According to the traditional image of the microbial loop, heterotrophic nanoflagellates (HNFs) are the major consumers of bacteria and picophytoplankton (Azam et al. 1983, Caron and Goldman 1990; Fig.



3.1 a). They respire about 40 % of the energy bound in their prey (Fenchel 1982) and excrete a considerable share of the nutrients they ingest with their prey (Caron and Goldman 1990). By using nutrients from the picoplankton directly for photosynthesis, a mixotrophic flagellates represents a shortcut within the microbial loop (Fig. 3.1 b). If light is sufficient, but nutrients are limiting, the mixotroph should retain the limiting nutrient for photosynthetic growth (Rothhaupt 1997).

If a single nutrient is limiting (e.g. soluble nitrogen), bacteria, mixotrophic and autotrophic phytoplankton compete for this limiting resource. The mixotroph competes at the same time with the HNF for bacteria and picophytoplankton (Fig 3.1 b). If light is saturating, and picoplankton feeding by the mixotroph driven by the need for nutrient gain, the following predictions can be made: (1) a mixotrophic flagellate reduces nutrient remineralization in the microbial loop compared to a food web without mixotrophs. (2) a mixotrophic flagellate may affect productivity of the system. If bacterial productivity is high and the mixotroph is primarily bacterivorous, mixotrophy should enhance primary productivity, since nutrients bound in bacteria are directly used for primary production. Otherwise, if the mixotroph is primarily algivorous, it might have a neutral effect or even reduce primary production, since the nutrients utilized by the mixotroph originate from another primary producer (Stickney et al. 2000). The strength of any effect of the mixotroph should depend on its competitive abilities relative to its pure heterotrophic and pure autotrophic competitors.

In order to investigate the effects of mixotrophy on dynamics of a microbial food web, artificial food webs with and without a mixotrophic flagellate were assembled. The scope of this study was to see whether the mixotrophic flagellate may persist at steady state with specialised competitors, and how its presence affects overall productivity of the system. To see whether possible effects depend on the degree of bacterial productivity, the food webs were exposed to a gradient of DOC in the form of glucose enrichment.

## 3.2 Materials and methods

The experiment was performed in a factorial design: artificial food webs without and with a mixotroph were assembled from monocultures (Fig. 3.1). Both food web configurations were run under three different levels of glucose enrichment (Table 3.1). Each of the resulting six different treatments was twice replicated.

All cultures were grown non-axenically under same light and nutrient conditions as applied in the experiment (without glucose addition, except for the cultivation of the heterotrophic flag-

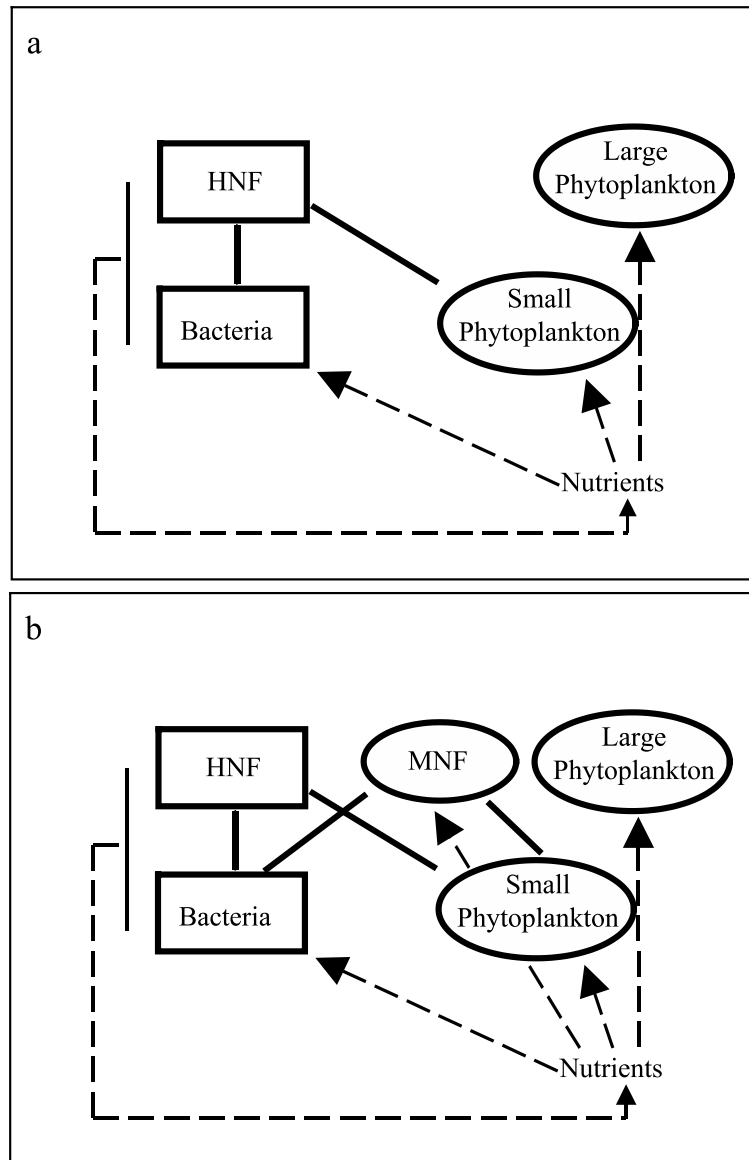


Figure 3.1: Schemes of the microbial loop (a) without and (b) with a mixotrophic flagellate. HNF, heterotrophic nanoflagellate; MNF, mixotrophic nanoflagellate. Solid lines represent fluxes of matter (grazing), dashed lines represent fluxes of dissolved nutrients.

Table 3.1: Nutrient concentrations of the medium. Gradient of daily glucose addition in milligram  $L^{-1} day^{-1}$  and equivalent amount of carbon (C) in micromol  $L^{-1} day^{-1}$ .

| Major nutrients $\mu mol L^{-1}$ |            | Glucose enrichment $mg L^{-1} day^{-1}$<br>( $\mu mol C L^{-1} day^{-1}$ ) |          |          |
|----------------------------------|------------|--|----------|----------|
| Nitrogen                         | Phosphorus | zero   | low      | high     |
| 40                               | 6          | 0 (0)  | 0.3 (10) | 1.5 (50) |

ellate *Spumella* where glucose was added to stimulate growth of bacteria). *Synechococcus* sp. originates from the Caribbean Sea (strain-no. CCMP 1282, Provasoli-Guillard Culture Center, USA) and has been cultivated on a Baltic Sea medium for several years (Markus Reckermann, pers. comm.). The euryhaline cryptophyte *Rhodomonas salina* is a strain originally isolated from the North Sea, that has been cultivated for many years on a Baltic Sea medium at the IFM (appr. 15 PSU). The heterotrophic chrysophyte *Spumella* sp. was isolated from the Baltic Sea (Klaus Jürgens, pers. comm.). The mixotrophic chrysophyte *Ochromonas minima* originates from the Kattegat (Jahn Thronsen, pers. comm.). It is worthwhile mentioning that this *Ochromonas* strain cannot survive on bacterivory alone, but needs to be grown in the light. In this respect it differs considerably from the photosynthetic abilities of most described *Ochromonas* strains, that are mainly heterotrophic (Anderson et al. 1989, Sibbald and Albright 1991, Rothhaupt 1996 a, b, Sanders et al. 2001). Bacteria were not grown separately, but were contained in all protist cultures.

Cultivation of the protists and the experiment itself were done in an environmental walk-in chamber at a 16-L-8-D cycle at 16 °C. The medium used was prepared from sterile filtered surface water collected from the Kattegat in summer 2002 (salinity 25 PSU). Nitrogen (N) and phosphorus (P) were added to final concentrations as given in Table 3.1, minor nutrients as given in Rick and Dürselen (1995). The atomic N:P ratio of the medium was 6.7, i.e. nitrogen was the growth limiting nutrient. The light intensity at surface of the experimental containers was about  $60 \mu E m^{-2} s^{-1}$  (LICOR Quantum Photometer LI-185B).

In the experiment, bacterial productivity was stimulated by daily additions of glucose (Table 3.1). The additions were chosen in such a way to equal 2.5 (treatment 'low') or 12.5 (treatment 'high') % of the expected seston biomass ( $400 \mu mol$  carbon (C)  $ml^{-1}$  for an expected C:N-ratio of about 10; Table 3.1).

The experiment was performed in a batch design: 1 L autoclaved Erlenmeyer flasks were filled with sterile medium and inoculated with the protist cultures to a final volume of 600 ml. The Erlenmeyer flasks were closed with sterile cellulose stoppers and placed below the light bench on a shaking table. In addition to the automatic shaking, the flasks were gently shaken by

hand every day and prior to each sampling. No conspicuous sediment layer has been observed in the experimental containers throughout the experiment.

The experiment lasted for 12 days. On day 4 and 8, 10 per cent fresh medium (relative to the current volume) were supplemented to each experimental container. Water samples for dissolved nutrient, microscopical and flow cytometric analysis were taken on every second day (nutrients: day 4, 8 and 10). Samples for nutrient analysis were immediately frozen and stored at -20 °C, samples for flow-cytometric and microscopical analysis were preserved with 2 % formaldehyde and stored in the dark at 5 °C until being analysed. On day 12, 150 ml samples for particulate carbon and 100 ml for chlorophyll *a* analysis were taken. These samples were filtered on pre-combusted WHATMAN GF/F filters and stored at -20 °C. Sampling volume exceeded the volume of the supplemented medium, thus the volume in the experimental containers decreased over time from 600 to 390 ml (day 12).

**Chemical and biological analysis** The filters for carbon analysis were dried at 60 °C and analysed by heat combustion on an FISON NA 1500 N analyser. Dissolved nutrient in water samples were analysed on a SKALAR SCANPLUS SYSTEM autoanalyser with standard methods. Chlorophyll filters were extracted overnight in 90 % acetone. Chlorophyll content was estimated photometrically on a SHIMADZU UV-160 spectral photometer according to Lorenzen (1967). Microscopic analysis of the plankton samples was done with an inverted fluorescence microscope (LEITZ DMIRB). 10 ml sample volume were transferred to Utermöhl chambers (Utermöhl 1958; height of the chamber 2.2 cm) and stained with 0.01  $\mu\text{g ml}^{-1}$  DAPI (Porter and Feig 1980). After 48 hours of sedimentation, first the smallest fraction (picophytoplankton, heterotrophic nanoflagellates) were counted at 1000x magnification under oil immersion and fluorescent light. This method allowed reliable differentiation between bacteria, picophytoplankton and heterotrophic nanoflagellates. The larger fractions were counted at lower magnifications under normal light. Except for cases of extreme rarity, at least 100 cells of each species were counted per sample by scanning a minimum of two perpendicular transects on the bottom side of the chamber or 20 distinct areas randomly distributed on two such transects. Additionally, bacterial flocs and filaments were counted and measured microscopically in all samples of day 12. Filaments were counted at a minimum length of 6  $\mu\text{m}$ , colonies at a minimum diameter of 5  $\mu\text{m}$ . Per sample, at least 50 filaments and 50 flocs were counted, and their length (filaments) and average diameter (flocs) were measured. Volume of the flocs was calculated by assuming that the 3rd dimension (height) of a floc was two thirds of its diameter, since particles should sediment on their broadside. By counting bacteria in several flocs, a factor was obtained for conversion of

floc volume to bacterial abundances (15 cells per 1000  $\mu\text{m}^3$ ). Total filament length per sample was converted to bacterial abundances by assuming a length of 1  $\mu\text{m}$  per bacteria cell.

**Calculations** Growth rates ( $\mu$ ) of the protists were calculated assuming exponential growth

$$\mu = \frac{\ln\left(\frac{N_t}{N_0}\right)}{t}$$

with  $N_0$  and  $N_t$  as initial and final cell concentrations at the beginning and at the end of time interval  $t$ , respectively (Sommer 1994).

**Flow cytometric analysis** Bacterial abundances were obtained from sample 12 by flow cytometry. 2 ml sample volume were prefiltered by a 64  $\mu\text{m}$  syringe membrane filter and stained with SYBR-I green (Marie et al. 1997). Samples were analyzed on a Becton Dickinson FACSCalibur flow cytometer. Using a side-scatter detector, 50,000 particles were counted per sample. Counts were discriminated by using WinMDI freeware (<http://facs.scripps.edu/software.html>). The bulk of the counted particles was considerably smaller than the flagellates and was defined as bacteria. This group should represent single cells as well as very short filaments. Picophytoplankton was not differentiated from bacteria. Its abundances were 3 orders of magnitude below bacterial abundances (see results) and therefore negligible in this analysis.

### 3.3 Results

**Development over time** Abundances of all species except the picophytoplankton *Synechococcus* increased after inoculation. In the treatments without glucose addition, *Synechococcus* was the most important prey for the heterotrophic nanoflagellate *Spumella*, and therefore the strong decline of *Synechococcus* can only be explained by grazing of *Spumella* (Šimek et al. 1997, Dolan and Šimek 1999). Concentrations of dissolved nitrogen ( $\text{NO}_3^{2-}$ ,  $\text{NH}_4^+$ ) decreased with increasing glucose enrichment on day 4 (Fig. 3.2) and were below detection limit ( $0.1 \mu\text{mol L}^{-1}$ ) on day 8. The steep decline in dissolved nitrogen with glucose enrichment (Fig. 3.2) indicates that the bacteria effectively consumed soluble nitrogen.

In the glucose enriched treatments, bacterial flocs became conspicuous in the second week and accumulated until the end of the experiment. These flocs were not attached to the bottom,

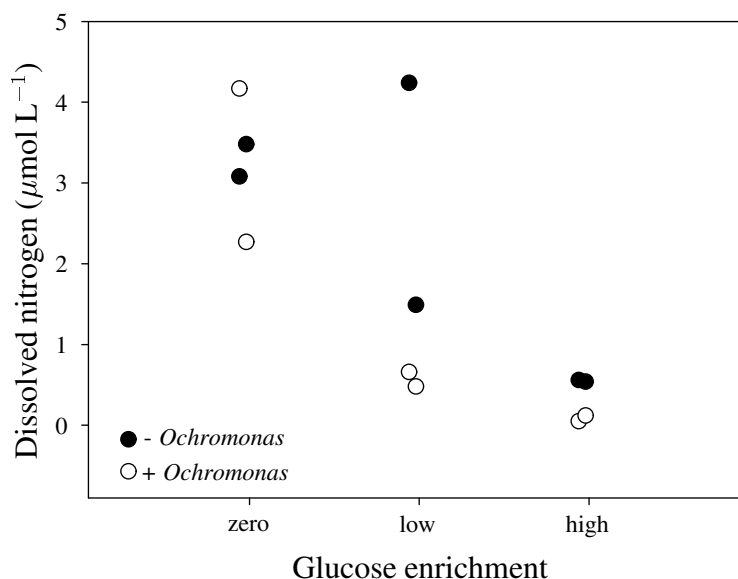


Figure 3.2: Concentration of soluble nitrogen (sum of  $\text{NO}_3^{2-}$ ,  $\text{NH}_4^+$ ) on day 4. On day 8 and 12 concentrations were below detection limit (soluble nitrogen was measured only on day 4, 8 and 12).

and got resuspended at each manual shaking.

**Species abundances** The comparison of species abundances is based on the data of day 12 (last sample), after fluctuations in most systems have become low (Fig. 3.3, Table 3.2).

Abundances of single celled bacteria were reduced by about 1 order of magnitude by the mixotrophic *Ochromonas minima* compared to treatments without the mixotroph, while the effects of glucose enrichment on single celled bacteria was comparatively small (Fig. 3.4, Table 3.3). In contrast, abundances of bacteria in filaments and flocs increased strongly with increasing glucose enrichment, but mixotrophy had no or only minor effects on the formation of flocs and filaments, respectively (Fig. 3.5). Total numbers of filaments were not significantly different between treatments without and with mixotrophs (data not shown), but the average filament length in mixotrophy treatments was lower in unenriched, and larger in the 'high' enriched treatments, as indicated by a significant interaction between glucose enrichment and mixotrophy (Table 3.2, 3.3). Formation of flocs and filaments cannot be interpreted as a result of glucose enrichment alone; rather, since numbers of single celled bacteria were controlled by the phagotrophic flagellates *Spumella* and *Ochromonas*, floc and filament formation is interpreted as an escape from

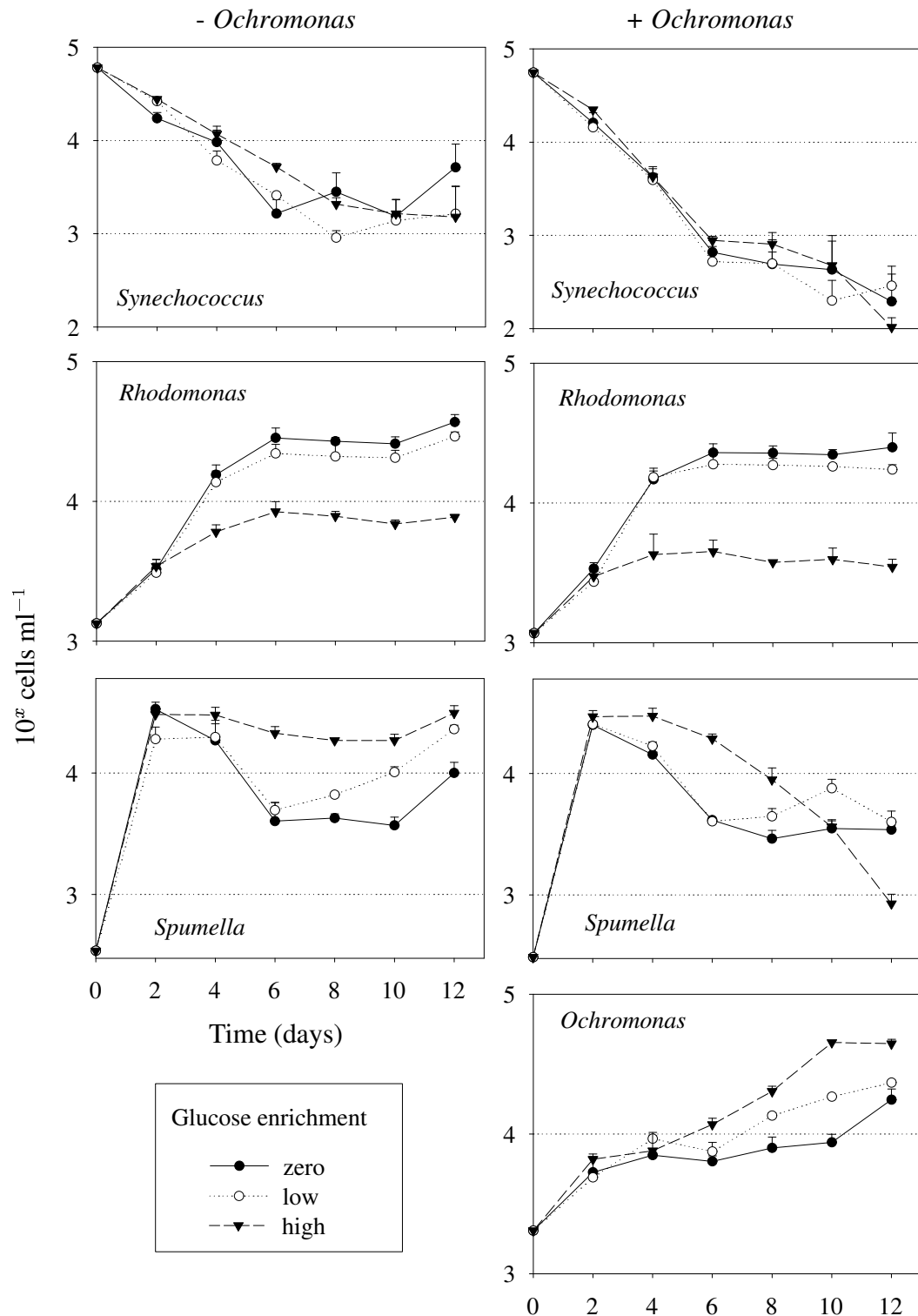


Figure 3.3: Abundances over time. Means of both replicates are shown, error bars indicate standard deviation (only upper direction).

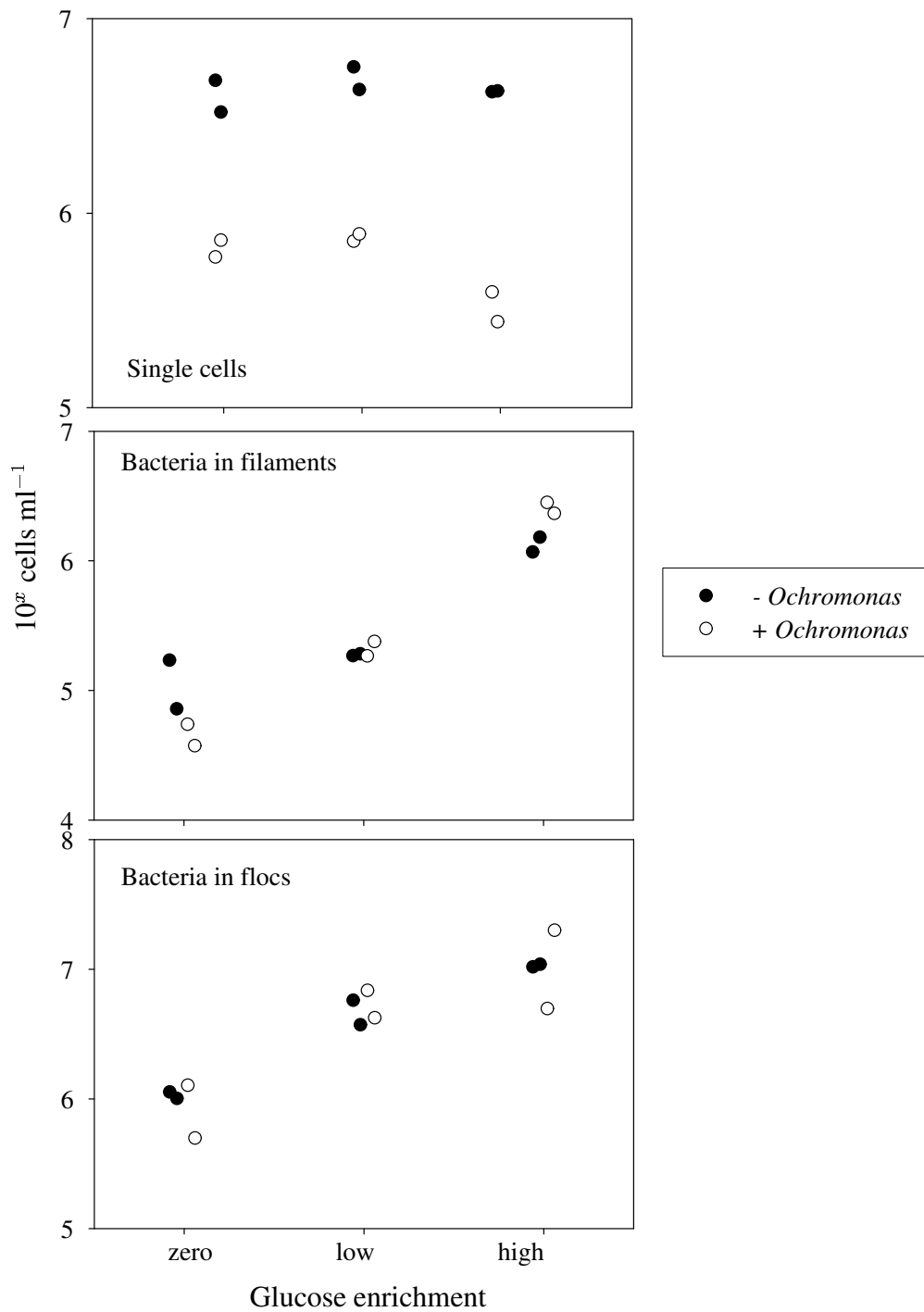


Figure 3.4: Abundances of bacteria in last sample (day 12). Abundances of single cells were obtained by flow cytometric analysis, abundances in filaments and flocs from microscopical measurements (see material and methods).



Table 3.2: Average dimensions of bacterial filaments and flocs for both replicates (day 12).

| -/+ <i>Ochromonas</i>                             | Glucose enrichment |            |            |            |            |            |
|---|--------------------|------------|------------|------------|------------|------------|
|   | zero               |            | low        |            | high       |            |
|   | -                  | +          | -          | +          | -          | +          |
| Filaments<br>(length, $\mu\text{m}$ )             | 41.2; 37.8         | 20.8; 22.7 | 43.7; 47.7 | 39.3; 45.6 | 49.4; 50.2 | 70.0; 68.1 |
| Flocs<br>(volume, $\times 10,000 \mu\text{m}^3$ ) | 2.55; 2.64         | 3.74; 0.99 | 10.4; 10.2 | 19.3; 11.3 | 4.21; 6.91 | 4.74; 1.5  |

Table 3.3: Results of a two-way ANOVA analysing the effects of mixotrophy (presence of *Ochromonas*), glucose addition and interaction of both factors (Glc.  $\times$  Mixotr.) on the log-transformed abundances of bacteria (single cells, filaments and flocs) and on the average dimensions of filaments (length) and flocs (volume) in the last sample (day 12). Homogeneity of variances for each species/parameter were tested prior to ANOVA by Box-M tests (no significant results).

| Bacteria group            | ANOVA |          | Mixotrophy | <i>p</i> factor |                       |
|---------------------------|-------|----------|------------|-----------------|-----------------------|
|                           | $r^2$ | <i>p</i> |            | Glucose         | Glc. $\times$ Mixotr. |
| Single cells              | 0.99  | <0.01    | <0.01      | 0.02            | 0.05                  |
| Cells in filaments        | 0.98  | <0.01    | 0.81       | <0.01           | 0.03                  |
| - average filament length | 0.98  | <0.01    | 0.02       | <0.01           | <0.01                 |
| Cells in flocs            | 0.89  | <0.01    | 0.85       | <0.01           | 0.08                  |
| - average floc volume     | 0.79  | 0.05     | 0.62       | 0.01            | 0.60                  |

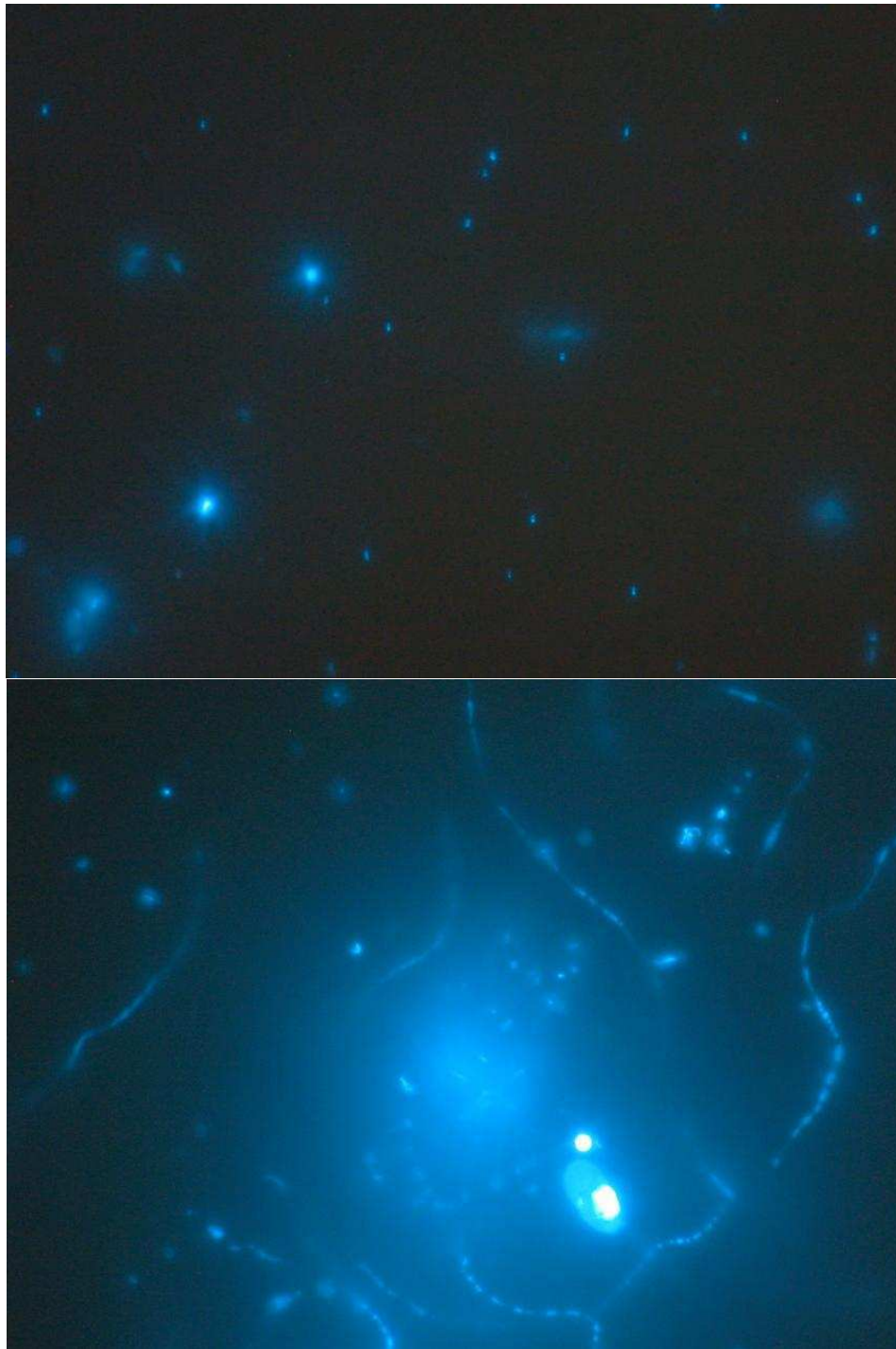


Figure 3.5: Photographs of DAPI samples from unenriched (upper picture) and highly enriched (lower picture) treatments. Upper picture: Single celled bacteria (small dots) and HNFs (bright large dots). Lower picture: Bacteria in single cells, filaments and flocs. Oval spot: *Rhodomonas* cell.

Table 3.4: Results of a two-way ANOVA analysing the effects of mixotrophy (presence of *Ochromonas*), glucose addition and interaction of both factors (Glc. x Mixotr.) on the log-transformed abundances of all species and on particulate organic carbon (POC) and chlorophyll *a* (chl *a*) on the last sampling date (day 12).  $n = 12$  except chlorophyll *a* ( $n = 11$ ; one filter got lost during analysis). Effects of glucose addition on the mixotrophic flagellate *Ochromonas* were analysed by a one-way ANOVA ( $n = 6$ ). Homogeneity of variances for each species / parameter was tested prior to ANOVA by Box-M tests (no significant results).

| Species,<br>Parameter | ANOVA |          | <i>p</i> factor |         |               |
|-----------------------|-------|----------|-----------------|---------|---------------|
|                       | $r^2$ | <i>p</i> | Mixotrophy      | Glucose | Glc x Mixotr. |
| <i>Synechococcus</i>  | 0.76  | 0.07     | < 0.01          | 0.48    | 0.47          |
| <i>Rhodomonas</i>     | 0.99  | < 0.01   | < 0.01          | < 0.01  | 0.22          |
| <i>Spumella</i>       | 0.99  | < 0.01   | < 0.01          | < 0.01  | < 0.01        |
| <i>Ochromonas</i>     | 0.95  | 0.01     | -               | 0.01    | -             |
| Chl <i>a</i>          | 0.99  | < 0.01   | < 0.01          | < 0.01  | < 0.01        |
| POC                   | 0.68  | 0.14     | 0.12            | 0.60    | 0.07          |

strong grazing pressure (Jürgens and Güde 1994, Jürgens et al. 1996). The observed shifts in bacterial morphology in respect to the absence/presence of *Ochromonas* are in accordance with studies about the effects of different species of heterotrophic nanoflagellates on bacterial community composition (Jürgens and Güde 1994, Posch et al. 1999)

The picophytoplankton *Synechococcus* sp. was strongly reduced by the mixotroph (Table 3.4). The comparatively high variability between the corresponding replicates in this species (error bars in Fig. 3.3) are most likely a result of the low absolute numbers that were counted during microscopical analysis. Possible effects of glucose enrichment may be confounded by this high variability.

Abundances of the autotrophic nanoflagellate *Rhodomonas salina* were also reduced by the mixotroph. Additionally, glucose enrichment caused lower abundances in the autotrophic nanoflagellate. Since food webs contained no grazers except bacterivorous flagellates, *Rhodomonas* was only controlled by resources, and therefore changes in its abundances should reflect availability of the limiting nutrient nitrogen (see discussion).

In the absence of the mixotroph, the heterotrophic nanoflagellate *Spumella* sp. was enhanced by glucose enrichment. In its presence, glucose enrichment had no (low enrichment) or even a negative effect (high enrichment) on the heterotrophic flagellate. The abundances of the mixotrophic flagellate *Ochromonas minima* were enhanced by glucose enrichment.

**Chlorophyll concentrations and overall microbial biomass** While glucose enrichment decreased chlorophyll concentrations of the seston, the presence of the mixotroph enhanced them

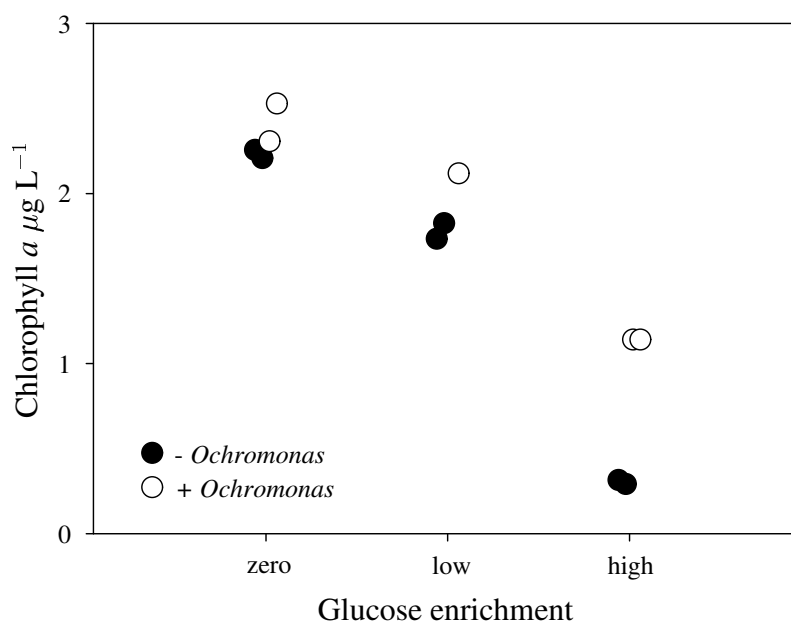


Figure 3.6: Chlorophyll *a* concentrations on day 12 (last sample).

(Fig. 3.6). The positive effect of the mixotroph increased with increasing glucose enrichment (significant interaction term enrichment  $\times$  mixotrophy, Table 3.2).

In the two-way ANOVA, glucose enrichment and mixotrophy had no significant effect on particulate organic carbon (POC; Fig. 3.7, Table 3.4). However, visual inspection of carbon concentrations indicates a general difference between the unenriched and the enriched treatments. When differentiating only between unenriched and enriched treatments, an otherwise identical two-way ANOVA gives a significant result with a highly significant interaction term between enrichment and mixotrophy ( $n = 12$ ;  $r^2 = 0.61$ ,  $p_{ANOVA} = 0.05$ ;  $p_{enrichment} = 0.71$ ,  $p_{mixotrophy} = 0.43$ ,  $p_{enrich. \times mixotr.} = 0.02$ ; Box-M test n.s.). When testing the effects of mixotrophy on carbon content in two-tailed independent t-tests, mixotrophy has a marginally significant negative effect in treatments without glucose enrichment ( $n = 4$ ;  $p = 0.053$ ), and a clearly significant positive effect in the treatments with glucose enrichment ( $n = 8$ ;  $p = 0.03$ ). Hence, microbial biomass was affected by mixotrophy in a contrasting manner, depending if bacterial productivity was enhanced by glucose enrichment or not.

**Growth rates of *Rhodomonas* and *Ochromonas*** Both phototrophic flagellates exhibited their highest growth rates between day 0 and 4, when the availability of nitrogen was high (aver-

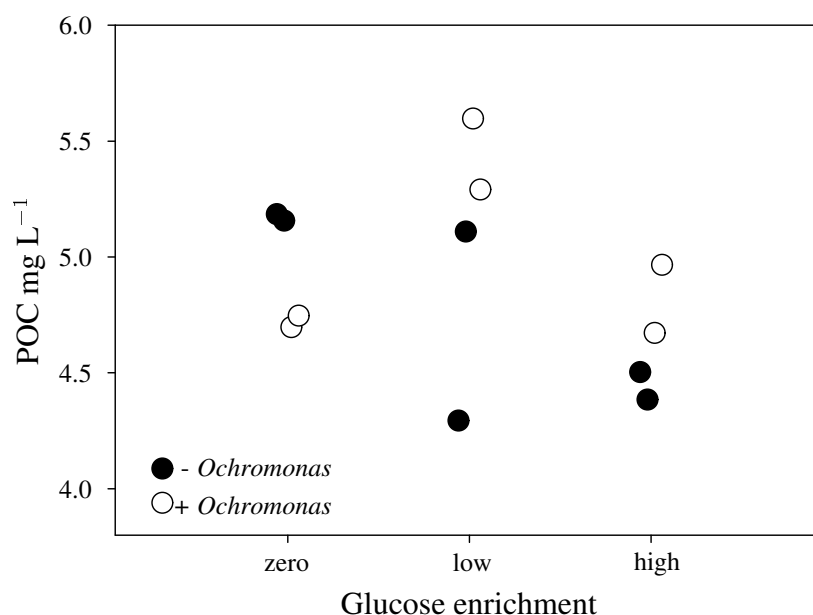


Figure 3.7: Concentrations of particulate organic carbon on day 12 (last sample).

age growth rate (plus standard deviation) in the *Ochromonas* treatments: 0.53 (0.25) and 0.34 (0.23) day<sup>-1</sup> for *Rhodomonas* and *Ochromonas*, respectively). Conversely, in the last four days when nitrogen was below detection limit, growth rates of *Rhodomonas* were close to zero, while *Ochromonas* still had considerable growth rates (-0.01 (0.06) and 0.18 (0.16) for *Rhodomonas* and *Ochromonas*, respectively).

**Numerical response of the heterotrophic and the mixotrophic flagellate** In order to compare the competitive abilities of the phagotrophic flagellates *Ochromonas* and *Spumella* with respect to their prey, the treatments without glucose addition shall be analysed, where *Synechococcus* was probably the major prey for both flagellates, because abundances of *Synechococcus* are available over the whole experimental period, whereas bacterial abundances are only available for the last sampling date.

During the initial growth phase, the heterotrophic flagellate *Spumella* increased within only 2 days by two orders of magnitudes (from appr. 300 to 30,000 cells ml<sup>-1</sup>; Fig. 3.3, 3.8). This corresponds to an average growth rate in this time interval of 2.4 day<sup>-1</sup>, that is still well below maximal observed growth rates of small heterotrophic nanoflagellates (3.6 - 6 day<sup>-1</sup>, Fenchel 1982). Growth rates of the mixotrophic *Ochromonas* were much smaller (never above 0.4 day<sup>-1</sup>;

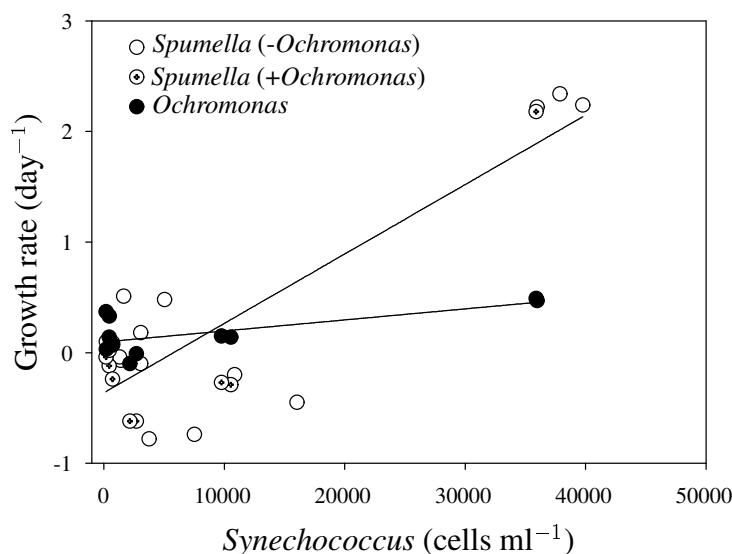


Figure 3.8: Growth rates of the heterotrophic *Spumella* and the mixotrophic *Ochromonas* plotted against the concentrations of the picophytoplankton *Synechococcus* (treatments without glucose addition).

Fig. 3.8). However, *Ochromonas* increased in abundances until the end of the experiment, whereas *Spumella* stopped growth or even decreased after day 2. Additionally, in the presence of *Ochromonas*, *Synechococcus* and single celled bacteria were reduced to lower levels than in treatments without *Ochromonas*. These findings indicate clearly different numerical response patterns between these two flagellates and their prey: the heterotrophic flagellate seems to reach higher growth rates than the mixotroph at high prey levels, while the mixotroph seems to do relatively better when prey is at low levels. When growth rates of *Ochromonas* and *Spumella* in the treatments without glucose enrichment are plotted against abundances of their prey *Synechococcus* (means between adjacent time intervals), the heterotrophic *Spumella* exhibits much higher growth rates than the mixotrophic *Ochromonas* at high prey levels, whereas *Ochromonas* reaches higher growth rates than *Spumella* at low prey levels (Fig. 3.8). In both flagellates there is a significant positive linear relationship between specific growth rates and abundances of *Synechococcus* (Fig. 3.8, Table 3.5). However, in both species this relationship is mainly due to the big differences in growth rates between the first and all later time intervals (Fig. 3.8), and no significant relationship exists if growth rates from the very first time interval are excluded from the analysis. The variability in growth rates after day 2 that cannot be explained by abundances of *Synechococcus* may be related to an increase in bacterial productivity, and therefore an increase

Table 3.5: Results of linear regressions between flagellate growth rates and abundances of *Synechococcus* (simple, a) or abundances of *Synechococcus* and time (multiple, b and c; method enter). In (c), the first time interval was excluded from analysis.

| Species               | Regression |       |              | <i>Synechococcus</i> |         | time     |        |
|-----------------------|------------|-------|--------------|----------------------|---------|----------|--------|
|                       | <i>p</i>   | $r^2$ | intersection | <i>p</i>             | coeff.  | <i>p</i> | coeff. |
| HNF (a)               | < 0.01     | 0.74  | -0.36        | < 0.01               | 6.3 E-5 | -        | -      |
| HNF (b)               | < 0.01     | 0.91  | -1.85        | < 0.01               | 1.0 E-4 | < 0.01   | 0.19   |
| HNF (c)               | < 0.01     | 0.61  | -1.19        | 0.08                 | 3.3 E-5 | < 0.01   | 0.13   |
| <i>Ochromonas</i> (a) | 0.01       | 0.49  | 0.10         | 0.01                 | 1.0 E-5 | -        | -      |
| <i>Ochromonas</i> (b) | < 0.01     | 0.82  | -0.30        | < 0.01               | 2.1 E-5 | < 0.01   | 0.05   |
| <i>Ochromonas</i> (c) | 0.01       | 0.71  | -0.48        | 0.02                 | 4.0 E-5 | < 0.01   | 0.07   |

in bacterivory with time: production of exudates by phytoflagellates may strongly increase under nutrient limitation (Guillard and Wangersky 1958), and since nutrient limitation increased with time, bacterial productivity possibly increased over the experimental period. When time as a surrogate parameter for increasing bacterial productivity is included into the regressions, the fit of the regressions increases substantially, and both regressions are significant even when the growth rates of the first time interval are excluded (Table 3.5). In all regressions with growth rates of *Ochromonas*, the intercepts with the y-axis are higher, and in all except one cases, the slopes of the *Synechococcus*-terms are lower than in the corresponding regressions of *Spumella*, confirming the suggested differences in the numerical responses of *Ochromonas* and *Spumella*.

### 3.4 Discussion

**Nutrient dynamics** Dissolved inorganic nitrogen ( $\text{NO}_3^{2-}$ ,  $\text{NH}_4^+$ ) decreased rapidly and fell below the detection limit between day 4 and day 8 in all treatments. Therefore, availability of the limiting nutrient nitrogen cannot be assessed directly. However, since the autotrophic *Rhodomonas* was only limited by mineral nutrients, its abundances provide an indication of the availability of dissolved nitrogen. *Rhodomonas* was clearly reduced by glucose addition as well as by the presence of the mixotrophic flagellate. Glucose addition led to accumulation of bacterial biomass (ungrazable flocs and filaments) and therefore to a sink of nitrogen. It may further be assumed, that without nutrient regeneration by the heterotrophic flagellate *Spumella*, less dissolved nitrogen would have become available for the primary producers (Goldman et al. 1985, Rothhaupt 1992). In addition to the effects of glucose addition, *Rhodomonas* was reduced by the presence of the mixotrophic *Ochromonas*. The mixotroph competed for picoplankton with the heterotrophic *Spumella*, and obviously retained the bulk of the nitrogen contained in its prey

(Rothhaupt 1997).

**Effects on seston biomass** In absence of the mixotroph, seston biomass tended to decrease with increasing glucose addition. This is somewhat counterintuitive, since it means that less biomass was produced when more (organic) carbon was supplied to the systems. The explanation, however, is simple: glucose addition drove the systems from a dominance by phytoplankton to a dominance by bacteria. Since bacterial biomass has got a lower carbon to nitrogen ratio than nutrient limited phytoplankton (Kohl and Nicklisch 1988, Fukuda et al. 1998, Sterner and Elser 2002), they can build up less biomass per limiting nutrient unit than phytoplankton. The pattern is more complicated in the presence of the mixotroph: compared to systems without *Ochromonas*, seston biomass was reduced by *Ochromonas* in the unenriched, but enhanced in the enriched treatments. The reduction in the unenriched treatments is in accordance with reduced abundances of the autotrophic *Rhodomonas* and *Synechococcus*. In the enriched treatments conversion from bacterial to mixotrophic biomass probably outweighed the decrease in autotrophic biomass.

#### **Competition between the heterotrophic *Spumella* and the mixotrophic *Ochromonas***

Rothhaupt (1996 b) investigated the numerical responses of a heterotrophic and a mixotrophic nanoflagellate (*Spumella* sp. and *Ochromonas* sp.). Under light, the heterotrophic flagellate reached higher growth rates than the mixotroph at high resource (= bacteria) levels, while the mixotroph was characterized by a lower minimum resource concentration to achieve zero net growth ( $R^*$ , Tilman 1990, Grover 1997) and reduced their common resource bacteria to lower levels than the heterotrophic flagellate, similar to results in this study (Fig. 3.8). Rothhaupt (1996 b) concluded, that the mixotroph took advantage of its photosynthetic abilities (utilization of light and dissolved nutrients) at low prey levels, and may therefore grow at lower resource (= bacteria) levels than its heterotrophic competitor. In Rothhaupt's experiments (1996 a, b), nutrients were available in excess, and the mixotroph substantially took up dissolved nutrients at low prey levels. In contrast, in this study dissolved nitrogen became limiting after several days. Nevertheless the growth rates of *Ochromonas* were considerably higher than the growth rates of *Spumella* when their common prey picoplankton was reduced to very low levels. This shows that a mixotrophic flagellate may compete successfully with a heterotrophic flagellate even under nutrient limitation, i.e. that availability of light may be sufficient to make a mixotrophic flagellate a superior competitor for picoplankton compared to a heterotrophic flagellate.



**Coexistence of the mixotroph with its specialized competitors** Especially in the treatments without and with low glucose enrichment, the abundances of the two specialists *Rhodomonas* and *Spumella* were relatively stable over time, indicating stable coexistence of the mixotrophic *Ochromonas* with both specialists. This is somewhat surprising, since according to resource competition theory, constant conditions permit only coexistence of two species for two limiting resources (e.g. light and nitrogen; Grover 1997). According to theoretical investigations in systems with bacteria, a pure heterotrophic, a pure autotrophic and a mixotrophic flagellate, a mixotroph can only coexist with either the heterotrophic or the autotrophic competitor, but coexistence with both competitors at the same time should be impossible (Thingstad et al. 1996). Coexistence of the mixotrophic *Ochromonas* with the autotrophic *Rhodomonas* can be explained by utilization of different resources: the autotroph utilized dissolved nutrients, while the mixotroph utilized particulate nutrients bound in its prey. Coexistence of the mixotroph with the heterotrophic flagellate is less intuitive, since the data indicate that the mixotroph was the superior competitor for their common prey at low prey levels (see above). It can only be speculated that the coexistence is a result of resource partitioning due to morphological diversity among the picoplankton (bacteria and *Synechococcus*). Heterotrophic nanoflagellates may differ considerably in their prey size spectra (Chrzanowski and Šimek 1990, Posch et al. 1999).

Ingestion of cells similar to its own size is common in the genus *Ochromonas* (J. Vrba, pers. comm.), and it is therefore likely that *Ochromonas* ingested *Spumella*. At comparable prey levels, a negative effect beyond competition should result in lower growth rates of the heterotrophic flagellate in the presence of the mixotroph. However, growth rates of *Spumella* did not differ considerably in the treatments with and without *Ochromonas* (Fig. 3.8). Therefore, predation of *Ochromonas* on *Spumella* was probably negligible.

**Concluding remarks** The presented results help to understand why mixotrophs are an inherent part of plankton communities, competing successfully with specialised autotrophic and heterotrophic competitors. Exhibiting relatively low maximum growth rates at favourable conditions, the combination of phototrophy and phagotrophy allows for successful competition with pure auto- and heterotrophic flagellates when nutrients and picoplankton are at low levels (provided that light is sufficient). According to these results, one would expect mixotrophs to be an important constituent of the plankton especially in steady-state like situations where light is sufficient, but dissolved nutrients are limiting and overall productivity is rather low, as it is the case in surface layers after a longer period of stratification. Under such conditions, external import of nutrients is low, and recycling is the primary source for mineral nutrients. Growth rates of

pure autotrophs are well below their possible maxima, and mixotrophs might take full advantage of their strategy. Havskum and Riemann (1996) found that mixotrophic nanoflagellates were the major bacterivores as well as the major primary producers in the the surface layer of a fjord in the Baltic Sea during summer stratification. In a recent study by Tittel et al. (unpublished), mixotrophic flagellates controlled the autotrophic fraction near the surface in an acidified lake. Due to vertically increasing light limitation, the grazing impact of the mixotrophs decreased with depth, resulting in a pronounced subsurface chlorophyll maximum. Relatively high concentrations of mixotrophs were also reported by Arenovski et al. (1995) and Sanders et al. (2000) in the stratified surface layer of the oligotrophic Sargasso Sea.

## Chapter 4

# Manipulation of omnivory and mixotrophy in an experimental planktonic food web

This chapter is currently submitted to *Limnology & Oceanography*

**Abstract** - The trophic role of two protist groups, microzooplankton and mixotrophic flagellates, was investigated in artificial, lifelike food webs with calanoid copepods as top predators. Microzooplankton has recently received increased attention as an important trophic link between the microbial loop and calanoid copepods. Based on food size spectra overlap in some microzooplankton groups and calanoid copepods, however, such microzooplankton could function as a competitor rather than as a link for calanoid copepods. Mixotrophic flagellates are discussed to represent an effective link between the microbial loop and the micro- and mesozooplankton. These hypotheses were tested by altering the presence of a heterotrophic dinoflagellate and of a mixotrophic nanoflagellate in artificial food webs. The heterotrophic dinoflagellate reduced drastically the nanophytoplankton, and enhanced the reproduction of the copepods, suggesting that its role as competitor is negligible compared to its function as trophic link. In spite of the presence of heterotrophic nanoflagellates, the mixotroph had a strong negative effect on the picophytoplankton and (presumably) on bacterial biomass. At the same time, the mixotroph enhanced the atomic C:N ratio of the seston, indicating a higher efficiency in overall primary production. Offspring of the copepods was enhanced in presence of the mixotrophic nanoflagellate.

## 4.1 Introduction

The importance of omnivory in planktonic food webs became increasingly obvious during the last two decades (Sherr et al. 1986, France 1997, Gurney et al. 2001). In particular, it was found that virtually all calanoid copepod species, formerly viewed as herbivorous (Paffenhöfer et al. 1982, Wong 1988), also feed substantially on heterotrophic organisms (e.g. Stoecker and Egloff 1987, Kleppel 1993, Zeldis et al. 2002). Specifically microzooplankton seem to be an important food for calanoid copepods (Kleppel et al. 1998, Klein Breteler et al. 1999, Bonnet and Carlotti 2001). In spite of conspicuous size differences between calanoid copepods and microzooplankton (1,000 - 2,000  $\mu\text{m}$  and 20 - 200  $\mu\text{m}$ , respectively), their food size spectra may overlap considerably (Sherr et al. 1986, Sanders and Wickham 1993). This is mainly caused by oligotrich ciliates and heterotrophic dinoflagellates ingesting prey that is negligibly smaller than themselves (Hansen et al. 1994). Microzooplankton utilizing a food source of similar size act as competitor and prey for the copepods at the same time ('intraguild predation', Mylius et al. 2001; compare with positions of copepods, microzooplankton and nanophytoplankton in Fig. 4.1). On the one hand, by feeding on such an 'intermediate consumer' (Diehl and Feissel 2000), the copepods control its abundances (Thingstad et al. 1996), but on the other hand they have an energetic disadvantage, since they are feeding on a higher trophic level (Oksanen et al. 1981). Alternatively, if microzooplankton utilize prey too small in size for the copepods, the microzooplankton should act as trophic link, providing indirect access to the biomass produced in the microbial loop (Sherr et al. 1986, Calbet and Landry 1999).

The energy transfer efficiency from the microbial loop (Fig. 4.2 a) to the mesozooplankton is generally believed to be low according to the intermediate trophic levels between small phytoplankton and the mesozooplankton (Ducklow et al. 1986, Sherr and Sherr 1988). However, there is increasing awareness that mixotrophic protists compose a considerable portion of planktonic communities and that they are important consumers of bacteria and small phytoplankton in the marine plankton (Riemann et al. 1995, Havskum and Riemann 1996). Mixotrophy is here used in the restricted sense of combining photosynthesis and phagotrophy in a single organism (Sanders 1991, Jones 1994). By combining photosynthesis and phagotrophy, mixotrophs should represent a more effective trophic link between the microbial loop and the micro- and mesozooplankton than heterotrophic protists (Fig. 4.2 b; Jones 1994, Riemann et al. 1995). Though this hypothesis seems important for the understanding of the microbial loop, to the best of our knowledge it has not yet been tested.

In this study, the effects of mixotrophy and omnivory on trophic structure of a planktonic food web and on the productivity of its top consumer are investigated. Artificial food webs were as-

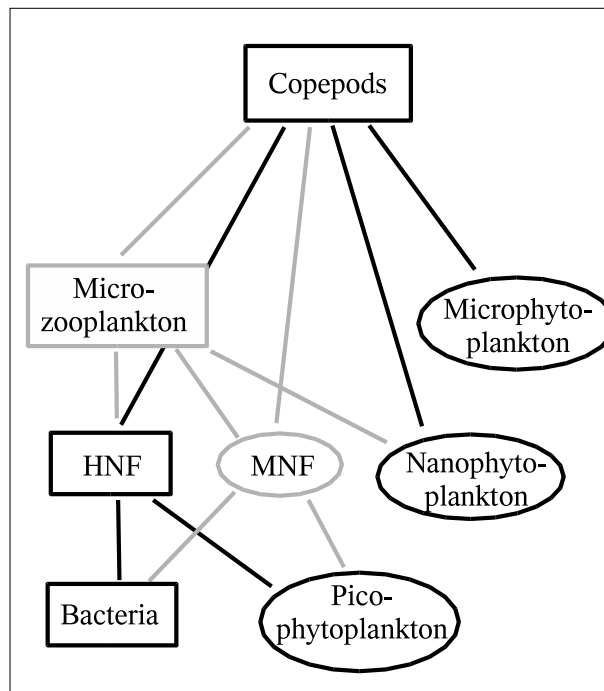


Figure 4.1: Sketch of the experimental food web. Black lines represent links that were present in all food webs, while grey lines represent facultative links that were generated by the addition of the microzooplankton and the mixotrophic nanoflagellate (MNF). HNF, heterotrophic nanoflagellate. For clarity, the weak links between microzooplankton and bacteria and picophytoplankton are not displayed.

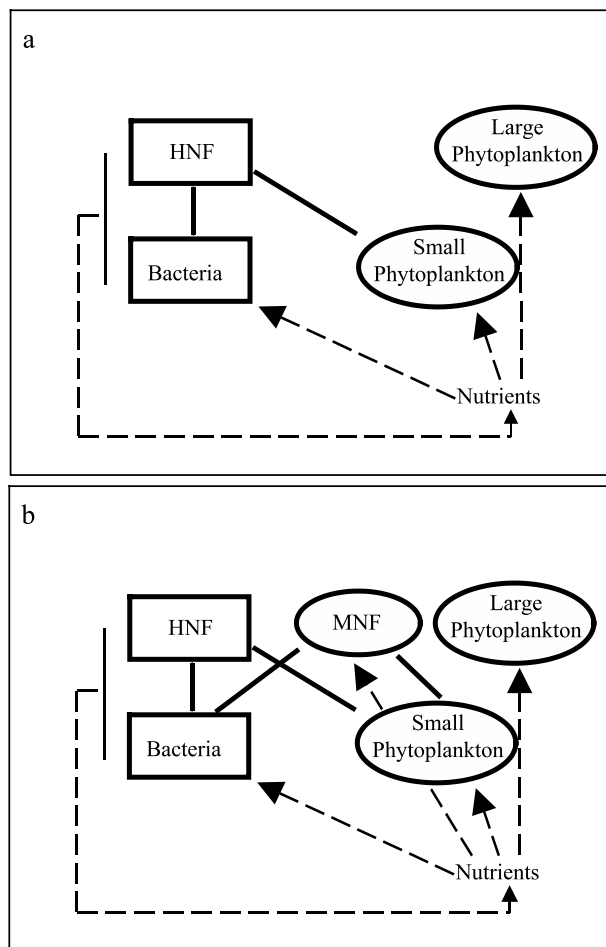


Figure 4.2: Microbial loop (a) without and (b) with a mixotrophic flagellate. Solid lines represent fluxes of particulate matter (grazing), dotted lines represent fluxes of dissolved nutrients. HNF, heterotrophic nanoflagellate; MNF, mixotrophic nanoflagellate.

Table 4.1: Food web configurations and nutrient levels. Microzoo., microzooplankton; mixotr., mixotrophic nanophytoplankton.

| Food web configurations |           |         |           | Nutrient levels                   |               |
|-------------------------|-----------|---------|-----------|-----------------------------------|---------------|
| B                       | BO        | BM      | BMO       | low                               | high          |
|                         | Microzoo. | Mixotr. | Microzoo. | 9:3:5                             | 28.5:9.5:15.8 |
|                         |           |         | Mixotr.   | N:Si:P ( $\mu\text{mol L}^{-1}$ ) |               |

all: bacteria, HNFs, autotr. pico-, nano- and microphytoplankton, copepods

sembled, that consisted of typical representatives of a marine plankton community with calanoid copepods as top predators (Fig. 4.1). Within this food web, presence and absence of omnivory and mixotrophy were manipulated. Omnivory in copepods was altered by the absence/presence of a microzooplankton species with an optimal prey size in the size range of the nanophytoplankton. The copepods should be mainly herbivorous in the food webs without microzooplankton, but compete with and feed on the microzooplankton when it is present. In this manner it should be tested whether the microzooplankton is functioning as a competitor or a trophic link to the copepods. Mixotrophy was manipulated by the absence/presence of a mixotrophic nanoflagellate (Fig. 4.1). Nutrient enrichment was included as an additional factor to test whether possible top-down effects and the relative importance of the link and competitor effects, respectively, are influenced by productivity.

## 4.2 Materials and methods

The experiment was carried out in June 2001 in a walk-in environmental chamber that was set to a 16-L-8-D-cycle at a temperature of 16 °C. The water used for the preparation of the medium was collected from the mixed surface layer of the Kiel Bight (western Baltic Sea, salinity 15 PSU) one week prior to the experiment and stored in the dark at 16 °C. It was then filtered into sterile experimental containers by a 0.45  $\mu\text{m}$  filter capsule (SARTORIUS Sartobran-P Capsule). This pore width was chosen to exclude all eukaryotic protists, but permit passing of smaller bacteria from the natural bacterial assemblage. Major nutrients were added to final concentrations as given in Table 4.1, minor nutrients as given in Rick and Dürselen (1995). The nitrogen to phosphorus ratio was about two, i.e. for all phytoplankton nitrogen should have been the limiting nutrient (except for possible silica-limitation in the diatoms). The protists were grown as non-axenic monocultures under same salinity and under a similar light and nutrient regime as applied

in the experiment. The euryhaline cryptophyte *Rhodomonas salina* is a strain originally isolated from the North Sea, that has been cultivated for many years on a Baltic Sea medium at the IFM (appr. 15 PSU). The diatom *Thalassionema nitzschioides* and the heterotrophic dinoflagellate *Oxyrrhis marina* were isolated from the Kiel Fjord (western Baltic Sea) a few months before the experiment. After isolation, *Oxyrrhis* was grown on *Rhodomonas salina*. The heterotrophic nanoflagellate *Cafeteria rosenbergensis* (*Silicoflagellidae*) was isolated from the Baltic proper (K. Jürgens, pers. comm.). The mixotrophic nanoflagellate *Chrysochromulina polylepis* (*Haptophyceae*) is a strain from the SCCAP Copenhagen, Denmark (K-0617), that originally has been isolated from the Kattegat, North Sea. The cyanobacterium *Synechococcus* sp. (picophytoplankton) originates from the Caribbean Sea (strain-no. CCMP 1282, Provasoli-Guillard Culture Center, USA) and was cultivated on a Baltic Sea medium for several years (Markus Reckermann, pers. comm.). The copepods were collected by vertical net hauls (250  $\mu\text{m}$  mesh size) from the Kiel Bight two weeks before the start of the experiment. During this time they were kept in two 300 L containers with little food addition. Rotifers and nauplii disappeared during this period, mainly as a result of predation by copepods (Stoecker and Egloff 1987). Before adding the copepods to the experimental containers they were washed twice with sterile filtered water over a 64  $\mu\text{m}$  mesh. The final inoculum consisted of an assemblage of various copepodid stages and adults of *Acartia tonsa*, *Pseudocalanus elongatus*, *Paracalanus parvus*, and *Centropages hamatus*, no other mesozooplankton was observed at this time, nor later during the experiment.

**Experimental containers** The experimental containers consisted of circular 30 L polypropylene buckets that were covered by a transparent lid to reduce contamination (Fig. 4.3), and placed under a light bench. Atmospheric air was pumped into the airspace between the lid and water surface. A filter at the connection between tube and lid prevented contamination by the airflow. The medium was mixed by a kind of Archimedes' screw: a small electric motor was mounted on the lid and connected to a glass baton through a small hole in the lid. The baton carried a Polyvinyl Chloride (PVC) screw on its bottom end (diameter 10 cm). A PVC cylinder with a slightly larger diameter than the screw was placed on the bottom of the container, enclosing the whole thread of the screw. The cylinder stood on three knobs, leaving approximately 1 cm between the bottom end of the cylinder and the base of the container. The motor was adjusted to approximately one turn per second, and the rotation of the screw resulted in the water moving down and through the slit between cylinder and base. This induced a current just above the base of the container, impeding sedimentation of the phytoplankton. Aside from this effect, mixing improved gas exchange of the medium and evenly distributed the food.



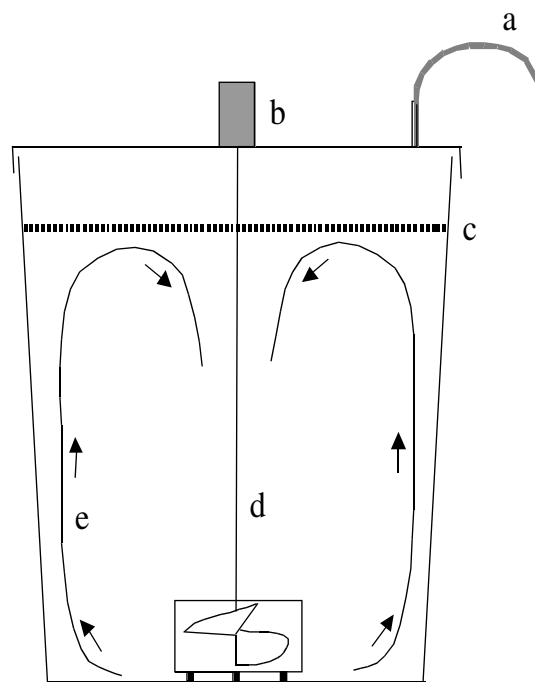


Figure 4.3: Experimental container. a - aeration tube; b - motor; c - water level; d - glass stick with screw; e - induced current. For further explanation see text.

Containers were arranged in groups of three per light bench. Each light bench consisted of two parallel 36 watt neon lamps with a length of 120 cm (STARLICHT 36 W 020 cool white and OSRAM L 36W/77 Fluora (plant light)). The light intensity was  $100 \mu\text{E m}^2 \text{ s}^{-1}$  in mid-depth of the containers under pure water (LICOR Quantum Photometer LI-185B).

**Experimental design and sampling** The experimental set-up was a factorial design. Three factors were varied (presence of microzooplankton, presence of mixotrophs, and nutrient level), leading to 4 different food web configurations at 2 different nutrient levels (Table 4.1, Fig. 4.1). Each of the 8 resulting treatments was twice replicated.

First the containers were filled with medium as given in Table 4.1 and inoculated with the protists (except the microzooplankton). Initial sampling was done five days later (start of the experiment, day 0), one day later the copepods and the microzooplankton were added. The final volume was 25 liters per container.

The experiment was run 24 days with 10 % of the medium being exchanged every 6 days. 1.5 to 2 liters of the exchanged water were filtered by a  $64 \mu\text{m}$  mesh to retain copepods of all developmental stages. They were immediately counted under a dissecting microscope and returned to the experimental containers (without the old medium). Copepods were classified as nauplii and copepodids (including adults). The rest of the exchanged volume was filtered by a  $100 \mu\text{m}$  mesh and used for further analysis (though the  $100 \mu\text{m}$  mesh did not retain all nauplii, it was used for phytoplankton and seston analyses, because the  $64 \mu\text{m}$  mesh retained a considerable fraction of the diatoms). For analysis of particulate carbon and nitrogen (C, N), 100 ml of medium were filtered on precombusted WHATMAN GF/F-filters, dried at  $60^\circ\text{C}$  and stored in a desiccator until analysis. Samples for microscopic analysis were preserved with 2 % glutar aldehyde and kept dark at  $5^\circ\text{C}$  until analysis. In addition to the 6-day interval sampling, samples for phytoplankton and C- and N-filters were taken in the middle of each 6-day interval. The volume lost from the containers by this additional sampling was taken into consideration at each subsequent exchange of water.

**Chemical and biological analysis** Particulate carbon and nitrogen were analysed by heat combustion on a FISON NA 1500 N analyser.

Microscopic analysis of the plankton samples was done on an inverted fluorescence microscope (LEITZ DMIRB). 10 ml sample volume were transferred to Utermöhl chambers (Utermöhl 1958; height of the chamber 2.2 cm) and stained with  $0.01 \mu\text{g ml}^{-1}$  DAPI (Porter and Feig 1980). After 48 hours of sedimentation, first the smallest fraction (picophytoplankton, heterotrophic nanoflagellates) was counted at 1000x magnification under oil immersion and fluores-

Table 4.2: Functional groups and their representatives in the food webs. For each protist (single cell), its equivalent spherical diameter (ESD), calculated biovolume and carbon (C) content are given.

| Functional group            | Species in food web                | ESD ( $\mu\text{m}$ ) | Biovol. ( $\mu\text{m}^3$ ) | C-content (pg)  |
|-----------------------------|------------------------------------|-----------------------|-----------------------------|-----------------|
| Picophytoplankton           | <i>Synechococcus</i> sp.           | 1.3                   | 1.15                        | 0.25            |
| Autotroph. nanophytopl.     | <i>Rhodomonas salina</i>           | 6.4                   | 136                         | 21.8            |
| Mixotroph. nanophytopl.     | <i>Chrysochromulina polylepis</i>  | 4.14                  | 71                          | 11.8            |
| Microphytoplankton          | <i>Thalassionema nitzschioides</i> | 11.6                  | 820                         | 66.4            |
| Heterotroph. nanoflagellate | <i>Cafeteria rosenbergensis</i>    | 3.05                  | 14.8                        | 2.72            |
| Microzooplankton            | <i>Oxyrrhis marina</i>             | 14.5                  | 1590                        | 219             |
| Mesozooplankton             | average nauplius                   |                       |                             | $2 \times 10^5$ |
|                             | average copepodid                  |                       |                             | $1 \times 10^6$ |

cent light. This method allowed reliable differentiation between bacteria, picophytoplankton and small heterotrophic nanoflagellates. The larger fractions were counted at lower magnifications under normal light. Except for cases of extreme rareness, at least 100 cells of each species per sample were counted by scanning a minimum of two perpendicular transects on the bottom side of the chamber or 20 distinct areas randomly distributed on two such transects.

To compare the relative share of all functional groups, the carbon content for each group was estimated (Table 4.2). For the protists, dimensions of 30 cells of each species were measured under the inverted microscope in a variety of samples (*Cafeteria* was selected as representative for the HNFs). Biovolume was calculated by using simple geometric bodies. Carbon content of each species was then derived from the biovolume by the formula given in Menden-Deuer and Lessard (2000). The copepods belonged to various species, and were only classified as nauplii and copepodids including adults, so only a rough estimate was possible here. Carbon contents of an average nauplia and copepodid were estimated from data for *Acartia tonsa* (Berggreen et al. 1988).

**Statistical analysis** For a statistical analysis of treatment effects on the food web compartments, abundances of all groups and the atomic C:N ratio of the seston were averaged over the last three (copepods and C:N-ratio: last two) sampling dates (day 18 to 24). Copepodids (including adults) and nauplii were treated as individual groups since they differ considerably in their food size spectra (Hansen et al. 1994).

Overall effects of the three treatments (enrichment, omnivory and mixotrophy) were analysed in a redundancy analysis (RDA, Jongmann et al. 1987). RDA is a form of direct gradient

analysis that assumes linear relationships between the experimental treatments and the species. Contrary to MANOVA, RDA is not limited to situations where the number of dependent variables is smaller than the number of replicates. RDA allows for an assessment of the amount of total variation in species abundances among replicates that can be explained by each treatment. Additionally, ordination diagrams based on RDA can be used to interpret the relationships between the species and the applied treatments. RDA was done with CANOCO for Windows (ter Braak and Šmilauer 1998). Abundances were  $\log(x+1)$  transformed to normalize each groups dataset. Factors were included into the model depending on a forward selection method ( $p < 0.05$ ), based on a Monte-Carlo permutation test.

In a three-way full factorial ANOVA the effects of the treatments and treatment-interactions on the single functional groups and on the C:N-ratio were analysed. For the ANOVA, data were log transformed (nauplii:  $\log(x+1)$  transformed).

### 4.3 Results

**Contaminations** The absence of contaminations by mixotrophs and microzooplankton was a major prerequisite for our experimental design, particularly for treatments without these organisms. Such contaminations were never observed during the experiment. However, small heterotrophic nanoflagellates (HNFs; 2 to 6  $\mu\text{m}$ ) of species other than *Cafeteria* appeared in week two in all containers, belonging mainly to *Choanoflagellidea* and *Kinetoplastidea*. Since they appeared everywhere, they were probably introduced with the inoculum of the copepods. HNFs were counted as one functional group, containing *Cafeteria* and other species. Additionally, picoeukaryotes were found from week two on in all containers. They were of similar size as *Synechococcus* and counted together as picophytoplankton.

**Community effects of mixotrophs, microzooplankton and enrichment** Overall effects of the applied treatments on all functional groups and on the C:N-ratio (below referred to as parameters) were investigated in a redundancy analysis (RDA, Table 4.3, Fig. 4.4). In a forward selection process, mixotrophy and omnivory gave significant results ( $p < 0.05$ ) and explained together 54 % of the total observed variance (sum of canonical eigenvalues, Table 4.3). In the ordination diagram (Fig. 4.4), the length of the parameters' axes indicate the degree of variation in each parameter explained by the analysis. The more a parameters' arrow is parallel to a factors' arrow, the more its variance is correlated with this factor (positively, if both arrows point to the same direction; negatively, if they point to opposite directions). Most species' arrows are more

Table 4.3: Results from the redundancy analysis (RDA).  $\lambda$ , eigenvalue of the concerning factors in this analysis. Factors were selected by a forward selection process ( $p < 0.05$ ), based on 1999 Monte-Carlo Permutations. The analysis included all 7 parameters displayed in Fig. 4.4.  $n = 16$  for each parameter.

| Variable   | $p$   | $F$ -ratio | $\lambda$ |
|------------|-------|------------|-----------|
| Omnivory   | 0.01  | 9.5        | 0.43      |
| Mixotrophy | 0.005 | 3.9        | 0.11      |
| Enrichment | 0.06  | 2.05       | -         |
| together   |       |            | 0.54      |

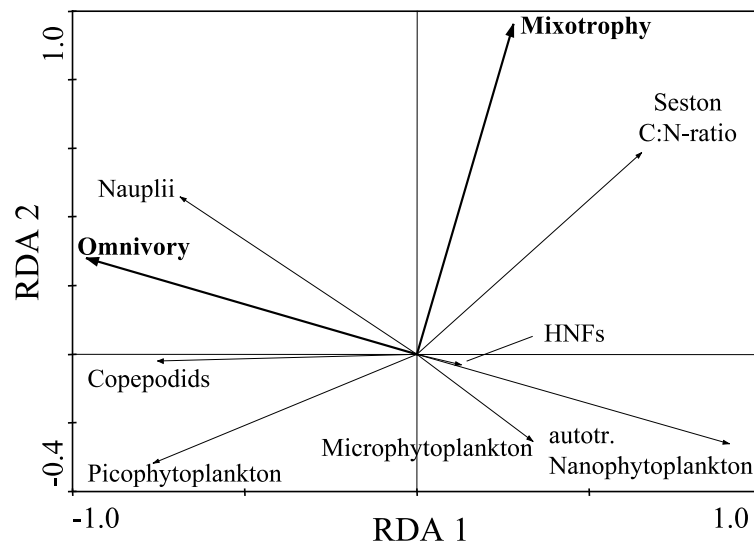


Figure 4.4: Redundancy analysis (RDA) of the species abundances (means of days 18 to 24) in relation to the treatments mixotrophy and omnivory. RDA 1 and 2, first and second canonical axes.

or less parallel with omnivory; only *Synechococcus* and the carbon to nitrogen (C:N) ratio were strongly affected by mixotrophy.

**Treatment effects on the single functional groups and on seston stoichiometry** In a three-way ANOVA the effects of omnivory, mixotrophy and enrichment and their interactions on the single functional groups and on the C:N ratio were investigated (Table 4.4, Fig. 4.5).

**Picophytoplankton** (*Synechococcus* sp. and picoeukaryotes) - Among all protist groups, the picophytoplankton turned out to be most sensitive to the applied treatments (Table 4.4). It was clearly reduced by the mixotroph *Chrysochromulina*. Since *Chrysochromulina* did not reduce the nano- and microphytoplankton, nutrient competition cannot explain this effect; therefore, *Chrysochromulina* obviously grazed effectively on the picophytoplankton. Especially in the un-enriched treatments, picophytoplankton profited from the presence of the microzooplankton, that obviously remineralized/remineralised nutrients of the ingested nanophytoplankton and reduced the mixotrophic *Chrysochromulina*.

**Autotrophic nanophytoplankton** (*Rhodomonas salina*) - The autotrophic nanophytoplankton experienced a strong negative effect from the microzooplankton. This effect was strongest in the high nutrient levels, where the abundances of *Rhodomonas* were 3 orders of magnitude lower in the presence of the microzooplankton than in its absence (Fig. 4.5). *Rhodomonas* was enhanced by enrichment, but only in the treatments without microzooplankton. This effect was not significant in the full factorial model (Table 4.4), but was significant when testing the effect of enrichment in the B and BM treatments alone (ANCOVA, factor enrichment and covariable mixotrophy;  $p < 0.01$ ,  $r^2 = 0.87$ ,  $p_{enrichment} < 0.01$ ,  $p_{mixotrophy} = 0.15$ ).

**Microphytoplankton** (*Thalassionema nitzschioides*) - From week two on, filaments of this diatom became attached to the container walls. Wall growth was removed at each sampling (after taking the samples) by a scraper, but on average, a considerable fraction of the diatom remained attached to the walls and was therefore unavailable for the zooplankton. The abundances given in Fig. 4.5 represent only the suspended algae, that are of major interest since they were available for the zooplankton. Similar to the HNFs, the within-treatment variation was higher than the among-treatment effects. This 'noise' was probably caused by uneven distribution of the diatoms in the containers. The share of the diatom on overall (suspended) phytoplankton biomass was low (Fig. 4.6), and its importance as prey for the copepods was probably low.

**Mixotrophic nanophytoplankton** (*Chrysochromulina polylepis*) - *Chrysochromulina* reached considerable abundances, but was close to detection limit near the end of the experiment (Fig. 4.7). Since this happened in all containers irrespective of the treatment, aging of the medium

Table 4.4: Results from a full-factorial three-way ANOVA testing the effects of mixotrophic flagellates (M), microzooplankton (O), and enrichment (E), as well as their interactions on the log-transformed abundances of the various groups and on the C:N ratio (means of days 18 to 24). Nauplii contained zero values and were log (x+1) transformed. n = 16, except for mixotrophic nanophytoplankton and microzooplankton (8). Prior to analysis data of each group has been tested on homogeneity of variances (Box-M test, n.s.)

| Functional group / parameter  | <i>p</i> | <i>r</i> <sup>2</sup> | E      | M      | O      | EM   | EO     | MO   | EMO  |
|-------------------------------|----------|-----------------------|--------|--------|--------|------|--------|------|------|
| Picophytoplankton             | < 0.01   | 0.89                  | 0.38   | < 0.01 | < 0.01 | 0.69 | 0.23   | 0.06 | 0.02 |
| Autotrophic nanophytoplankton | < 0.01   | 0.92                  | 0.52   | < 0.01 | < 0.01 | 0.69 | 0.12   | 0.50 | 0.56 |
| Microphytoplankton            | 0.054    | -                     | -      | -      | -      | -    | -      | -    | -    |
| Mixotrophic nanophytoplankton | 0.045    | 0.84                  | 0.62   |        | 0.2    |      | 0.06   |      |      |
| Heterotrophic nanoflagellates | 0.89     | -                     | -      | -      | -      | -    | -      | -    | -    |
| Microzooplankton              | 0.12     | -                     | -      | -      |        | -    |        |      |      |
| Copepodids                    | < 0.01   | 0.89                  | < 0.01 | 0.09   | < 0.01 | 0.81 | 0.33   | 0.66 | 0.74 |
| Nauplii                       | < 0.01   | 0.96                  | < 0.01 | < 0.01 | < 0.01 | 0.38 | < 0.01 | 0.08 | 0.81 |
| C:N ratio of the seston       | 0.01     | 0.84                  | 0.37   | < 0.01 | 0.72   | 0.48 | 0.40   | 0.31 | 0.39 |

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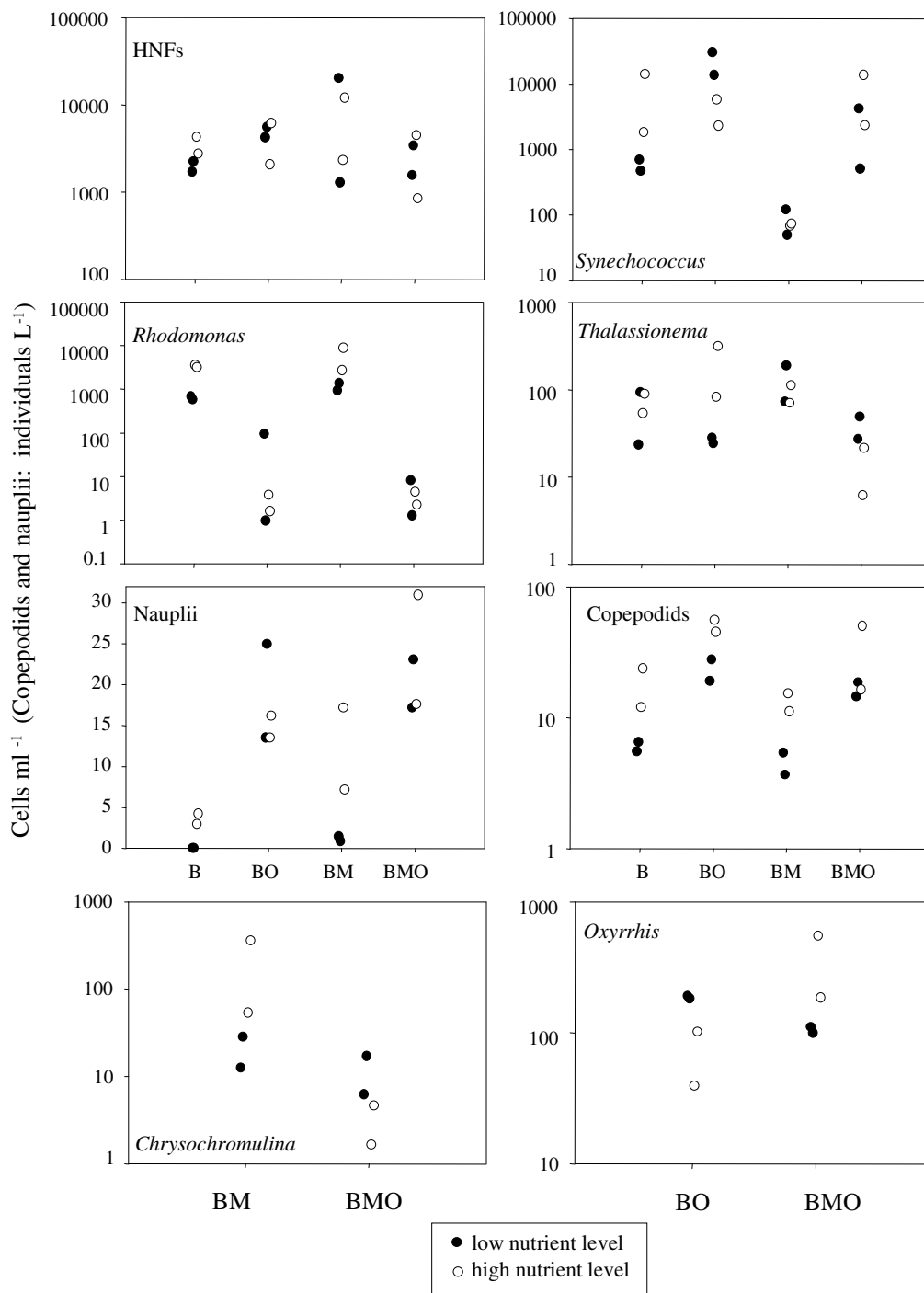


Figure 4.5: Abundances of all functional groups, means of days 18 to 24. Log-scale except nauplii (contained zero-values). Codes of food web configurations are explained in Table 4.1.



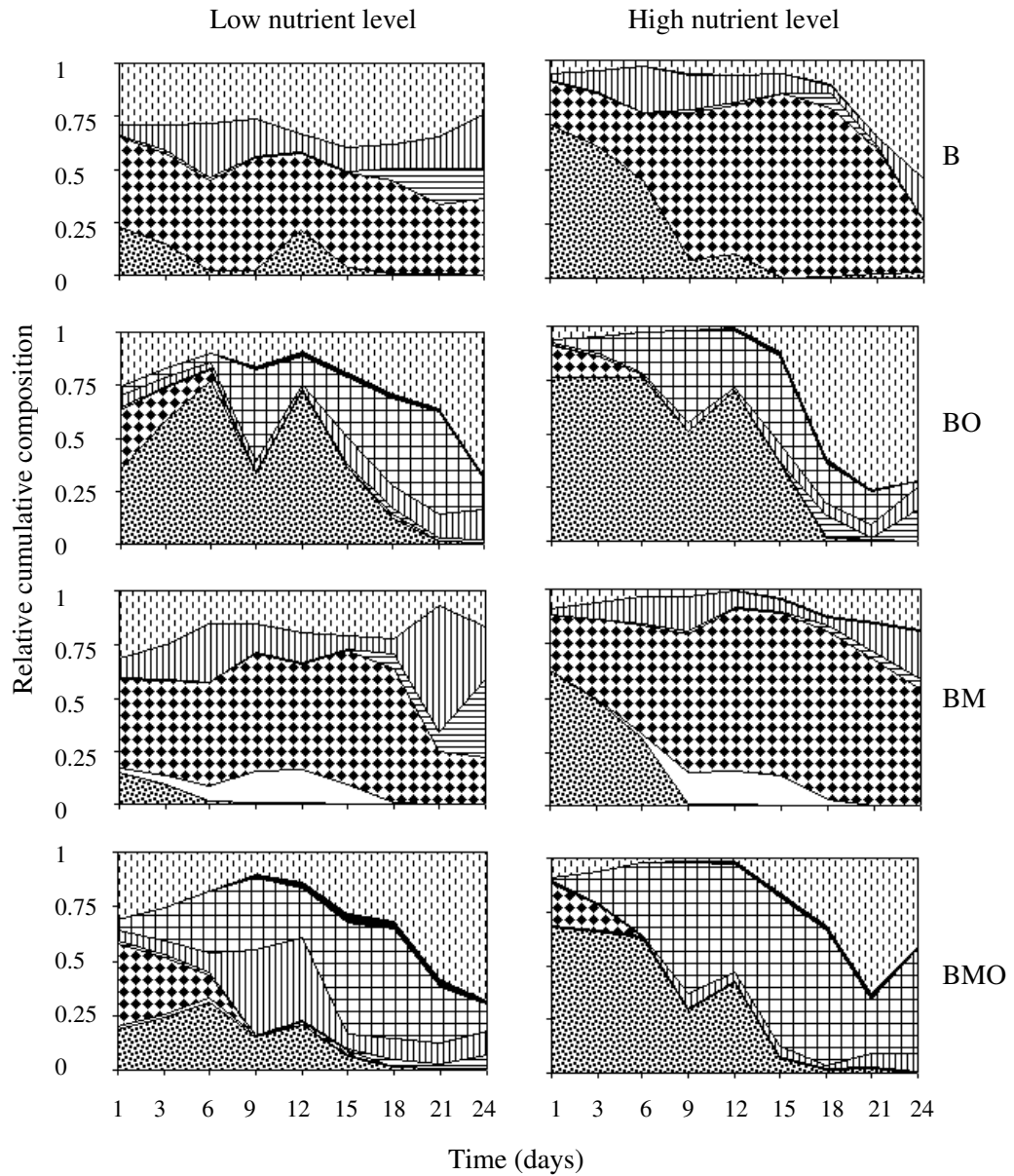


Figure 4.6: Relative share of all functional groups over time (means of the corresponding replicates). Codes of food web configurations are explained in Table 4.1.

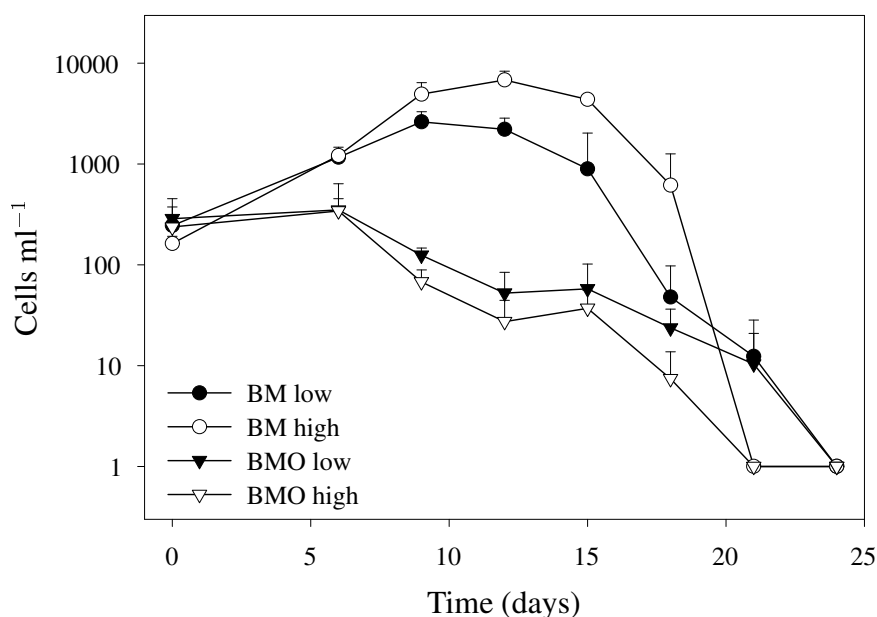


Figure 4.7: Time series of the abundances of the mixotrophic flagellate *Chrysochromulina polylepis* (log-scale). Means of both replicates and standard deviation (only one direction). Codes of food web configurations are explained in Table 4.1.

probably caused its disappearance. Similarly to the autotrophic nanophytoplankton, the mixotrophic nanophytoplankton was clearly reduced by the microzooplankton, especially in the high nutrient treatments. *Chrysochromulina* grazed very effectively on the picophytoplankton. Therefore, it seems obvious that it grazed also on similar-sized bacteria.

**Heterotrophic nanoflagellates** - This group represents all nano-sized heterotrophic flagellates, including *Cafeteria rosenbergensis*. Since these organisms varied in size, the abundances are only roughly correlated to the overall HNF biomass. This may partly explain the comparatively small among-treatment effects. Moreover, since in this group morphologically differing taxa were merged (see Contaminations), effects on functional diversity are obscured. No treatment had a significant effect on the heterotrophic nanoflagellates.

**Microzooplankton** (*Oxyrrhis marina*) - The heterotrophic dinoflagellate grazed mainly on the nanoflagellates *Rhodomonas* and *Chrysochromulina* (where present), as visible from the strong decline in these species in all corresponding treatments (Fig. 4.5, 4.6, 4.7). However, since *Oxyrrhis* persisted after it reduced the nanophytoplankton to very low abundances, other prey must have sustained its growth by then (Fig. 4.6). Though the optimal food size spectrum of *Oxyrrhis* is around 7  $\mu\text{m}$  equivalent spherical diameter (Hansen et al. 1996), it can feed also

on picoplankton (Schumann et al. 1994). Therefore, in the absence of nanoflagellates, *Oxyrrhis* probably grazed on HNFs, picophytoplankton and bacteria.

**Calanoid copepods** - The copepods reproduced in all treatments, but their reproductive success was highly variable among treatments (Fig. 4.8). Abundances of both nauplii and copepodids including adults were enhanced by the presence of the microzooplankton (treatments BO, BMO) and by enrichment (Fig. 4.5, 4.8; Table 4.4); additionally, the number of nauplii was significantly enhanced in the BM treatments compared to the controls. Differences between treatments with and without *Oxyrrhis* were most pronounced at the low nutrient level: the copepods (sum of nauplii and copepodids) decreased below  $5 \text{ L}^{-1}$  in the absence of *Oxyrrhis*, but were above  $20 \text{ L}^{-1}$  in the corresponding treatments with *Oxyrrhis* on day 24 (Fig. 4.8).

**Seston stoichiometry** - According to the low nitrogen to phosphorus ratio in the supplied medium, phosphorus was available in excess. The atomic C:N ratio of the seston was between 7 and 10 (Fig. 4.9), that is above the Redfield ratio and indicates that phytoplankton production was limited by nitrogen (Goldman et al. 1979). In all treatments containing the mixotroph *Chrysochromulina*, the C:N ratio was enhanced compared to the corresponding treatments without (Fig. 4.9, Table 4.4), indicating higher nutrient limitation in the presence of the mixotroph.

**Relative composition over time** The systems without microzooplankton were dominated by nanophytoplankton (*Rhodomonas*) for the longest time, and *Rhodomonas* still had a considerable share on overall biomass at the end of the experiment (Fig. 4.6). Conversely, in the systems with microzooplankton, *Rhodomonas* and the similar sized mixotroph *Chrysochromulina* vanished soon, and the picophytoplankton became the dominant primary producer. Whereas the share of copepods stayed at rather constant levels in most B and BM treatments (except B, high nutrient level), their share increased over time in the BO and BMO treatments. Towards the end of the experiment the share of copepods on overall biomass was considerably larger in the treatments containing microzooplankton. The change in relative composition in the BO and BMO treatments (from nanophytoplankton to picophytoplankton) indicates a shift in the diet of the microzooplankton, since *Oxyrrhis* did not vanish after the strong decline of the nanophytoplankton.

## 4.4 Discussion

### Reproduction of the copepods and interaction with the microzooplankton

There are two possible explanations for the observed positive effect of the microzooplankton (Figs. 4.6, 4.8). One being *Oxyrrhis marina* enhanced nutritional food quality for the cope-

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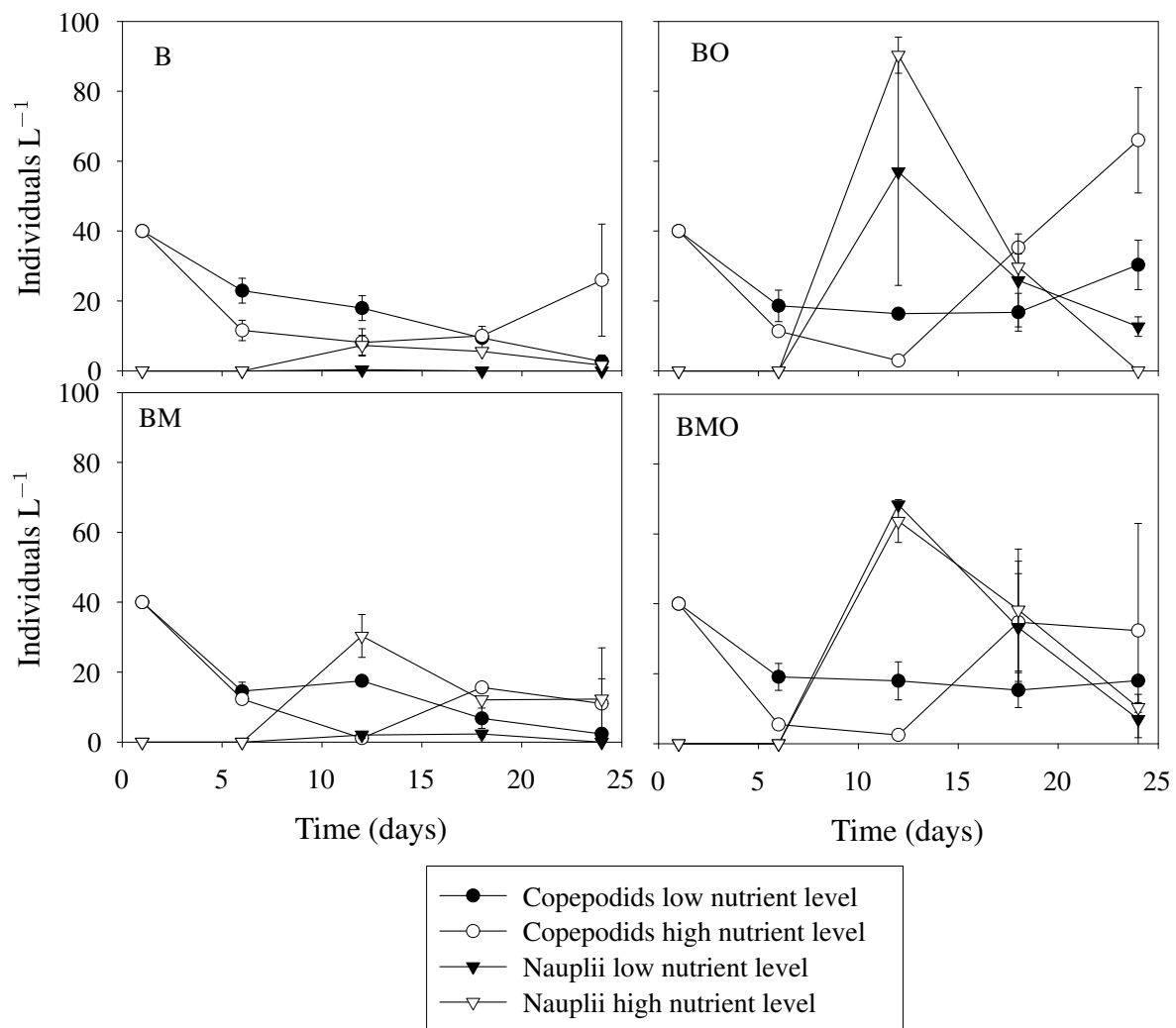


Figure 4.8: Time series of the abundances of nauplii and copepodids including adults. Means of both replicates and standard deviations are shown. Codes of food web configurations are explained in Table 4.1.

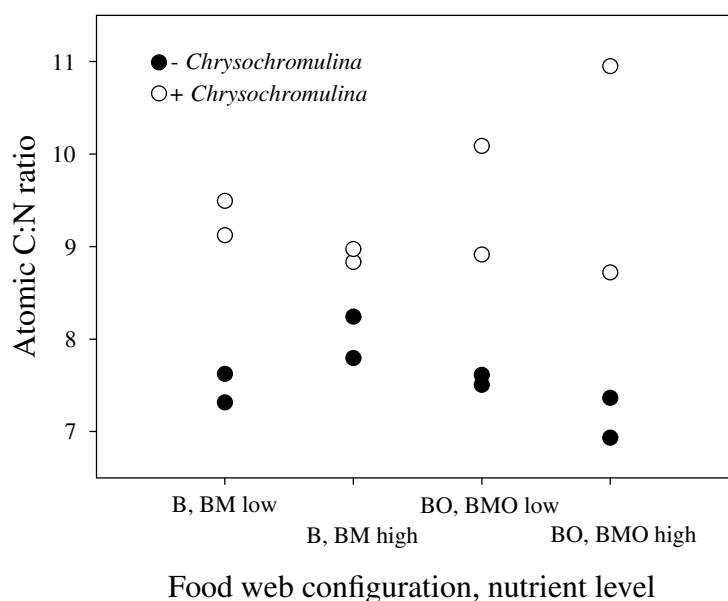


Figure 4.9: Atomic carbon to nitrogen ratio of the seston, means of days 18 and 24. Data of corresponding treatments with/without the mixotroph *Chrysochromulina* (e.g. B, BM) are depicted one upon another. low, low nutrient level; high, high nutrient level. Codes of food web configurations are explained in Table 4.1.

Pods. In several studies this heterotrophic dinoflagellate enhanced growth and/or reproduction in calanoid copepods when it was offered as an additional prey to a phytoplankton diet since it provided essential nutrients that were lacking in the phytoplankton diet (Kleppel et al. 1998, Klein Breteler et al. 1999, Chapter 2). However, in absence of the microzooplankton, *Rhodomonas salina* was the most abundant phytoplankton and likely the most important prey for the copepods. Several species of the genus *Rhodomonas* with a similar size (ESD 6-7  $\mu\text{m}$ ) are known to be a good prey for all life stages of small calanoid copepods (Støttrup et al. 1986, Berggreen et al. 1988, Klein Breteler et al. 1999). In addition, in studies by Klein Breteler et al (1990) and Koski et al. (1998), *Oxyrrhis marina* did not improve prey quality of *Rhodomonas* sp. for the calanoid copepods *Temora longicornis* and *Pseudocalanus elongatus*. It is therefore unlikely that the observed impact of the microzooplankton is an effect of chemical food quality. Alternatively, prey size could be the reason. Optimal prey size in copepodids and adults of small calanoid copepods ranges between 14 and 30  $\mu\text{m}$  ESD (equivalent spherical diameter; Hansen et al. 1994). With its ESD of 14  $\mu\text{m}$ , *Oxyrrhis* was the largest prey and closest to the optimal prey size of small calanoids. Therefore, the presence of the microzooplankton possibly resulted in a higher feeding efficiency of the copepodids (Hansen et al. 1994), and thus could explain the enhanced repro-

duction and development in the presence of the microzooplankton. This explanation is supported by the observation that *Oxyrrhis* had a stronger effect on the copepods at the low than at high nutrient level: if feeding efficiency was the reason for the observed improvements in copepod reproduction, both factors, enhancing prey concentration (enrichment) and enhancing prey size (presence of *Oxyrrhis*) should have comparable effects on copepod reproduction.

The microzooplankton *Oxyrrhis* preyed preferentially on the nanophytoplankton and therefore competed with the copepods, since the nanophytoplankton must have been the most important prey in the absence of the microzooplankton (Fig. 4.6). Nevertheless, the positive effects of the microzooplankton presence outweighed the reduction of the nanophytoplankton. The results show, that the possible energetic disadvantage of microzooplankton as an intermediate consumer for calanoid copepods is less important than its role as an important trophic link between phytoplankton and calanoid copepods.

**Effects of the mixotroph on food web structure and on seston stoichiometry** *Chrysochromulina* had a strong negative effect on the picophytoplankton, and most likely also on bacteria (see below). Enrichment enhanced this negative effect, but only in those treatments where *Chrysochromulina* was not controlled by the microzooplankton (BM). Despite the question concerning the role of the HNFs on abundances of the picophytoplankton (see below), the reduction of the picophytoplankton is consistent with the expectation that an omnivorous top consumer (i.e. here the mixotroph) reduces its intermediate consumer (picoplankton), and that such an effect is enhanced by enrichment (Thingstad et al. 1996, Diehl and Feissel 2000, Mylius et al. 2001).

HNFs were present in all treatments. Since they are usually regarded as the most important consumers of picoplankton (Azam et al 1983, Caron and Goldman 1990), an additional negative effect of *Chrysochromulina* on the picophytoplankton cannot be expected automatically. The reduced abundances of the picophytoplankton in the presence of the mixotroph indicates that *Chrysochromulina* can reduce the (common resource) picophytoplankton to lower concentrations than its competitors (HNFs). According to resource competition theory (Tilman 1990) *Chrysochromulina* therefore has got a lower  $R^*$  (minimum resource concentration for zero net growth) than the HNFs in respect to their shared resource picophytoplankton. Under nutrient limited and light sufficient conditions, nutrient gain from the prey biomass is the main benefit of phagotrophy for *Chrysochromulina* (Stibor and Sommer 2003). HNFs, however, have also to cover their energy demands by phagotrophy. Since the stoichiometry of bacteria and phytoplankton in respect to organic carbon and nutrients as phosphorus and nitrogen does not match with the stoichiometric demands of HNFs, they have to ingest more prey than necessary in respect

to their nutrient demands, and therefore excrete a considerable share of the ingested nutrients (Caron and Goldman 1990). Consequently, *Chrysochromulina* needs less picoplankton to cover its demands in nutrients than a HNF needs to cover its demands in energy, and that might in turn explain the lower  $R^*$  in *Chrysochromulina*. Resource competition theory predicts also competitive exclusion of the inferior competitor, but a reduction in abundances of the HNFs by the mixotroph has not been observed in this experiment. This deviation is probably caused by the missing taxonomic resolution of the group 'HNF', which included several species.

The observed shift in the C:N ratio of the seston can only be explained by bacterivory in *Chrysochromulina* (Fig. 4.2): the atomic C:N ratio of heterotrophic bacteria is generally lower (4 - 6) than the C:N ratio of phytoplankton, including cyanobacteria like *Synechococcus* (depending on nitrogen limitation between 6 and 20; Kohl and Nicklisch 1988, Biddanda and Benner 1997, Fukuda et al. 1998, Liu et al. 1999). Therefore, since *Chrysochromulina* converted bacterial biomass into phytoplankton biomass, the observed shift in the C:N ratio indicates a shift in the ratio of bacterial to phytoplankton biomass. Consequently, the mixotrophic *Chrysochromulina* enhanced primary production, since more biomass was built up per limiting nutrient unit.

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## Chapter 5

# The mixotrophic *Ochromonas minima* affects primary and secondary production in opposing ways

**Abstract** - In artificial microbial food webs with rotifers as top predators, the effects of a mixotrophic chrysophyte were investigated. The mixotroph had opposing effects on primary and secondary production: while seston biomass was enhanced by the mixotroph, biomass on the mesozooan trophic level was reduced due to low food quality of the mixotroph. In addition, the mixotroph had strong negative effects on picophytoplankton, but positive effects on nano- and microphytoplankton. The latter were caused by predatory release from the mesozooplankton. The results underline that mixotrophs may have strong shaping effects on various levels in microbial food webs.

### 5.1 Introduction

According to general theory, heterotrophic nanoflagellates and ciliates are the major consumers of picoplankton in planktonic food webs (Azam et al. 1983, Ducklow et al. 1986, Smetacek 2002). Generally they are believed to be ineffective links between picoplankton and higher trophic levels, since they respire a major share of the energy that they ingest with their prey (Fenchel 1981, Ducklow et al. 1986). Therefore, the energy transfer from the picoplankton to the mesozooplankton is generally believed to be low according to the intermediate trophic levels between small phytoplankton and the mesozooplankton (Ducklow et al. 1986, Sherr and Sherr

1988). However, there is increasing awareness that mixotrophic protists compose a considerable portion of planktonic communities and that they may be important consumers of bacteria and small phytoplankton in the marine plankton (Riemann et al. 1995, Havskum and Riemann 1996). Mixotrophy is here used in the restricted sense of combining photosynthesis and phagotrophy in a single organism (Sanders 1991, Jones 1994). By combining photosynthesis and phagotrophy, mixotrophs should represent a more effective trophic link between the microbial loop and the micro- and mesozooplankton than heterotrophic protists (Jones 1994, Riemann et al. 1995).

In nature-like experimental food webs with rotifers as top-predators, the impact of mixotrophy has been studied by manipulating the presence of the mixotrophic chrysophyte *Ochromonas minima* (Fig. 5.2). In addition to mixotrophy, productivity was manipulated to see whether the performance of the mixotroph depends on productivity, and if possible link-effects are stronger under lower productivity.

## 5.2 Materials and methods

This experiment has been performed in a very similar way as described in Chapter 4, so only a brief description of the methods is given here.

A scheme of the assembled food web is given in Fig. 5.2. Picophytoplankton was represented by a small chlorophyte, *Chlorella* sp. (diameter appr. 2 - 4  $\mu\text{m}$ ), that originates from the Indic Ocean (U. Sommer, pers. comm.). Autotrophic nanophytoplankton was represented by the cryptophyte *Rhodomonas salina*, that originally has been isolated from the North Sea (U. Sommer, pers. comm.). The diatom *Skeletonema costatum* (microphytoplankton) is a strain from the British Culture Collection of Algae and Protozoa, isolated from the North Sea (CCAP, strain-no. 1077/1-C). Heterotrophic nanoflagellates were represented in the food webs by the heterotrophic chrysophyte *Spumella* sp., that originates from the Baltic Sea (K. Jürgens, pers. comm.). It has been cultivated on a North Sea medium several weeks prior to the experiment. The rotifer *Brachionus plicatilis* originates from the Western Baltic and has been cultivated originally on a Baltic Sea medium. Four weeks prior to the experiment the rotifer has been acclimatized to the higher salinity.

Medium was prepared from surface water from the North Sea (33 PSU), enriched to final nutrient concentrations as shown in Table 5.1. Experimental containers and environmental conditions were identical to the preceding experiment (Chapter 4), except for that there was no automatic mixing (Fig. 5.2). Instead, containers were placed every second day in a clean bench and stirred manually by gentle upward and downward movements of a disc mounted on the bot-

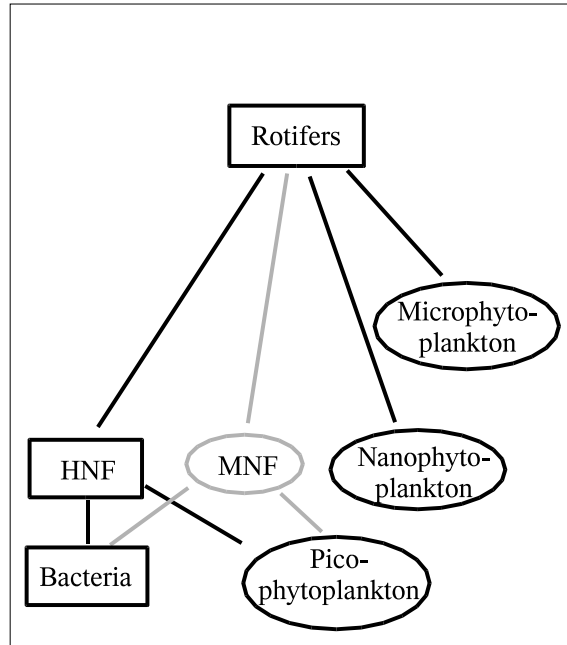


Figure 5.1: Sketch of the experimental food web. HNF, heterotrophic nanoflagellate; MNF, mixotrophic nanoflagellate.

Table 5.1: Initial nutrient concentrations.

|            | Nutrient level |                  |          |
|------------|----------------|------------------|----------|
|            | 1 - low        | 2 - intermediate | 3 - high |
| Nitrogen   | 15             | 45               | 150      |
| Phosphorus | 1              | 3                | 10       |
| Silicate   | 9              | 27               | 90       |

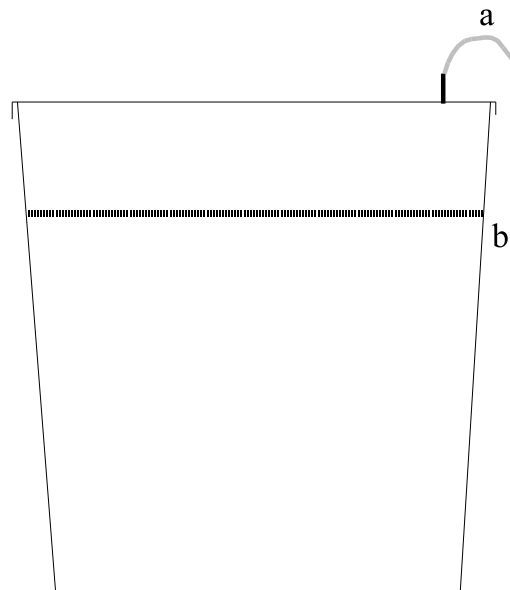


Figure 5.2: Experimental container. a - aeration tube; b - water level. Volume of the medium was 25 L.

tom end of a stick. The medium was mixed also prior to each sampling. All organisms were cultivated on the same medium as applied in the experiment.

The sterile containers were first filled with  $0.45 \mu\text{m}$  filtered sea water, and then inoculated with the protists (day 0). Six days later, the rotifers were added to the systems. The experiment lasted for 25 days.

Samples for microscopical and nutrient analysis were taken on every third day, starting on day 5. On day 13 and on every next but one sampling, 10 % of the overall medium was replaced by fresh medium. About 1.5 liters of the replaced volume were filtered over a  $30 \mu\text{m}$  mesh to retain the rotifers and preserved with Lugol's solution. The rest was prefiltered by a  $64 \mu\text{m}$  mesh and filtered on precombusted WHATMAN GF/F filters for analysis of particulate carbon and nitrogen of the seston fraction (the  $64 \mu\text{m}$  still retained virtually all rotifers, but permitted passing of *Skeletonema* filaments). Plankton (including rotifers), nutrient in water samples and filters were treated and analysed in the same way as described in Chapter 4.

### 5.3 Results

The analysis of the treatment effects is based on the last two sampling dates (Figs. 5.3, 5.4; day 19 and 25 for abundances of protists and rotifers, day 22 and 25 for carbon:nitrogen ratio of

Table 5.2: Results from a two-way ANOVA analysing treatment effects on average log-transformed (*Brachionus*: log-log) abundances of the last two sampling dates. Abundances of *Skeletonema* and the C:N ratio violated assumption of homogenous variances (Box-M test) and were analysed separately per nutrient level by one-way ANOVA (see text).

|                               | <i>p</i> - values |            |                    |
|-------------------------------|-------------------|------------|--------------------|
|                               | Mixotrophy        | Enrichment | Mixotr. x Enrichm. |
| <i>Chlorella</i> sp.          | < 0.01            | 0.52       | 0.88               |
| <i>Rhodomonas salina</i>      | 0.02              | < 0.01     | 0.01               |
| Heterotrophic nanoflagellates | 0.17              | < 0.01     | 0.21               |
| <i>Ochromonas minima</i>      | -                 | < 0.01     | -                  |
| <i>Brachionus plicatilis</i>  | < 0.01            | < 0.01     | < 0.01             |

the seston biomass). By doing so, transient effects during the phase of initial growth should be largely excluded from the analysis.

**Effects on the single food web compartments** Species abundances and chemical parameters were analysed by a full factorial two-way ANOVA (Table 5.2). In cases when test on homogeneity of variances revealed significant results (*Skeletonema*, C:N ratio), effects of mixotrophy were analysed separately within each enrichment level.

**Picophytoplankton** - While abundances of *Chlorella* sp. were not affected by enrichment, the picophytoplankton has been strongly reduced by the mixotroph.

**Nanophytoplankton** - *Rhodomonas salina* was clearly enhanced by enrichment. In addition, it reached higher concentrations in the presence of the mixotroph than in its absence.

**Microphytoplankton** - Abundances of the diatom *Skeletonema costatum* are probably biased by aggregation. From week two on, the diatom formed large aggregates that sedimented on the bottom of the experimental containers. The abundances in Fig. 5.3 refer only to the suspended fraction of *Skeletonema* and therefore do not correspond to overall biomass of the diatom. Since the data of *Skeletonema* significantly violated the assumption of homogenous variances (Table 5.2), mixotrophy effects were analysed separately for each nutrient level by a one-way ANOVA. In the intermediate and high nutrient levels, abundances of *Skeletonema* were significantly higher in the mixotrophy treatments ( $p = 0.013$  and  $0.026$ ; Cochran's test on homogeneity of variances n.s.), whereas no significant effect was evident in the intermediate nutrient level.

**Heterotrophic nanoflagellates** - Similar to Chapter 4, several species of heterotrophic nanoflagellates appeared in all treatments, making 'heterotrophic nanoflagellates' a diverse group, contrary to all other functional groups, that were represented by a single species in the food webs. Despite the high variability in their abundances, heterotrophic nanoflagellates showed a

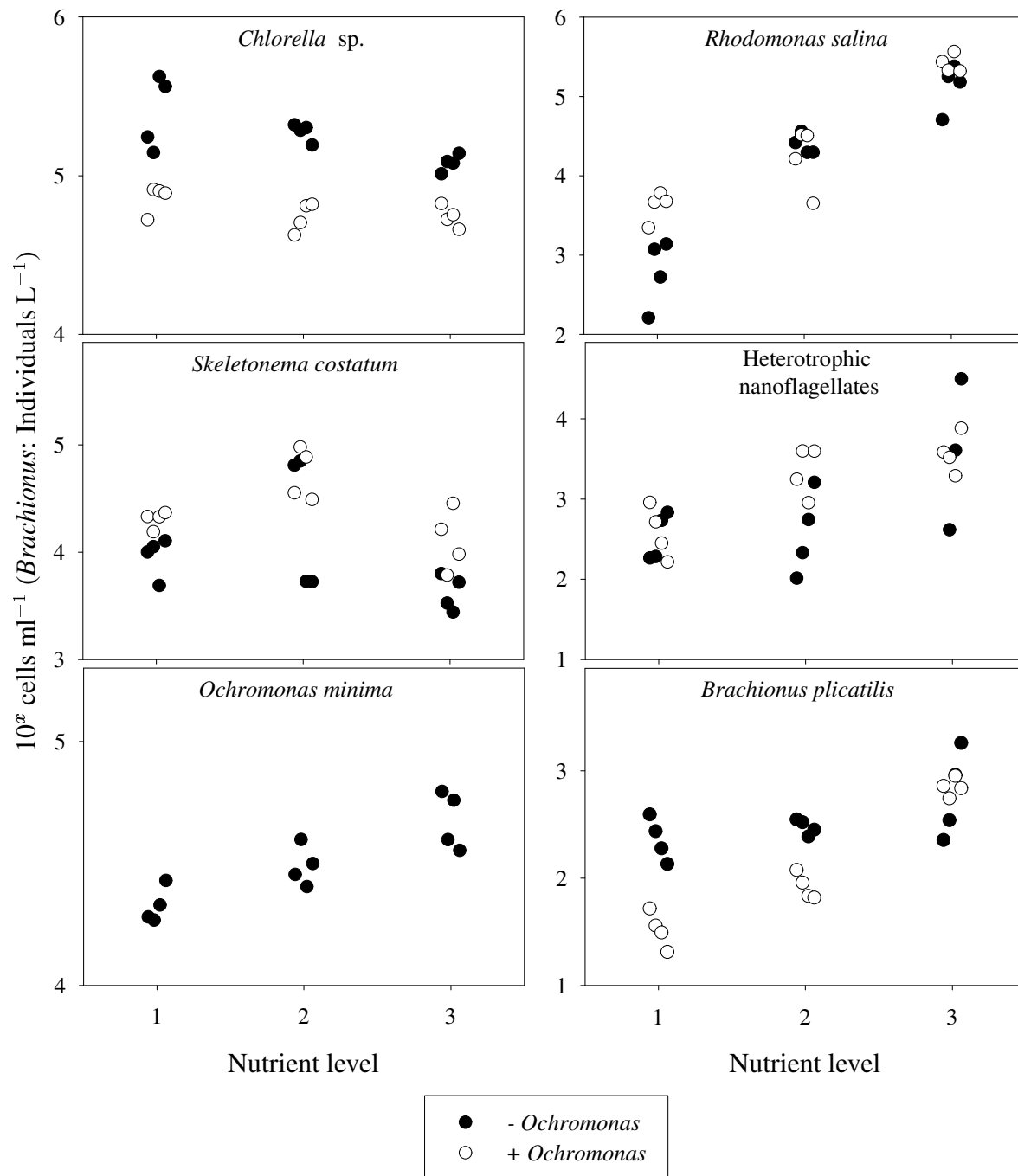


Figure 5.3: Abundances of the single food web compartments, averages of the last two samples (day 19 and 25). Nutrient levels as given in Table 5.1.

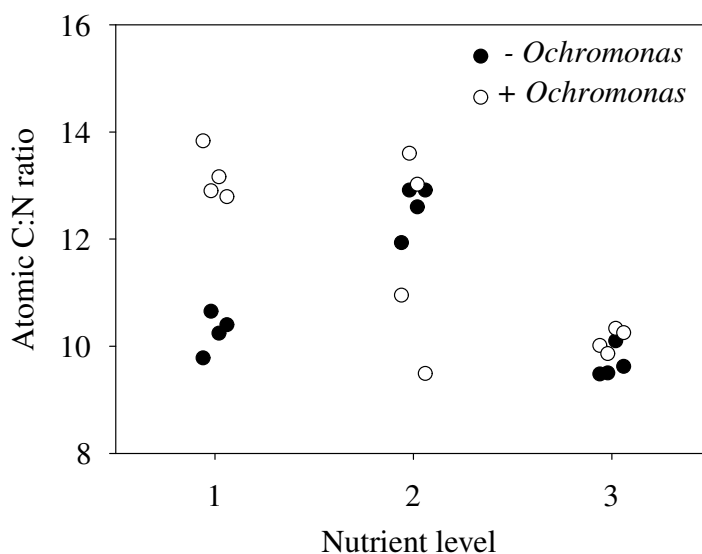


Figure 5.4: Atomic carbon:nitrogen ratio of the seston biomass, averages of the last two samples (day 22 and 25).

significant positive relationship to enrichment, but no relationship to mixotrophy (Fig. 5.4, Table 5.2).

**Mixotrophic nanoflagellate** - *Ochromonas minima* increased linearly with increasing nutrient enrichment (Fig. 5.4, Table 5.2).

**Mesozooplankton** - The rotifer *Brachionus plicatilis* was clearly enhanced by nutrient enrichment, but negatively affected by the presence of the mixotroph (Table 5.2). In feeding experiments with *Brachionus* and various algae (unpublished data), *Brachionus* exhibited negative net growth on a monospecific diet of *Ochromonas minima*. Therefore, the reduced abundances of *Brachionus* in the presence of *Ochromonas* result from nutritional inadequate food quality of the mixotroph.

**Effects on seston stoichiometry** The nutrient concentration of the medium was close to Redfield ratio (16:1). However, already few days after the start of the experiment, N:P ratio of soluble nutrients fell below this ratio and stayed at low levels. In the final time interval the N:P ratio ranged from 0.5 to 5, indicating nitrogen limitation of the phytoplankton. Therefore the carbon to nitrogen ratio of the seston biomass (exclusive rotifers) may be used as a measure for nutrient limitation (Fig. 5.4). The C:N-ratio generally decreased with increasing nutrient enrichment, ranging from about 13 (low nutrient level, mixotrophy treatment) to about 10 (all high nutrient levels). Mixotrophy tended to enhance the C:N ratio, but there was a high variance

among replicates. In independent two-tailed t-tests, mixotrophy had a highly significant effect in the unenriched treatments ( $p < 0.01$ ; assumption of the homogeneity of variances was not violated in the unenriched treatments), and no effect in both enriched treatments ( $p = 0.45$  and  $p = 0.52$ , respectively).

## 5.4 Discussion

Results of this experiment confirm the effects of mixotrophs on microbial food webs, that were found in Chapter 3 and 4. Despite a variable community of heterotrophic nanoflagellates, the presence of a mixotrophic nanoflagellate caused a steep decline in the abundances of the picophytoplankton, and very likely also a reduction of bacteria. The reduced abundances of the picoplankton indicate a lower minimum resource concentration for zero net growth with respect to picoplankton, compared to the pure heterotrophic nanoflagellates that were present in the systems. In addition, the C:N ratio of the seston biomass was enhanced by the mixotrophs, indicating enhanced nutrient limitation and a higher efficiency of primary production (synthesized biomass per limiting nutrient unit). However, in contrast to Chapter 3 where the mixotrophs caused a decline in the autotrophic nanoplankton, here the autotrophic nano- and microplankton were enhanced by the mixotroph. While reduced nutrient remineralization was the reason for the negative effect in Chapter 3, here the mixotrophs caused an indirect release from predation by the rotifer, since the rotifer developed worse in the presence of the mixotroph (see below).

A marked difference to the previous experiment consists on the mesozooplankton level: while *Chrysochromulina* tended to enhance the copepods, *Ochromonas* had clearly negative effects on the rotifer *Brachionus plicatilis*. The taxonomic, morphological and functional variability among mixotrophic flagellates is almost as big as the variability among the whole phytoplankton community (there exist mixotrophic flagellates in almost every taxonomic group, excluding only diatoms and cyanobacteria). Therefore, general species-independent effects (positive/negative) of mixotrophs on higher trophic levels are not likely to be found. In addition, a variety of marine mixotrophic flagellates can be toxic (Riemann et al. 1995, Graneli et al. 1999). Hence, as evident from the comparison of this and the previous experiment, food quality must be considered as an important parameter when assessing the effects of mixotrophs on higher trophic levels.

Effects of *Ochromonas* on seston stoichiometry (Fig. 5.4) and mesozooplankton (Fig. 5.3) decreased with increasing enrichment, indicating that the relative importance of the mixotroph decreased with enrichment. This could be related to decreased nutrient limitation with increasing enrichment, that should reduce the competitive abilities of the mixotroph (Chapter 3).



## Chapter 6

# Continuous breeding of calanoid copepods in a system based on autochthonous primary production

**Abstract** - A method is described for the continuous cultivation of marine calanoid copepods on a small scale to be used for laboratory experiments. Autochthonous food production in the cultivation containers is initiated regularly by a protist culture consisting of *Rhodomonas* sp. and *Oxyrrhis marina*.

### 6.1 Introduction

Calanoid copepods are important organisms in marine plankton and often the focus of laboratory experiments. Most researchers depend on field catches, either adults or diapause eggs (e.g. Ban et al. 2000, Bonnet and Carlotti 2001; see also Chapter 4). Permanent cultivation of calanoid copepods under constant conditions comparable to protist cultures for laboratory studies is desirable, but hardly done, though generation times of calanoid copepods can be short provided the proper food is supplied (approx. 20 days for various species; Landry 1983, Gillooly 2000). It is usually difficult to find a suitable diet, since most calanoid copepods rarely grow on phytoplankton monocultures, contrary to e.g. *Daphnia* (Koski et al. 1998, Klein Breteler et al. 1999; but see Støttrup et al. 1986 for cultivation of *Acartia tonsa* on *Rhodomonas baltica*). In contrast, there is clear evidence that calanoid copepods are omnivorous organisms. A mixture of phytoflagellates and microzooplankton provides a good basis for the growth and reproductive success of most species (Kleppel 1993, Klein Breteler et al. 1999, Bonnet and Carlotti 2001).

As a consequence, a continuous flow system for the cultivation of copepods must consist of three stages, phytoplankton, phytoplankton plus microzooplankton, and copepods. Additionally, when growing copepods in light to allow autochthonous algal production in the cultivation containers, one must cope with contamination, wall growth and sedimentation of the food.

Klein Breteler (1980), Klein Breteler et al (1990) and Støttrup et al. (1986) describe methods to grow calanoid copepods for many generations. They show effective ways to cultivate calanoid copepods on a large scale. Phytoplankton has to be supplied continuously to the copepods, since they are kept in the dark. These methods work well, but are rarely applied, probably because most researchers consider the required effort prohibitive.

Here a simple method is described to grow calanoid copepods with low technical expenditure and a minimal requirement on space. Contrary to the methods mentioned above, autochthonous algal production within the copepod stage is utilised as the basic food source. Using this method, it is possible to grow copepods throughout the year making them available for experiments during all seasons. The described method has originally been designed for a food web experiment (Chapter 4), and the conditions for cultivation in the first 30 days (see Chapter 4, Materials and methods) differed in some way from the subsequent time (this Chapter).

## 6.2 Materials and methods

The copepod culture was initiated by field catches from the Kiel Bight, consisting mainly from the genera *Acartia*, *Centropages*, *Pseudocalanus* (all Calanoida) and *Oithona* (Cyclopoida). These catches also contained a large number of rotifers (*Synchaeta* sp.). Copepods were kept in temporary containers without additional food for two weeks prior to adding them to cultivation containers. In this time rotifers disappeared completely due to predation by the starving copepods (Stoecker and Egloff 1987). Immediately before transferring the copepods to the cultivation containers, they were washed over a 100  $\mu\text{m}$  mesh with filtered water (0.45  $\mu\text{m}$ ) to reduce contaminants.

The copepods were kept in circular 25 liter containers covered by a transparent lid that reduced contamination (Fig. 6.1). They were placed under a light bench in a walk-in environmental chamber (16 °C; 16L - 8D cycle). Atmospheric air was pumped into the airspace between the lid and water surface. The medium was mixed by a kind of Archimedes' screw: a small electric motor was mounted on the lid and connected to a glass baton through a small hole in the lid. The baton carried a PVC screw on its bottom end (diameter 10 cm). A PVC cylinder with a slightly larger diameter than the screw was placed on the bottom of the container, enclosing the whole

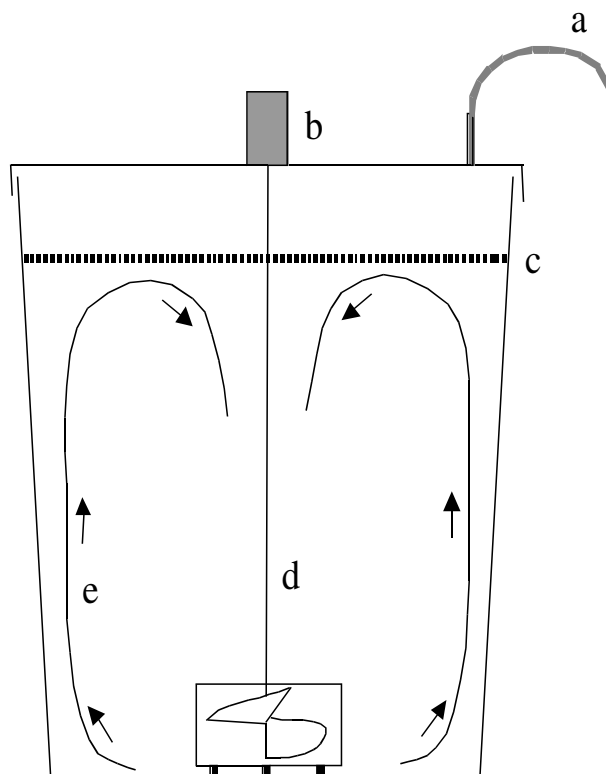


Figure 6.1: Container for permanent cultivation of calanoid copepods. Volume of the medium appr. 25 L. a - aeration tube; b - engine; c - water level; d - glass stick with screw; e - induced current.

## CHAPTER 6. CONTINUOUS BREEDING OF CALANOID COPEPODS IN A SYSTEM BASED ON AUTOCHTHONOUS PRIMARY PRODUCTION

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thread of the screw. The cylinder stood on three knobs, leaving approximately 1 cm between the bottom end of the cylinder and the base of the container. The motor was adjusted to approximately one turn per second, and the rolling direction of the screw was such a transferring way that the water moved downwards and through the slit between cylinder and base. This resulted in a current just above the base of the container, impeding sedimentation of the phytoplankton. Aside from this effect, mixing improved gas exchange of the medium and evenly distributed the food. Filtered water from the Kiel Fjord ( $0.45 \mu\text{m}$ ) was used as medium. In summer the water was enriched to enhance phytoplankton production (see below), in winter no enrichment was necessary because of the naturally high nutrient concentrations in the water.

The copepods were fed with a mixture of the autotrophic flagellate *Rhodomonas salina* (*Cryptophyta*, length 10-15  $\mu\text{m}$ ) and the heterotrophic dinoflagellate *Oxyrrhis marina* (length 20-25  $\mu\text{m}$ ). The same containers used for the copepods were used for the cultivation of the food: a container was cleaned with alcohol to reduce the number of possible contaminants. After filling it with filtered water ( $0.45 \mu\text{m}$ ) nutrients were added to yield a final concentration of about 200  $\mu\text{mol}$  nitrate and 15  $\mu\text{mol}$  phosphorus. No micronutrients were added. After fertilisation, the medium was inoculated with *Rhodomonas* sp. One week later *Oxyrrhis marina* was added to the food container. After another week a community of *Rhodomonas* and *Oxyrrhis* emerged and was ready to be fed to the copepods.

Between 1 and 2 liters of the *Rhodomonas-Oxyrrhis* suspension was added every week, depending on the density of the copepods, and each time when the water was exchanged. Turbidity of the medium gave a rough estimate of the availability of food. However, from time to time availability of food was microscopically determined because turbidity may be caused solely by contaminating picophytoplankton that cannot be grazed by the copepods. When copepod abundances were low or when just maintaining the copepod cultures, no additional food was necessary since the autochthonous production sustained growth of the copepods (see below).

The whole water volume was exchanged every two weeks. The old medium was filtered by a 64  $\mu\text{m}$  mesh to retain copepods of all developmental stages including eggs. They were transferred into a clean container filled with fresh medium. Exchange of the containers was important to remove detritus and periphyton which favours growth of metazoan contaminants such as harpacticoid copepods. Direct negative effects by the harpacticoid copepods on the calanoid copepods have not been observed; however, if they become abundant, separation of the calanoid copepods gets too time consuming.

The medium in the copepod containers always contained other flagellates and some ciliates. However, those contaminants did not affect the growth and reproduction of the copepods. A big

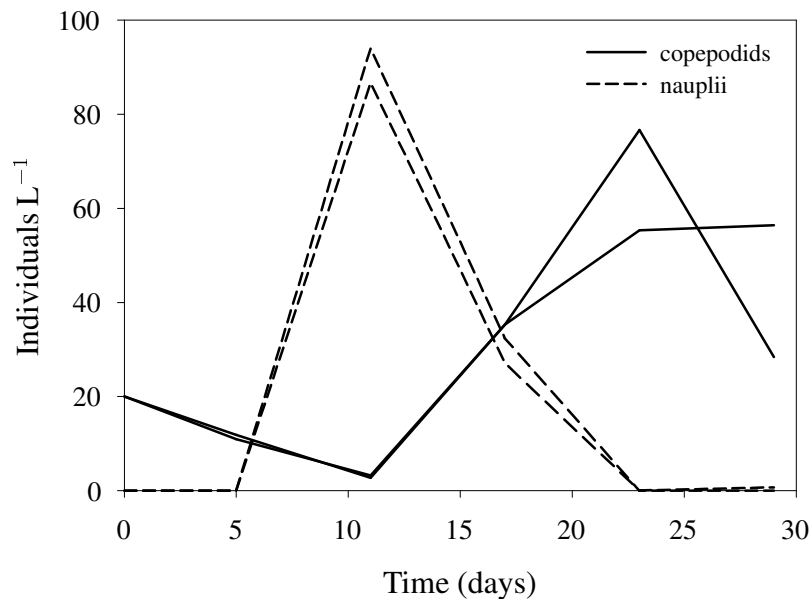


Figure 6.2: Abundances of the mixed copepod community in two cultivation containers in the first 30 days after inoculation.

advantage in using *Oxyrrhis marina* as 'trophic link' was that it can grow on a diet of various nanoflagellates and even on picoplankton (Hansen et al. 1996, Schumann et al. 1994; Chapter 4).

### 6.3 Results and discussion

With the described method copepods have successfully been grown for 2 years. In the first generations that hatched in the experimental containers, adults of *Acartia*, *Centropages* and *Pseudocalanus* were observed. The development of the first 29 days in two containers is given in Fig. 6.2 (corresponds to the BO treatments, high nutrient level, in Chapter 4). Nauplii were first observed five days after addition of the copepods and had highest abundances at approximately day 11. Embryonic development time of small calanoid copepods is short (between 16 and 30 hours at 15 °C; Landry 1983). Thus, the copepods recovered from starvation before egg production began (see above). The second generation of copepods peaked between day 23 and 29 (sum of adults and all copepodids). In the subsequent time *Acartia tonsa* excluded the other species, becoming the only copepod in the cultivation containers. Distinct generations disappeared, and a continuum of different life stages remained in the containers (Table 6.1).

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Table 6.1: Abundances of copepods in 5 cultivation containers (cont.), individuals per liter (sum of all stages; copepodids include adults).

|         | <i>Acartia tonsa</i> |            | <i>Harpacticoida</i> |            |
|---------|----------------------|------------|----------------------|------------|
|         | Nauplii              | Copepodids | Nauplii              | Copepodids |
| Cont. 1 | 36                   | 12.5       | 5                    | 4.5        |
| Cont. 2 | 60                   | 23.5       | 16.5                 | 40.5       |
| Cont. 3 | 74.5                 | 8.5        | 0                    | 2.5        |
| Cont. 4 | 11                   | 63.5       | 7                    | 12.5       |
| Cont. 5 | 36                   | 85         | 0                    | 0          |

For pure maintenance of the copepod cultures no additional food was necessary. *Acartia tonsa* survived in the containers over more than 6 weeks (at low abundances) by autochthonous production only. Abundances of copepods in 5 cultivation containers after 4 weeks without exchange of water and containers are shown in Table 6.1. Food was supplied only twice during this interval. In all containers nauplii of *Acartia tonsa* were present, indicating nutrition sufficient for reproduction of the adults. At this time (approximately 6 months after initiation of the culture), most containers were contaminated with harpacticoid copepods. As mentioned above, they are favoured when medium and containers are not exchanged regularly.

Interactions between copepods, microzooplankton and phytoplankton resulted in fluctuating abundances of protists and copepods. The densities of the copepods (all stages) were usually between 40 and 80 individuals L<sup>-1</sup> (at maximum 120 ind. L<sup>-1</sup> were observed). Although it might sometimes be desirable to produce denser copepod suspensions using more dense food cultures, this may result in more pronounced predator-prey cycles (Rosenzweig 1971, Abrams and Roth 1994; own data, unpublished). Total nutrient concentrations of about 40:3 μmol L<sup>-1</sup> nitrogen:phosphorus in the copepod cultivation containers works well (see also Chapter 4).

The method may also work well with other taxa such as *Centropages* and *Pseudocalanus*, since they too were present in the first generations.

# Chapter 7

## Discussion

### 7.1 General effects of omnivory

According to pure energetical considerations, the addition of a heterotrophic intermediate consumer should reduce productivity of a top consumer in a food web. However, in the studies presented here (Chapters 2, 4), intermediate consumers facilitated the productivity of the top consumers. In spite of reduction of the basal resource (esp. Chapter 4), enhancement of food quality (size and nutrient composition) outweighed thermodynamic constraints. In Chapter 4, *Oxyrrhis* had an additional effect in providing a link for the copepods to picoplankton and HNFs, which otherwise were too small for direct ingestion by copepods. At first glance the results seem similar to previous studies, where phytoplankton diets were enriched with or replaced by microzooplankton that was grown on the same phytoplankton prey as offered to the copepods (Chapter 2, Table 2.1). These studies confirm that microzooplankton, such as the heterotrophic dinoflagellates *Oxyrrhis* and *Gyrodinium*, may enhance the nutritional quality of phytoplankton for calanoid copepods by providing essential nutrients that are lacking in several phytoplankton taxa ('trophic upgrading', Klein Breteler et al. 1999). However, energetical constraints that arise from the presence of an intermediate consumer are not taken into considerations by the studies given in Table 2.1. In contrast, the results from Chapters 2 and 4 show, that enhancement by microzooplankton may work also in closed systems, where the presence of an intermediate consumer inevitably will reduce the availability of the common basal resource.

Overall productivity is a function of individual growth and abundances. In Chapter 4, grazing by the intermediate consumer *Oxyrrhis* reduced abundances of the nanophytoplankton *Rhodomonas*, but it is very likely that grazing caused nutrient release and thereby enhanced cell specific growth in *Rhodomonas*: due to differences in metabolic rates and excretion, nutrient-

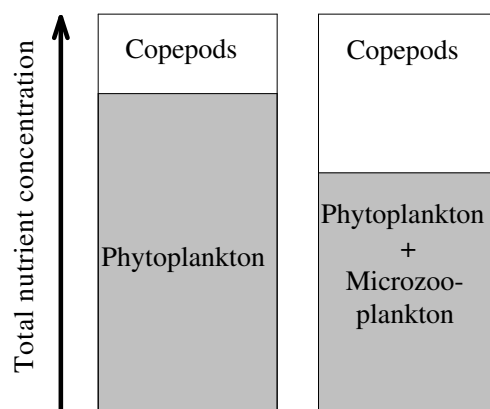


Figure 7.1: Effect of microzooplankton on ecological efficiency in the experimental food webs

rem mineralisation rates of phagotrophic protists are considerably higher than of mesozooplankton (Ikeda et al. 1982, Dolan 1997). Hence, grazing effects by the microzooplankton were probably partly compensated by stimulated phytoplankton growth. In addition, if phytoplankton growth is enhanced by nutrient regeneration, faster growth is accompanied by a higher cell quota of the limiting nutrient which leads to an increased nutritional value for the metazoan grazers. The effect may be direct, if zooplankton growth is limited by mineral nutrients (Sterner and Elser 2000), or indirect, if essential organic substances (e.g. polyunsaturated fatty acids) reach higher concentrations in faster growing algae (Kleppel et al. 1998, Klein Breteler et al. 1999). In spite of providing essential nutrients by itself, the microzooplankton in Chapters 2 and 4 may therefore have had additional positive effects on nutritional quality of the phytoplankton.

The effects of the microzooplankton on abundances of phytoplankton and copepods are sketched in Fig. 7.1. At identical total nutrient concentrations, the prey:predator ratio was shifted towards the predators in presence of the microzooplankton. Though microzooplankton shifted the trophic position of the copepods, the ecological efficiency (production ratio between adjacent trophic levels) was enhanced by the intermediate consumers. This result has two important implications for length of food chains in planktonic food webs: (1) by providing suitable prey for calanoid copepods and by selective feeding of copepods, microzooplankton increases the trophic position of the copepods. (2) due to 'trophic upgrading' (Klein Breteler et al. 1999), microzoo-



plankton may enhance ecological efficiency to such a degree that outweighs the energy losses normally associated with elongation of food chains. Thus, productivity of the higher trophic levels can even be increased by food chain elongation.

Recent theoretic investigations predict that an omnivorous predator is likely to exclude its intermediate consumer under sufficient productivity. Intermediate consumers were represented by picophytoplankton (relative to the mixotrophs; Chapters 3, 4 and 5) and by microzooplankton (Chapters 2 and 4). Picophytoplankton has been reduced, but never excluded by the mixotrophs, even under extremely high bacterial productivity (Chapter 3). The microzooplankton in Chapter 4 was not eliminated by the copepods. Only in Chapter 2 the microzooplankton was excluded by the copepods within few days. However, in this short-term experiment, zooplankton abundances and therefore grazing pressure were far above natural concentrations. In addition, the small dimensions of the experimental containers in combination with intensive mixing prevented from any spatial separation of copepods and dinoflagellates. Nevertheless, the latter results show that selective feeding by copepods may cause severe grazing pressure on a single group within a mixed protist community, and hence that copepods potentially may reduce microzooplankton. This is in accordance with field experiments, where microzooplankton abundances were reduced in presence of calanoid copepods (Sommer et al. 2001, 2003).

## 7.2 Specific effects of mixotrophy

Mixotrophs may be considered omnivorous (Thingstad et al. 1996), because they feed on abiotic resources and organisms at the base of the food web, instead of feeding on two trophic levels. If the abiotic resources are treated like a trophic level, bacteria and algae might be considered as analogous to intermediate consumers, because they compete with mixotrophs for inorganic nutrients (and light) and are potential prey of the mixotrophs at the same time. According to Stickney et al. (2000), mixotrophy should reduce primary production, since mixotrophs reduce abundances of pure autotrophs. This was true for the treatments without glucose enrichment in Chapter 3. However, in the enriched treatments mixotrophy enhanced production of seston biomass, indicating enhanced primary production because of redistribution of nutrients from bacteria to photosynthetic production. Similar results were obtained from the experiments containing top predators (Chapters 4, 5). Zooplankton grazing is a major source for DOC in aquatic food webs (Ikeda et al. 1982) and favours bacterial productivity. According to the conflicting results in experiments with and without glucose enrichment (Chapter 3), in the complex food webs (Chapters 4 and 5) mixotrophs were more important as bacterivores than as algivores.

An important conclusion from the performed experiments is that mixotrophic flagellates may persist in complex food webs under nutrient limitation and steady-state like conditions, competing successfully with pure heterotrophic and pure autotrophic protists at the same time. This contrasts considerably with the common view that mixotrophs should be inferior competitors compared to specialised auto- and heterotrophs (Thingstad et al. 1996). Maximum growth rates of mixotrophs seem to be considerably lower than of auto- and heterotrophic flagellates (Chapter 3, Rothhaupt 1996 b). However, in situations when resources become limiting, mixotrophs obviously may maintain higher growth rates than their specialised competitors (Chapter 3, Rothhaupt 1996 b).

Consequently, such situations should favour mixotrophic flagellates in natural systems. Especially the low productive areas like the subtropical Atlantic Ocean are characterized by nutrient limitation (Mann and Lazier 1996). In addition, severe nutrient limitation may also occur seasonally in more productive areas as a consequence of prolonged stratification in combination with high primary production (Mann and Lazier 1996). Arenovski et al. (1995) and Sanders et al. (2000) give examples for the importance of mixotrophs in the oligotrophic Sargasso Sea. Similarly, Pitta et al. (2000) found that mixotrophic ciliates were a major constituent of the protistan plankton community in the ultraoligotrophic eastern part of the Mediterranean Sea. An example for the importance of mixotrophs in stratified eutrophic waters is given by Havskum and Riemann (1996). They found that mixotrophs were both the major primary producers and the major phagotrophic consumers in the surface waters in the Bay of Aarhus (Baltic Sea). Nutrient limitation may also be a consequence of high DOC levels that promote bacterial production. Due to the results from glucose enrichment (Chapter 2), high bacterial productivity should favour mixotrophic protists. Indeed, mixotrophic flagellates were found to be the dominating bacterivores in humic lakes (Isaksson et al. 1999, Blomqvist et al. 2001).

These results diverge from the traditional view, that has acknowledged the existence of mixotrophy but not considered it important enough to deserve detailed study. If mixotrophs can successfully compete with pure auto- and heterotrophs, it is rather surprising that, compared to the bulk of plankton studies, there are only few studies reporting considerable share of mixotrophs in plankton communities (see above). This discrepancy is probably caused by methodological difficulties in the identification of mixotrophic flagellates in the field. In most plankton analyses, mixotrophs are not distinguished from autotrophs. In order to identify mixotrophs in a natural plankton community, a sample must be incubated in presence of fluorescent tracers (fluorescent labeled bacteria (FLBs) or algae (FLAs)), and the samples have to be analysed by fluorescent microscopy (e.g. Havskum and Riemann 1996, Sanders et al. 2000) or flow

cytometry (T. Hansen, pers. comm.). Mixotrophs are identified as pigmented protists with ingested fluorescent tracers. Still, it is unlikely that all mixotrophs in a sample will ingest tracers in the experimental period (Boraas et al. 1992), especially if experimental conditions favour pure autotrophic growth (e.g. lack of nutrient limitation; Nygaard and Tobiesen 1993, Stibor and Sommer 2003). Hence, by conventional methods, only a minimum estimate can be obtained for the share of mixotrophs on a natural plankton community.



# Chapter 8

## Summary - Zusammenfassung

### Summary

Omnivory, feeding on two or more different trophic levels by one consumer, is a common phenomenon in aquatic food webs. A terminal predator is competing with a so-called intermediate consumer for a common basal resource (Fig. 1.1., p. 4). At the same time, the terminal predator preys upon the intermediate consumer, making the intermediate consumer suffering from competition and predation at the same time. According to recent dynamical models, omnivory should lead to exclusion of the intermediate consumer when productivity of the common basal resource is high (Diehl and Feissel 2000, Mylius et al 2001). In addition, the presence of an intermediate consumer should reduce the productivity of the top predator, since in its presence, the top predator is feeding on a higher trophic level than in its absence. Mixotrophy is a special case of omnivory: Here, a phototrophic protist is additionally consuming particulate prey (usually small phytoplankton and bacteria) by phagotrophy. Phytoplankton and bacteria compete with the mixotroph on the shared resources nutrients and light (only phytoplankton) and are its potential prey at the same time.

In order to investigate the impact of omnivory and mixotrophy on aquatic food webs, artificial food webs were assembled from monocultures. Within these food webs, mixotrophy was manipulated by the absence / presence of mixotrophic flagellates. Omnivory was manipulated in calanoid copepods by the absence / presence of microzooplankton. In the absence of the latter, the copepods were mainly herbivorous, while in its presence, the copepods were feeding additionally on the microzooplankton. Productivity of the phytoplankton was manipulated by different degrees of nutrient enrichment. In one experiment, bacterial productivity was manipulated by different degrees of dissolved organic carbon (DOC) addition to investigate the effect of bacterial productivity on the performance of mixotrophs. Experiments were set up in a factorial

design (-/+ target organisms, -/+ enrichment) and treatments were twice or four times replicated. The experiments lasted for 12 to 25 days.

The presence of mixotrophic flagellates led in all experiments to a marked reduction of picophytoplankton and (where counted) bacteria. Effects on other food web compartments and on overall productivity were context dependent. Seston biomass and biomass per limiting nutrient unit (nitrogen in all experiments) were enhanced by the mixotrophs in experiments where mesozooplankton was present or where bacterial productivity was enhanced by addition of DOC. In absence of mesozooplankton and glucose addition, seston biomass was reduced by the mixotrophs. It is concluded, that the mixotrophs may enhance primary production, provided that bacterial productivity is relatively high, either due to external DOC input or due to internal DOC production by zooplankton. Effects of mixotrophs on secondary production were species-dependent: The chrysophyte *Ochromonas minima* reduced productivity of the rotifer *Brachionus plicatilis*, while the haptophyte *Chrysochromulina polylepis* enhanced reproduction in calanoid copepods.

In a complex food web with calanoid copepods as terminal consumers, the presence of the heterotrophic dinoflagellate *Oxyrrhis marina* clearly enhanced copepod reproduction. In addition to that, the presence of the dinoflagellates drastically reduced the nanophytoplankton, the major food of the copepods in the absence of the dinoflagellates. Hence, the intermediate consumer *Oxyrrhis* enhanced copepod nutrition in spite of reducing the phytoplankton prey of the copepods. In another experiment, calanoid copepods were fed either with the diatom *Skeletonema costatum*, or with the diatom and the heterotrophic dinoflagellate *Gyrodinium dominans*, that was feeding itself on the diatom (intermediate consumer). Again, reproduction of the copepods was clearly enhanced by the dinoflagellates, though abundances of the dinoflagellates were low compared to the diatoms (appr. 200 and 20,000 cells ml<sup>-1</sup>, respectively). Fast disappearance of the dinoflagellates in the mixed treatments indicated strong selective feeding by the copepods. The results are in agreement with other studies where addition of microzooplankton to phytoplankton diets enhanced copepod reproduction. However, the results presented here show for the first time, that an enhancement by microzooplankton also works in closed systems, where microzooplankton acts as an intermediate consumer and inevitably reduces the phytoplankton prey of the copepods. In addition to that, the results of the latter experiment may explain, why negative effects of diatoms on calanoid copepods, that were found in several laboratory studies, are not found in the field during diatom blooms: Due to selective feeding by copepods, even low relative abundances of microzooplankton may significantly enhance nutritional quality of the diet for the copepods.

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Exclusion of intermediate consumers by top predators has not been observed in the experimental food webs except for the experiment with diatoms, the heterotrophic dinoflagellate *Gyrodinium* and calanoid copepods. Here, the latter reduced the intermediate consumer *Gyrodinium* below detection limit within few days. However, this result was mainly due to small containers size (600 ml) and high copepod densities ( $70 \text{ L}^{-1}$ ).

### **Zusammenfassung**

Das Fressen auf zwei oder mehr verschiedenen trophischen Ebenen durch einen Konsumenten, Omnivorie genannt, ist eine verbreitete Erscheinung in aquatischen Nahrungsnetzen. Ein terminaler Räuber konkurriert mit einem sogenannten 'intermediären Konsumenten' um eine gemeinsame Ressource (Fig. 1.1., S. 4). Gleichzeitig frisst der terminale Räuber auch den intermediären Konsumenten, wodurch der intermediäre Konsument sowohl unter Prädation als auch unter Konkurrenz leidet. Nach neueren dynamischen Modellen sollte Omnivorie in Verbindung mit hoher Produktivität der basalen Ressource zum Ausschluss des intermediären Konsumenten führen (Diehl und Feissel 2000, Mylius et al. 2001). Darüber hinaus sollte ein intermediären Konsument die Produktivität des terminalen Räubers reduzieren, da letzterer bei Anwesenheit des intermediären Konsumenten auf einer höheren trophischen Stufe steht als bei seiner Abwesenheit. Mixotrophie stellt einen Sonderfall von Omnivorie dar: Ein phototropher Protist ist zugleich in der Lage, Partikel zu ingestieren (Phagotrophie). Kleines Phytoplankton und Bakterien konkurrieren mit dem Mixotrophen um die gemeinsamen Ressourcen Nährstoffe und Licht (letzere nur Phytoplankton), und sind zugleich Beute des Mixotrophen.

Um die Auswirkungen von Omnivorie und Mixotrophie auf aquatische Nahrungsnetze zu untersuchen, wurden künstliche Nahrungsnetze aus Monokulturen zusammengesetzt. Mixotrophie wurde durch die wahlweise Zugabe von mixotrophen Flagellaten zu den Nahrungsnetzen gesteuert. Omnivorie wurde bei calanoiden Copepoden durch die Zugabe eines Mikrozooplankters beeinflusst: In Abwesenheit des Mikrozooplankters waren die Copepoden weitgehend herbivor, während sie in dessen Anwesenheit sowohl Phytoplankton als auch Mikrozooplankton fraßen. Die Produktivität des Phytoplanktons wurde durch unterschiedliche Nährstoffniveaus variiert. Um die Rolle der bakteriellen Produktivität für die Entwicklung der Mixotrophen zu untersuchen wurde darüber hinaus in einem Experiment die bakterielle Produktivität durch Zugabe von gelöstem organischem Kohlenstoff ('dissolved organic carbon', DOC) variiert. Die Experimente wurden jeweils in einem faktoriellen Design durchgeführt (-/+ Zielorganismus, -/+ Nährstoffe oder DOC) und alle Behandlungen jeweils zwei- oder vierfach repliziert. Die Versuchsdauer betrug 12-25 Tage.

Die Anwesenheit der mixotrophen Flagellaten führte in allen Experimenten zu einer klaren Reduktion des Picophytoplanktons und (wo gezählt) der Bakterien. Effekte auf andere Nahrungskompartimente und die Gesamtproduktivität waren Kontext-abhängig. Die Sestonbiomasse und Biomasse je limitierender Nährstoffeinheit (Stickstoff in allen Experimenten) wurden in allen Experimenten mit Mesozooplankton sowie in den Ansätzen, in denen die bakterielle Produktivität durch DOC Zugabe gefördert wurde, durch die Mixotrophen erhöht. In Abwesenheit von Mesozooplankton und DOC Zugabe hingegen reduzierten die Mixotrophen die Sestonbiomasse. Daraus wird geschlossen, dass Mixotrophe die Primärproduktion bei gleichzeitig hoher bakterieller Produktivität erhöhen können, wobei die bakterielle Produktivität sowohl durch externe DOC Zugabe, oder durch Grazing von Zooplankton und somit interne DOC Freisetzung gefördert werden kann. Effekte der Mixotrophen auf die Sekundärproduktion waren artabhängig: Während die Chrysophyce *Ochromonas minima* sich negativ auf die Produktivität des Rotators *Brachionus plicatilis* auswirkte, erhöhte die Haptophyce *Chrysochromulina polylepis* die Reproduktion von calanoiden Copepoden.

In einem komplexen Nahrungsnetz mit calanoiden Copepoden als terminalen Konsumenten führte die Anwesenheit des Mikrozooplanktons *Oxyrrhis marina* (Dinophyce) zu einer deutlich erhöhten Reproduktion der Copepoden. Gleichzeitig reduzierte *Oxyrrhis* das Nanophytoplankton, das die wichtigste Futterquelle in Abwesenheit des Dinoflagellaten darstellte, um bis zu 3 Größenordnungen. Somit verbesserte der intermediäre Konsument *Oxyrrhis* die Nahrungsgrundlage der omnivoren Copepoden, obwohl er die gemeinsame Ressource Nanophytoplankton reduzierte. In einem anderen Experiment wurden calanoide Copepoden entweder nur mit Diatomeen, oder mit einer gemischten Kultur von Diatomeen und dem heterotrophen Dinoflagellaten *Gyrodinium dominans*, der Diatomeen ingestiert, inkubiert. Wiederum wurde die Reproduktion der Copepoden deutlich durch das Mikrozooplankton gefördert, obwohl es gegenüber den Diatomeen deutlich weniger häufig war (200 und 20.000 Zellen ml<sup>-1</sup>). Ein rasches Verschwinden der Dinoflagellaten aus den gemischten Ansätzen deutete auf selektives Fressen der Copepoden hin. Das Ergebnis stimmt mit anderen Studien überein, in denen die Zugabe von Mikrozooplankton zu einer Phytoplanktondiät zu einer erhöhten Reproduktion bei calanoiden Copepoden führte. Die hier dargestellten Ergebnisse zeigen allerdings erstmals, dass dieser positive Effekt auch in geschlossenen Systemen auftritt, in denen die Anwesenheit des intermediären Konsumenten Mikrozooplankton unweigerlich mit einer Reduktion des Phytoplanktons verbunden ist. Darüber hinaus erklären die Ergebnisse des letzteren Experimentes, warum negative Effekte von Diatomeen auf den Reproduktionserfolg von Copepoden, wie sie in mehreren Laborstudien auftraten, nicht im Freiland während Diatomeenblüten gefunden wurden: Aufgrund selektiven



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Grazings können selbst relativ geringe Abundanzen von Mikrozooplankton zu einer Verbesserung der Nahrungsgrundlage von Copepoden führen.

Mit Ausnahme des letzten Experimentes, in dem die Copepoden die Dinoflagellaten innerhalb weniger Tage bis unter die Nachweisgrenze reduzierten, wurde in keinem Experiment ein Ausschluss eines intermediären Konsumenten beobachtet. Der beobachtete Ausschluss der heterotrophen Dinoflagellaten in diesem Experiment war wohl vor allem auf die geringe Größe der Inkubationsflaschen (600 ml) sowie die hohe Copepodendichte ( $70 \text{ L}^{-1}$ ) zurückzuführen.



# Chapter 9

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# Chapter 10

## Danksagung

Viele Leute haben mich in den zurückliegenden drei Jahren bei meiner Arbeit unterstützt. Ihnen gilt mein herzlicher Dank.

Meinem Betreuer Uli Sommer danke ich für Freiheit, die er mir bei der Bearbeitung des Themas gelassen hat, und für seine zuverlässige und rasche Unterstützung.

Thomas Hansen und Marcel Sandow danke ich für diverse technische Hilfe und für viel Motivation zu allen Zeiten meiner Dissertation.

Herzlichen Dank an Volker Martens, Heidi Gonschior und Peter Fritsche, die mir vielfältig technische Hilfe leisteten, sowie an Herwig Stibor und Jaroslav Vrba, von denen ich viele nützliche Anregungen bekommen habe.

Vielen Dank an Steffi Moorthi, Silke Rick, Todd Hubbard, Matthew Mills und Karel Šimek, die mir beim Zusammenschreiben viele nützliche Kommentare gaben, sowie an Daniela Brepohl und Isabella Galvao für praktische Hilfe bei den Versuchen.

Meiner Freundin Radka danke ich für ihre tatkräftige Unterstützung zu jeder Zeit, besonders für ihre nimmermüde Hilfe während der letzten Tage vor der Abgabe :-)

- Meinen Eltern -