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Microzooplankton grazing and phytoplankton growth in marine mesocosms with increased CO_2 levels

K. Suffrian[1,*], P. Simonelli[2], J. C. Nejstgaard[3], S. Putzeys[4], Y. Carotenuto[5], and A. N. Antia[1]

[1]Leibniz Institute for Marine Sciences (IFM-GEOMAR), Düsternbrooker Weg 20, 24105 Kiel, Germany
[2]University of Bergen, Department of Biology, P.O.Box 7800, 5020 Bergen, Norway
[3]Department of Biology, UNIFOB, P.O. Box 7800, 5020 Bergen, Norway
[4]Facultad de Ciencias del Mar, Universidad de las Palmas de Gran Canaria, 35017 Tafira Baja – Las Palmas, Spain
[5]Stazione Zoologica "A. Dohrn", Villa Comunale 1, 80121 Naples, Italy
[*]also at: CAU Kiel, Institute for Physiology, Hermann-Rodewald-Straße 5, 24118 Kiel, Germany

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Abstract. Microzooplankton grazing and algae growth responses to increasing pCO_2 levels (350, 700 and 1050 μatm) were investigated in nitrate and phosphate fertilized mesocosms during the PeECE III experiment 2005. Grazing and growth rates were estimated by the dilution technique combined with taxon specific HPLC pigment analysis. Microzooplankton composition was determined by light microscopy. Despite a range of up to 3 times the present CO_2 levels, there were no clear differences in any measured parameter between the different CO_2 treatments. During days 3–9 of the experiment the algae community standing stock, measured as chlorophyll a (Chl-*a*), showed the highest instantaneous grow rates (k=0.37–0.99 d^{-1}) and increased from ca. 2–3 to 6–12 $\mu g\,l^{-1}$, in all mesocosms. Afterwards the phytoplankton standing stock decreased in all mesocosms until the end of the experiment. The microzooplankton standing stock, that was mainly constituted by dinoflagellates and ciliates, varied between 23 and 130 $\mu g\,C\,l^{-1}$ (corresponding to 1.9 and 10.8 $\mu mol\,C\,l^{-1}$), peaking on day 13–15, apparently responding to the phytoplankton development. Instantaneous Chl-*a* growth rates were generally higher than the grazing rates, indicating only a limited overall effect of microzooplankton grazing on the most dominant phytoplankton. Diatoms and prymnesiophytes were significantly grazed (12–43% of the standing stock d^{-1}) only in the pre-bloom phase when they were in low numbers, and in the post-bloom phase when they were already affected by low nutrients and/or viral lysis. The cyanobacteria populations appeared more affected by microzooplankton grazing which generally removed 20–65% of the standing stock per day.

Correspondence to: K. Suffrian
(k.suffrian@physiologie.uni-kiel.de)

1 Introduction

Atmospheric CO_2 levels have increased from about 280 to 380 μatm since the beginning of the industrial revolution. They are furthermore projected to reach values as high as 700 μatm by the end of the 21st century (IPCC, 2001). This increase in climate-relevant atmospheric gases including CO_2 is predicted to result in e.g. increasing global temperatures, rising sea level and accelerating extreme weather events (IPCC, 2007) Increased atmospheric CO_2 levels have already led to increased ocean acidity with a pH drop of 0.1 in the surface ocean since the beginning of the industrial revolution. Before the end of this century, a drop of a further 0.4 units is predicted (Caldeira and Wicket, 2003). Therefore, the carbonate saturation in the ocean is decreasing, likely affecting a number of organisms. Especially those with calcareous skeletons such as coccolithophorids, corals and molluscs are expected to be affected (see discussion and references in Schulz et al., 2007).

Auto- and mixotrophic protists play a key role in the global carbon cycle since they fix inorganic carbon. This fixed carbon is transferred to the higher trophic levels through grazing, and/or exported to deeper ocean layers through sedimentation. However, it is still unclear, how and to what extent the alteration in the ocean chemistry affects, and is affected by the phytoplankton growth and grazing interaction.

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As shown in previous experiments, the decreasing pH and hence decreasing carbonate saturation in the ocean may have a negative effect on the calcite ($CaCO_3$) production by coccolithophores and foraminifera (Riebesell et al., 2000; Russell et al., 2004). On the other hand algal species which are favoured by higher dissolved CO_2 concentration for photosynthesis, might instead benefit from an increase in the surface ocean CO_2 concentration (e.g. Barcelos e Ramos et al., 2007; Fu et al., 2007; Iglesias-Rodriguez et al., 2008; Riebesell, 2000; Rost et al., 2003). To our knowledge, not much is known about the impact of an increased pCO_2 on the performance of microzooplankton, nor on the grazing pressure exerted on microphytoplankton either.

CO_2 perturbations at an ecosystem level may provoke very complex responses in phytoplankton species composition and succession. Structure and functioning of the marine food web may be affected in different ways: Firstly, by cascading effects on elemental recycling by viruses and bacteria, secondly, by affecting the carbon fluxes through the grazing food web, and thirdly, by affecting the export through sedimentation.

As such complicated effects are best studied with natural populations, mesocosms are a powerful tool to study close to natural populations yet in controlled environments. These may be both replicated and simultaneously compared for different treatments. Mesocosms thus can be used to better understand complex responses of marine systems to increasing CO_2 levels and its feedback effects on carbon cycle (Riebesell et al., 2008).

Thus, to investigate how increased CO_2 levels in the atmosphere could affect the phytoplankton-grazer interactions we conducted a series of dilution experiments to quantify microzooplankton grazing during the 2005 Pelagic Ecosystem CO_2 Enrichment study (Schulz et al., 2007). During the study a typical bloom developed in all different CO_2 setups, the phytoplankton biomass was dominated by diatoms and coccolithophorids (i.e. Schulz et al., 2007, this issue). In the present study, we examined whether an increase in pCO_2 might result in measurable systematic changes in algal growth and/or grazing by microzooplankton. Our expectation was that the growth of specific, especially the calcifying, algae would be decreased under high CO_2 conditions. If so, we would expect this to impact the microzooplankton grazing on a taxonomic level: Either, affected algae would be grazed more, as they are weakened by CO_2 stress already. Alternatively, affected algae might grow so slow, that other algae outcompete them, and they are hardly grazed.

2 Material and methods

2.1 Mesocosm setup and sampling

The Pelagic Ecosystem CO_2 Enrichment experiment (PeECE III) was carried out at the National Mesocosm Centre, University of Bergen, Norway, from 16 May (day 1, d1) to 10 June 2005 (d25). Details are given in Schulz et al. (2007), while a general description of the mesocosm facility is available at: www.ifm.uib.no/LSF/inst2.html. Briefly summarized: nine floating 27 m^3 polyethylene seawater enclosures were filled in situ with unfiltered nutrient poor post bloom water from the surface of the surrounding fjord (Raunefjord, 60°16′ N, 5°14′ E). To initiate a phytoplankton bloom all the mesocosms were fertilized with NO_3^- and PO_4^{3-} to initial concentrations of 15 and 0.6 μmol l^{-1}, respectively (N:P $\approx$25:1). The mesocosms were manipulated (in triplicates) to three pCO_2 levels (350, 700 and 1050 μatm) by aerating with ambient or CO_2-enriched air. These CO_2 concentrations represented one (1×), two (2×) and three (3×) times the present atmospheric CO_2 conditions, respectively. The mesocosms were well mixed by an aquarium pump system that recirculated the entire volume ca. 5 times per day (ca. 40 l min^{-1}) during the overall experiment (Jacobsen et al., 1995).

2.2 Setup and sampling of dilution experiments and nutrient analysis

Phytoplankton growth and microzooplankton grazing rates were assessed by dilution experiments (Landry and Hassett, 1982). The experiments were performed using water from the mesocosm 2 (1150 μatm CO_2=3×), 5 (700 μatm CO_2=2×), and 8 (350 μatm CO_2=1×). Each mesocosm was sampled on 4 occasions, corresponding to pre algal bloom (d1–3), bloom (d7–9) and post bloom conditions (d13–15 and d20–22; Schulz et al., 2007). Due to logistical reasons, experiments for mesocosms 2, 5, and 8 could not be conducted on the same day but rather on consecutive days. Thus mesocosm 8 was sampled on days 1, 7, 13, and 20, mesocosm 5 on days 2, 8, 14, and 21, and mesocosm 2 on days 3, 9, 15, and 22. Water for the dilution experiments was collected by submerging 25 l polycarbonate bottles $\approx$30 cm from the water surface. The main opening was covered by a 200 μm nylon mesh to exclude mesozooplankton. The spigot was turned open to let air out of the bottle in order to sample with minimal turbulence and sheer-stress of the delicate protists. The experimental bottles were screened optically for mesoplankton. Particle free seawater was produced via filtration through 0.2 μm cellulose acetate filter (Whatman, 142 mm), using tissue culture hoses and low pressure (<50 hPa). Filtration was conducted in a cold room at in situ temperature (ca. 10°C) immediately before the setup of the experiments. Ambient seawater was mixed with particle free seawater to achieve target concentrations of the microplank-

Table 1. Name and abbreviation of the pigments used as algae taxon-specific markers. "Taxon" denotes the major taxon the pigment was considered to reflect here, while the "Additional Taxon" denotes other groups that potentially could contribute to the pool of the specific pigment (based on the reference given). However, the pigment was not used to characterize the additional taxon in this study.

Pigment	Abbreviation	Taxon	Additional Taxon	Reference
Chlorophyll a	Chl-*a*	Community		Mackey et al., 1996
4-keto-19′-hexanoyloxyfucoxanthin	4-keto-hex	Prymnesiophytes		Zapata et al., 2004
19′-hexanoyloxyfucoxanthin	19-hex	Prymnesiophytes		Mackey et al., 1996
Prasinoxanthin	Pras	Prasinophytes	Prymnesiophytes	Mackey et al., 1996
Fucoxanthin	Fuco	Diatoms	Prymnesiophytes	Mackey et al., 1996
Chlorophyll b	Chl b	Chlorophytes	Prasinophytes	Mackey et al., 1996
Peridinin	Peri	Dinoflagellates		Mackey et al., 1996
Zeaxanthin	Zea	Cyanobacteria		Mackey et al., 1996

tonic community. Concentrations used in this study were 25, 50, 75 and 100% of the ambient organismic concentration. They were mixed in 10 l polycarbonate carboys and distributed to triplicate 2 l polycarbonate incubation bottles by siphoning bubble free. The bottles were filled alternating the flow into each bottle until they were all topped off at about the same time. Absolute dilutions were checked by Chl-*a* concentrations at start in the 10 l carboys.

To assure that the experiments were not biased by nutrient limitation, nutrients were measured in the 100% sea water bottles at the start and the end of the incubations. Nutrient samples were frozen and stored at −20°C until analysis according to Hansen and Koroleff (1999) and Holmes et al. (1999) as described in detail by Schulz et al. (2007). Nutrients were added to the experimental bottles only when nutrient levels were below 2 μmol l^{-1} of nitrate or 0.2 μmol l^{-1} of phosphate (i.e. from d13 on). Final concentrations of nutrients added (N:P=10:1) were 1 μM (NO_3^-, NH_4^+), 0.1 μM (PO_4^{3-}) and trace metals corresponding to $f/40$ medium according to Guillard and Ryther (1962). Due to logistical restrictions no extra bottles without nutrients were used. Samples for initial concentration of pigments and microzooplankton were taken from the 10 l carboys at start (t_0) and filtered under low vacuum (200–300 hPa) onto 25 mm GF/F filters (Whatman).

The 2 l bottles were tightly capped avoiding air bubbles and incubated in situ outside the mesocosms for 24 h, by hanging them horizontally on strings from a surface floating ring to 6 m depth. This setup reproduced light conditions comparable to the average conditions inside the upper mixed layer of the mesocosms. This was measured with a horizontally mounted LI-192 underwater quantum sensor (not shown). The incubation setup also created a gentle irregular tipping movement, which prevented sedimentation in the flasks. After 24 h (t_{24}) bottles were collected, kept dark, and taken to the laboratory for immediate processing at in-situ temperature (ca. 10°C). Samples for microzooplankton counts and HPLC analysis were taken from the 2 l incubation bottles. For this bottles were gently mixed and siphoned off, while slowly stirring with the hose. Aliquots for HPLC analysis (400–500 ml) were filtered under low vacuum (200–300 hPa) onto 25 mm GF/F filters (Whatman) at t_0 and t_{24} of each experiment (n=3). Filters were put in Eppendorf tubes, shock frozen with liquid nitrogen, and stored at −80°C until further analysis. Subsamples (100–300 ml) for microzooplankton analyses were fixed with Lugol's iodine (1–2% final concentration) and stored in brown glass bottles at ambient temperature (ca. 15°C).

2.3 Algal pigment analysis

Phytoplankton pigments were analysed with high performance liquid chromatography (HPLC) to obtain growth and grazing rates. Pigments were extracted in 1 ml of 100% acetone. Additionally 100 μl of an internal standard (canthaxanthin) and glass beads were added before sonication (4°C, 5 min). Subsequently the samples were centrifuged (6000 rpm, 4°C, 15 min) and the supernatant was filtered through 0.2 μm PTFE-syringe filters into Eppendorf reaction tubes. The samples were then stored at −20°C until measurement in a Waters HPLC combined with a Waters 474 Scanning Fluorescence Detector and a Waters 2996 Photodiode Array Detector. Pigments were separated at a flow rate of 1 ml min^{-1} by a linear solvent gradient (min, % solvent A, % solvent B): (0, 65, 35), (1, 50, 50), (10, 15, 85), (15, 0, 100), (20, 0, 100), and (22, 65, 35), modified after Barlow et al. (1997). Pigments were detected by absorption at 440 nm and identified by comparison of their retention times and spectra with retention times and spectra of pigment standards.

Chl-*a* was used as a proxy for the whole phytoplankton community while taxon specific marker pigments were analysed to obtain specific growth and grazing coefficients for different algal groups (Table 1). 19′-hexanoyloxyfucoxanthin (19-hex) could not be used as a marker for prymnesiophytes during the bloom phase, as it could not be well separated from prasinoxanthin in the HPLC measurements of the samples. To separate this important

group, pure *E. huxleyi* samples from Bergen (cells isolated from the mesocosms by M. N. Müller, IFM-GEOMAR) were screened by HPLC to find an alternative marker. A clear 19′-hexanoyloxyfucoxanthin-like peak was found in the samples. This peak was regarded as being typical for prymnesiophytes or even specific for coccolithophorids (Zapata et al., 2004). It corresponded to 4-keto-19′-hexanoyloxyfucoxanthin (4-keto-hex), recently reported by Airs and Llewellyn (Airs and Llewellyn, 2006). Both markers, 19-hex and 4-keto-hex, were found at stable ratios to each other and to Chl-*a* in the pure *E. huxleyi* samples from Bergen. Thus, one or both of these pigments were used to identify the prymnesiophytes in each experiment and for simplicity called 19-hex*.

2.4 Microzooplankton abundance estimates

Samples were settled for 24 h in 50 ml sedimentation chambers (Utermöhl, 1958). Microphytoplankton was determined qualitatively (data not shown). We counted microzooplankton on one to two transects of each chamber at 200× magnification (Zeiss Axiovert 100, inverted microscope). Additional transects at 400x magnification were used to count smaller cells. A total of 120–1000 cells were enumerated in each sample. Cell sizes were measured with an ocular scale and used to calculate bio-volume, using formulas for spherical (Eq. 1) and prolate spheroid shapes (Eq. 2), with diameter (d) and height (h).

$$\mathrm{Vol_{Sphere}}=\pi/6\cdot d^3 \quad (1)$$

$$\mathrm{Vol_{Spheroid}}=\pi/6\cdot d^2\cdot h \quad (2)$$

Plankton biovolume (except for ciliates) was converted to carbon biomass (Eq. 3) according to Menden-Deuer and Lessard (2000):

$$\log \mathrm{pg\,C\,cell^{-1}}=-0.665+0.939\cdot \log \mathrm{vol} \quad (3)$$

Ciliate biovolume was converted to carbon (C) biomass using a conversion factor of 0.19 pg C per μm^3 (Putt and Stoecker, 1989).

The microplankton was differentiated into autotrophic microplankton and microzooplankton (the latter including both heterotrophic and mixotrophic organisms). This was done by comparison of morphological features to literature (Kuylenstierna and Karlson, 1996–2006; Strüder-Kypke et al., 2000–2001; Throndsen and Eikrem, 2005; Throndsen et al., 2003). The microzooplankton was grouped into dinoflagellates, ciliates and "Other". All ciliates were regarded as heterotrophic by morphological features (only apical cilia, no visible chloroplasts). The group named "Other" consisted mainly of microflagellates that were both scarse and of very low biomass (Fig. 1). We considered all microflagellates to be heterotrophic due to the absence of chloroplasts, and because they are known to be common in this area.

2.5 Calculation of growth and grazing rates

Changes in pigment concentrations over the incubation period were used to calculate the apparent phytoplankton growth rate (μ) and the mortality losses due to microzooplankton grazing (g). Assuming exponential growth:

$$\mu=\ln(P_t/P_0)/t=k-cg \quad (4)$$

P_0 and P_t are the initial and final pigment concentrations respectively, t is the incubation time ($t=t_{24}-t_0$), k is the instantaneous coefficient of phytoplankton growth, g the coefficient of grazing mortality and c is the dilution factor expressed as percentage of ambient seawater. The growth rate μ is plotted against c and a linear regression is applied to calculate g (negative slope of the regression) and k (Y-axis-intercept). It can be inferred that μ is linearly related to the dilution factor c (Landry, 1993). Changes in grazer density were monitored in the 100% bottle at start (t_0) and end (t_{24}) of the experiment. Since such changes accounted always for less than 10% (±) of the community (not shown), and were always below the methodological error, no correction for grazer density was applied to the calculations (cf. Landry, 1993). Regressions were tested with ANOVA (Sigmaplot version 9, Systat Software Inc.).

The percentage (% d^{-1}) of initial pigment standing stock grazed daily (Φ) by microzooplankton was calculated according to:

$$\Phi=1-e^{-g\cdot 100} \quad (5)$$

3 Results

3.1 Development of the overall phytoplankton community, growth and grazing

Nutrients became limiting in the mesocosms from d14 onwards. To assure that growth was unlimited in the bottle experiments nutrients were added thenceforward. Nutrients in the bottles thus were always replete, as shown in the nutrient measurements at the end of the incubations (Table 2). Since nutrients were limiting in the mesocosms after d13, our results after d13 represent maximal potential growth rates, which were nonetheless very low. Overall phytoplankton growth and grazing estimates based on Chl-*a* showed similar patterns in the three CO_2 treatments during the incubation experiments (Table 3). The in situ surface water temperature increased from ca. 9°C to ~11.5°C over the 25 experimental days.

Three phases can be observed. After an initial lag phase (d1 and d2) immediately after nutrient addition to the mesocosms we saw highest algal growth rates (k=0.37 to 0.99 d^{-1}) before and during the peak of the bloom (d3 to d9). The microzooplankton community grazing rates also were highest ($g=0.35-0.49\,d^{-1}$) during the phase of high growth, with

Table 2. Nutrient data measured on the day of experiment (d) in the 100 % seawater treatments at beginning (t_0) and end (t_{24}) of the dilution experiments. Data are shown for 1×, 2×, and 3× CO_2-treatments. Nutrients were added to the experimental bottles from d13 onwards (see text). The PO_4^{3-} concentration on d13 is regarded as an error in the measurement.

	d		NO_3^-	PO_4^{3-}	SiO_2
1×CO_2			μmol l^{-1}		
	1	t_0	14.29	0.68	3.08
		t_{24}	14.22	0.67	2.93
	7	t_0	7.40	0.27	0.28
		t_{24}	6.15	0.11	0.08
	13	t_0	1.93	(1.31)	0.63
		t_{24}	2.03	0.19	0.30
	20	t_0	1.89	0.18	0.34
		t_{24}	1.84	0.13	0.28
2×CO_2					
	2	t_0	14.41	0.67	2.96
		t_{24}	13.62	0.69	2.33
	8	t_0	6.94	0.19	0.16
		t_{24}	5.04	0.14	0.15
	14	t_0	1.73	0.13	0.25
		t_{24}	1.73	0.11	0.38
	21	t_0	1.72	0.15	0.31
		t_{24}	1.65	0.09	0.32
3×CO_2					
	3	t_0	12.72	0.73	2.47
		t_{24}	12.61	0.69	1.56
	9	t_0	5.51	0.18	0.16
		t_{24}	4.61	0.14	0.15
	15	t_0	1.61	0.15	0.46
		t_{24}	1.55	0.08	0.33
	22	t_0	1.32	0.13	0.34
		t_{24}	1.17	0.08	0.30

a daily Chl-*a* standing stock removal of 29–39%. Nonetheless, the algal community reached a maximum standing stock (6.54–12.23 μg Chl-*a* l^{-1}, Table 3) between d8 to d13. Thus, by consuming between a quarter and a third of the algal standing stock per day, the microzooplankton prolonged the temporal progression of nutrient depletion in the mesocosms (Table 1). The excess of growth over grazing seen in Fig. 2 is also consistent with the net accumulation of autotrophic biomass. Between days 9 and 13, there was a decrease in instantaneous Chl-*a* growth rates in all CO_2 treatments. During the third period (d14 to 22, post bloom) all treatments showed a marked decrease in Chl-*a* standing stock to reach 2.06-2.54 μg l^{-1} at d20-22 (Table 3). In this period phytoplankton growth rates decreased (k=0.02–0.29 d^{-1}) and overall microzooplankton grazing pressure stayed relatively low (5–12% Φ d^{-1}, Table 3). An exception was detected on

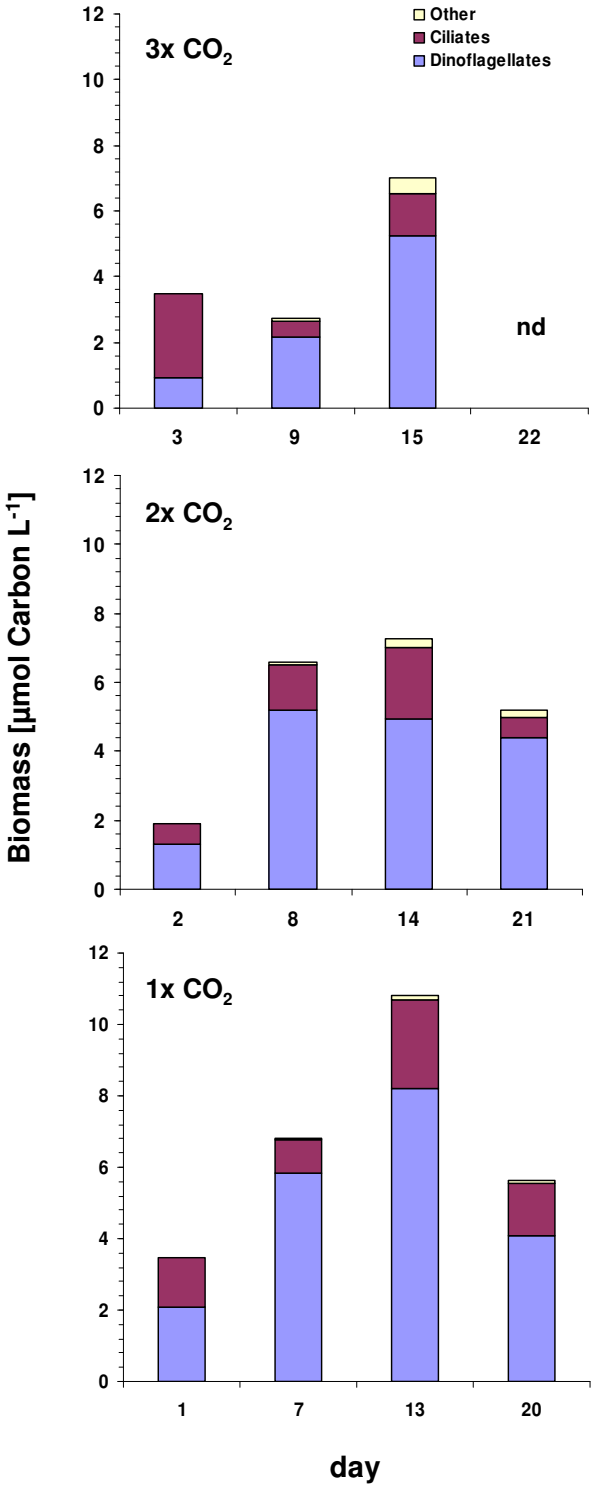

Fig. 1. Relative biomasses (μmol C l^{-1}) of major groups of heterotrophic protists at start (t_0) of the respective experiments in the ambient mesocosm water. Data are shown for CO_2-treatments 1×, 2×, 3× at the respective day of experiment (1-22). Data from 3× CO_2 on day 22 are missing (=not determined, nd).

Table 3. Compilation of pigment-based results from dilution experiments for all 3 treatments ($1\times CO_2$, $2\times CO_2$, and $3\times CO_2$). Day of experiment (d), standing stock in μg at time 0 (SS), instantaneous coefficient of phytoplankton growth (k), instantaneous coefficient of grazing mortality (g), standard error of the regression coefficients (SE), significance level ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$), correlation coefficient (R^2), number of means used for the calculation of k and g (n), percent standing stock grazed daily (Φ), not detectable (nd), not calculated (nc).

	d	SS	k		SE	g		SE	R^2	n	Φ
$1\times CO_2$		μg pigm l^{-1}	d^{-1}			d^{-1}					%
Chl-*a*	1	1.99	0.56	±	0.05***	0.28	±	0.08**	0.67	9	25
(Phytoplankton Community)	7	5.80	0.99	±	0.11***	0.49	±	0.15**	0.51	12	39
	13	6.54	0.08	±	0.04	0.19	±	0.06*	0.58	9	17
	20	2.54	0.16	±	0.02***	0.06	±	0.03*	0.31	12	5
19-hex/4-keto-hex	1	0.01	1.53	±	0.12***	0.44	±	0.17*	0.42	11	36
(Prymnesiophytes)	7	0.51	1.23	±	0.05***	0.29	±	0.08**	0.55	12	25
	13	0.77	−0.39	±	0.03***	0.07	±	0.04	0.20	12	7
	20	0.08	0.20	±	0.07*	0.16	±	0.11	0.17	12	14
Fuco	1	0.42	1.15	±	0.06***	0.41	±	0.10**	0.73	9	34
(Diatoms)	7	4.47	1.12	±	0.06***	0.35	±	0.08**	0.73	9	29
	13	2.12	−0.31	±	0.03***	0.25	±	0.05***	0.71	12	22
	20	0.24	0.29	±	0.071**	0.55	±	0.10***	0.73	12	42
Peri	1	0.00	nd	±	nd	nd	±	nd	nd	nd	nc
(Dinoflagellates)	7	0.11	−0.08	±	0.27***	−1.01	±	0.35*	0.55	9	nc
	13	0.34	0.78	±	0.14***	0.64	±	0.21*	0.49	12	47
	20	1.13	0.49	±	0.08***	0.32	±	0.14*	0.35	12	28
Zea	1	0.14	0.71	±	0.14***	0.55	±	0.20*	0.42	12	42
(Cyanobacteria)	7	0.03	2.25	±	0.12***	1.04	±	0.17***	0.85	9	65
	13	0.19	0.74	±	0.12***	0.65	±	0.17**	0.62	12	48
	20	0.30	0.86	±	0.05***	0.32	±	0.07***	0.68	12	28
$2\times CO_2$											
Chl-*a*	2	3.12	0.12	±	0.04*	0.29	±	0.06**	0.68	12	25
(Phytoplankton Community)	8	10.02	0.55	±	0.03***	0.43	±	0.04***	0.92	12	35
	14	4.64	0.02	±	0.04	0.06	±	0.06	0.09	12	6
	21	2.52	0.37	±	0.03***	0.28	±	0.05**	0.75	12	24
19-hex/4-keto-hex	2	0.02	1.10	±	0.10***	0.45	±	0.14*	0.49	12	36
(Prymnesiophytes)	8	0.89	0.63	±	0.05***	0.10	±	0.07	0.18	12	9
	14	0.41	−0.07	±	0.01***	0.11	±	0.01**	0.86	12	10
	21	0.08	0.38	±	0.04***	0.42	±	0.06***	0.82	12	34
Fuco	2	0.64	1.15	±	0.04***	0.56	±	0.06***	0.89	12	43
(Diatoms)	8	8.02	0.31	±	0.02***	0.07	±	0.03*	0.35	12	7
	14	0.88	−0.22	±	0.03***	0.07	±	0.05	0.19	12	7
	21	0.31	−0.09	±	0.06	0.44	±	0.09***	0.10	12	36
Peri	2	0.05	1.05	±	0.17***	1.09	±	0.25**	0.65	12	66
(Dinoflagellates)	8	0.30	0.10	±	0.05	0.21	±	0.08*	0.43	11	19
	14	0.32	0.81	±	0.04***	0.14	±	0.06*	0.37	12	13
	21	0.86	0.39	±	0.03***	0.30	±	0.04***	0.83	12	26
Zea	2	0.13	0.79	±	0.07***	0.77	±	0.10***	0.86	12	54
(Cyanobacteria)	8	0.06	1.02	±	0.04***	0.43	±	0.06***	0.84	12	35
	14	0.22	0.70	±	0.07***	0.66	±	0.11***	0.79	12	49
	21	0.27	0.71	±	0.05***	0.23	±	0.08*	0.45	12	20

Table 3. Continued.

	d	SS	k		SE	g		SE	R^2	n	Φ
3×CO_2											
Chl-*a*	3	2.75	0.58	±	0.05***	0.49	±	0.08***	0.81	12	39
(Phytoplankton Community)	9	12.23	0.37	±	0.08**	0.35	±	0.12*	0.47	12	29
	15	3.78	0.22	±	0.06**	0.13	±	0.09	0.16	12	12
	22	2.06	0.29	±	0.02***	0.03	±	0.03	0.10	12	3
19-hex/4-keto-hex	3	0.05	1.19	±	0.16**	0.55	±	0.21*	0.50	9	43
(Prymnesiophytes)	9	1.11	0.37	±	0.03***	0.10	±	0.05	0.27	12	9
	15	0.36	0.03	±	0.05	0.07	±	0.08	0.07	12	7
	22	0.14	0.30	±	0.03***	0.17	±	0.05**	0.51	12	15
Fuco	3	1.16	0.85	±	0.03***	0.13	±	0.04**	0.51	12	12
(Diatoms)	9	8.36	0.05	±	0.04	0.02	±	0.05	0.01	12	2
	15	0.79	−0.04	±	0.06	0.23	±	0.09*	0.40	12	20
	22	0.22	0.29	±	0.03***	0.30	±	0.05***	0.78	12	26
Peri	3	0.07	0.77	±	0.14**	0.88	±	0.18**	0.72	10	58
(Dinoflagellates)	9	0.42	−0.67	±	0.12***	−0.37	±	0.17	0.35	11	nc
	15	0.38	0.23	±	0.06**	0.17	±	0.09	0.23	12	15
	22	0.55	0.28	±	0.04*	0.03	±	0.07*	0.33	12	3
Zea	3	0.15	0.19	±	0.08*	0.62	±	0.11***	0.75	12	46
(Cyanobacteria)	9	0.09	0.88	±	0.10***	0.20	±	0.14	0.20	11	19
	15	0.19	0.91	±	0.06***	0.76	±	0.09***	0.87	12	53
	22	0.23	0.47	±	0.06***	0.02	±	0.09	0.00	12	2

d21 in mesocosm 2×CO_2, where higher growth and grazing rates were seen. Since these specific rates are determined at such low ambient pigment concentrations, a significant biomass increase could not be detected. Thus, in the latter part of the experiment low ambient nutrient concentrations were reflected in small and slower turnover in the microbial food web.

3.2 Microzooplankton community composition and development

Experimental bottles were screened optically for mesozooplankton, in 1 sample (2×d8) out of 19 microzooplankton samples two larger nauplii (315×200 μm) were found. As these larvae can flatten, they thus pass through the 200 μm mesh. Compared to other experiments no significant differences were found. Other nauplii found occasionally were smaller than 200 μm, and thus per definition, microzooplankton.

There was no measurable difference in microzooplankton community composition between the three different CO_2 treatments (Fig. 1). Dinoflagellates were the most abundant group dominated numerically by *Gymnodinium* spp. (length, l=8–29 μm) and *Minuscula* sp. (l=10–18 μm). The larger *Gyrodinium* sp. (l=43–86 μm) dominated the dinoflagellate biomass. Ciliates made up ca. one third of the community with species of the genus *Strombidium* (l=27–57 μm) and *Lohmaniella* (l=16–35 μm) as the main biomass contributors. The group named "Other" consisted mainly of microflagellates (l=16–23 μm), of which all were considered heterotrophic although we cannot rule out that some were autotrophic. Although the biomass of the heterotrophs thus may have been overestimated, it had negligible quantitative effect since the total biomass of "Other" was only 0–6.5% of the total microzooplankton biomass (Fig. 1). *Gyrodinium* spp. were largest during the pre-bloom and bloom (l=65–86μm), and decreased in size thereafter (l=43–54 μm). The other genera did not show a significant change in size. We did not detect any significant differences in size or development of size over time between microzooplankton in the 3 different CO_2-treatments. The total heterotrophic biomass reached its maximum (90–130 μg C l^{-1}, corresponding to 7.5–10.8 μmol C l^{-1}) during the experiment on d13–15, while it decreased again at the time of the last experiments (60–70 μg C l^{-1}, corresponding to 5.0–5.8 μmol C l^{-1}). While dinoflagellates increased in abundance during the first 8–15 days, ciliates did not show any clear trend of development through the experiment.

3.3 Development of standing stock, growth and grazing of specific algae groups

Samples were optically screened for chain-forming and other large phytoplankton. Mesophytoplankton was found to be excluded effectively. Only fragments of *Ceratium* spp. were seen in microscopic samples. Diatoms and prymnesiophytes dominated the phytoplankton community. No calculations for relative contributions of genera to bulk Chl-*a* were made in this study, for information refer to CHEMTAX data of the main contributing genera in Schulz et al. (2007).

Of the seven analysed specific algal pigments (Table 1) only the following pigments were assumed to characterize some of the most dominant groups; Prymnesiophytes (4-keto-19′-hexanoyloxyfucoxanthin and 19′-hexanoyloxyfucoxanthin, 19-hex*), Diatoms (fucoxanthin, Fuco), Dinoflagellates (peridinin, Peri) and Cyanobacteria (zeaxanthin, Zea) yielded significant growth or grazing rates in most of the experiments (Table 3). Thus, data on the other pigments are not further discussed.

It is not surprising that the general pattern observed for the total phytoplankton community (Chl-*a*) was mirrored in the effect of the microzooplankton grazing on Fuco and 19-hex*, as these pigments reflect the dominant diatoms and prymnesiophytes. Grazing on diatoms and prymnesiophytes also showed similar patterns. During the first ten days, the growth rates of these algae were generally higher than the grazing rates. Grazing rates exceeded the growth rates during d13–15 while they were generally in balance at the end of the experiment (d20–22).

The grazing pressure on cyanobacteria standing stock was higher compared with that on the larger autotrophs, ranging between 19% and 65% of standing stock during the first ten days (Table 3). This intense feeding activity was balanced by higher instantaneous growth rates (k=0.19–2.25 d^{-1}). Thus, analysis of Zea showed the more rapid turnover in this compartment of the phytoplankton than for bulk algae. The apparent patterns of growth and grazing on dinoflagellates were more inconsistent, and few conclusions may be drawn from these data.

As observed for Chl-*a*, the microzooplankton grazing on phytoplankton as indicated by the change in pigments concentrations did not seem to be influenced by the different CO_2 treatments. The highest daily percentages of standing stock removed by microzooplankton were 42% for diatoms, 43% for prymnesiophytes and 65% for cyanobacteria.

4 Discussion

4.1 General

The general temporal dynamics of the phytoplankton community, observed in our bottle incubation experiments, mirrored the dynamics observed within the mesocosms by other studies (Egge et al., 2007; Paulino et al., 2007; Schulz et al., 2007). The main effects of CO_2 addition were an increased carbon uptake relative to nutrient uptake, resulting in an increased carbon drawdown, increased subsurface O_2 consumption and decreased ammonium regeneration in the higher CO_2 treatments (Riebesell et al., 2007).

Following nutrient addition algae grew fast during d3-9 of the experiment (Schulz et al., 2007). During this period microzooplankton was grazing actively (g=0.28–0.49 d^{-1}) on the autotrophic biomass and increased its biomass during the same period. However, microzooplankton did not inhibit net phytoplankton growth or the bloom development. Their activity rather prolonged the temporal progression of nutrient depletion and decreased the ultimate phytoplankton biomass accumulation.

After onset of nutrient depletion (d10), the blooms in all mesocosms rapidly declined. This decline was probably due to a combination of reasons: Firstly, the nutrient depletion as reported by Schulz et al. (2007) led to reduced growth and increased algal mortality, as well as sedimentation. Secondly, a high abundance of approx. $10–14 \cdot 10^{14}$ viruses ml^{-1} at the end of the coccolithophorid bloom (Larsen et al., 2007) could also have contributed to the decline. As the mortality due to viral lysis compromises the assumption of the dilution approach, namely that algal cells are under non-limited growth, viral activity might explain some of the close-to-zero and negative growth rates in the dilution experiments. In fact, the few negative growth rates (Table 3) coincided with the high viral abundances, so that the viral mortality might have added up with the grazing mortality, leading to a net decrease in standing stock. Nevertheless this remains speculative, as only one virus could yet be clearly assigned to specific algae growing in the mesocosm experiments (Larsen et al., 2007).

At the beginning of PeECE III a N:P ratio of ≈25:1 was used to stimulate growth of the coccolithophorid *Emiliania huxleyi* (Egge, 1993; Egge and Jacobsen, 1997). When nutrients were depleted in the mesocosms, they were added to our experimental bottles to ensure unlimited algal growth rates. During the post-bloom phase only NO_3^- could have been limiting. As nutrients were never completely depleted in our experimental bottles (Table 2), nutrients were added in a more natural N:P ratio of ≈10:1. Nevertheless, although growth rates stayed low during this phase of the experiments, they have to be considered maximal potential growth rates. The addition of nutrients in a different ratio did not change the species composition by favouring other algae than before. This is in agreement with data from Paulino et al. (2007) and Schulz et al. (2007), which show the same species composition as our results.

The turnover of algal biomass by growth and subsequent microzooplankton grazing suggests a close coupling of algal growth and loss factors in the mesocosms. The cyclical progression of the specific rates of growth and grazing (Fig. 2) show an initial excess of growth, allowing for the build-up of a bloom, followed by a phase of excess grazing

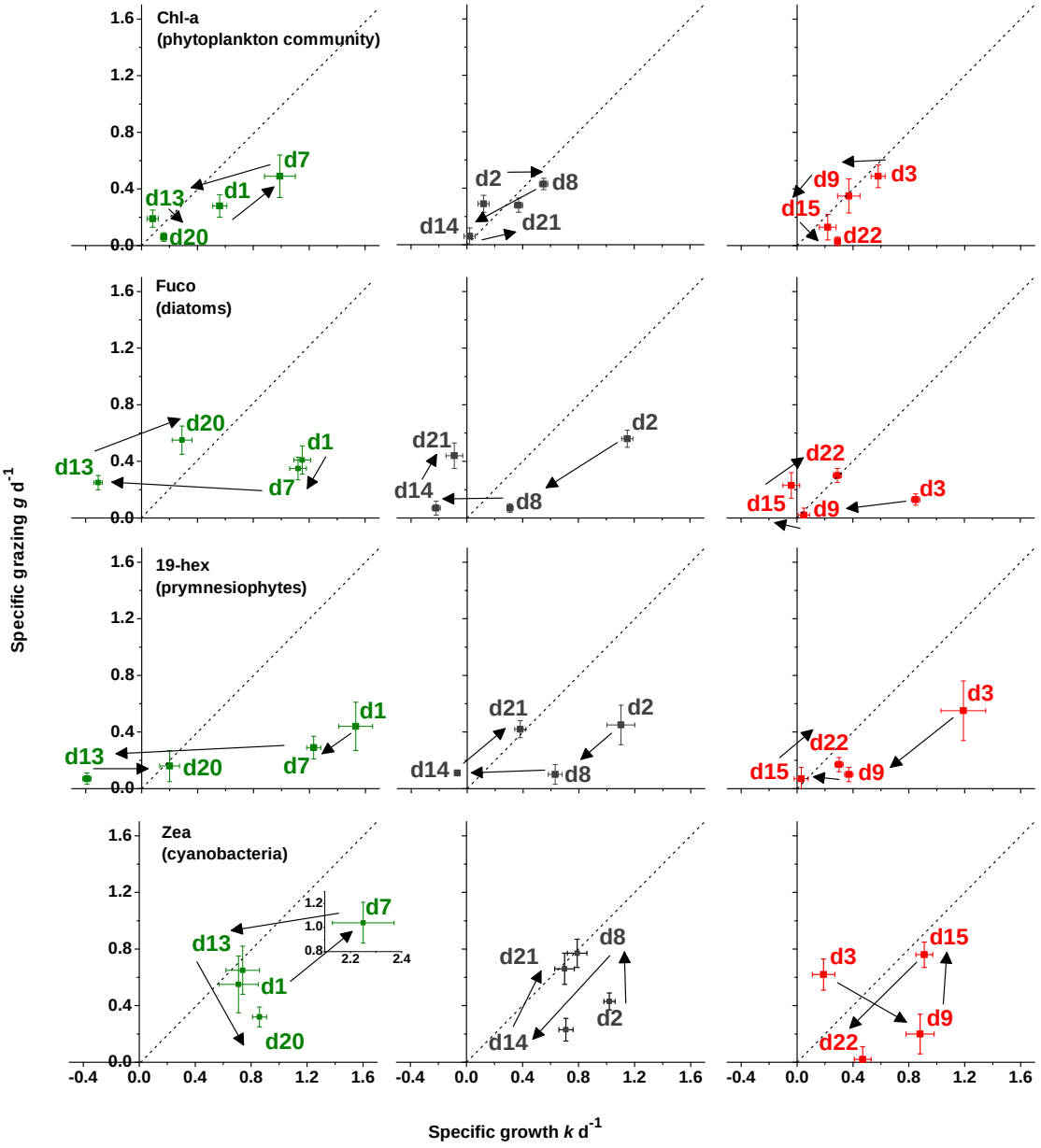

Fig. 2. Instantaneous grazing mortality coefficient (g) against instantaneous phytoplankton growth coefficient (k) based on chlorophyll (Chl-a), fucoxanthin (Fuco, diatoms), 19′-hexanoyloxyfucoxanthin or 4-keto-19′-hexanoyloxyfucoxanthin (19-hex*, prymnesiophytes), and zeaxanthin (Zea, cyanobacteria). The dotted lines indicate steady state, arrows indicate the temporal order of the experiments, and data points are labelled with the respective day of experiment. $1\times CO_2$ (green), $2\times CO_2$ (grey), $3\times CO_2$ (red).

over growth, coinciding with the period of bloom decline. Irrespective of the dimension of the standing stocks, this close coupling is a characteristic of most pelagic systems, since within the microbial compartment response times to changes in prey and predator can be extremely rapid. Microzooplankton biomass (7.5–10.9 μmol C l^{-1}) comprised 10%–20% of the total particulate organic carbon in the mesocosms during the bloom (30–100 μmol C l^{-1}; Schulz et al., 2007), However, even though they had a low contribution to the biomass they showed high turnover rates and significant grazing on the algal stocks.

4.2 Use of specific marker pigments as a proxy for different algae.

When using pigments as markers for individual taxa it is important to verify against direct optical observations. (Antajan et al., 2004; Irigoien et al., 2004). We compared the pigment data to microscopic counts (data not shown), as well as to plankton analyses from the mesocosms (Egge et al., 2007; Schulz et al., 2007), and found it to be consistent. Due to the initial high silicate concentrations in all mesocoms (Table 2) the phytoplankton community biomass rapidly became dominated by diatoms while the silicate became significantly reduced (Egge et al., 2007; Schulz et al., 2007). Thus, the development of the fucoxanthin standing stock (Table 3) closely mirrors the biomass accumulation of the diatoms. For fucoxanthin, the same cyclical changes in the relative coefficients of growth and grazing, reflecting biomass increase and subsequent decrease, could be seen (Fig. 2). Diatoms are generally not considered the preferred prey of many microzooplankton species. The presence, indeed dominance, of heterotrophic dinoflagellates among the microzooplankton may be due to their ability to envelop food particles and digest them internally, something which ciliates cannot do. Indeed, dinoflagellates have been found to be major grazers of diatoms (Sherr and Sherr, 2007).

The calcifying prymnesiophyte *Emiliania huxleyi* only reached moderate numbers. Other prymnesiophytes were also present in the mesocosms (Engel et al., 2007; Paulino et al., 2007; Schulz et al., 2007, Egge and Larsen, personal communication, 2006), corroborating the use of 19-hex* as an indicator of coccolithophores in all the CO_2 treatments. Also the development of the dominating cyanobacteria *Synechococcus* sp. (Paulino et al., 2007) appeared to follow the same pattern as the development of the zeaxanthin measured here. Although we observed autotrophic dinoflagellates (not shown), while analysing the heterotrophic dinoflagellates, the development of the peridinin concentration and rates (Table 3) is less clear, and may be obscured by the problem of defining mixotrophy in this group. The smallest group of phytoplankton, the cyanobacteria, clearly showed the highest growth and grazing rates (Fig. 2) here. This clearly demonstrates, that individual phytoplankton groups can have turnover rates differing significantly from that of the bulk phytoplankton.

4.3 Effects of the CO_2 treatments on phytoplankton growth and grazing.

The main aim of this study was to compare microzooplankton grazing and algae growth interactions in different CO_2-environments.

The time lag of one to two days due to consecutive experiments in the different CO_2 levels is unfortunate, considering that the entire duration for the bloom to develop was $\approx$7 days. However, although the experiments in each round could not be conducted the same day, due to the labour intensive method necessary to use for determination of detailed grazing rates (see e.g. Båmstedt et al., 2000), they were conducted successively directly after each other. The time lag between experiments in the same round (1–2 days) was less than between each round of experiments ($\sim$6 days). Four rounds of experiments were conducted throughout the mesocosm experiment in similar phases in the bloom development in the different mesocosms. This suggests that if there were substantial differences in grazing responses between the CO_2 treatments this should be reflected in the overall results.

The grazing showed a highly dynamic course over the time of the mesocosm experiment. However a similar temporal development was detected in the three investigated mesocosms, which can be related to the development of the blooms in the mesocosms (as would be expected from previously published data e.g. Riebesell et al., 2007; Schulz et al., 2007). Therefore we conclude that no effect of CO_2 on microzooplankton grazing or phytoplankton growth was detectable when comparing the plankton community in the three CO_2-treatments over a three-week period.

In contrast to some laboratory studies which have shown a number of acute effects on single planktonic organisms (even if sometimes conflicting and contradictory, as discussed in Schulz et al., 2007), we suggest from our results that either:

1. complex, close to natural systems such as investigated here may show such a complex response pattern to increasing CO_2, that it needs more detailed studies (including e.g. biogeochemical studies of the material transport between the trophic compartments) to be disclosed. or

2. such complex systems may have large "buffering capacities" enabling them to absorb increased CO_2, at least under certain conditions.

Riebesell et al. (2007) describe that the DIC-consumption increased with an increased pCO_2, whereas the nutrient uptake remained stable. However, such CO_2 over-consumption would lead to offset Redfield ratios, and possibly significant deterioration of the content of essential constituents in the prey of the microzooplankton. This has not been investigated here. If the observed CO_2 over-consumption observed by Riebesell et al. (2007) in this system leads to a deterioration of the food quality this may not be readily visible on the first trophic level. This is based on the fact that at least some microzooplankton, e.g. some dinoflagellates, may have the capacity to upgrade low quality prey (Veloza et al., 2006), such as carbon rich algae. If this is true, the trophic cascade response may thus not be visible until higher levels in the marine food web, such as copepods. However, effects on higher trophic levels may need longer experimental duration than a few weeks to be clearly manifested.

It is also interesting to notice that while the ciliates did not change substantially in biomass over the course of time, the

heterotrophic dinoflagellates did (Fig. 1). This may be explained by that many dinoflagellates feed on diatoms (compare e.g. feeding guilds discussed in Nejstgaard et al., 1997, 2001). Diatoms were the phytoplankton group showing the highest growth and grazing rates here.

In contrast to dinoflagellates, ciliates may have a wider prey-size range, reaching from 2 to 50 μm (e.g. Burkill and Mantoura, 1987 and references therein). This flexibility could be favourable during an algal succession as in the PeECE III experiment. Ciliates may also be a high quality prey for larger organisms (Stoecker and Capuzzo, 1990), although it is not clear whether this is due to their biochemical composition or size alone (Klein Breteler et al., 1999; Koski, 2007; Koski and Rampen, 2004; Koski and Wexels Riser, 2006). Thus, if the two main contributing genera of the microzooplankton represented favourable food for the copepods this may help explain the lack of large differences in copepod egg production, hatching success, and naupliar recruitment rates in copepods incubated in water from different CO_2 treatments in this mesocosm experiment (Carotenuto et al., unpublished data).

To our knowledge, this is the first microzooplankton study in such marine systems and more data are required before such conclusions can be drawn. Further studies, especially on the possible effects on food quality vs. quantity for higher trophic levels, such as copepods, and perhaps fish, are needed.

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