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Microzooplankton grazing and phytoplankton growth in marine mesocosms with increased CO₂ levels

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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. Phytoplankton and microzooplankton composition were determined by light microscopy. Despite a range up to 3 times the present CO$_2$ levels, there were no clear differences in any measured parameter between the different CO$_2$ treatments. Thus, during the first 9 days of the experiment the algae community standing stock (SS), measured as chlorophyll a (Chl a), showed the highest instantaneous grow rates (0.02–0.99 d$^{-1}$) and increased from ca 2–3 to 6–12 µg l$^{-1}$, in all mesocosms. Afterwards the phytoplankton SS decreased in all mesocosms until the end of the experiment. The microzooplankton SS, that was mainly dinoflagellates and ciliates varied between 23 and 130 µg 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 (14–43% of the SS d$^{-1}$) only in the pre-bloom phase when they were in low numbers and in the post-bloom phase when they were already limited by low nutrients and/or virus lysis. The cyanobacteria populations appeared more affected by microzooplankton grazing, generally removing 20–65% of the SS d$^{-1}$.

1 Introduction

Atmospheric CO$_2$ levels have increased from about 280 to 380 µatm since the beginning of the industrial revolution, and are projected to reach values as high as 700 µatm by the end of the 21st century (IPCC, 2001). This increase in atmospheric CO$_2$ (and other gases) is predicted to result in e.g. increasing global temperatures, rising sea
level and accelerating extreme weather incidences (IPCC, 2007). Increased atmospheric CO$_2$ levels have lead to increased ocean acidity with a pH drop of 0.1 since the beginning of the industrial revolution and with a predicted drop of another 0.4 units already before the end of this century (Caldeira and Wicket, 2003). As a consequence, the carbonate saturation in the ocean is decreasing, likely effecting a number of organisms, especially those with calcareous skeletons such as coccolithophorids, corals and molluscs (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 that is either transferred to the higher trophic levels through grazing or exported to deeper ocean layers through the biological pump and sedimentation. But 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. 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), while other algal species which rely on dissolved CO$_2$ concentration for photosynthesis, might benefit from an increase in the surface ocean CO$_2$ concentration. Thus, CO$_2$ perturbations at an ecosystem level may provoke very complex responses in phytoplankton species composition and succession, and thereby affect the structure and functioning of the marine food web by cascading effects on elemental recycling by virus and bacteria as well as carbon fluxes through the grazing food web and export through sedimentation. While such complicated effects can not be studied in laboratory, mesocosm experiments provide a powerful tool to better understand complex responses of marine systems to increasing CO$_2$ levels and its feedback effects on carbon cycle and global climate. 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).
2 Material and methods

2.1 Mesocosm setup and sampling

The Pelagic Ecosystem CO₂ Enrichment experiment (PeECE III) was carried out at the National Mesocosm Centre, University of Bergen, Norway, from 16 May (day 1) to 10 June 2005 (day 25). Details are given in Schulz et al. (2007), while a general description of the mesocosm facility is available at: http://www.ifm.uib.no/LSF/inst2.html. Briefly summarized: nine floating 27 m³ 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, 05°14′ E). To initiate phytoplankton blooms all the mesocosms were fertilized with NO₃ and PO₄ to initial concentrations of 15 and 0.6 µmol l⁻¹, respectively. The mesocosms were manipulated (in triplicates) to three pCO₂ levels (ca 350, 700 and 1050 µatm) by aerating with normal or CO₂-enriched air. These CO₂ concentrations represented one (1×), two (2×) and three (3×) times the present atmospheric CO₂ conditions, respectively.

2.2 Setup and sampling of dilution experiments and nutrient analysis

Phytoplankton growth and microzooplankton grazing rates were assessed by a total of 12 dilution experiments (Landry, 1993; Landry and Hassett, 1982), listed in Table 1. The experiments were performed using water from one of each of the three CO₂ treatments at 4 occasions corresponding to pre algal bloom (day 1–3), bloom (day 7–9) and post bloom conditions (day 13–15 and 20–22) (Schulz et al., 2007). Water for the dilution experiments was collected by submerging 25 l polycarbonate bottles with the main opening covered by a 200 µm nylon mesh to exclude mesozooplankton, and with the spigot open to let air out of the bottle in order to sample with minimal turbulence and shear-stress of the delicate protists. An aliquot was filtered 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 immediately before the
setup of the experiments. Target concentrations for the dilution of 25, 50, 75 and 100% undiluted sea water were carefully mixed in 10 l polycarbonate bottles and distributed to triplicate 2 l polycarbonate incubation bottles by siphoning. 2 l 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 bottles. 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 (Table 1). Nutrient samples were frozen and stored at −20°C until analysis according to Grasshoff and Kremling (1999) as described in detail by Schulz et al. (2007). In order to avoid unnecessary changes in the experimental nutrient conditions (e.g. Landry, 1993), 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 day 13 and on). Final concentrations of nutrients added were 1 µM (NO₃⁻, NH₄⁺), 0.1 µM (PO₄³⁻) and trace metals corresponding to f/40 medium according to Guillard and Ryther (1962). Nutrients were never depleted in the experiments (Table 1). The 2 l bottles were tightly capped and incubated in situ outside the mesocosms for 24 h hanging horizontally on strings from a floating ring at 6 m depth. This setup reproduced light conditions comparable to the average conditions inside the mesocosm (measured with a horizontally mounted underwater LI-192 underwater quantum sensor). The incubation setup also created a gentle irregular tipping movement which prevented sedimentation in the flasks. Samples for microzooplankton counts and HPLC analysis were taken from the 10 l bottles at start (t₀) and from the 2 l incubation bottles at end (t₂₄), by gently siphoning off while slowly stirring with the hose.

2.3 Algal pigment analysis

Phytoplankton pigments were analysed with high performance liquid chromatography (HPLC) to obtain growth and grazing rates for the entire community and for selected algal groups based on their marker pigments (Table 2). Aliquots for HPLC analysis (400–500 ml) were filtered under low vacuum (200–300 hPa) onto 25 mm GF/F filters
(Whatman, nominal pore size 0.4 µm) 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 \(-80^\circ C\) until further analysis.

Pigments were extracted in 1 ml of 100% acetone. Additionally 100 µl of an internal standard (canthaxanthin) and glass beads were added before sonication (\(4^\circ C\), 5 min). Subsequently the samples were centrifuged (\(4^\circ C\), 15 min) and the supernatant was filtered through 0.2 µm PTFE-syringe filters into Eppendorf reaction tubes. The samples were then stored at \(-20^\circ C\) 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 gradient (minutes, % 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 well-characterized pigments.

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 2). 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 get genuine values for this important group pure \(E. huxleyi\) samples from Bergen (provided by M. N. Müller, IFM-GEOMAR) were screened by HPLC to find an alternative marker. A 19’-hexanoyloxyfucoxanthin-like peak, which was regarded typical for prymnesiophytes or even specific for coccolithophorids (Zapata et al., 2004), was found in the samples, corresponding to 4-keto-19’-hexanoyloxyfucoxanthin (4-keto-hex) recently reported by 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.
2.4 Microzooplankton abundance estimates

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). Samples were settled for 24 h in 50 ml sedimentation chambers (Utermöhl, 1958). One to two transects of each sample was counted with a Zeiss Axiovert 100 inverted microscope at 200x magnification. Additional transects at 400x magnification were used to determine smaller cells. A total of ca 120–1000 cells were enumerated in each sample. Cell sizes were measured with an ocular scale and used to calculate biovolume, using formulas for spherical (1) and prolate spheroid shapes (2), with diameter ($d$) and height ($h$).

$$V_{\text{Sphere}} = \frac{\pi}{6} \cdot d^3 \quad (1)$$

$$V_{\text{spheroid}} = \frac{\pi}{6} \cdot d^2 \cdot h \quad (2)$$

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

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

Ciliate biovolume was converted to carbon biomass using a conversion factor of 0.19 pg C/µm³ (Putt and Stoecker, 1989).

The microplankton was differentiated into autotrophic plankton and microzooplankton (including both heterotrophic and mixotrophic organisms) 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 (ciliates only apical, no visible chloroplasts etc.). The group named “other” consisted mainly of microflagellates that were both scarce and of very low biomass (Fig. 1), thus for simplicity all microflagellates were considered heterotrophic.
2.5 Calculation of growth and grazing rates

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

$$ P_0 \text{ and } P_t \text{ are the initial and final pigment concentrations respectively; } t \text{ is the incubation time (} t = t_{24} - t_0 \text{), } k \text{ is the instantaneous coefficient of phytoplankton growth, } g \text{ the coefficient of grazing mortality and } c \text{ is the dilution factor expressed as percentage of ambient seawater. It can be inferred that } \mu \text{ is linearly related to the dilution factor } c, \text{ that the negative slope is the grazing coefficient } g \text{ and that the Y-intercept is the phytoplankton growth rate } k \text{ (Landry 1993). Changes in grazer density were monitored in the 100% bottle at start (} t_0 \text{) and end (} t_{24} \text{) of the experiment. Since such changes accounted always for less than 10% (±) of the community (not shown), 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 of initial pigment SS daily grazed by microzooplankton (% d$^{-1}$) was calculated according to:

$$ SS = 1 - e^{-g \cdot 100} $$ (4)

3 Results

3.1 Microzooplankton community composition and development

There was no clear 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. and Minuscula sp., while the larger Gyrodinium sp. dominated the dinoflagellate biomass. Ciliates made up ca. one third of the community with species of the genus Strombidium and Lohmaniella as the main biomass contributors. The group named “other” consisted mainly of microflagellates,
whereof all were considered heterotrophic, for simplicity. Although the biomass of the heterotrophs thus was overestimated, it has no practical quantitative effect since the total biomass of “Other” was only 0–6.5% of the total “microzooplankton” biomass (Fig. 1). The total heterotrophic biomass reached its maximum (90–130 µg C l⁻¹) during the experiment starting days 13–15, while it decreased again at the time of the last experiments (65±5 µg C l⁻¹). Although dinoflagellates increased in abundance during the first 8-15 days, ciliates did not show any clear trend of development through the experiment.

3.2 Development of the overall phytoplankton community, growth and grazing

Overall phytoplankton community biomass, growth and grazing estimates based on Chl a showed similar patterns in the three CO₂ treatments during the incubation experiment (Tables 3a–c). Two phases can be observed: the first from day one to nine was characterized by the highest algal growth rates (0.12 to 0.99 d⁻¹). Although the microzooplankton community grazing rates also were the highest (0.28–0.49 d⁻¹) with a daily Chl a SS removal of 25–39% during this first period, the algal community reached the maximum SS (1.99–12.23 µg Chl a l⁻¹, Tables 3a–c). Thus, microzooplankton only appeared to have a minor effect on the overall phytoplankton development when nutrients were abundant (Table 1) during the first 9 days, this is also apparent from the general distribution of the data points below the 1:1-lines in Fig. 2.

Between days 9 and 13 there was a significant decrease in instantaneous Chl a growth rates (k) in all CO₂ treatments. While the 2× and 3× CO₂ treatments showed a marked decrease in Chl a SS (Tables 3b and c), the 1×CO₂ treatment Chl a SS was relatively stable in that period (Table 3a). However, after day 13 the algal Chl a SS declined in all three mesocosms to 2.1–2.5 µg l⁻¹ at day 20–22 (Tables 3a–c). During this latter period phytoplankton growth rates decreased (0.02–0.37 d⁻¹) and overall microzooplankton grazing pressure stayed relatively low (5–24% SS d⁻¹, Tables 3a–c). Thus, the microzooplankton grazing impact on the overall phytoplankton community was limited.
3.3 Development of SS, growth and grazing of specific algae groups

Of the seven analysed specific algal pigments (Table 2) only the pigments assumed to characterize some of the most dominant groups; Prymnesiophytes (4-keto-19’-hexanoyloxyfucoxanthin and 19’-hexanoyloxyfucoxanthin), Diatoms (fucoxanthin), Dinoflagellates (peridinin) and Cyanobacteria (zeaxanthin) yielded significant growth or grazing rates in most of the experiments (Tables 3a–c). 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 the pigments assumed to 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 feeding rates indicating that microzooplankton feeding was not a factor significantly limiting their blooming. Grazing rates overcame the growth rates during the days 13–15 while they were comparable in the end of the experiment (20–22).

The grazing pressure on cyanobacteria SS was higher compared with the ones on the larger autotrophs, ranging between 19% and 65% (Tables 3a–c). This intense feeding activity was balanced by higher instantaneous growth rates (0.19–2.25 d⁻¹) and did not seem to limit the increase of the SS during the last two experiments. 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 the specific pigments did not seem to be influenced by the different CO₂ treatments, and neither did the grazing pressure seem to have any major effect on the development of the bloom of the different groups, except perhaps in the very beginning of the experiment when the standing stocks of the phytoplankton were generally low. The highest percentages of SS removed by microzooplankton were 42% for diatoms, 43% for prymnesiophytes and 65% for cyanobacteria.
4 Discussion

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

When using specific pigments as markers for individual taxa it is of great importance to know what species are physically present and dominant (Antajan et al., 2004; Irigoien et al., 2004). Due to the initial high silicate concentrations in all mesocosms (Table 1) the phytoplankton community biomass rapidly became dominated by diatoms while the silicate became significantly reduced (Schulz et al., 2007; Egge et al., 2007). Thus, the development of the fucoxanthin showed a similar development as the draw down of the silicate and observations of phytoplankton samples from the mesocosms, and this pigment should thus be considered to closely mirror the development of the diatoms.

Although the calcifying prymnesiophyte *Emiliania huxleyi* only reached moderate numbers, other prymnesiophytes were abundant in the mesocosms corroborating our use of 4-keto-hex and 19-hex as indicators of prymnesiophytes in all the CO$_2$ treatments (Engel et al., 2007; Paulino et al., 2007; Schulz et al., 2007, J. K. Egge and A. Larsen, personal communication). 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 (Tables 3a–c) is less clear, and may be obscured by the problem of defining mixotrophy in this group. The dinoflagellates will therefore not be further discussed here.

In conclusion, as the presence of the major phytoplanktonic groups – diatoms, prymnesiophytes and cyanobacteria (*Synechococcus* sp.) – was verified against flow cytometry and microscopy we consider the HPLC data as trustworthy for these three groups.
4.2 Effects of the CO$_2$ treatments on phytoplankton growth and grazing

The major aim with this investigation was to compare microzooplankton grazing and algae growth interactions in different CO$_2$ environments. However, we found no clear effects on microzooplankton grazing or phytoplankton growth when comparing the three CO$_2$-treatments over a three week period. Despite that previous laboratory studies 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 patterns 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 simply have large “buffering capacities” making them able to absorb increased CO$_2$, at least under certain conditions, such as described in Riebesell et al. (2007). However, as described in Riebesell et al. (2007) such CO$_2$ over-consumption would lead to offset Red field 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, because at least some microzooplankton may have the capacity to upgrade low quality prey (Veloza et al., 2006) such as the carbon rich algae in the 3×CO$_2$-treatment, and if this is true, the trophic cascade response may thus not be visible until higher levels in the marine food web, such as e.g. for copepods. But 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, the heterotrophic dinoflagellates did so (Fig. 1). This may be explained by that many dinoflagellates feed on diatoms (compare e.g. feeding guilds discussed in Nejstgaard et al., 1997 and 2001), the phytoplankton group showing the highest growth and grazing rates here. It has also been hypothesized that at least some dinoflagellates may...
trophically upgrade the food for higher trophic levels such as copepods (see discussion and references in Veloza et al., 2006). These are potentially interesting aspects that need to be investigated in future studies. However, to our knowledge, this is the first study in such marine systems and more data is needed before such conclusions can be drawn. It should especially be focused on the possible effects on food quality vs. quantity for higher trophic levels, such as copepods, and perhaps fish.

The general temporal dynamic of the phytoplankton community, observed in our bottle incubation experiments, mirrored the dynamic observed inside the mesocosms by other studies (Egge et al., 2007; Paulino et al., 2007; Schulz et al., 2007). They grew during the first ten days of the experiment as a consequence of the nutrients addition (Schulz et al., 2007). During this period microzooplankton was grazing actively (0.28–0.49 d⁻¹) on the autotrophic compartment but without limiting the development of the bloom. The effect of this trophic activity was evident from the microzooplankton biomass increase during the same period. The decline of the bloom after day 10, is therefore due to the nutrient depletion as reported by Schulz et al. (2007), or perhaps viral activity (Larsen et al., 2007) more than a result of grazing. It was only during the initial phase of the experiments when phytoplankton biomass was low, and possibly during the post bloom phase, when the instantaneous growth rates were close to zero or negative, that the grazing became more significant and the microzooplankton biomass reached its maximum. Neither did the cyanobacteria appear to be significantly limited by the microzooplankton. Despite of a high daily removal (19%–65% of the SS), the microzooplankton did not control the biomass increase registered during the last two incubation experiments. The lack of microzooplankton grazing may also explain the increase in the cyanobacteria community observed in the mesocosms by Paulino et al. (2007). The general relatively low levels of microzooplankton grazing activity may explain the observed lack of a net heterotrophic phase in this PeECE III mesocosm experiment (Egge et al., 2007), and support the hypothesis by Riebesell et al. (2007) that such a system may favour a high export of organic material through the pycnocline.

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References


Table 1. Nutrient data measured at beginning and end of the dilution experiments. Data are shown for 1×, 2× and 3× CO₂ treatments at \( t_0 \) and \( t_{24} \).

<table>
<thead>
<tr>
<th>DATE</th>
<th>DAY</th>
<th>EXP</th>
<th>( t_0 )</th>
<th>( t_{24} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>17/05</td>
<td>1</td>
<td>1×d1</td>
<td>0.22 14.29 0.68 3.08</td>
<td>0.23 14.22 0.67 2.93</td>
</tr>
<tr>
<td>24/05</td>
<td>7</td>
<td>1×d7</td>
<td>0.13 7.40 0.27 0.28</td>
<td>0.13 6.15 0.11 0.08</td>
</tr>
<tr>
<td>29/05</td>
<td>13</td>
<td>1×d13</td>
<td>0.20 1.93 1.31 0.63</td>
<td>0.11 2.03 0.19 0.30</td>
</tr>
<tr>
<td>05/06</td>
<td>20</td>
<td>1×d20</td>
<td>0.10 1.89 0.18 0.34</td>
<td>0.07 1.84 0.13 0.28</td>
</tr>
<tr>
<td>18/05</td>
<td>2</td>
<td>2×d2</td>
<td>0.22 14.41 0.67 2.96</td>
<td>0.20 13.62 0.69 2.33</td>
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<tr>
<td>25/05</td>
<td>8</td>
<td>2×d8</td>
<td>0.14 6.94 0.19 0.16</td>
<td>0.13 5.04 0.14 0.15</td>
</tr>
<tr>
<td>30/05</td>
<td>14</td>
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<td>0.09 1.73 0.13 0.25</td>
<td>0.09 1.73 0.11 0.38</td>
</tr>
<tr>
<td>06/06</td>
<td>21</td>
<td>2×d21</td>
<td>0.05 1.72 0.15 0.31</td>
<td>0.06 1.65 0.09 0.32</td>
</tr>
<tr>
<td>19/05</td>
<td>3</td>
<td>3×d3</td>
<td>0.18 12.72 0.73 2.47</td>
<td>0.19 12.61 0.69 1.56</td>
</tr>
<tr>
<td>26/05</td>
<td>9</td>
<td>3×d9</td>
<td>0.15 5.51 0.18 0.16</td>
<td>0.10 4.61 0.14 0.15</td>
</tr>
<tr>
<td>31/05</td>
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<td>0.02 1.32 0.13 0.34</td>
<td>0.05 1.17 0.08 0.30</td>
</tr>
</tbody>
</table>
**Table 2.** 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); the pigment was not used to characterize the additional taxon in this study.

<table>
<thead>
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<th>Pigment</th>
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<th>Taxon</th>
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**Table 3a.** Compilation of pigment key data for dilution experiment based run with water from mesocosm 1×CO$_2$ (a), 2×CO$_2$ (b) and 3×CO$_2$ (c). Day of experiment (DAY), standing stock 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), dynamic standing stock grazed daily (SS GRAZ %), not detectable (nd), not calculated (nc).

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<tr>
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Table 3b. Compilation of pigment key data for dilution experiment based run with water from mesocosm 2×CO\textsubscript{2} otherwise as Table 3a.

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<th>SE</th>
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<td>0.29</td>
<td>± 0.06**</td>
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<td>± 0.04***</td>
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<td>± 0.05</td>
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<td>± 0.08*</td>
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Table 3c. Compilation of pigment key data for dilution experiment based run with water from mesocosm 3×CO₂ otherwise as Table 3a.

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<th>R²</th>
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<td>0.03 ± 0.07*</td>
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<tr>
<td>Zea (Cyanobacteria)</td>
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<td>0.19 ± 0.08*</td>
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<td>0.20 ± 0.14</td>
<td>0.20</td>
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Fig. 1. Relative biomasses (µg C L⁻¹) of major groups of heterotrophic protists at start (t₀) of the respective experiments. Data from the 3×d22 experiment is missing (= not determined, nd).
Fig. 2. Instantaneous grazing mortality coefficient ($g$) against instantaneous phytoplankton growth coefficient ($k$) based on chlorophyll (Chl) $a$ (panel a), fucoxanthin (Fuco, diatoms) (panel b), 19'-hexanoyloxyfucoxanthin (19-hex, prymnesiophytes) (panel c), and zeaxanthin (Zea, cyanobacteria) (panel d). 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.