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Changes in the phytoplankton community and microbial food web of Blanes Bay (Catalan Sea, NW Mediterranean) under prolonged grazing pressure by doliolids (Tunicata), cladocerans or copepods (Crustacea)

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ABSTRACT: We report how different zooplankton groups (doliolids, cladocerans and copepods) are able to influence the coastal pelagic food web, including the microbial food web, in waters of the NW Mediterranean. We studied the effect of grazing and of grazing-induced nutrient recycling mediated by different types of zooplankton grazing on a natural phytoplankton community. Experiments were conducted in semicontinuous 2-stage chemostats. The 1st stage vessels contained seawater from Blanes Bay, Spain (NW Mediterranean) including its natural phytoplankton community; the 2nd stage vessels contained the same seawater and copepods, cladocerans or doliolids. At daily intervals we transferred part of the medium from the 2nd to the 1st stage flasks, which contained ungrazed algae and excreted nutrients. In this way, the zooplankton could influence phytoplankton dynamics both by selective grazing and by differential excretion of limiting nutrients. In the 2nd stage flasks grazing changed the algal community composition. Doliolids and cladocerans promoted the growth of large algae and copepods shifted the size spectrum towards small sizes. This effect was transferred to the 1st stage flasks. Doliolids, cladocerans and copepods also affected the microbial food web in different ways. Size-selective grazing led to differences in the nanoplankton concentrations. These in turn affected bacterial concentrations in a trophic cascade. The potential to modify a given algal population increased with increasing selectivity of the grazer.

KEY WORDS: Doliolids · Cladocerans · Copepods · Grazing · Marine pelagic food web · Microbial food web · Trophic cascade

INTRODUCTION

The grazing behaviour of herbivorous mesozooplankton is one of the critical factors structuring pelagic food webs. Herbivores distribute the organic matter synthetized by autotrophs to higher trophic levels. In spite of some recent controversy (Miralto et al. 1999, Tang & Dam 2001), the energy flow from diatoms via crustaceans to fishes is considered particularly efficient (Cushing 1975, Officer & Ryther 1980, Iverson 1990, Sommer et al. 2002). In contrast, gelatinous zooplankton are considered a poor food base for commercial fish stocks (Verity & Smetacek 1996), due to their high volume to plasma ratio and their low protein content (Cushing 1975).

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There are many studies concerning grazing by marine copepods and their influence on the marine pelagic food web structure (e.g. Kiørboe 1998). Gelatinous mesozooplankton and cladocerans are poorly investigated in this context, although seasonally they may dominate zooplankton communities at times (e.g. Alldredge & Madin 1982, Deibel 1982a,b, 1998, Crocker et al. 1991, Paffenhöfer et al. 1991, Andreu & Duarte 1996).

Besides exerting direct grazing pressure, zooplankton may also influence the phytoplankton community indirectly (Gismervik et al. 1996 review, Andersen 1997). Whilst feeding on algae, herbivores release nutrients through excretion and sloppy feeding. The regeneration of dissolved nutrients may influence the gross growth rate of the algal community. A changing nutrient-stoichiometry can alter its composition (e.g. Officer & Ryther 1980, Tilman 1982, Sommer 1983, 1994a, 1996, 1998a, Tilman et al. 1986, Hessen & Andersen 1992, Escaravage et al. 1996, Schöllhorn & Granéli 1996). This might feed back on the competition within the herbivorous zooplankton and affect the energy transfer in the pelagic food web (e.g. Sommer 1998b).

To study the effects of grazing and grazing-induced nutrient regeneration, we conducted experiments with mesozooplankton from Blanes Bay (Catalan Sea, NW Mediterranean) feeding on a natural phytoplankton assemblage. Three zooplankton groups dominated in Blanes Bay in summer: copepods, cladocerans and doliolids. Short-term grazing experiments (several hours) with these zooplankton groups showed that they differ in their size preference for algae; therefore, they can influence the competition between different-sized algal groups (Katechakis 1999). This makes them well-suited for longer experiments (several weeks) to investigate how copepods, cladocerans and doliolids influence the algal community over several phytoplankton generations.

MATERIALS AND METHODS

Experimental setup. Experiments were performed in semicontinuous 2-stage chemostats, consisting of 600 ml tissue culture flasks. The 1st stage flasks were filled with the natural phytoplankton community occurring in summer in Blanes Bay (Catalan Sea, NW Mediterranean, 42° 18′ 26″ N, 3° 18′ 11″ E); water was filtered through a plankton net with a mesh size of 100 µm to exclude mesozooplankton. To the 2nd stage flasks (reaction chambers) we added 20 copepods (*Acartia* sp.), 20 cladocerans (*Penilia avirostris*) or 15 doliolids (solitary gonozooids of *Doliolum denticulatum*), at higher densities than those in summer in Blanes Bay (natural densities: 500 to 780 copepods m^{-3}, 750 to 1250 cladocerans m^{-3}, 90 doliolids m^{-3}: Andreu & Duarte 1996). We were careful to incubate similar biovolumes of grazers in the various flasks. We estimated biovolumes from size measurements. All treatments were replicated 3 times, including controls without grazers. The replicates were placed randomly in a water bath at a temperature between 21 and 23°C. The *in situ* surface temperature in Blanes Bay was 25 to 26°C (measured with a WTW LF 20 temperature sensor). The 1st stage flasks were ventilated with air pumps and illuminated with 6 fluorescent tubes (3× Osram light code 77, 3× Osram light code 21-840, 36 W each). The reaction chambers remained dark and were not ventilated—preliminary experiments had shown that bubbling affected especially cladocerans and doliolids adversely. We took 150 ml from the 1st and 2nd stage flasks daily (dilution rate, $D = 0.25\ d^{-1}$). The 150 ml from the 1st stage flasks were transferred to the 2nd stage flasks. Of the 150 ml taken from the 2nd stage flasks, 75 ml were returned to the 1st stage flasks, together with uneaten algae and recycled nutrients but without transferring mesozooplankton grazers; 75 ml were used for sampling or discarded. Sampling was done 4 times during the experiment: at the beginning, after 6 d, after 12 d and at the end. Sampling of 1st stage flasks after 6 d and after 12 d resulted in dilution rates higher than $0.25\ d^{-1}$; however we estimated this to be no problem taking into account the duration of the experiment. Sampling at the beginning and at the end did not influence the dilution rate. The 75 ml deficits in 1st stage flasks were made up with fresh medium (Fig. 1) consisting of sterile-filtered seawater (0.2 µm cellulose-acetate filters) enriched with nutrients (N, 21 µM: 50% $NaNO_3$ and 50% NH_4Cl; P, 1 µM: $Na_2HPO_4 \cdot 2H_2O$; Si, 7 µM: $Na_2O_3Si \cdot 5H_2O$), which is similar to the *in situ* supply from natural terrestial and human sources during summer in Blanes Bay (Y. Olsen unpubl. data). During the experimental period of 17 d we visually controlled whether grazers were intact several times a day by observing their swimming behaviour in the flasks. Injured individuals were replaced if necessary. Animals were not reproducing during the experiment.

Sample preparation and analysis. The recirculating design permitted the zooplankton to influence the phytoplankton community in 2 ways—directly through grazing impact and indirectly through excretion of limiting nutrients. To determine grazer-induced changes in abundance, species composition, biovolume and the biomass of the nano- and microplankton, we preserved samples with Lugol's iodine (5 g I_2 + 10 g KI ad 100 ml aq. dest.). We counted the samples using an inverse microscope (Leica DMIL; Utermöhl 1958). If present, we counted at least 400 cells of each species to

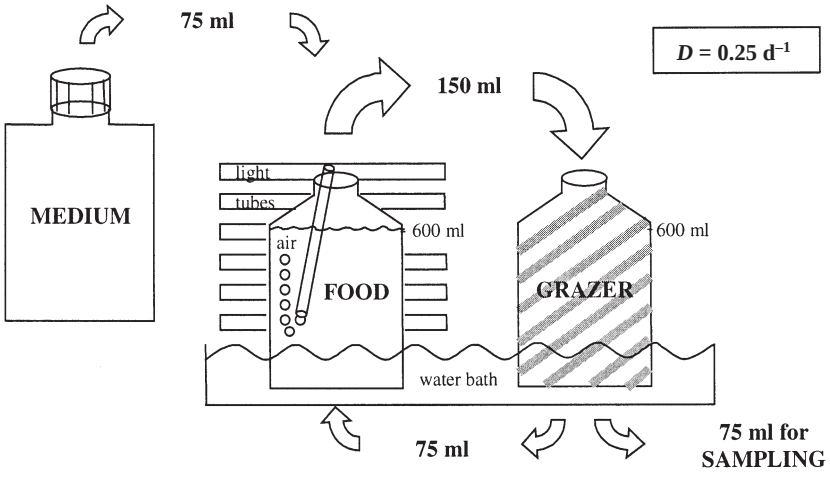

Fig. 1. Scheme illustrating the experimental setup (for details see 'Materials and methods'). *D*: dilution rate

achieve an error of <10% (Lund et al. 1958). Biovolumes were calculated using the equations of Hillebrand et al. (1999); for this purpose we measured the linear dimensions of 20 specimens of each species. Carbon contents were estimated after Strickland & Parsons (1972).

Booth et al. (1982) and Reid (1983) criticized the Utermöhl method, suggesting it underestimates pico- and small nanoplankton abundances drastically. Therefore we determined the abundances of bacteria, naked flagellates <5 µm and dinoflagellates <10 µm by staining with DAPI (4,6-diamidino-2-phenylindol) (Porter & Feig 1980). We fixed samples in formalin (final concentration: 2%) and stained them with a final concentration of 1.76 µg DAPI ml^{-1} for bacteria and 2.45 µg DAPI ml^{-1} for flagellates. After 10 min, the samples were filtered onto black 0.2 µm polycarbonate filters (Millipore) and 0.8 µm filters (Nuclepore), respectively. Filters were rinsed with 5 ml washing solution (sterile filtered tap water, 2% formaldehyde). Counts were done using an epifluorescence microscope (Leitz DMRB) equipped with a blue light and an UV-light filter set. For bacteria, at least 400 cells of each morphotype were enumerated if present. Bacteria attached to particles were counted as 'particle-bound bacteria cells', independent of particle size and abundance measurements. Naked flagellates and dinoflagellates were divided into 3 size classes: 2.5 to 5 µm, >5 to 7.5 µm and >7.5 to 10 µm, of which at least 400, 200 or 100 cells, respectively, were counted. To calculate biovolumes we measured the linear dimensions of 50 specimen of each morphotype (Fuhrmann & Azam 1980, Bjørnsen 1986). Flagellate biovolumes were calculated on the base of the respective interval means of every size class (3.75, 6.3, 8.8 µm). The carbon content of bacteria was calculated by multiplying cell numbers with 23.3 fgC $cell^{-1}$ (Simon & Azam 1989). Flagellate biomass was estimated with 0.22 pgC $µm^{-3}$ according to Bøsheim & Bratbak (1987), that of ciliates with 0.15 pgC $µm^{-3}$ (DeBiase et al. 1990). Moreover, under blue light stimulation, the differentiation of autotrophic cells (chlorophyll *a*: red autofluorescence) and heterotrophic cells (green coloration) was possible, as well as the detection of cyanobacteria (chl *a* + accessory phycobilines: yellow-orange coloration). DAPI-countings were done for 1st stage flasks.

Dissolved inorganic nutrients were analyzed with a continuous flow analyser using the methods of Grasshoff et al. (1983) for silicate, nitrate, ammonium and phosphate. For the determination of particulate carbon and nitrogen we filtered samples onto precombusted Whatman GF/C filters and measured them with a Fisons CN-analyser (NA 1500N).

Similarities between the resulting communities at the end of the experiment in the 1st stage flasks were expressed as Euclidean distances (Eq. 1), based on the following groups: cyanobacteria, naked flagellates, ciliates, dinoflagellates, diatoms and amoeba.

$$\Delta_{jk} = \sqrt{\sum_{i=1}^{n} (X_{ij} - X_{ik})^2} \quad (1)$$

where Δ_{jk} = Euclidean distance between Chemostats j and k; X_{ij} = proportion of Group i of total biovolume in Chemostat j (X_{ik} analog) and n = total number of groups. Δ_{jk} increases with increasing n. To compensate for this we calculated the average distance d_{jk} (Eq. 2):

$$d_{jk} = \sqrt{\frac{\Delta_{jk}^2}{n}} \quad (2)$$

Both Δ_{jk} and d_{jk} vary from 0 to +∞; the larger the distance, the less similar are the 2 communities.

Data analysis. For statistical analysis, SigmaStat 2.0 and SPSS 10.0.5 software was used.

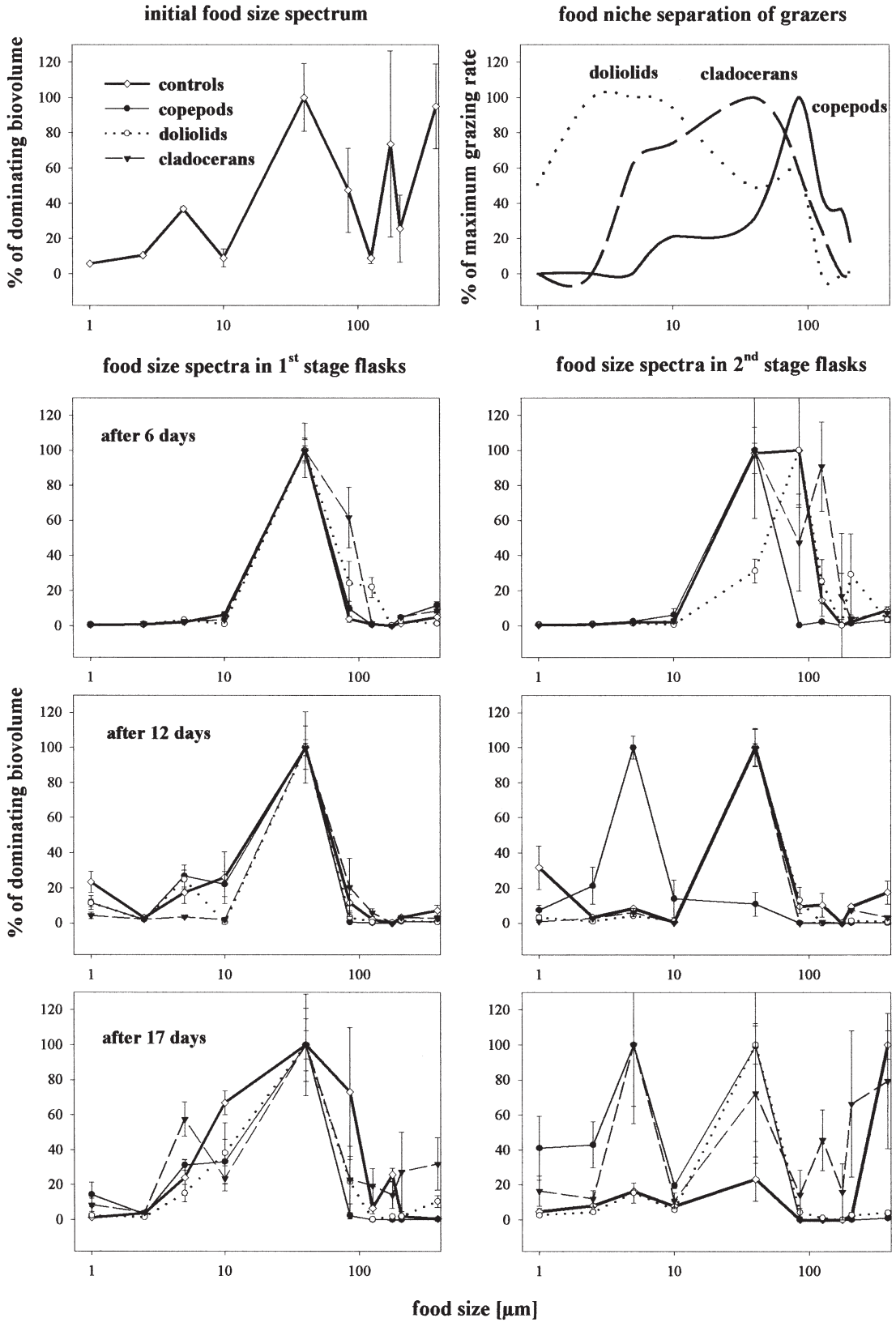

Fig. 2. Changes in the size composition of the summer plankton community of Blanes Bay (NW Mediterranean) under prolonged grazing pressure by doliolids, cladocerans or copepods in semicontinuous 2-stage chemostats (1st stage: food without grazer, 2nd stage: reaction chamber). Top left: initial seawater; top right: grazing spectra of doliolids, cladocerans and copepods as evaluated in grazing experiments (Katechakis 1999). Data points are means of triplicates; error bars represent ±SE of the means. Note logarithmic scale of particle-size axes

RESULTS

Changes in composition of food guilds

Size composition

Fig. 2 shows how the size composition within the food changed under the persisting grazing pressure of copepods, cladocerans or doliolids. We subdivided the plankton community into 9 size classes with mean intervals of 1, 2.5, 5, 10, 40, 85, 125, 175 and 205 µm. Colonial species were assigned to classes according to the biggest linear dimension of colonies. For the following comparisons of size classes we set the biovolume of the most abundant size class at 100% and refer to it as the 'dominating biovolume'.

Initial seawater

The size spectrum of food types initially presented to grazers was dominated by organisms between 15 and 70 µm (for comparitive purposes we set the biovolume at 100 ± 19.2% SE of the means: Fig. 2). Microplankton from 150 to 200 µm and >210 µm made up 73.7 ± 52.7% of the dominating biovolume and 95.0 ± 24.0% of the dominating biovolume, respectively. Among small size classes only individuals around 5 µm were important (36.6 ± 1.4%).

Second stage flasks (reaction chambers)

In 2nd stage flasks copepods, cladocerans and doliolids caused size compositions according to their respective grazing spectra, as evaluated in grazing experiments (Katechakis 1999 and present Fig. 2: right), with the following exceptions: After 17 d, chemostats with cladocerans showed high biovolumes of intermediate food sizes between 15 and 70 µm (72.4 ± 40.0% of the dominating biovolume) compared to controls, and in chemostats with doliolids large food items >100 µm were efficiently reduced. Controls were dominated by intermediate food sizes after 6 d and 12 d. Lastly, organisms >210 µm prevailed in control flasks. Differences among treatments were tested for significance using 2-way ANOVAs with the factor grazer type as a fixed factor and food size as a random factor. For percentages of dominating biovolume original data were arcsine-transformed. The interaction between different grazers and phytoplankton size composition was significant ($p \leq 0.001$, $F_{9,24} = 4.904$).

First stage flasks

Shifts in food size composition were transferred to 1st stage flasks ($p \leq 0.05$, $F_{9,24} = 2.443$) through recurrent inoculation with small amounts of material from the 2nd stage flasks.

Taxonomic composition

Initial seawater: The initial community was dominated by diatoms, ciliates and organisms <5 µm. Dinophyceae and naked flagellates were of little importance. Abundances of amoebae lay below the detection limit initially but became detectable later. The most important species were *Rhizosolenia fragilissima* and *Skeletonema costatum*. Together they accounted for more than 55% of the total food biovolume (for details see Table 2). The whole taxonomic spectrum is listed in Table 1.

Second stage flasks (*reaction chambers*)*:* By Day 6, the taxonomical composition of the various chemostats differed little. Diatoms extended their dominance in all treatments. Compared to the initial community, pico- and nanoplankton showed substantial decreases in all flasks. Ciliates decreased in the copepod and doliolid treatments (Tables 2 & 3). After 12 d the communities had changed radically. In all flasks with grazers, non-siliceous species had become predominant: naked flagellates in chemostats with copepods, dinoflagellates (mainly *Peridinium* sp. accompanied by *Prorocentrum micans*) in those with doliolids or cladocerans. After 17 d, communities with different treatments differed greatly from each other. By Day 12 of the experiment, the prevailing naked flagellates had declined in the copepod chambers, while pico- and nanoplankton <5 µm and *Peridinium* sp. increased. *Peridinium* sp. was also the outstanding taxon under the influence of doliolids. In both chemostats with cladocerans and controls, diatoms gained importance, whereas dinophyceaes declined slightly. For details see Table 3.

First stage flasks: Here the central characteristic was the rise in dinophyceaens at the expense of diatoms. The change was expressed by the shift from *Rhizosolenia* spp. and *Skeletonema costatum* to *Peridinium* sp. and *Prorocentrum micans* as the most important species. Except for cladoceran treatments this was valid for all chemostats, although most evident in copepod systems.

Similarity of communities: The most dissimilar communities resulted from the influence of selective grazers (copepods) on the one hand and unselective filter-feeders (cladocerans or doliolids) on the other hand (Table 4). The latter were more similar to each other. The most similar communities were chemostats with doliolids and those serving as controls.

Changes in composition of microbial food web (1st stage flasks)

Bacteria and cyanobacteria

Solitary bacteria abundances: Solitary bacteria (diameter 0.3 µm, biovolume 0.014 μm^3) increased in all

Table 1. Taxonomic list of all plankton food in chemostat experiments. Biovolumes were calculated using the equations of Hillebrand et al. (1999). Carbon contents were estimated after Strickland & Parsons (1972) for phytoplankton, after Bøsheim & Bratbak (1987) for flagellates and after DeBiase et al. (1990) for ciliates. ANF: autotrophic nanoflagellates; HNF: heterotrophic nanoflagellates

Taxon	Geometrical shape	Cell dimension (µm) biggest extension	Biovolume (μm^3 $cell^{-1}$)	Biomass (pg C $cell^{-1}$)
Picoplankton				
1 µm	Sphere	1	0.52	0.07
Nanoplankton				
2.5 µm	Sphere	2.5	8.2	1.2
5 µm	Sphere	5	65	9.2
Cyanobacteria				
Coccal	Sphere	0.5	0.07	0.01
Filamentous	Cylinder	7.0–140	0.88–17.6	1.4–28
Bacillariophyceae				
Centrales				
Biddulphia sp.	Elliptic prism	15	442	39
Coscinodiscus sp.	Cylinder	12.5–40	920–12566	81–1100
Chaetoceros sp. A	Elliptic prism	5	79	6.9
Chaetoceros sp. B	Elliptic prism	20	707	62
Leptocylindrus sp.	Cylinder	45	884	77
Rhizosolenia deliculata	Cylinder	28	2160	189
Rhizosolenia fragilissima	Cylinder	18–75	344–5890	30–515
Rhizosolenia stolterfothii	Cylinder	38–200	1657–62832	145–5498
Rhizosolenia sp. A	Cylinder	70	3093	271
Rhizosolenia sp. B	Cylinder	100–500	1964–9817	172–859
Skeletonema costatum	Cylinder + 2 halfspheres	7.5–25	94–1104	8–97
Thalassiosira sp.	Cylinder	20	3534	309
Pennales				
Licmophora sp.	Gomphonemoid	75	10000	875
Navicula sp.	Elliptic prism	15	147	13
Nitzschia closterium	Prism on parallelogram	30	94	8.2
Nitzschia longissima	Prism on parallelogram	75	125	11
Nitzschia sp. A	Prism on parallelogram	17.5–30	47–156	4.1–14
Nitzschia sp. B	Prism on parallelogram	70	125	11
Thalassionema nitzschioides	Box	40	785	69
Dinophyceae				
Dinophysiales				
Dinophysis sp.	Ellipsoid	50	10472	1466
Peridiniales				
Ceratium tripos	3 cones + cylinder	50	25000	3500
Gymnodinium sp.	Ellipsoid	10	654	92
Peridinium sp.	Ellipsoid	15–30	1767–9425	247–1319
Prorocentrales				
Prorocentrum micans	Cone + halfsphere	30–50	2209–6283	309–880
Prymnesiophyceae				
Coccolithus sp.	Sphere	7.5	221	31
Phaeocystis pouchetii	Sphere	7.5	221	73
Other flagellates				
ANF spp.	Sphere	2.5–10	8.2–523	1.8–73
HNF spp.	Sphere	2.5–10	8.2–523	1.8–73
Ciliata				
Ciliate sp.	Ellipsoid	25	29452	4123
Amoeba				
Amoeba sp.	Irregular	7.5–15	331–2651	46–371

Table 2. Taxonomic composition (% of total food guild biovolume and SE of means in chemostats) of the food presented to grazers in the initial seawater at the beginning of the experiment

Functional group	%
Pico/nanoplankton <5 µm	15.26 ± 0.87
Diatoms	
Total	57.21 ± 6.70
Skeletonema costatum	20.66 ± 0.38
Rhizosolenia fragilissima	35.21 ± 6.00
Dinophyceae	2.52 ± 1.39
Naked flagellates	2.51 ± 2.54
Ciliates	22.49 ± 5.25
Amoeba	0

treatments. At the end of the experiment, controls showed lower values than chemostats with grazers. Biovolumes were highest in cladoceran-influenced systems followed by those systems affected by doliolids or copepods (Fig. 3). The cell numbers in the 1st stage chemostats differed significantly from each other (1-way ANOVA, $p \leq 0.001$, $F_{4,14} = 16.64$). Post hoc Tukey-test analyses showed a significant difference between the cladoceran and all the other treatments.

Particle-bound bacteria: We could not find any of these in natural seawater; 17 d later, in the chambers with doliolids most bacteria were attached to particles (2.81×10^5 cells ml^{-1}), while the remaining chambers had densities of 55 200 cells ml^{-1} (cladocerans), 51 886 cells ml^{-1} (controls), and 8529 cells ml^{-1} (copepods). Differences among treatments were significant (1-way ANOVA, $p \leq 0.001$, $F_{4,14} = 15.132$). Post hoc Tukey-test analyses showed that the doliolid treatments formed a separate group.

Coccal cyanobacteria abundances: Coccal cyanobacteria (diameter 0.5 µm, biovolume 0.065 µm^3) were below the detection limit in the initial samples and did not occur in chemostats with doliolids. They reached highest abundances (5.66×10^5 cells ml^{-1}) in controls, followed by the treatments with cladocerans and with copepods, in that order (Fig. 3). Chemostats differed significantly from each other (1-way ANOVA, $p \leq 0.05$, $F_{4,14} = 4.109$). Post hoc Tukey-test analyses showed that systems with doliolids and the initial sample represented separate groups.

Filamentous cyanobacteria: These could not be found at the beginning of the experiment but occurred in all treatments at the end. Filamentous cyanobacteria had a diameter of 0.4 µm and covered lengths from 7 to 140 µm in all chemostats. The mean sizes of filamentous cyanobacteria were larger in treatments with doliolids as grazers (length 38.2 ± 1.6 µm SE, biovolume 4.8 ± 0.2 µm^3 SE) than in other treatments (copepods: 29.1 ± 1.8 µm and 3.7 ± 0.2 µm^3, cladocerans: 25.9 ± 1.1 µm and 3.3 ± 0.1 µm^3) and in controls (29.1 ± 0.6 µm and 3.7 ± 0.1 µm^3). Doliolid chambers also contained the most filamentous cyanobacteria (1.48×10^5 µm^3 ml^{-1}), followed by those with cladocerans or copepods and controls. Differences between treatments were significant (1-way ANOVA, $p \leq 0.05$, $F_{(4;14)} = 3.495$). Post hoc Tukey-test analyses showed that doliolid treatments formed a separate group.

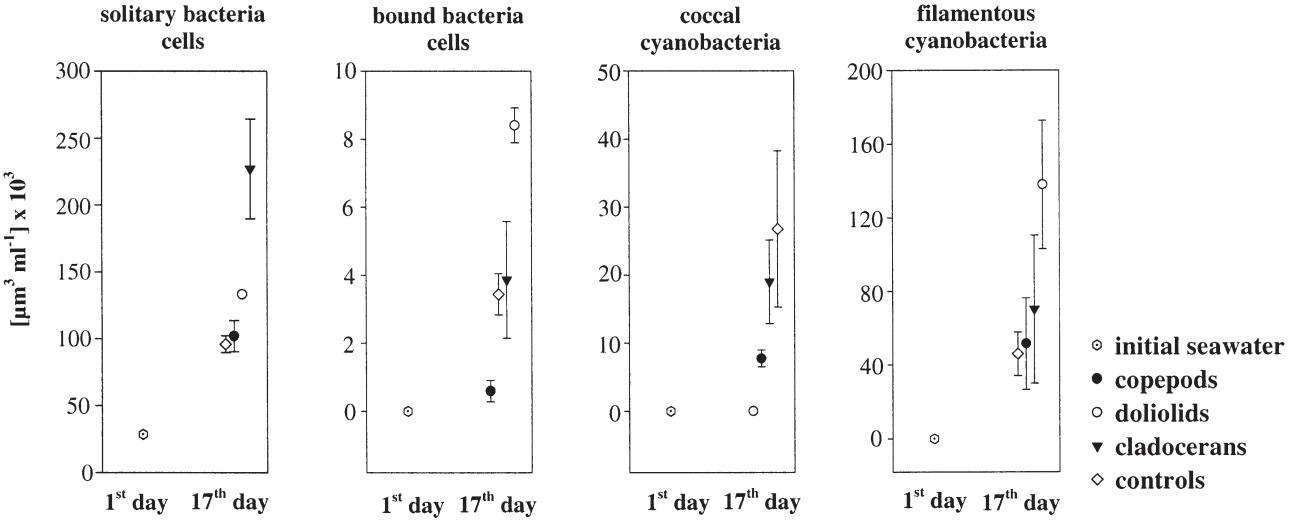

Fig. 3. Changes in the composition of the bacterial community of Blanes Bay (NW Mediterranean) under prolonged grazing pressure by doliolids, cladocerans or copepods (1st stage flasks). Data points are means of triplicates; error bars represent ±SE of the means

Flagellates

We found a significant negative correlation between bacterial abundance (bacteria + coccal cyanobacteria) and the appearance of heterotrophic nanoflagellates (HNF) from 5.1 to 10 µm size (Fig. 4). At the end of the experiment, chemostats with cladocerans as grazers showed the lowest HNF biovolumes (3.68×10^5 µm^3

Table 3. Changes in taxonomic composition (% of total food guild biovolume and SE of the means in chemostats) of the summer plankton community in Blanes Bay (NW Mediterranean) under prolonged grazing pressure by doliolids, cladocerans or copepods in semicontinuous 2-stage chemostats (1st stage: food without grazers, 2nd stage: reaction chamber)

Functional group	1st stage after			2nd stage after		
	6 d	12 d	17 d	6 d	12 d	17 d
Copepods						
Pico/nanoplankton <5 µm	2.19 ± 0.27	9.23 ± 1.59	5.60 ± 1.20	3.55 ± 0.94	16.91 ± 5.62	60.51 ± 18.54
Diatoms total	59.33 ± 3.29	5.89 ± 1.36	3.40 ± 1.60	88.39 ± 2.97	7.84 ± 4.28	3.98 ± 1.07
Skeletonema costatum	3.75 ± 1.99			33.09 ± 3.33		
Rhizosolenia fragilissima	53.52 ± 4.79			46.01 ± 4.42		
Dinophyceae total	15.13 ± 3.48	63.40 ± 7.63	52.33 ± 11.22			28.92 ± 18.68
Peridinium sp.		54.13 ± 2.25	50.14 ± 10.70			0.75 ± 0.61
Prorocentrum micans		2.90 ± 0.20	1.64 ± 1.06			
Naked flagellates	4.44 ± 1.34	21.38 ± 7.28	31.35 ± 7.52	5.18 ± 3.19	73.00 ± 10.46	6.47 ± 0.50
Ciliates	18.91 ± 4.38	0	0	1.68 ± 0.73	0	0
Amoeba	0	0.10 ± 0.09	0.66 ± 0.43	0	0	0
Doliolids						
Pico/nanoplankton <5 µm	2.79 ± 0.65	9.43 ± 1.86	3.63 ± 0.38	1.61 ± 0.54	5.57 ± 1.52	5.02 ± 1.06
Diatoms total	90.77 ± 1.70	18.22 ± 1.51	26.97 ± 11.86	86.59 ± 0.79	13.49 ± 3.22	12.89 ± 2.07
Skeletonema costatum	11.03 ± 5.40		18.45 ± 10.49	56.90 ± 11.26		
Rhizosolenia fragilissima	35.59 ± 2.67		7.04 ± 1.06	27.80 ± 12.34		
Dinophyceae total	3.93 ± 1.39	54.66 ± 3.26	43.63 ± 0.24	7.50 ± 2.37	64.20 ± 7.99	66.20 ± 6.79
Peridinium sp.		47.19 ± 4.57	38.31 ± 1.95		61.08 ± 8.78	59.86 ± 9.35
Prorocentrum micans		7.60 ± 3.92	6.84 ± 2.48		2.72 ± 0.91	6.34 ± 2.68
Naked flagellates	0.48 ± 0.16	16.84 ± 3.60	25.73 ± 12.49	0.33 ± 0.15	0.58 ± 0.38	14.42 ± 4.53
Ciliates	2.03 ± 1.66	0	0	3.97 ± 3.24	0	0
Amoeba	0	0.85 ± 0.70	0.03 ± 0.02	0	16.17 ± 7.31	1.49 ± 0.86
Cladocerans						
Pico/nanoplankton <5 µm	2.26 ± 0.36	6.26 ± 2.12	4.78 ± 0.39	0.98 ± 0.20	4.66 ± 0.09	14.83 ± 2.33
Diatoms total	60.58 ± 3.52	26.69 ± 10.76	41.15 ± 5.26	66.52 ± 6.07	15.66 ± 7.78	54.56 ± 5.05
Skeletonema costatum	6.20 ± 2.13		8.73 ± 6.67	56.90 ± 11.26		8.78 ± 3.15
Rhizosolenia fragilissima	51.60 ± 1.23		18.68 ± 8.86	27.80 ± 12.34		34.27 ± 2.73
Dinophyceae						
Total	19.23 ± 6.26	65.00 ± 12.31	28.11 ± 7.28	6.44 ± 1.61	54.65 ± 21.56	11.56 ± 7.93
Peridinium sp.		63.24 ± 11.85			54.23 ± 21.55	
Prorocentrum micans		1.83 ± 0.47			0.33 ± 0.05	
Naked flagellates	1.73 ± 0.40	1.83 ± 0.71	22.89 ± 4.01	0.63 ± 0.25	0.18 ± 0.10	16.78 ± 10.03
Ciliates	16.21 ± 4.65	0	0	25.43 ± 6.32	22.13 ± 12.78	0
Amoeba	0	0.33 ± 0.08	3.07 ± 2.44	0	2.74 ± 1.01	2.27 ± 1.00
Controls						
Pico/nanoplankton <5 µm	2.57 ± 0.08	16.48 ± 2.30	6.16 ± 0.40	1.25 ± 0.21	20.61 ± 7.12	15.80 ± 0.77
Diatoms total	64.89 ± 5.45	19.22 ± 2.70	22.33 ± 4.84	67.58 ± 10.54	44.10 ± 3.29	58.16 ± 3.35
Skeletonema costatum	16.90 ± 10.93			30.23 ± 10.09	14.88 ± 7.12	0.31 ± 0.02
Rhizosolenia fragilissima	42.49 ± 16.00			35.07 ± 1.50	27.18 ± 6.86	55.63 ± 3.66
Dinophyceae total	17.25 ± 1.45	43.47 ± 5.36	43.99 ± 5.32	3.90 ± 1.07	31.59 ± 6.93	21.97 ± 1.34
Peridinium sp.		30.89 ± 4.64	26.83 ± 2.81		21.36 ± 8.30	
Prorocentrum micans		12.77 ± 1.45	13.38 ± 0.24		9.77 ± 0.73	
Naked flagellates	4.60 ± 1.43	18.34 ± 7.61	27.53 ± 0.08	0.90 ± 0.22	1.62 ± 1.24	4.08 ± 1.24
Ciliates	10.69 ± 4.44	0.30 ± 0.24	0	26.36 ± 9.12	0	0
Amoeba	0	2.19 ± 1.13	0	0	2.07 ± 1.26	0

Table 4. Similarity between plankton communities at the end of the experiment expressed as average distances, d_{jk} (see 'Materials and methods': Eq. 2)

1st stage	Copepods	Doliolids	Cladocerans	Controls
Copepods	–	11.09	18.91	8.95
Doliolids		–	8.76	2.28
Cladocerans			–	10.32
Controls				–

ml^{-1}) and highest bacterial biovolumes (2.50×10^5 µm^3 ml^{-1}). In contrast, treatments with copepods showed the highest HNF biovolumes (1.05×10^6 µm^3 ml^{-1}) and lowest bacterial biovolumes (1.10×10^5 µm^3 ml^{-1}), with even fewer bacteria than the controls (1.26×10^5 µm^3 ml^{-1}). Under the influence of doliolids HNF had lower biovolumes (6.69×10^5 µm^3 ml^{-1}) and bacteria higher biovolumes (1.42×10^5 µm^3 ml^{-1}) than treatments with copepods and controls.

The ratio of autotrophic to heterotrophic flagellates (5.1 to 10 µm size) increased during the experimental term compared to the initial seawater (21% autotrophs). The increase was significant for all treatments (1-way ANOVA, $p \leq 0.05$, $F_{4,14} = 6.866$). Chemostats with doliolids had the highest proportion of autotrophs (63 ± 4% SE of the means, cladocerans: 43 ± 12%, copepods: 31 ± 4%, controls: 27 ± 5%). Post hoc Tukey-test analyses showed that systems with doliolids formed a separate group (Fig. 5).

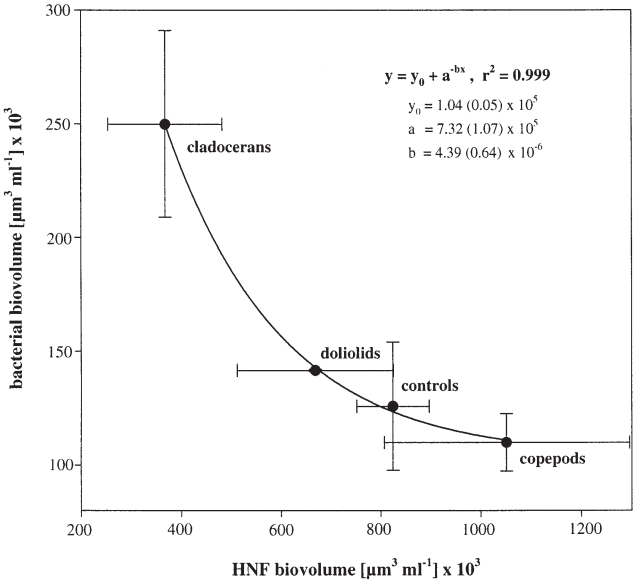

Fig. 4. Nonlinear regression analysis of heterotrophic nanoflagellate (HNF) biovolume (5.1 to 10 µm size) on bacterial biovolume (bacteria + coccal cyanobacteria). Values in parentheses are SE, $p < 0.05$. Data points are means of triplicates; error bars represent ±SE of the means

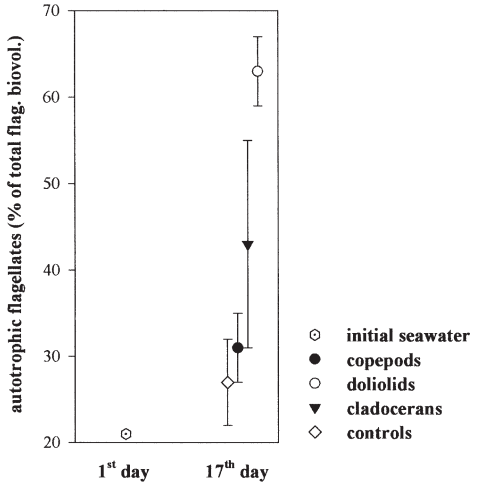

Fig. 5. Changes in the density of autotrophic flagellates as percentage total flagellate biovolume (5.1 to 10 µm size). Data points are means of triplicates; error bars represent ±SE of the means

Changes in nutrient regime

C:N ratios

The C:N ratios of copepods, cladocerans and doliolids did not differ significantly from each other (1-way ANOVA, $p = 0.576$, $F_{2,7} = 0.168$). At the end of the experiment, the particulate C:N ratios in 1st stage flasks approached the Redfield ratio (106:16, Redfield 1958; cf. Copin-Montegut & Copin-Montegut 1983) best in controls (117:16) and deviated most from it in treatments with cladocerans (141:16). Differences were not significant (1-way ANOVA, $p = 0.681$, $F_{4,13} = 0.586$). For results see Table 5.

Dissolved nutrients

At the end of the experiment silicate, nitrate, ammonium and phosphate concentrations were higher in chemostats influenced by grazers than in controls (Fig. 6). In 1st stage flasks differences were only significant for silicate (1-way ANOVA $p \leq 0.05$, $F_{3,9} = 15.176$) between copepod and doliolid treatments, between doliolid treatments and controls, and between controls and cladoceran treatments (post hoc Tukey-test $p < 0.05$). In 2nd stage flasks differences were only significant for ammonium (1-way ANOVA, $p \leq 0.001$, $F_{3,9} = 45.636$). Copepod treatments showed higher values than all other treatments and the ammonium concentrations in the cladoceran treatments were higher than in controls. These differences were significant (post hoc Tukey-test, $p < 0.05$).

Table 5. C:N ratio of grazers and of the summer plankton community in Blanes Bay (NW Mediterranean) before (initial seawater) and after prolonged grazing pressure by doliolids, cladocerans or copepods. Values in parentheses are SE of the means

Sample	C:N (SE)
Food guild	
Initial seawater	9.27 (0.36)
Copepod-chemostats	8.64 (0.40)
Doliolid-chemostats	8.72 (1.51)
Cladoceran-chemostats	8.79 (0.17)
Controls	7.32 (1.10)
Grazer	
Copepods	4.57 (0.25)
Doliolids	4.50 (0.05)
Cladocerans	4.84 (0.23)
Redfield ratio	6.63

At the end of the experiment, Si:N stoichiometry was more or less in accordance with the Redfield ratio (1:1 in all 1st stage flasks and in 2nd stage control flasks), but lower in chambers with grazers (Fig. 7). The N:P ratio was much lower than the Redfield ratio (16:1) in all 1st stage flasks. In 2nd stage flasks grazer exudates effected higher N:P values. Systems influenced by copepods corresponded best with the Redfield ratio. Chemostats with doliolids or cladocerans caused lower N:P ratios, although they were higher than those found in controls.

DISCUSSION

We used semicontinuous, re-circulating, 2-stage chemostats to study how doliolids, cladocerans and copepods can influence the phytoplankton community structure in Blanes Bay (Catalan Sea, NW Mediterranean). Semi-continuous chemostats permit a good approximation to the results gained from continuous designs (Sommer 1985), and have been proved to be effective tools in testing for direct and indirect effects of herbivore grazing on algae (e.g. Sommer 1988, 1998b).

Changes in composition of food guilds

Phytoplankton between 15 and 70 µm dominated both stages of all chemostats influenced by herbivores. This is surprising, because food size spectra of all grazer types span this size class (Katechakis 1999). Possible reasons could be unpalatability of organisms, toxicity, higher competitive abilities than other phytoplankton, or allelopathic effects. We will discuss these alternatives below.

Experiments were conducted in late August when copepods, cladocerans and doliolids are present in high abundances in Blanes Bay (500 to 780, 750 to 1250 and 90 ind. m^{-3}, respectively: Andreu & Duarte 1996), and the clear-water stadium has almost been reached (Mura et al. 1996, Satta et al. 1996). Hence, the natural (initial) community may already have been adapted to high grazing pressure. On the other hand, mostly Bacillariophyceae, mainly *Skeletonema costatum* and *Rhizosolenia fragilissima*, comprised the size class between 15 and 70 µm until Day 6 (Table 2) and both species are considered food suitable for copepods (e.g. Paffenhöfer & Knowles 1978, Ryther & Sanders 1980), cladocerans and doliolids (Katechakis 1999).

By Day 12, Dinophyceae dominated the same size class, principally *Peridinium* sp. and *Prorocentrum micans*. Both taxa are classified as potentially toxic. This possibly affected grazers adversely and benefited dinoflagellates compared to other plankton. Toxicity can be a potent instrument against grazers, particularly against selective feeders such as copepods (Granéli 1990). As we did not test for toxicity we do not know if species were really toxic. However, we did not observe any obvious detrimental effect on the grazers.

Another explanation for the observed dominance of dinoflagellates could have been allelopathic effects on other phytoplankton species. To test for allelopathy was not a topic of this work. Also, we could find no reports about possibly allelopathic effects of *Peridinium* or *Prorocentrum* species in the literature.

Organisms of intermediate size may also profit, by being (1) too large to be fed on by protozoans, but (2) small enough to be better competitors than larger algae for nutrients.

Only in 2nd stage flasks of the control treatments did large phytoplankton (>210 µm) dominate at the end of the experiment (Fig. 2). These were exclusively diatoms of the genus *Rhizosolenia* and sporadically *Nitzschia* spp. colonies (Table 1). They may have developed because of the high Si:N ratios in 2nd stage flasks in the control treatments (Fig. 7). A similar rise in large (and, due to their size, inedible) algae was observed during mesocosm experiments with high nutrient supplies in Blanes Bay (Y. Olsen unpubl. data). Differences between the 1st stage and 2nd stage control flasks may have resulted from higher Si concentrations in the 2nd stage flasks (Fig. 6). In addition it is conceivable that Bacillariophyceae benefited from dark incubation in the 2nd stage flasks, which contained high cell quotas of nitrogen and phosphorus. However, measurements cell quotas for different marine phytoplankton (Y. Olsen unpubl. data) provide no evidence for this assumption.

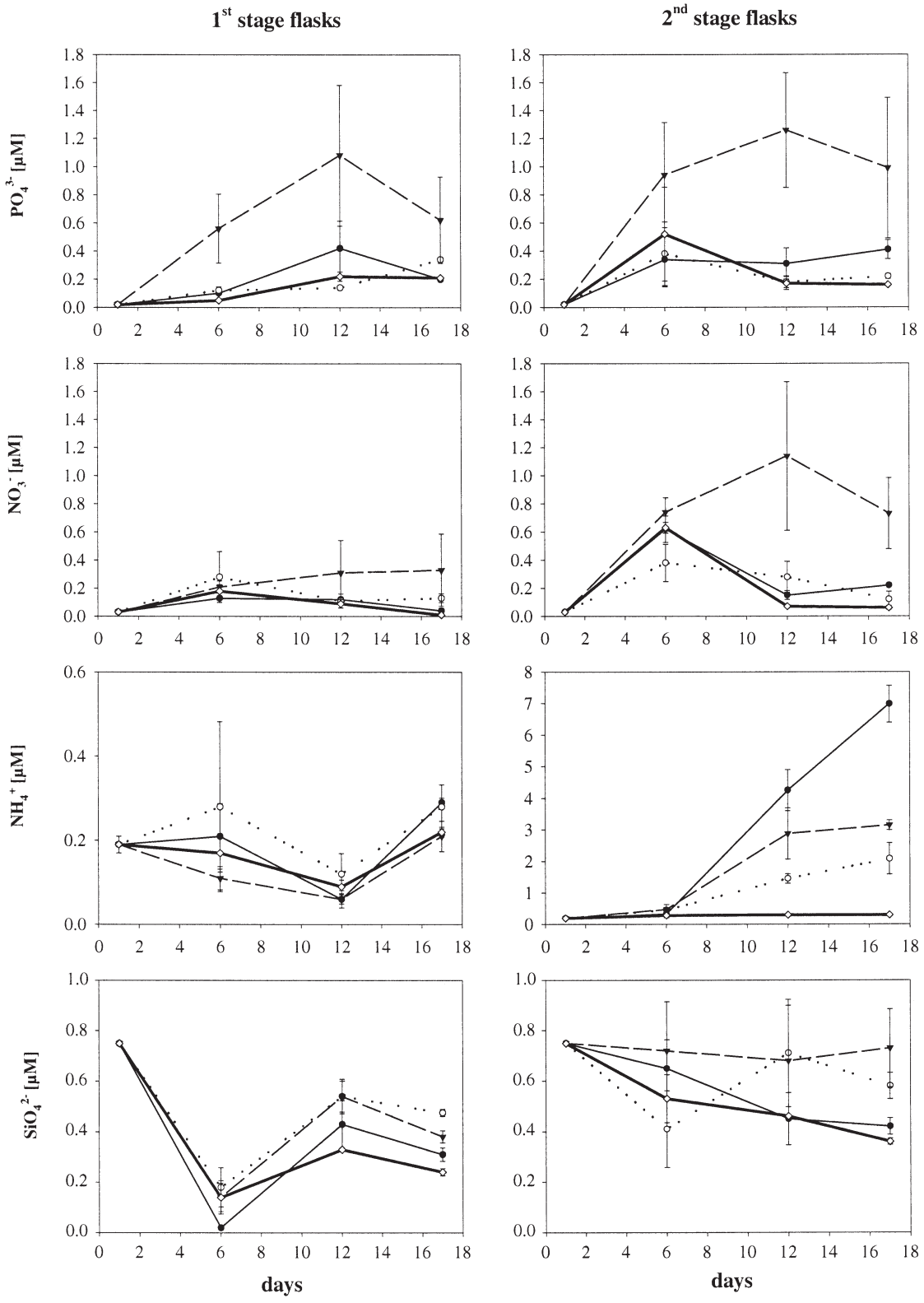

Fig. 6. Changes in the dissolved nutrient concentrations in 1st stage (left) and 2nd stage (right) flasks during the course of the experiment. Data points are means of triplicates; error bars represent ±SE of the means

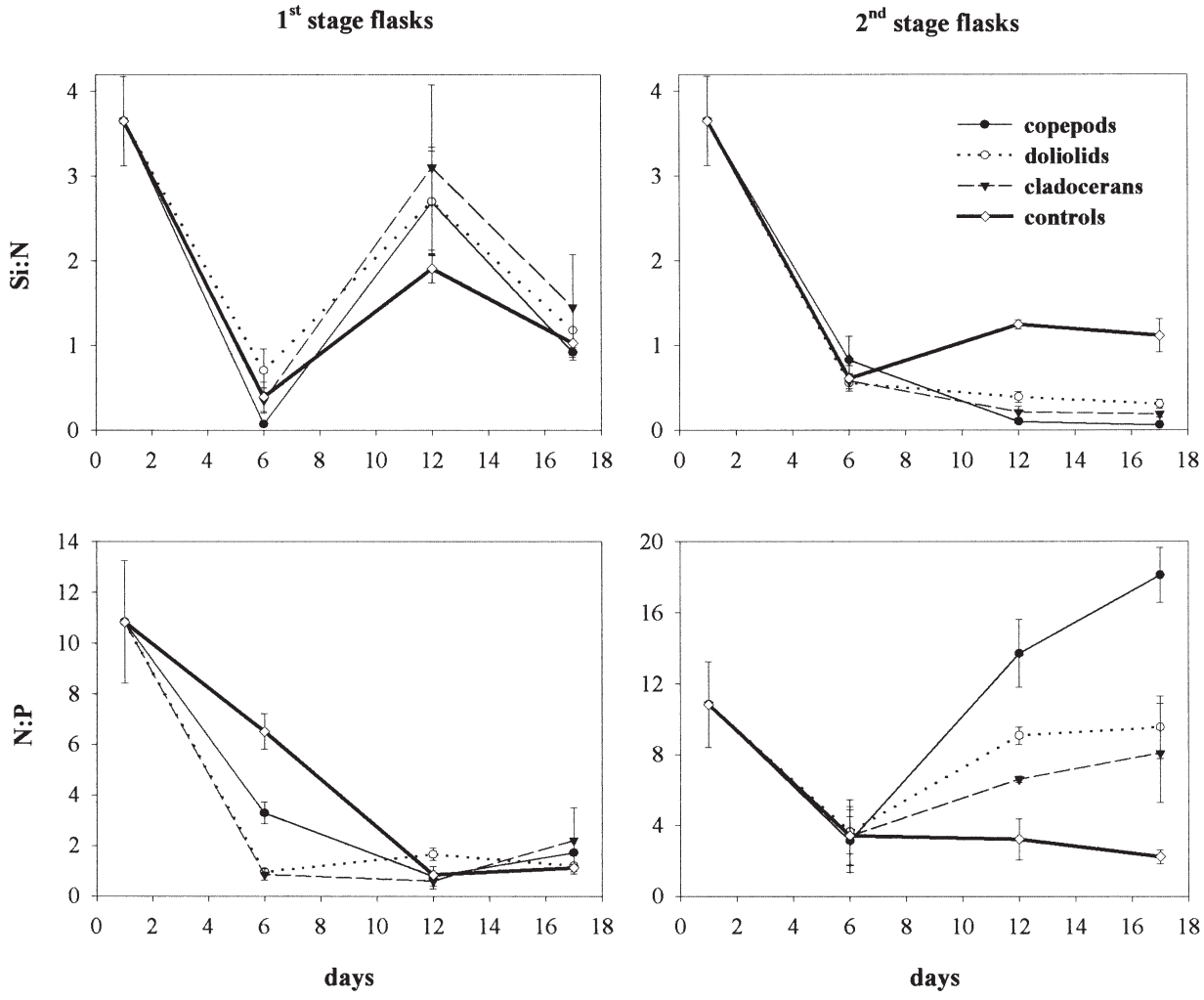

Fig. 7. Changes in the Si:N and N:P stoichometry in 1st stage (left) and 2nd stage (right) flasks during the course of the experiment. Data points are means of triplicates; error bars represent ±SE of the means

Various studies have suggested that competition between diatoms and flagellates is determined by the stoichiometry of dissolved nutrients. If Si is not limiting, diatoms usually dominate over non-siliceous species (e.g. Officer & Ryther 1980, Tilman et al. 1986, Cadée & Hegeman 1991, Sommer 1994a,b,c, 1998a,b). In the present study, grazers have influenced the nutrient regime in reaction chambers through their excretions; by increasing the dissolved nutrient concentrations, they changed the stoichiometry in relation to controls. This effect was weaker in 1st stage than in 2nd stage flasks. The stoichiometry of 1st stage flasks deviated little from that in controls at the end of the experiment (Fig. 7). It seems that the food organisms immediately assimilated the added dissolved nutrients indicating that their growth was nutrient-limited. The C:N data (Table 5) support this assumption. Biomass stoichiometry is an indicator of nutrient status (Droop 1974, 1975, Healey 1978, Healey & Hendzel 1980). All the plankton communities exposed to grazers had C:N ratios >8.3, indicating moderate N-limitation according to Healey & Hendzel (1980) and Hecky et al. (1993).

In size classes smaller and larger than 15 to 70 µm, grazers supported particle sizes outside their specific grazing spectra (Fig. 2). Unexpected distributions compared with controls occurred in chemostats influenced by doliolids, where large food-item levels (>100 µm) decreased, and in treatments with copepods, where picoplankton levels decreased. Doliolids efficiently decimated organisms >100 µm, although the maximum food size ingestible for *Doliolum denticulatum* was 75 µm in grazing experiments with natural plankton communities of Blanes Bay (Katechakis 1999). This can be explained by the circumstance that in this size class only long-chain diatoms (*Rhizosolenia* spp. and *Skeletonema costatum*) occurred, whose ingestibility depends on their orientation in the filtration stream.

Since their valve diameters are between 10 and 20 µm, it is possible that they were ingested.

The same grazing experiments showed that copepods were not able to pick up particles <7.5 µm. Yet temporarily, the proportion of picoplankton was lowest under the influence of copepods. This may be due to trophic cascade effects and will be discussed in the following section together with the implications for the microbial food web.

Measurements of similarity suggest that the potential to modify a given algal population increases with increasing selectivity of the grazer (Table 4).

Changes in composition of microbial food web (1st stage flasks)

The abundances of solitary bacteria determined in the initial samples correspond well with results of Vaqué (1996) for Blanes Bay. During the course of the experiment HNF abundances influenced the abundance of solitary bacteria. High HNF densities were accompanied by low densities of solitary bacteria, including coccal cyanobacteria (Fig. 4). The inability of copepods to ingest particles <7.5 µm led to higher HNF densities than in other treatments. This explains the low bacterial abundances in chemostats with copepods. Conversely, *Penilia avirostris* exerted the largest grazing pressure on HNF of all grazers, although this species cannot graze on the bacteria themselves (Turner at al. 1988, Katechakis 1999). Accordingly, the cladoceran treatment resulted in the highest bacteria numbers. Doliolids caused medium HNF and bacteria densities. Jürgens et al. (1994), Jürgens (1995), Jürgens & Jeppesen (2000) described similar cascading effects for limnic systems. In lakes, strong top-down effects in the pelagic are well known (Carpenter at al. 1985). It is still not clear whether such trophic cascades occur in the marine pelagic. The interactions in our experiments between mesozooplankton and the microbial food web suggest that a top-down transmission of effects can occur, at least in the lower trophic levels. Recent enclosure experiments with carnivorous mesozooplankton and natural algal communities in the NE Atlantic indicate that such effects can also occur at higher trophic levels (H.S. et al. unpubl. data).

The differences in the appearance of particle-bound bacteria and filamentous cyanobacteria between the treatments arise from an adaptation to the different kind of grazing pressures exerted by copepods, cladocerans or doliolids. Abundances of both bacterial groups increased most under the influence of doliolids (Fig. 3). Filamentous cyanobacteria of all sizes (7 to 140 µm) lay inside the food size spectrum of cladocerans and copepods, but not inside the food size spectrum of doliolids, as evaluated in grazing experiments with natural phytoplankton assemblages from Blanes Bay (Katechakis 1999). Indeed 18.9% (± 5.8% SE of the mean) of the total filamentous cyanobacterial biovolume lay over the maximum size doliolids can manipulate. As well, we found many more particles in treatments with doliolids than in other chemostats. The combination of a higher density of particles in chambers with doliolids and the production of particles larger than the ingestible food size for doliolids may explain the differences in particle-bound bacteria numbers between the doliolid treatments and the other chemostats. An elongated shape and attachment to particles can be an effective bacterial defence against grazing. Various authors have documented this for freshwater systems with respect to bacterivorous protists (e.g. Güde 1989, Šimek & Chrzanowski 1992, Jürgens et al. 1994, Jürgens 1995, Jürgens & Jeppesen 2000) and metazoan predation (Langenheder & Jürgens 2001). Little is known about similar processes in marine environments. In particular, the importance of bacterivorous metazooplankton such as appendicularians and doliolids may be underestimated.

The general increase of cyanobacteria in all 1st stage chemostats (Fig. 3) may be explained by the fact that the high light intensity was advantageous to cyanobacteria (Sommer 1994c).

The ratio of autotrophic to heterotrophic flagellates was highest in chemostats with doliolids, and these were the only treatments that differed significantly from controls (Fig. 3). The annual average autotrophic nanoflagellates (ANF) is 50.2% of the nanoflagellate community in Blanes Bay (Vaqué 1996). Under the influence of doliolids, the proportion was 13% higher. One conceivable cause is that doliolids competed with HNF for bacteria, so that the relative proportion of ANF increased. Comparable changes *in situ* might have consequences for the trophic interactions at lower trophic levels, as the grazing pressure on the microbial food web would be altered. Carbon demands should rise relative to production and, hence, lead to the potential for top-down control of bacterial biomass and production. Changes in food chain length and energetic transfer efficiency might follow.

Our results show that in marine systems direct and indirect effects of herbivores can result in trophic cascades and that the effects of herbivores on phyto-, protozoo- and bacterioplankton strongly depend on the taxonomic compostion of the herbivores. This has implications for the modeling of grazing effects in marine pelagic ecosystems.

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