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Effects of microzooplankton and mixotrophy in an experimental planktonic food web

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Abstract

Microzooplankton have received increased attention as an important trophic link between the microbial loop and calanoid copepods. On the basis of food size spectra overlap in some microzooplankton groups and calanoid copepods, however, such microzooplankton could function as competitors rather than as food for calanoid copepods (intraguild prey). Mixotrophic flagellates presumably represent a link between the microbial loop and the micro- and mesozooplankton. We investigated the effects of microzooplankton and mixotrophy by altering the presence of a heterotrophic dinoflagellate and of a mixotrophic nanoflagellate in artificial food webs with calanoid copepods as terminal consumers. Overall system productivity was manipulated by two levels of nutrient enrichment. The heterotrophic dinoflagellate drastically reduced the nanophytoplankton and enhanced the reproduction of the copepods, suggesting that its role as a competitor is negligible compared to its function as a 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 biomass, indicating a higher efficiency in overall primary production. Copepod reproduction was enhanced in the presence of the mixotrophic nanoflagellate. Results did not support predictions of the intraguild predation theory: The ratios of the intraguild predators and their preys were not affected by overall system productivity.

The importance of omnivory in planktonic food webs has been recognized during the last two decades. In particular, it was found that virtually all calanoid copepod species, formerly viewed as herbivorous, also feed substantially on heterotrophic organisms. Specifically, microzooplankton seem to be an important food for calanoid copepods (Kleppel et al. 1998; Klein Breteler et al. 1999; Bonnet and Carlotti 2001). Ciliates could represent an important link from small phytoplankton and bacteria to calanoid copepods (Calbet and Landry 1999). However, in spite of conspicuous size differences between copepods and microzooplankton, their food size spectra might overlap considerably (Sherr et al. 1986; Sanders and Wickham 1993). As a consequence, microzooplankton could act as competitors as well as food for copepods. This triangular interaction is referred to as intraguild predation (IGP; Holt and Polis 1997) and is a common feature in aquatic environments (Stoecker and Evans 1985; Diehl and Feissel 2000). Two important consequences arise from IGP. First, the intraguild (IG) predator can be at an energetic disadvantage relative to its IG prey because the IG predator must feed on a higher trophic level (Oksanen et al. 1981). Second, analytical models predict that coexistence of IG predator and prey is possible only over a limited range of system productivity (Holt and Polis 1997; Diehl and Feissel 2000). Within this range, the ratio of IG predator : IG prey is predicted to increase with increasing productivity, and at sufficiently high productivity the IG prey becomes excluded. Although several studies have highlighted the nutritional value of various microzooplankton for calanoid copepods (e.g., Klein Breteler et al. 1999; Bonnet and Carlotti 2001), the dynamic aspects of microzooplankton acting as IG prey between phytoplankton and copepods have received little attention.

The energy transfer efficiency from the microbial loop to the mesozooplankton is generally believed to be low because of the numerous trophic levels between small phytoplankton and the mesozooplankton (Ducklow et al. 1986). 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 (Havskum and Riemann 1996; Riemann et al. 1995). Mixotrophy is used here 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). Although this hypothesis seems important for the understanding of the microbial loop, to the best of our knowledge, it has not yet been tested. Algal mixotrophy represents a special case of IGP: The mixotroph competes with osmotrophic organisms (small phytoplankton and bacteria) for dissolved nutrients and preys on them at the same time. However, because the IG prey is a primary producer, the IG predator mixotroph should not have an energetic disadvantage from its IG prey.

In this study, we investigate the effects of mixotrophy and omnivory on the trophic structure of a planktonic food web and on the biomass of its terminal consumer. We assembled artificial food webs that consisted of typical representatives of a marine plankton community with calanoid copepods as terminal consumers and manipulated presence and absence of omnivory and mixotrophy (Fig. 1). We altered omnivory

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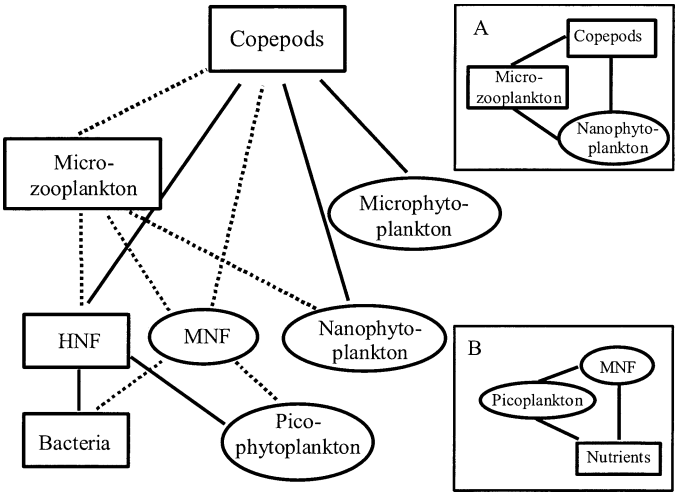

Fig. 1. Large figure shows the experimental food webs. Solid lines represent links that were present in all food webs, whereas dotted lines represent facultative links that were generated by the addition of the microzooplankton and the mixotrophic nanoflagellate (MNF). HNF, heterotrophic nanoflagellate. For clarity, the weak links between microzooplankton and bacteria and picophytoplankton are not displayed. Panels A and B highlight the IG predator copepods and mixotrophic nanoflagellates, respectively, together with their IG prey and common resources. Picoplankton comprises bacteria and picophytoplankton.

in copepods by the absence or presence of a microzooplankton species with an optimal food size in the size range of the nanophytoplankton (Fig. 1). The copepods should be mainly herbivorous in the food webs without microzooplankton but compete with and feed on the microzooplankton when they are present. In this manner, it should be tested whether the microzooplankton are acting as a competitor or as a trophic link for the copepods. Mixotrophy was manipulated by the absence or presence of a mixotrophic nanoflagellate (Fig. 1). The experiment was performed under two different nutrient regimes to test whether the relative abundances of IG predators and prey are affected by the overall system productivity. In addition, our experiment tested whether the presence of mixotrophs is affected by system productivity.

Materials and methods

The experiment was carried out in June 2001 in a walk-in environmental chamber that was set to a 16 : 8 light : dark 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‰) 1 week prior to the experiment and stored in the dark at 16°C. The water was then filtered into sterile experimental containers by a 0.45-μm filter capsule (Sartorius Sartobran-P capsule). This pore width was chosen to exclude all eukaryotic protists but to permit passing of smaller heterotrophic bacteria from the natural bacterial assemblage. The medium was enriched with nutrients to final concentrations as given in Table 1 (Rick and Dürselen 1995). The nitrogen to phosphorus ratio was about 3; that is, for all phytoplankton, nitrogen should have been the limiting nutrient (except for possible silica limitation in the diatom). The protists were grown as nonaxenic monocultures under the 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 and has been cultivated for many years on a Baltic Sea medium in our laboratory (~15‰). 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 *R. salina*. *Cafeteria rosenbergensis* was isolated from the Baltic proper. *Chrysochromulina polylepis* is a strain from the Scandinavian Culture Center for Algae and Protozoa (SCCAP), Copenhagen, Denmark (K-0617), that was isolated in 1988 from the Kattegat, North Sea. The *Synechococcus* sp. originates from the Caribbean Sea (strain CCMP 1282, Provasoli-Guillard Culture Center) and was cultivated on a Baltic Sea medium for several years (Reckermann pers. comm.). The copepods were collected by vertical net hauls (250 μm mesh) from the Kiel Bight 2 weeks before the start of the experiment. During this time, they were kept in two 300-liter containers with little food addition. Before adding the copepods to the experimental containers, they were washed twice with sterile filtered water over a 64-μm mesh. The final inoculum consisted of an assemblage of various copepodid stages of *Acartia tonsa*,

Table 1. Food web configurations and nutrient levels. Concentrations of trace elements were equal in both nutrient levels.

Food web configurations*					Nutrient levels	
Control	+O (Microzoo.)	+C (Mixotrop.)	+OC (Microzoo., Mixotrop.)		low	high
All: bacteria; heterotrophic nanoflagellates; autotrophic pico-, nano-, and microphytoplankton; copepods				N	9	28.5
				P	3	9.5
				Si	5	15.8
				EDTA, $FeSO_4$, Mn	0.33	1
				Biotin (vitamin H)	0.007	0.02
				Thiamine (vitamin B_1)	0.005	0.015
				Cyanocobalamin (vitamin B_{12})	0.0013	0.004

* Microzoo., microzooplankton; mixotrop., mixotrophic nanophytoplankton.

Pseudocalanus elongatus, Paracalanus parvus, and *Centropages hamatus,* with *A. tonsa* being most abundant. No other mesozooplankton were observed at this time or during the experiment.

Experimental containers—The experimental containers consisted of circular 30-liter polypropylene buckets that were covered by a transparent lid to reduce contamination 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 (height 6 cm). 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 distributed the food. A faucet mounted in midheight of the container wall allowed water sampling.

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 plant light (Starlicht 36W 020 cool white; Osram L 36W/77 Fluora). The light intensity was 100 μmol quanta m^{-2} s^{-1} in middepth of the containers under pure water (LICOR Quantum photometer LI-185B).

Experimental design and sampling—The experimental setup was a factorial design. We varied three factors (presence of the microzooplankton *Oxyrrhis* [+O], presence of the mixotrophic *Chrysochromulina* [+C], and nutrient level [high, low]), leading to four different food web configurations (controls, +O, +C, +OC) at two different nutrient levels (Table 1). Each of the eight resulting treatments was twice replicated.

First, the containers were filled with medium as given in Table 1 and inoculated with the protists (except the microzooplankton). Initial sampling was done 5 d later (start of the experiment, day 0); 1 d later the copepods and the microzooplankton were added. The final volume was 25 liters per container.

The experiment was run for 24 d. Ten percent of the medium was exchanged every 6 d within a large clean bench. Water was exchanged by removing 2.5 liters from the container with an autoclaved beaker and immediately replacing it with fresh medium; 1.5 to 2 liters of the water was filtered by a 64-μm 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. The rest of the exchanged volume was filtered by a 100-μm mesh and used for further analysis (although the 100-μm mesh did not retain all nauplii, it was used for phytoplankton and seston analyses because the 64-μm mesh retained a considerable fraction of the diatoms). For analysis of particulate carbon and nitrogen (C, N), 100 ml of medium was filtered on precombusted Whatman GF/F filters dried at 60°C and stored in a desiccator until analysis. Samples for microscopic analysis were preserved with 2% glutaraldehyde and kept in the dark at 5°C until analysis. In addition to the 6-d interval sampling, phytoplankton samples were taken in the middle of each 6-d interval. These samples were taken from the faucet mounted on the side of the containers. 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 analyzed by heat combustion on a Fisons NA 1500 N analyzer.

Microscopic analysis of the plankton samples was done on an inverted fluorescence microscope (Leitz DMIRB). Sample volumes (10 ml) were transferred to Utermöhl chambers (height of the chamber, 2.2 cm) and stained with 0.01 μg ml^{-1} DAPI (Porter and Feig 1980). After 48 h of sedimentation, we first counted the smallest fraction (picophytoplankton, heterotrophic nanoflagellates) at ×1,000 magnification under oil immersion and fluorescent light. This method allowed reliable differentiation between bacteria, picophytoplankton, and small heterotrophic nanoflagellates (HNFs). Because of the low sinking velocities of picoplankton, the method is likely to underestimate abundances of the picophytoplankton (Kemp et al. 1993); however, the recorded cell concentrations of this group turned out to be very sensitive to the applied treatments. We therefore assume that the obtained data at least gives a good estimate for the relative differences among treatments. The larger fractions were counted at lower magnifications under normal light. Excluding samples where some taxa were extremely rare, we counted at least 100 cells of each species 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.

To compare the relative share of all functional groups, we estimated the carbon content for each group (Table 2). For cyanobacteria and 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 with the use of 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, so only a rough estimate was possible here. Carbon content of an average nauplius and copepodid were estimated from data for *A. tonsa* because the copepod assemblage was dominated by this species (Berggreen et al. 1988).

Statistical analysis—To exclude transient dynamics from the analysis that might be related to the initial concentrations

Table 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 (μm)	Biovolume (μm^3)	C (pg)
Picophytoplankton	*Synechococcus* sp.*	1.3	1.15	0.25
Autotrophic nanophytoplankton	*Rhodomonas salina*	6.4	136	21.8
Mixotrophic nanophytoplankton	*Chrysochromulina polylepis*	5.14	71	11.8
Microphytoplankton	*Thalassionema nitzschioides*	11.6	820	66.4
Heterotrophic nanoflagellate	*Cafeteria rosenbergensis**	3.05	14.8	2.72
Microzooplankton	*Oxyrrhis marina*	14.5	1,590	219
Mesozooplankton	Average nauplius			1×10^5
	Average copepodid			2×10^6

* Plus contaminants.

of the organisms, we analyzed the data of the last three (copepods and the C : N ratio: last two) samples only (averages of days 18–24). This should also account for the long generation time of the copepods (~20 d; Landry 1983). Furthermore, as evident from the decline in copepods shortly after inoculation (Fig. 6), the copepods suffered from substantial mortality at the beginning of the experiment. This indicates a period of acclimatization that should be excluded from analysis. We calculated averages over the last three samples instead of using only data of the very last date to get a better estimate for groups of low abundance. Copepodids and nauplii were treated as individual groups because they differ considerably in their food size spectra (Hansen et al. 1994).

Overall effects of the three treatments (enrichment, *Oxyrrhis*, and *Chrysochromulina*) were analyzed in a redundancy analysis (RDA; Jongman et al. 1995). RDA is a form of direct gradient analysis that assumes linear relationships between the experimental treatments and the species. Unlike a MANOVA, RDA is not limited to situations in which 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). To normalize variations, each group's dataset was $\log(x + 1)$ transformed in this analysis. Factors were included in the model depending on a forward selection method ($P < 0.05$) on the basis of a Monte Carlo permutation test.

Table 3. Results from the redundancy analysis (RDA). Factors were selected by a forward selection process ($P < 0.05$) on the basis of 1999 Monte Carlo permutations. The analysis included all seven parameters displayed in Fig. 4. $n = 16$ for each parameter.

Variable	P	F-ratio	λ*
Oxyrrhis	0.01	9.5	0.43
Chrysochromulina	0.005	3.9	0.11
Enrichment	0.06	2.05	—
Together			0.54

* λ, eigenvalue of the corresponding factor in this analysis.

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

To test whether enrichment affected the abundances of the IG predators relative to their IG prey, we calculated the ratio between the two corresponding groups on the basis of each group's carbon content (copepod : *Oxyrrhis* and *Chrysochromulina* : *Synechococcus*). The log-transformed ratios were analyzed by ANCOVAS, with enrichment as the fixed factor and mixotrophy (+C) or omnivory (+O) as covariates.

Results

Contaminants—The absence of contaminants by mixotrophs and microzooplankton was a major prerequisite for our experimental design, particularly for those treatments without these organisms. Such contaminants were never observed during the experiment. However, small HNFs (2–6 μm) belonging mainly to *Choanoflagellidea* and *Kinetoplastidea* appeared after week 2 in all containers. Because 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 after week 2 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 (referred to below as parameters) were investigated by RDA (Table 3; Fig. 2). In a forward selection process, *Chrysochromulina* and *Oxyrrhis* gave significant results ($P < 0.05$) and together explained 54% of the total observed variance (sum of canonical eigenvalues, Table 3). In the ordination diagram (Fig. 2), the length of the parameters' axes indicate the degree of variation in each parameter explained by the analysis. The more a parameter's arrow is parallel to a factor's arrow, the more its variance is correlated with this factor (positively, if both arrows point in the same direction; negatively, if they point in opposite directions). Most species' arrows are more or less parallel with *Oxyrrhis*. Copepodids and nauplii were strongly enhanced by the presence of *Oxyrrhis*, whereas

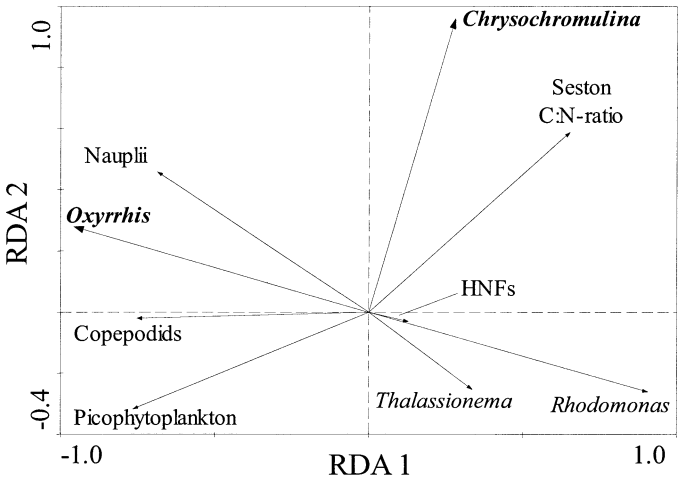

Fig. 2. Redundancy analysis (RDA) of the species abundances (means, days 18–24) in relation to the treatments *Chrysochromulina* and *Oxyrrhis.* RDA 1 and 2, first and second canonical axes. For explanation, *see Results.*

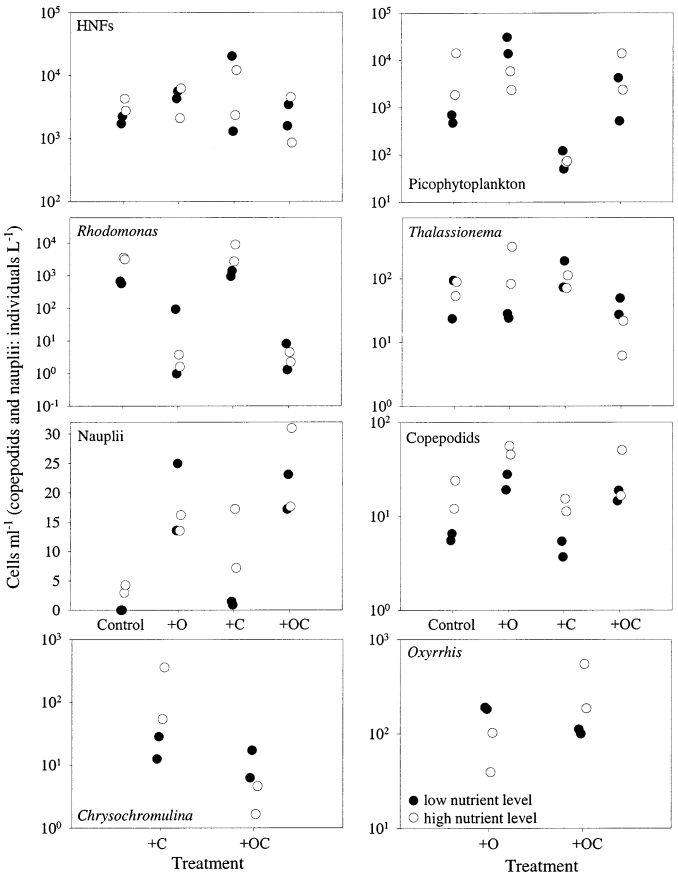

Fig. 3. Abundances of all functional groups in cells ml^{-1} (protists) and individuals L^{-1} (copepodids and nauplii), means of days 18–24. Log scale except for nauplii (contained zero values). Low and high nutrient levels are depicted as black and white circles, respectively. Codes of food web configurations as explained in Table 1.

Rhodomonas was drastically reduced. The picophytoplankton and C : N ratio were strongly affected by *Chrysochromulina.* Enrichment gave only a marginal significant result in this analysis ($P = 0.06$; Table 3); however, effects of enrichment were partly parallel to effects of *Oxyrrhis* (enhancement of copepods and nauplii; amplification of the negative effect of *Oxyrrhis* on *Rhodomonas, see* results from ANOVA, Table 4). Hence, the additional explanation provided by enrichment was low in this analysis.

Treatment effects on the single functional groups and on seston stoichiometry—In a three-way ANOVA the effects of *Oxyrrhis, Chrysochromulina,* and enrichment and their interactions on the single functional groups and on the C : N ratio were investigated (Table 4; Fig. 3).

Picophytoplankton (*Synechococcus* sp. and picoeukaryotes): Apart from the copepods, the picophytoplankton turned out to be the most sensitive group to the applied treatments (Table 4). Picophytoplankton were strongly reduced in the *Chrysochromulina* treatments. Especially in the unenriched treatments, picophytoplankton profited from the presence of *Oxyrrhis,* which reduced *Chrysochromulina* and probably remineralized nutrients of the ingested nanophytoplankton.

Table 4. Results from a full-factorial three-way ANOVA testing the effects of *Chrysochromulina* (C), *Oxyrrhis* (O), and enrichment (E), as well as their interactions on the log-transformed abundances of the various groups and on the C : N ratio (mean days 18–24). Significant *P*-values are in bold. Nauplii contained zero values and were $\log(x + 1)$ transformed. $n = 16$, except for *Chrysochromulina* and *Oxyrrhis* ($n = 8$).

	Overall model			*P*						
Taxon	*P*	*F*	r^2	E	C	O	E×C	E×O	C×O	E×C×O
Picophytoplankton	<**0.01**	9.72	0.89	0.38	<**0.01**	<**0.01**	0.69	0.23	0.06	**0.02**
Rhodomonas	<**0.01**	13.5	0.92	0.52	0.96	<**0.01**	0.69	0.12	0.50	0.56
Thalassionema	0.054	3.4	0.75	—	—	—	—	—	—	—
Chrysochromulina	**0.047**	6.82	0.84	0.62	—	**0.02**	—	0.06	—	—
Heterotrophic nanoflagellates	0.89	0.38	0.25	—	—	—	—	—	—	—
Oxyrrhis	0.12	3.71	0.73	—	—	—	—	—	—	—
Copepodids	<**0.01**	9.07	0.89	<**0.01**	0.09	<**0.01**	0.81	0.33	0.66	0.74
Nauplii	<**0.01**	29.9	0.96	<**0.01**	<**0.01**	<**0.01**	0.38	<**0.01**	0.08	0.81
C : N ratio of the seston	**0.02**	4.96	0.81	0.96	<**0.01**	0.8	0.88	0.87	0.19	0.24

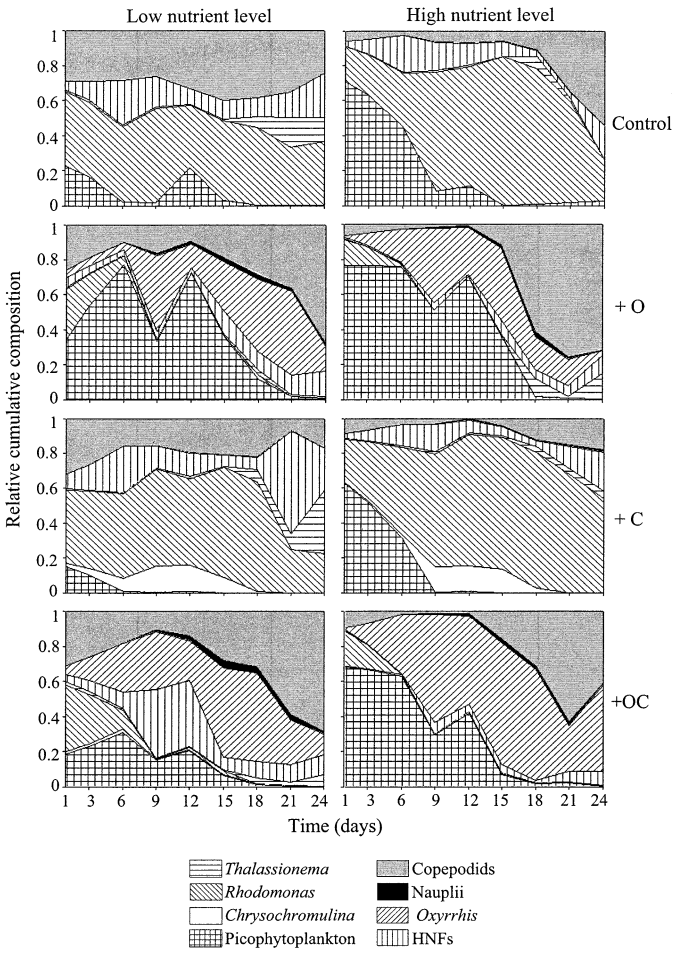

Fig. 4. Relative share of all functional groups over time based on each group's carbon content (means of the corresponding replicates). Left and right columns show low and high nutrient levels, respectively. Codes of food web configurations (right) are explained in Table 1.

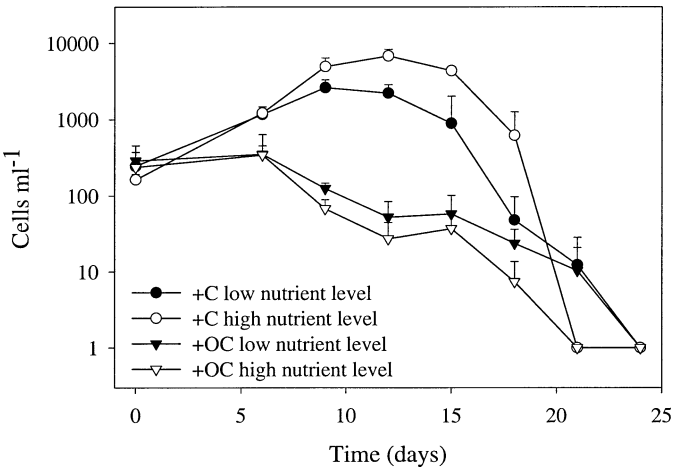

Fig. 5. Time series of the mixotrophic flagellate *Chrysochromulina polylepis* (cells ml^{-1}, log scale). Mean + SD of both replicates. Codes of food web configurations as explained in Table 1.

R. salina (autotrophic nanophytoplankton): *Rhodomonas* experienced a strong negative effect from the microzooplankton *Oxyrrhis.* This effect was strongest in the high nutrient levels, in which the cell concentrations of *Rhodomonas* were three orders of magnitude lower in the presence of *Oxyrrhis* than in its absence (Fig. 3). Although enrichment had no significant effect on *Rhodomonas,* this flagellate seems to have profited from enrichment in the treatments without *Oxyrrhis* (Fig. 3).

T. nitzschioides (microphytoplankton): From week 2 on, filaments of this diatom became attached to the container walls. We removed the wall growth following each sampling with a scraper, but over the experimental period, a considerable fraction of the diatom remained attached to the walls and therefore unavailable for the zooplankton. The cell concentrations given in Fig. 3 represent only the suspended algae that 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), and we therefore assume that its importance as prey for the copepods was low.

C. polylepis (mixotrophic nanophytoplankton): In the presence of *Chrysochromulina,* cell concentrations of the picophytoplankton were much lower than in its absence. Because *Chrysochromulina* did not reduce the nano- and microphytoplankton, nutrient competition or toxicity cannot explain this effect. Therefore, *Chrysochromulina* appears to have grazed effectively on the picophytoplankton and very likely also on similar-sized heterotrophic bacteria (Stibor and Sommer 2003). In addition, the C : N ratio of the seston biomass was enhanced by the mixotroph (*see below*). Similar to *Rhodomonas,* the mixotroph was clearly reduced by *Oxyrrhis,* especially in the high-nutrient treatments.

Heterotrophic nanoflagellates: This group represents all nano-sized heterotrophic flagellates, including *C. rosenbergensis.* Because these organisms varied in size, the cell concentrations are only roughly correlated to the overall HNF biomass. This could partly explain the comparatively small among-treatment effects. Also, because this group contains morphologically differing taxa (*see Contaminants*), effects on functional diversity are obscured. No treatment had a significant effect on the heterotrophic nanoflagellates.

O. marina (microzooplankton): The heterotrophic dinoflagellate grazed preferentially on the nanoflagellates *Rhodomonas* and *Chrysochromulina* (when present), as visible from the strong decline in these species in all corresponding treatments (Figs. 3–5). However, because *Oxyrrhis* persisted after it had reduced the nanophytoplankton to low abundances, it appears that other prey sustained its growth (Fig. 4). Although the optimal food size spectrum of *Oxyrrhis* is around 7 μm equivalent spherical diameter (ESD; Hansen et al. 1996), it can feed also on picoplankton (Schumann et al. 1994). Therefore, in the absence of *Rhodomonas* and *Chry-*

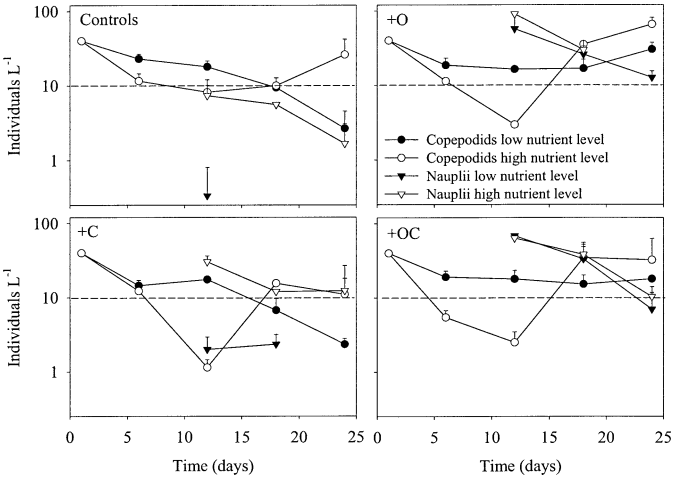

Fig. 6. Time series of the copepodids and nauplii (individuals L^{-1}, log scale). Means + SD of both replicates. Zero values of nauplii are not displayed. Codes of food web configurations as explained in Table 1.

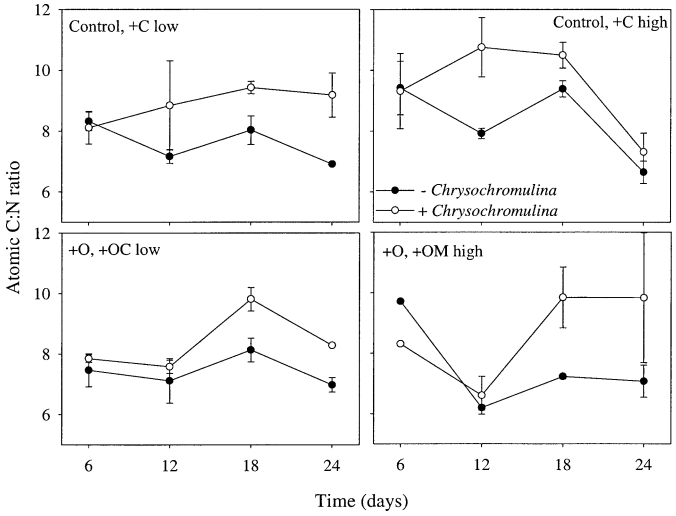

Fig. 7. Time series of the atomic carbon to nitrogen ratio of the seston. In each plot, the corresponding treatments ± the mixotrophic *Chrysochromulina* are shown. Nutrient levels are denoted as "high" and "low." One replicate of the +OC low treatment (day 24) has been omitted because it was obviously an error in measurement (value, 3.7). Codes of food web configurations as explained in Table 1.

sochromulina, Oxyrrhis probably grazed on HNFs, picophytoplankton, and bacteria.

Calanoid copepods: Copepods reproduced in all treatments, but their reproductive success was highly variable among treatments (Fig. 6). Numbers per volume of both nauplii and copepodids were enhanced by the presence of *Oxyrrhis* and by enrichment (Figs. 4, 6; Table 4); additionally, nauplii were significantly enhanced in the +C 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 to <5 individuals L^{-1} in the absence of *Oxyrrhis* but were >20 individuals L^{-1} in the corresponding treatments with *Oxyrrhis* on day 24 (Fig. 6).

Seston stoichiometry: According to the low nitrogen to phosphorus ratio in the supplied medium, phosphorus was available in excess. In the majority of cases, the atomic C : N ratio of the seston was between 7 and 10 (Fig. 7), which is above the Redfield ratio and indicates that phytoplankton production was limited by nitrogen (Goldman et al. 1979). In all treatments containing the mixotrophic *Chrysochromulina,* the C : N ratio was enhanced compared with the corresponding treatments without (Fig. 7, Table 4), indicating higher nutrient limitation in the presence of *Chrysochromulina.* Surprisingly, this effect persisted after the disappearance of *Chrysochromulina.* The C : N ratio was not significantly affected by nutrient enrichment.

Relative composition over time—The systems without microzooplankton were dominated by nanophytoplankton (*Rhodomonas*), and *Rhodomonas* still made up a considerable fraction of the overall biomass at the end of the experiment (Fig. 4). Conversely, in the systems with *Oxyrrhis, Rhodomonas* and the similar-sized mixotrophic *Chrysochromulina* quickly disappeared, and the picophytoplankton became the dominant primary producer. Whereas the share of copepods stayed at rather constant levels in most controls and +C treatments (except +C, high nutrient level), their share increased over time in the +O and +OC treatments. Toward the end of the experiment, the share of the copepods on overall biomass was considerably larger in the treatments containing microzooplankton. The change in relative composition in the +O and +OC treatments (from nanophytoplankton to picophytoplankton) indicates a shift in the diet of the microzooplankton because *Oxyrrhis* did not vanish after the strong decline of the nanophytoplankton.

Relative abundances of IG predators and IG prey—According to IGP theory, an IG predator should increase relative to an IG prey as the overall productivity of the system is increased (Holt and Polis 1997; Diehl and Feissel 2000). We tested this prediction by analyzing the log-transformed carbon-based ratios between the IG predators and their prey in ANCOVAS (Fig. 8). In the *Chrysochromulina–Synechococcus* interaction, *Oxyrrhis* had a significant negative effect on this ratio, but enrichment did not ($P_{\text{ANCOVA}} = 0.05$, $P_{Oxyrrhis} = 0.02$, $P_{\text{enrichment}} = 0.83$). The negative effect of *Oxyrrhis* reveals a trophic cascade, whereby *Oxyrrhis* reduced abundances of *Chrysochromulina,* and thus its negative effect on *Synechococcus.* The copepod : *Oxyrrhis* ratio was not significantly affected by any experimental treatment ($P_{\text{ANCOVA}} = 0.38$).

Discussion

Development of the copepods and role of the microzooplankton—The positive effects of the microzooplankton *Oxyrrhis* might partly be explained by an enhancement of the chemical food quality for the copepods. In several studies, this heterotrophic dinoflagellate enhanced growth, reproduc-

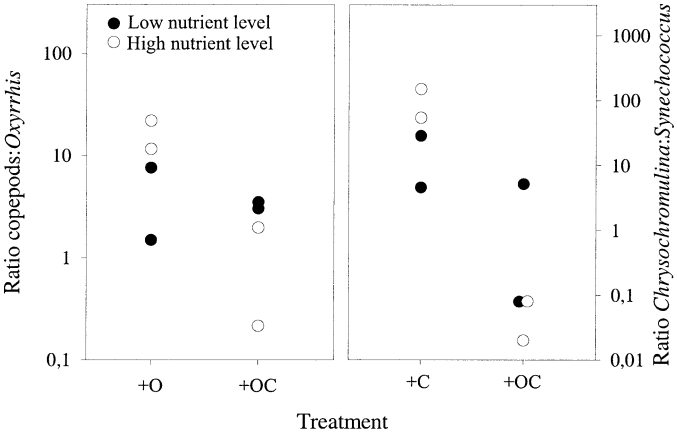

Fig. 8. Ratios of IG predator : IG prey (log scale) based on each group's carbon content. Ratios were calculated as averages of the period from day 18 to 24. Left panel, copepod : *Oxyrrhis*; right panel, *Chrysochromulina* : picophytoplankton.

tion, or both in calanoid copepods when it was added as an additional prey to a phytoplankton diet because it provided polyunsaturated fatty acids that were lacking in the phytoplankton diet (Kleppel et al. 1998; Klein Breteler et al. 1999). However, in absence of *Oxyrrhis, R. salina* was the most abundant phytoplankton by mass (Fig. 4) and very likely the most important prey for the copepods. Because several species of this genus with a similar ESD (6–7 μm) are known to be good prey for all life stages of small calanoid copepods (e.g., Berggreen et al. 1988; Klein Breteler et al. 1999), it is unlikely that the observed effect of the microzooplankton is solely an effect of chemical food quality. Additionally, prey size could be a reason. Optimal prey size in copepodids of small calanoid copepods ranges between 14 and 30 μm ESD (Hansen et al. 1994). With its ESD of 14 μm, *Oxyrrhis* was the largest prey and closest to the optimal prey size of the copepodids. Therefore, the presence of *Oxyrrhis* probably resulted in better chemical food quality in combination with higher feeding efficiency of the copepodids and thus could explain the enhanced reproduction and development in the presence of the microzooplankton.

Oxyrrhis preyed preferentially on the nanophytoplankton and presumably competed with the copepods, because the nanophytoplankton was likely the most important prey in the absence of the microzooplankton (Fig. 4). Nevertheless, the positive effects of the microzooplankton presence outweighed the reduction of nanophytoplankton. The results show that the possible energetic disadvantage of *Oxyrrhis* as an intermediate competitor for the calanoid copepods was less important than its role as a trophic link between phytoplankton and the copepods. Positive effects of microzooplankton on copepod growth and reproduction have been revealed in several feeding experiments (e.g., Klein Breteler et al. 1999; Bonnet and Carlotti 2001). In contrast to these previous studies, the results presented here demonstrate for the first time that such positive effects work in closed systems, where the presence of an IG prey such as *Oxyrrhis* inevitably reduces the availability of the common basal resource.

Effects of the mixotroph on food web structure and seston stoichiometry—Chrysochromulina had a strong negative effect on the picophytoplankton, and most likely also on bacteria (*see below*). HNFs, usually regarded as the most important consumers of picoplankton (Azam et al. 1983; Caron and Goldman 1990), were present in all treatments. Hence, an additional negative effect of *Chrysochromulina* on the picophytoplankton cannot be expected automatically. The reduced cell concentrations of the picophytoplankton in the presence of the mixotroph indicate 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 a lower R^* (minimum tolerable resource concentration) than the HNFs with respect to their shared resource, picophytoplankton (Rothhaupt 1996; Ptacnik et al. unpubl. data). Under sufficient light, *Chrysochromulina* ingests prey to enhance its gain in mineral nutrients (Stibor and Sommer 2003), whereas an HNF must cover all of its energy needs from its prey, while remineralizing a considerable share of the nutrients bound in the prey (Rothhaupt 1997). Hence, *Chrysochromulina* possibly needs less picoplankton to cover its nutrient demand than an HNF needs to cover its energy demand, and this might explain the lower R^* in *Chrysochromulina*. Resource competition theory also predicts competitive exclusion of the inferior competitor if both competitors are feeding on a single shared resource. However, cell concentrations of the HNFs were not reduced by the mixotroph. This deviation from the theoretical prediction could be caused by the missing taxonomic resolution of the group ''HNF.'' In addition, given the high morphological variety in natural bacterial assemblages (Jürgens and Güde 1994), bacterial prey possibly allowed for some resource partitioning between HNFs and *Chrysochromulina.* In a similar food web experiment with *Ochromonas minima* as mixotrophic and *Spumella* sp. as solely heterotrophic nanoflagellates, the mixotrophic flagellate also reduced picophytoplankton and single-celled bacteria to lower levels than did its heterotrophic counterpart. At the same time, cell concentrations of the heterotrophic flagellate were clearly reduced in the presence of the mixotroph (Ptacnik et al. unpubl. data).

The observed shift in the C : N ratio of the seston can only be explained by bacterivory in *Chrysochromulina* (Fig. 1). The atomic C : N ratio of heterotrophic bacteria is generally lower (5–6) than the C : N ratio of phytoplankton, including cyanobacteria like *Synechococcus* (depending on the degree of nitrogen limitation between 6 and 20; Sterner and Elser 2002). Because *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, and therefore an enhanced primary production, because more biomass was built up per limiting nutrient unit.

The presence of *Chrysochromulina* positively affected copepod reproduction as evidenced by increased numbers of nauplii. This effect was most pronounced in the middle of the experiment, when the mixotroph was most abundant (Figs. 5, 6) but was not reflected in higher numbers of copepods at the end of the experiment, when *Chrysochromu-*

lina was close to the detection limit (Fig. 4). The enhanced reproduction of the copepods in the +C treatments was probably caused by a higher number of grazable particles because *Chrysochromulina* converted (ungrazable) picoplankton into cells within the prey size range of nauplii and copepodids. Although not significant in the number of copepodids, the results indicate that mixotrophic nanoflagellates could have positive effects on transfer efficiency in planktonic food webs.

Chrysochromulina reached considerable abundances early in the experiment but declined to the detection limit toward the end of the experiment (Fig. 5). Because this effect occurred in no other group and independently from the applied treatments, depletion of some essential nutrient might have caused the disappearance of *Chrysochromulina. C. polylepis* has a requirement for selenium, which had not been added to our medium (Edvardsen et al. 1990; Table 1). Because our medium was based on natural seawater, initial concentrations of this nutrient were probably just enough to allow for temporary growth in *Chrysochromulina.*

The strain of *C. polylepis* that we used is a potential toxic strain. However, toxicity is not present permanently in this species but needs to be triggered (e.g., by phosphorus limitation; Edvardsen et al. 1990). Given the positive effects on copepod reproduction at times when *Chrysochromulina* was abundant, we assume that it was not toxic in this experiment.

Interactions of omnivores with their prey—According to IGP theory, coexistence of IG predator and prey is facilitated if the IG predator gains substantially from feeding on the IG prey and therefore feeds preferentially on this prey relative to the common basal resource. At the same time, the IG prey should be better at exploiting the common resource than the IG predator (Holt and Polis 1997). Both are true in the copepod–*Oxyrrhis* interaction. *Oxyrrhis* was more effective in exploiting *Rhodomonas* and *Chrysochromulina* than were the copepods. At the same time, *Oxyrrhis* enhanced reproduction in the copepods. Similarly, in the *Chrysochromulina*–picophytoplankton interaction, the picophytoplankton was most likely the superior competitor for dissolved nutrients (Mann and Lazier 1996) and probably served as an important source for mineral nutrients to the mixotroph. The same is likely to be true for heterotrophic bacteria that have not been recorded here (Stibor and Sommer 2003). Our results, however, do not support predictions from IGP theory regarding the effect of productivity on the relative abundances of an IG predator and its prey. *Oxyrrhis*, which acted as an IG prey between calanoid copepods and nanophytoplankton, persisted equally well under high and low productivity. Similarly, negative effects of *Chrysochromulina* on the picophytoplankton did not vary significantly between both nutrient regimes. The deviations from the predictions could be due to the high complexity of the food webs. In the copepod–*Oxyrrhis* interaction, *Oxyrrhis* fed on the common resource nanophytoplankton and on smaller organisms that were not accessible to the copepods. In the *Chrysochromulina*–picophytoplankton interaction, the IG predator *Chrysochromulina* was itself prey for higher trophic levels. Our results indicate that the interconnection of IG predators and IG prey in complex food webs might allow for their coexistence on a broader range of system productivity than predicted by IGP theory (Holt and Polis 1997; Diehl and Feissel 2000).

Generality of the results—C. polylepis is a common representative of bacterivorous phytoflagellates, a widespread group in marine and freshwater systems (Riemann et al. 1995). The contamination with HNFs other than *Cafeteria* highlights the generality of the observed effects because *Chrysochromulina* had to compete with a variety of heterotrophic nanoflagellates. In combination with the common distribution of mixotrophic flagellates, our results challenge the traditional view that bacteria and picophytoplankton are mainly consumed by heterotrophic protists (Azam et al. 1983). Furthermore, the effects of mixotrophs on a sum parameter such as seston stoichiometry have not yet been described and need further investigation. The discrepancy between results from field and experimental studies like this one might partly be explained by difficulties in estimating abundances and the effect of mixotrophs in the field. In most field studies, protists are only classified according to their pigmentation as auto- or heterotrophs. A quantification of mixotrophs in natural phytoplankton assemblages requires incubation of samples with fluorescent tracers in combination with epifluorescence microscopy or flow cytometry (Kemp et al. 1993). By this method, an underestimation of their real abundances is likely obtained because it is improbable that all potential mixotrophic flagellates will have ingested tracers and therefore be labeled simultaneously (Boraas et al. 1992). Our results show also that mixotrophs are not restricted to oligotrophic systems because they had a similar effect under both nutrient regimes. Thus, not biomass limitation of the system, but growth rate limitation because of low concentrations of dissolved nutrients seems to favor algal mixotrophy (Rothhaupt 1996; Ptacnik et al. unpubl. data). Limitation of dissolved nutrients might even occur in highly productive systems as a result of thermal stratification (Mann and Lazier 1996) or, especially in some freshwater and coastal systems, high influx of dissolved organic carbon that favors bacterial productivity (Isaksson et al. 1999; Ptacnik et al. unpubl. data).

Larger mixotrophs, such as many dinoflagellates and some ciliates, are mainly algivorous and might have different effects on system productivity. According to a theoretical model by Stickney et al. (2000), algivorous mixotrophic flagellates should reduce overall productivity.

Negative effects of IG prey on IG predators, as predicted by the IGP theory, were observed in microbial food webs where IG predators and prey were represented by heterotrophic protists (Stoecker and Evans 1985; Diehl and Feissel 2000) but do not seem to be important in the interaction between microzooplankton and calanoid copepods. The functional role of bacterivorous microzooplankton as a link between picoplankton and calanoid copepods is evident. Our results show that also microzooplankton, which reduce potential prey for calanoid copepods, still might improve the copepods' food environment. If phytoplankton growth is nutrient limited, grazing by microzooplankton might additionally influence the nutritional value of the phytoplankton for metazoan grazers, because nutrient remineralization by the

microzooplankton should result in a higher cell quota of the limiting nutrient in the corresponding phytoplankton (Sterner and Elser 2002). Furthermore, essential organic substances such as polyunsaturated fatty acids reach higher concentrations in faster growing algae (Otero et al. 1997). Thus microzooplankton might enhance the food environment for calanoid copepods, even when reducing numbers of potential prey.

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