



Ciliate and mesozooplankton community response to increasing CO₂ levels in the Baltic Sea: insights from a large-scale mesocosm experiment

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Abstract. Community approaches to investigating ocean acidification (OA) effects suggest a high tolerance of micro- and mesozooplankton to carbonate chemistry changes expected to occur within this century. Plankton communities in the coastal areas of the Baltic Sea frequently experience pH variations partly exceeding projections for the near future both on a diurnal and seasonal basis. We conducted a large-scale mesocosm CO₂ enrichment experiment (~55 m³) enclosing the natural plankton community in Tvärminne–Storfjärden for 8 weeks during June–August 2012 and studied community and species–taxon response of ciliates and mesozooplankton to CO₂ elevations expected for this century. In addition to the response to *f*CO₂, we also considered temperature and chlorophyll *a* variations in our analyses. Shannon diversity of ciliates significantly decreased with *f*CO₂ and temperature with a greater dominance of smaller species. The mixotrophic *Myrionecta rubra* seemed to indirectly and directly benefit from higher CO₂ concentrations in the post-bloom phase through increased occurrence of picoeukaryotes (most likely Cryptophytes) and Dinophyta at higher CO₂ levels. With respect to mesozooplankton, we did not detect significant effects for either total abundance or for Shannon diversity. The cladocera *Bosmina* sp. occurred at distinctly higher abundance for a short time period during the second half of the experiment in three of the CO₂-enriched mesocosms except for the highest CO₂ level. The ratio of *Bosmina* sp. with empty to embryo- or resting-egg-bearing brood chambers, however, was significantly affected by CO₂, temperature, and chlorophyll *a*. An indirect CO₂ ef-

fect via increased food availability (Cyanobacteria) stimulating *Bosmina* sp. reproduction cannot be ruled out. Although increased regenerated primary production diminishes trophic transfer in general, the presence of organisms able to graze on bacteria such as cladocerans may positively impact organic matter transfer to higher trophic levels. Thus, under increasing OA in cladoceran-dominated mesozooplankton communities, the importance of the microbial loop in the pelagic zone may be temporarily enhanced and carbon transfer to higher trophic levels may be stimulated.

1 Introduction

Since the industrial revolution, anthropogenic CO₂ emissions have increased at an unprecedented rate and caused a concomitant increase of CO₂ concentration in the surface oceans. Thereby, ocean carbonate chemistry is altered, with the main changes being reduced carbonate ion concentrations [CO₃²⁻] and increased proton concentrations [H⁺], causing a pH decrease. Today this phenomenon is well recognized as ocean acidification (OA). Ocean pH has decreased by approx. 0.1 units already and projections suggest a further decrease of 0.14–0.43 units by the end of the century (IPCC, 2013). The Baltic Sea, one of the largest brackish water systems, is sensitive to CO₂ changes because it naturally has low alkalinity and thus carbonate buffer capacity. Models project a drop of 0.5 pH units for the Baltic Sea by the year 2100 (Hjalmarsson et al., 2008; Havenhand, 2012; Omstedt et al.,

2012). Eutrophication specifically affects coastal areas and can add to the $f\text{CO}_2$ fluctuations by provoking low-oxygen partial pressure due to increased degradation processes as well as respiration. Therefore, diel and seasonal variations of carbonate chemistry parameters, particularly of coastal areas of the Baltic Sea, are already huge today. Furthermore, the amplitude of fluctuations has even increased since the beginning of industrialization and concomitant eutrophication (Omstedt et al., 2009; Melzner et al., 2013; Jansson et al., 2013). Consequently, zooplankton in the coastal Baltic Sea naturally experience large pH fluctuations on a daily and seasonal basis and are possibly adapted to these highly variable abiotic conditions (Melzner et al., 2013; Almén et al., 2014).

Ocean acidification is suspected to have severe consequences for marine organisms and acts synergistically with the concurrent temperature increase due to greenhouse gas emissions (Riebesell et al., 2009). Until now, most attempts to test for sensitivity of marine organisms to OA were conducted as single-species experiments under controlled laboratory conditions. Such an approach cannot account for community interactions in natural environments, and thus application of results to natural environments is limited. Laboratory experiments suggest calcifying organisms to be most vulnerable to OA because the formation and preservation of calcareous structures is hindered (e.g., Riebesell et al., 2000; Hoegh-Guldberg et al., 2007; Lischka et al., 2011). Non-calcareous microzooplankton (MiZP) and mesozooplankton (MZP) are generally considered quite robust to elevated CO_2 concentrations. Effects on the microzooplankton level seem to be of a more indirect nature through changes in primary production, phytoplankton community composition, and stoichiometry (Suffrian et al., 2008; Feng et al., 2009; Rossoll et al., 2012). Mesozooplankton is often dominated by copepods (Longhurst, 1985), which are relatively insensitive to $f\text{CO}_2$ and/or pH changes expected for this century and direct negative effects do not usually occur unless exposed to much higher $f\text{CO}_2$ levels projected only much later (Kurihara et al., 2004; IPCC, 2013). More recent evidence suggests, however, that nauplii stages may be the weak point in copepod life cycles (Cripps et al., 2014). As for microzooplankton, studies on copepods and cladocerans suggest that CO_2 effects may be more indirectly mediated to the zooplankton level through CO_2 -induced changes in the biochemical and/or stoichiometric composition of their food (Urabe et al., 2003; Rossoll et al., 2012).

Holistic approaches to studying CO_2 effects on entire natural plankton communities, including zooplankton, are still rare. In a similar preceding mesocosm experiment, Aberle et al. (2013) and Niehoff et al. (2013) found no effects on Arctic micro- and mesozooplankton communities either with respect to abundance of single species or total numbers or with respect to change in community diversity. In terms of ciliates, these communities were dominated by large-sized forms ($> 30 \mu\text{m}$). In terms of mesozooplankton, communities were dominated by copepods and cirripede larvae.

The Tvärminne–Storfjärden area is an open archipelago on the eastern side of the Hanko peninsula on the south-west coast of Finland. Among microzooplankton, ciliates and heterotrophic dinoflagellates dominate in summer in Tvärminne–Storfjärden. Among mesozooplankton rotifers, copepods and cladocerans dominate (Kivi, 1986; Viitasalo, 1992; Koski et al., 1999). In the Tvärminne–Storfjärden area during late summer and autumn, the microbial food web (MFW) is of particular importance when filter-feeding cladocerans mediate carbon transfer to higher trophic levels including fish (Koski et al., 1999, and references therein). Summer dynamics of the planktonic food web were described in more detail by Uitto et al. (1997). In general, omnivores dominate across all trophic groups, but the importance of herbivory and feeding on heterotrophs varies in summer. Earlier in summer, heterotrophic nanoflagellates (HNF) transfer carbon from picoplankton to ciliates, and ciliates constitute the link from nano- to metazooplankton. Later in summer, HNF were largely bacterivorous, transferring bacterial carbon to ciliates and metazooplankton, when phytoplankton $> 10 \mu\text{m}$ was grazed by metazooplankton and heterotrophic dinoflagellates. In July, $< 10 \mu\text{m}$ phytoplankton increased and protists became the most important herbivores. The efficiency of the MFW in transferring bacterial carbon to metazooplankton was also the highest measured. However, the amount of carbon transferred to higher trophic levels also depends on the mesozooplankton species composition (Hansen et al., 1994). Elevated CO_2 concentrations can be beneficial for some phytoplankton groups, in particular picoeukaryotes. For micro- and mesozooplankton communities, so far no effects have been shown, at least for CO_2 ranges projected to occur within this century (Aberle et al., 2013; Niehoff et al., 2013; Schulz et al., 2013).

As part of the KOSMOS (Kiel Off-Shore Mesocosms for future Ocean Simulation) Tvärminne mesocosm experiment, we examined CO_2 effects on the enclosed ciliate and mesozooplankton community. A map showing the study site and mesocosm moorings is included in Paul et al. (2015). Between June and August 2012, an $f\text{CO}_2$ gradient was set up in six approximately 55 m^3 mesocosms covering $f\text{CO}_2$ projections for this century or beyond (IPCC, 2013). Abundance and community composition were followed through enumeration of regularly taken water and net samples. Per definition, mesozooplankton includes metazoa ranging between 0.2 and 20 mm (200–20 000 μm) in size. In this study, we do not follow this classification strictly because we also included the smaller juvenile life stages ($< 200 \mu\text{m}$) in the MZP category. In the case of protozoa we focus on ciliates only. Other protozoa, including Dinophyta and their response to CO_2 elevations, are included in Bermúdez et al. (2016). Ciliates in our study include some species that can be facultative autotrophs or obligate mixotrophs (*Myrionecta rubra* for instance).

Temperature can have a general effect on MiZP abundance and community composition and governs the dynamics of crustacean species (it affects productivity of cladocer-

ans for instance) in late summer in our study area (Nanazato and Yasuno, 1985; Koski et al., 1999; Rose et al., 2009; Aberle et al., 2013). Furthermore, warming ocean temperature changes are underway concurrently with ocean acidification with the potential to impact pelagic communities by providing suboptimal temperature conditions for species (IPCC, 2013). To consider possible impact of temperature variation and/or CO₂-driven chlorophyll *a* differences (Schulz et al., 2013), we also included temperature and chlorophyll *a* as explanatory variables in our statistical analyses.

2 Methods

To study the effect of elevated $f\text{CO}_2$ on a natural plankton community in the Baltic Sea, nine KOSMOS offshore pelagic mesocosms were deployed and moored from 12 June 2012 until the middle of August in the Tvärminne–Storfjärden archipelago area on the southwest coast of Finland at 59°51.5' N and 23°15.5' E. The water depth at the mooring site was approximately 30 m. The mesocosm bags extended down to 17 m and were closed with 2 m long sediment traps at the bottom of the bags to enclose an isolated water body with its natural plankton community. After deployment, the mesocosm bags were initially kept open and submerged ~0.5 m below the surface to allow for a free exchange of the water and plankton community in the bags with the surrounding water masses. Organisms > 3 mm, such as fish and Cnidaria, were excluded by 3 mm nets at the top and bottom openings of the bags during the first 5 days. These nets were removed on t_{-7} (i.e., 7 days before the first CO₂ addition on t_0), the sediment traps were attached to the bottom, and the top ends of the mesocosm bags were pulled up to 1.5 m above the surface to isolate the enclosed pelagic community from the Baltic Sea. The final volumes of the mesocosms ranged between 53.1 and 55.1 m³ (Paul et al., 2015). The nine mesocosms were enriched with different amounts of CO₂-saturated seawater to set up an initial gradient of $f\text{CO}_2$ from 240 µatm (ambient control mesocosms) up to ~1650 µatm. Three mesocosms (M2, M4, M9) were lost during the course of the experiment due to leakage. $f\text{CO}_2$ values in the six remaining mesocosms averaged over the sampling period (t_1 – t_{43}) were 365 (M1 control), 368 (M5, control), 497 (M7), 821 (M6), 1007 (M3), and 1231 µatm (M8). CTD (conductivity, temperature, depth) profiles and samples for dissolved inorganic nutrients (silicate, phosphate, nitrate, nitrite, ammonium) and carbonate chemistry system parameters (DIC, TA, pH_T) were either taken daily or every second day. For more technical details about the experimental setup, the CO₂ manipulations, and sampling procedures for various analyses, see Paul et al. (2015). Sampling days were enumerated consecutively with t_{-3} indicating 3 days before CO₂ manipulation, t_0 as the day of the first CO₂ manipulation, and t_{1+X} as the days following the first CO₂ manipulation.

2.1 Microzooplankton sampling

Water samples for the enumeration of ciliates were taken every second day with a depth-integrating sampler (0–17 m), IWS (HYDRO-BIOS, Kiel, Germany), between 9:00 and 12:00 CET from six mesocosms. After careful mixing, 250 mL of seawater was filled into brown glass bottles and preserved in acidic Lugol's iodine (1 % final concentration). We transferred 50 mL of the sample to Utermöhl sedimentation chambers. After 24 h settling time, ciliates were counted with a Zeiss Axiovert 100 inverted microscope at 200× magnification (Utermöhl, 1958). At high cell numbers (>400 cells), half the bottom plate area was counted. If less than 400 cells were found in the first half of the bottom plate area, the entire chamber was counted. Rare species were counted on the whole bottom plate. Ciliates were identified to the lowest possible taxonomic level (genus or species) according to Setälä et al. (1995) and according to descriptions found at the planktonic ciliate project (<http://ciliate.zooplankton.cn/>). In total, 138 samples were analyzed. Abundances were calculated as cells L⁻¹.

2.2 Mesozooplankton sampling

Mesozooplankton samples from six mesocosms were taken with an Apstein net of 17 cm diameter and 100 µm mesh size. Zooplankton were sampled between 8:00 and 11:00 CET by towing the net vertically from 17 m depth to the mesocosm surface. In total, on 11 sampling days vertical net hauls were done from the mesocosms: prior to the CO₂ addition (t_{-3} , t_{-2} , t_{-1}), on the day of the first CO₂ addition (t_0), and after the first CO₂ addition (t_3 , t_{10} , t_{17} , t_{24} , t_{31} , t_{38} , t_{45}). After collection, the samples were brought back to the laboratory in the Tvärminne zoological station (University of Helsinki) and preserved in 70 % ethanol. Zooplankton abundance was calculated assuming 100 % filtering efficiency of the net. The samples were divided with a Folsom plankton splitter (1 : 2, 1 : 4, 1 : 8, 1 : 16, 1 : 32) and the aliquots of the samples were counted. Organisms were counted and determined to the lowest taxonomical level possible under a stereo microscope (WILD M3B). Abundant species or taxa (>30 individuals in an aliquot) were only counted from subsamples, while less abundant species or taxa were counted from the whole sample. Juvenile bivalves did not distribute equally in the Folsom splitter due to their relatively large mass and were therefore counted from the whole sample. Copepods (*Acartia* spp., *Eurytemora* spp., *Temora* spp.) were identified according to different stages (adult females, adult males, copepodite stages CI–CV). Copepod nauplii were counted but not determined to species level. The counting of the cladoceran species (*Bosmina* spp., *Evadne* spp., *Podon* spp.) was distinguished according to organisms with empty or filled brood chambers (i.e., organisms that had empty brood chambers or bore embryos and/or resting eggs in their brood chambers) and categorized as “empty” or “filled”. For data analyses, the

ratio of organisms with empty brood chambers to organisms with filled brood chambers was calculated for each mesocosm and sampling day, i.e., a small ratio stands for a higher proportion of reproducing organisms in the population in a particular mesocosm on a particular sampling day. A total of 66 samples were analyzed. Abundances were calculated as individuals m^{-3} .

2.3 Data analysis and statistics

To assure equally spaced data, some sampling days were excluded from statistical analyses. For the ciliate data this applied to t_{-3} , t_0 , t_2 , and t_4 , and for the mesozooplankton this applied to t_{-3} , t_{-2} , t_{-1} , and t_0 . However, for demonstration purposes only, the data of these sampling days were included in the figures.

As explanatory variables, $f\text{CO}_2$, temperature, and chlorophyll a were used to test for effects on different response variables (see below). Collinearity was checked prior to analyses. To account for the change in $f\text{CO}_2$ over time due to ingassing or outgassing, as well as temperature and chlorophyll a changes over time, all explanatory variables were used as continuous variables for each t -day included in the analyses. All analyses were carried out with R using the packages nlme, mgcv, Hmisc, and MASS. All plots were done in ggplot (R Developmental Core Team, 2012).

The Shannon index (H) was calculated as a measure of diversity in each of the mesocosms and to estimate changes in the relative contribution of single species or groups in the whole ciliate and mesozooplankton community over time and in response to different abiotic parameters such as the $f\text{CO}_2$ levels. When all considered species or groups contribute equally to the community in terms of their abundances, H calculated on the natural logarithm becomes 2.3. The more a community is dominated by a single species or group, the smaller the Shannon index gets. Calculations of H were performed in the vegan package of the R environment (Oksanen et al., 2012).

For the ciliates, 14 species or groups were included to calculate H : *Balanion comatum*, *Strombidium* cf. *epidemum*, *Mesodinium* sp., *Myrionecta rubra* ($\leq 10\ \mu\text{m}$), *M. rubra* ($11\text{--}20\ \mu\text{m}$), *M. rubra* ($> 20\ \mu\text{m}$), *Rimostrombidium* sp., *Spathidium* sp., *Strobilidium* spp. ($\leq 20\ \mu\text{m}$), *Strobilidium* spp. ($> 20\ \mu\text{m}$), *Strombidium* sp., tintinnids, cysts (*Strobilidium* sp., unidentified cysts), and ciliates sp. (*Euplotes* sp., *Lacrymaria* sp., *Strobilidium* sp., unidentified ciliates). *Lohmaniella* sp. could not be clearly separated from other Strobilids and was therefore included with *Strobilidium* spp. ($\leq 20\ \mu\text{m}$). However, most of the Strobilids found, were probably *Lohmaniella* sp..

For the mesozooplankton, 17 species or taxonomic groups were included in the calculation of H : copepodite stages and larval stages of *Balanus* sp. (nauplii and cypris larvae) were summarized on the genus level (Copepoda: *Acartia* sp., *Eurytemora* sp., *Temora* sp., Harpacticoida sp., copepod nau-

plii; Cladocera: *Bosmina* sp., *Daphnia* sp., *Evadne* sp., *Podon* sp.; Rotifera: *Asplanchna* sp., *Keratella* sp., *Synchaeta* sp., *Rotifera* sp.; larvae of *Balanus* sp., juvenile bivalves, juvenile gastropods, and larvae of polychaetes).

2.3.1 Ciliates

Statistical analyses were done on total cell numbers, the Shannon index H , as well as the abundance of particular groups that showed distinct differences, such as small size class *Myrionecta rubra*, *Balanion comatum*, *Strombidium* cf. *epidemum*, and small *Strobilidium* sp.. Linear mixed effect modeling (LME) was applied on a Gaussian distribution to determine the effects of CO_2 , temperature, and chlorophyll a . Actually, count data should be modeled on a Poisson distribution, but model selection (s.b.) yielded convergence problems in R for Poisson distribution. Therefore, we used a Gaussian distribution, which can also be applied to count data (Zuur et al., 2009). If preceding data exploration suggested interactions between the factors, respective interaction terms were included in the model. Model selection was based on the Akaike information criterion (AIC) by removing non-significant terms to find the simplest adequate model. However, missing values for chlorophyll a occurred for M3/ t_{25} and for M5/ t_{23} ; these values were estimated as means of the preceding and following days. Chlorophyll a values were also missing for t_{41} and t_{43} . A polynomial fit curve applied to phase III (according to temperature variations, three experimental phases (I, II, III) were defined, which are thoroughly introduced in Paul et al. (2015); phase III lasted from t_{31} until t_{43}) resulted in no meaningful values; therefore, these values were estimated as phase III means.

The different response variables were modeled as function of the daily change in $f\text{CO}_2$, temperature, and chlorophyll a , and if suggested, with interaction terms as mentioned above. To account for the time dependency and the nested nature of the data, GLM (generalized mixed effect) models were applied to a Gaussian distribution using $f\text{CO}_2$ (values on a continuous scale for each sampling day) and sampling day nested in the mesocosm as a random intercept. In case of violation of the assumptions for linear models yielding untrustworthy p values, the GLM model was reapplied as a GAMM (generalized additive mixed model) and a smoother for the sampling day was included to prove the validity of the GLM model outcome. In some cases, some residual patterns, mostly due to sampling day, still remained even after applying the GAMM. However, GAMM is as much as can be done with current hardware and software. Therefore, for highly significant p values, our results should still be reasonably robust, and p values that are not highly significant should be taken with some caution (Zuur et al., 2009).

2.3.2 Mesozooplankton

The statistical approach with respect to MZP corresponded with the description in Sect. 2.2.1. Total abundance, Shannon index H , total abundance of species that suggested distinct differences (such as *Bosmina*), and ratio of *Bosmina* with empty brood chambers to those with full brood chambers (i.e., either bearing embryos and/or resting eggs in their brood chambers) were analyzed statistically. Missing values for $f\text{CO}_2$ occurred on t_{24} , t_{38} , and t_{45} , and they occurred for temperature and chlorophyll a on t_{38} and t_{45} . Missing observations for t_{24} and t_{38} were estimated by building the mean of values measured at t_{23} and t_{25} and t_{37} and t_{39} respectively. t_{45} was the last sampling day; hence, it was not possible to estimate a mean from the preceding and following days. Therefore, missing values for t_{45} were estimated from a polynomial fit curve applied to phase III values (Paul et al., 2015).

2.3.3 Predator–prey relationships

Pearson correlation was used to investigate possible trophic relationships between ciliates and MZP, and bacteria, nanoeukaryotes, picoeukaryotes (total bacteria, low-DNA bacteria, high-DNA bacteria, Cyanobacteria, particle-associated bacteria, *Synechococcus*, pico- and nanoeukaryotes), and phytoplankton groups (Prasinophytes, Cryptophytes, Chlorophytes, Cyanobacteria, Diatoms, Euglenophytes, auto- and heterotrophic dinoflagellates, and heterotrophic dinoflagellates excluding *Ebria* sp.). For these correlations, data from Crawford et al. (2016), Paul et al. (2015), and A. Stühr (unpublished data from this study) were used.

3 Results

3.1 Ciliates

3.1.1 Ciliate total abundance

Total abundance of ciliates at the experiment start (t_0) varied between 78 120 (M5) and 52 360 cells L^{-1} (M3) and more or less continually decreased from the beginning over time until t_{17} when a plateau was reached with low cell numbers between 7080 (M8) and 10 940 (M3) until t_{33} . During the last 5 sampling days (t_{35} – t_{43}), total cell numbers were more variable again, with some small ups and downs, and reached minimum values between 900 (M6) and 3580 cells L^{-1} (M8) on the last sampling day (Fig. 1).

3.1.2 Abundance of *Myrionecta rubra*

Myrionecta rubra was (by far) the most dominant ciliate species during the entire period (Fig. 2). *M. rubra* occurred in three different size classes (≤ 10 , 11–20, $> 20 \mu\text{m}$), of

which organisms of the smallest size range made up the highest numbers. On t_0 cell numbers of *M. rubra* of the smallest size class varied between 26 720 and 44 520 cells L^{-1} . Cell numbers stayed relatively high until t_{11} and t_{13} (16 600–37 400 cells L^{-1}) when they strongly declined to values below 10 000 cells L^{-1} on t_{17} . They further decreased with some fluctuations until the end of the experiment to reach final values between 130 and 1740 cells L^{-1} among all mesocosms. However, a striking difference occurred between t_{25} and t_{35} when abundance in the three highest CO_2 mesocosms was higher compared to the two controls and the lowest CO_2 enriched mesocosm (mean: 4518 cells L^{-1} (SD 1,082) and mean: 3459 cells L^{-1} (SD 383), respectively). *M. rubra* of the medium size class also had maximum numbers on t_0 ranging from 17 600 to 25 680 cells L^{-1} . From the experiment start, numbers more or less continually decreased and reached minimum values of between 480 and 0 cells L^{-1} from t_{19} on. The largest *M. rubra* occurred only rarely but as in the other two size classes, the highest numbers were found during the first few sampling days, varying between 2680–5800 cells L^{-1} on t_0 and already reaching very low numbers on t_7 and t_9 (1080–280 cells L^{-1}). After t_{19} , *M. rubra* $> 20 \mu\text{m}$ occurred only exceptionally.

3.1.3 Abundance of other species–genera–groups

Other dominant groups or species that contributed to the total cell numbers of ciliates were *Balanion comatum*, *Strombidium* cf. *epidimum*, *Strombidium* sp. ($< 20 \mu\text{m}$ and $> 20 \mu\text{m}$), *Mesodinium* sp., *Rimostrombidium* sp., *Strombidium* sp., tintinnids, *Spathidium* sp., cysts, and ciliates that could not be identified (Fig. 2). Among those, *Strombidium* cf. *epidimum* was most dominant and showed three peaks, around t_9 and t_{11} , t_{23} , and t_{37} . On t_9 and t_{11} a distinct difference occurred between the control and CO_2 -enriched mesocosms (mean: 1250 cells L^{-1} (SD 180) and mean: 2205 cells L^{-1} (SD 851), respectively). *Balanion comatum*, *Rimostrombidium* sp., *Strombidium* sp. ($< 20 \mu\text{m}$), *Spathidium* sp., and Tintinnids were of some importance during the first days of the experiment, showing peaks in cell numbers between t_0 and t_{11} . Most interestingly, peak abundance of *Balanion comatum* diverged from CO_2 concentration, with higher mean cell numbers in the control and lowest-enriched mesocosms compared to the three high- CO_2 mesocosms (mean: 1680 cells L^{-1} (SD 139) and mean: 880 cells L^{-1} (SD 223), respectively). Likewise, small *Strombidium* sp. developed some CO_2 -related differences with mean abundance of 1360 (SD 170) and 2400 cells L^{-1} (SD 872) in the two controls and the CO_2 -enriched mesocosms, respectively. *Mesodinium* sp., *Strombidium* sp. $> 20 \mu\text{m}$, cysts, and unidentifiable ciliates always occurred in relatively low cell numbers (mostly < 850 cells L^{-1}).

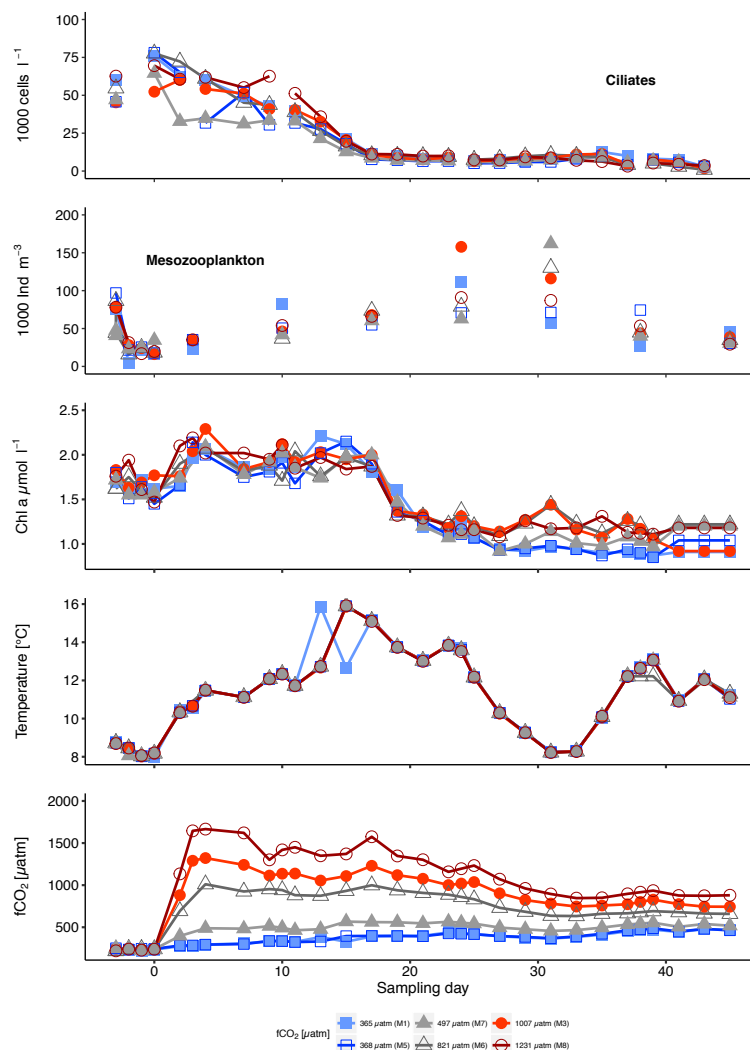


Figure 1. Total cell numbers of ciliates and total abundance of mesozooplankton during the course of the experiment as well as chlorophyll *a* succession, temperature, and $f\text{CO}_2$ development. According to temperature variations and the first CO_2 manipulation, different experimental phases were defined: phase 0 = t_{-5} to t_0 , phase I = t_1 to t_{16} , phase II = t_{17} to t_{30} , phase III = t_{31} to t_{43} . Note that there is one missing value in M1 on t_{13} .

3.1.4 Percent contribution of numerically dominant species–genera–groups to total cell numbers

Figure 3 shows the percent contribution of dominant species–genera–groups to the total cell numbers over time for each of the mesocosms. For better clarity, *Myrionecta rubra* size classes, *Strombidium* sp. size classes together with *Rimostrombidium* sp. and *Strombidium* spp., and cysts together with ciliates sp. were combined. *M. rubra* dominated the ciliate community in all mesocosms most of the time. During the first days of the experiment, *M. rubra* contributed ~90 % to the total cell numbers in all mesocosms and stayed above 50 % until t_{21} . Minimum contributions occurred on t_{37} when *M. rubra* had a share of only 6–24 %. After t_{37} , *M. rubra* proportions ranged between 18 and 67 %. The second most

important group was *Strombidium* sp., consisting mostly of *Strombidium* cf. *epidemum*. *Strombidium* sp. had the highest shares during the second half of the experiment, varying between 58 % and 69 % during t_{35} – t_{39} . All remaining groups usually had contributions below 15 %.

The Shannon diversity index H ranged from 0.58 to 1.66 over the whole period of time (Fig. 3). In general, it showed a slightly increasing trend varying between 1.04 and 1.23 on t_{-3} and 1.30 and 1.66 on t_{43} and was generally lower during higher temperature phases (I + II) (Fig. 3).

3.1.5 Statistical analyses ciliates

GAMM determined significant synergistic effects for total abundance of small size class *Myrionecta rubra*

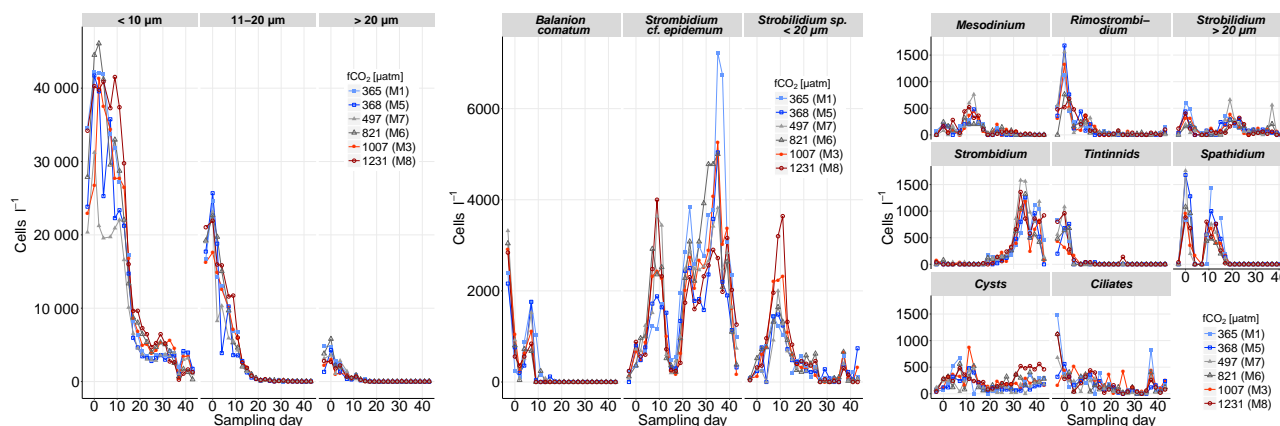


Figure 2. Left: abundance of different size classes of *Myrionecta rubra*. Middle–right: abundance of other ciliate species–genera–groups. Note that there is one missing value in M1 on t_{13} .

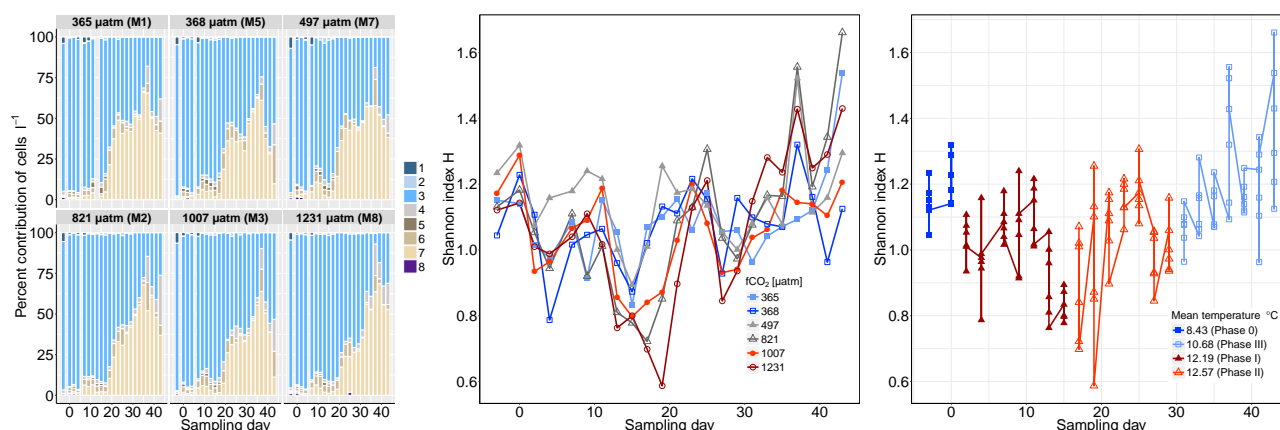


Figure 3. Left: percent contribution of abundance of major taxonomic species–genera–groups to the ciliate community. Numbers in the figure legend indicate: 1 = *Balanion comatum*, 2 = *Mesodinium* sp., 3 = *Myrionecta rubra*, 4 = *Others*, 5 = *Spathidium* sp., 6 = *Strombidium* sp., 7 = *Strombidium* sp., 8 = *Tintinnidae* sp. Note that there is one missing value in M1 on t_{13} . Middle: Ciliates, daily change of the Shannon diversity index H at the different $f\text{CO}_2$ levels in the mesocosms. Right: Ciliates, daily change of the Shannon diversity index H during the four different defined temperature phases. Color legend gives mean temperature during phase 0 (12.57 °C), phase I (8.43 °C), phase II (10.68 °C), and phase III (12.19 °C).

in response to $f\text{CO}_2 \times \text{temperature}$ ($p = 0.024$) and $f\text{CO}_2 \times \text{chlorophyll } a$ ($p = 0.004$). Total abundance of *Balanion comatum* was affected by temperature and $f\text{CO}_2$ ($p_{\text{temperature}} = 0.022$, $p_{f\text{CO}_2} = 0.03$). Total abundance of *Strombidium cf. epidemum* by chlorophyll a ($p = 0.002$). Total abundance of *Strombidium* sp. showed synergistic responses to the combination of the factors $f\text{CO}_2 \times \text{temperature}$ and $f\text{CO}_2 \times \text{chlorophyll } a$ ($p = 0.0005$ and $p = 0.0002$, respectively), and for the Shannon index H a synergistic effect of $f\text{CO}_2 \times \text{temperature}$ was determined ($p = 0.0008$). Depiction of the statistical results of H showed a non-monotonic relationship with a slightly increasing trend at lower $f\text{CO}_2$ and a decreasing trend the more $f\text{CO}_2$ increased, as well as a decreasing trend with temperature (Fig. 4). Statistical results are shown in more detail in Table 1. Model validation showed some residual

pattern in all cases, but most of the obtained p -values are highly significant and are therefore reasonably trustworthy (Zuur et al., 2009). Only with respect to *Balanion comatum*, should p values be seen with some caution since they are not highly significant.

3.2 Mesozooplankton

3.2.1 Mesozooplankton total abundance

After a sharp initial decrease, total abundance of mesozooplankton increased continuously until peak abundances were reached between t_{24} and t_{31} (Fig. 5). M7, M6, and M3 (497–1007 µatm) had the highest peak values ranging between 130 276 and 162 082 ind. m⁻³, whereas abundance in M1 and M8 was somewhat lower with 111 980 and 90 975 ind. m⁻³,

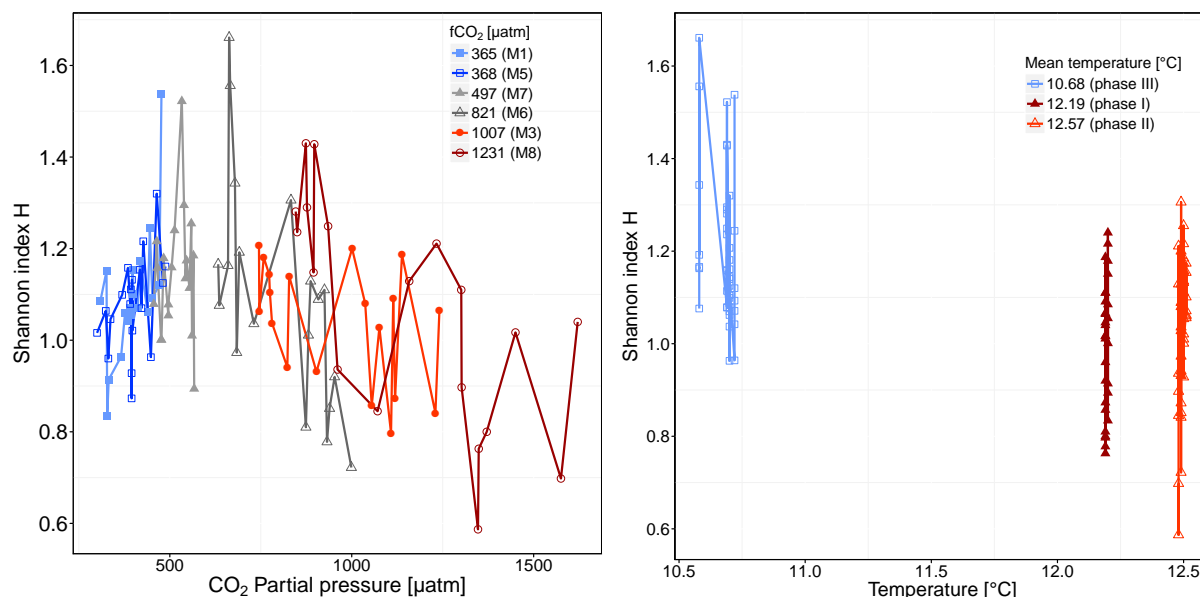


Figure 4. Left: ciliates, graphical depiction of statistical results for Shannon diversity index H as a function of $f\text{CO}_2$. H is shown in relation to the daily change of $f\text{CO}_2$. Symbols and colors identify the mean $f\text{CO}_2$ for each mesocosm. Right: ciliates, graphical depiction of statistical results for Shannon diversity index H as a function of temperature. For better visibility, H is plotted against the mean phase (I, II, III) temperature of each mesocosm. Symbols and colors identify mean phase temperature across all mesocosms.

respectively. In M5, no abundance peak occurred, but zooplankton developed a plateau between t_{24} and t_{38} of around $70\text{--}74\,000\text{ ind. m}^{-3}$. Towards the end of the experiment, zooplankton total abundance returned to about the initial values ($29\,325$ and $44\,824\text{ ind. m}^{-3}$ in M8 and M1, respectively).

3.2.2 Community composition

The mesozooplankton community was dominated by five taxonomic groups, i.e., Cladocera (*Bosmina* sp., *Daphnia* sp., *Evadne* sp., *Podon* sp.), Copepoda (*Acartia* sp., *Eurytemora* sp., *Temora* sp., copepod nauplii, Harpacticoida, Cyclopoida, Copepoda sp.), Crustacea (*Balanus* sp., including nauplii and cyprid larvae), Mollusca (juvenile Bivalvia and Gastropoda), and Rotifera (*Asplanchna* sp., *Keratella* sp., *Synchaeta* sp., Rotifera sp.). The group “others” comprises larvae of Bryozoans (cyphonautes), juvenile Polychaeta, and unidentifiable organisms (Fig. 6). Among these groups, cladocerans and copepods dominated the zooplankton community during the entire experimental period. Cladocerans contributed mostly between 50 and 95 % to the total abundance. Copepods had their highest share halfway through the experiment when they constituted 74–84 % (t_{17}) of the whole community. Rotifera were a major part of the zooplankton only during the first days of the experiment with about 11 to 42 % between t_{-1} and t_3 . Among the group Mollusca, gastropods always had a smaller share than bivalves with usually below 2 % (max. 5 %) contribution to the total abundance of the group. Juvenile bivalves mainly occurred from the start until day t_{10} and had maximum contributions of 17–45 % to the

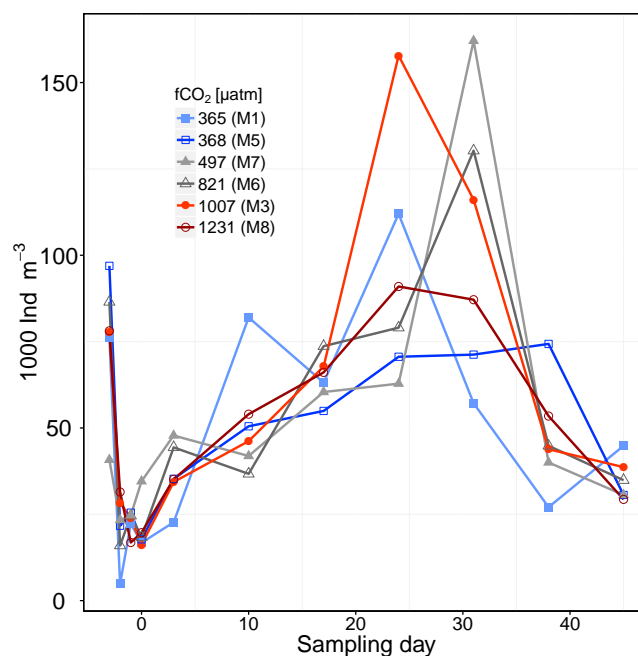


Figure 5. Mesozooplankton total abundance. According to temperature variations and the first CO_2 manipulation, different experimental phases were defined: phase 0 = t_{-5} to t_0 , phase I = t_1 to t_{16} , phase II = t_{17} to t_{30} , phase III = t_{31} to t_{43} .

total zooplankton community between t_{-2} and t_0 . The group “Crustacea” comprises mainly larvae of *Balanus* sp. (nau-

Table 1. Statistics summary table of retained fixed effects of the GLM models and GAMMs. Significant *p* values are indicated in bold (Temp: temperature).

	Explanatory variable	DF	t	<i>p</i> value	Model
Ciliates					
Ciliate total abundance	Temp	1	−3.506	0.0007	GAMM
<i>Myrionecta rubra</i> , ≤ 10 µm	Temp	1	2.376	0.019	GAMM
<i>Myrionecta rubra</i> , ≤ 10 µm	<i>f</i> CO ₂ × Temp	1	−2.298	0.024	GAMM
<i>Myrionecta rubra</i> , ≤ 10 µm	<i>f</i> CO ₂ × Chl <i>a</i>	1	2.936	0.004	GAMM
<i>Balanion comatum</i>	Temp	1	2.320	0.022	GAMM
<i>Balanion comatum</i>	<i>f</i> CO ₂	1	−2.210	0.030	GAMM
<i>Strombidium</i> cf. <i>epidemum</i>	Chl <i>a</i>	1	−3.229	0.002	GAMM
<i>Strobilidium</i> sp., < 20 µm	Temp	1	2.811	0.006	GAMM
<i>Strobilidium</i> sp., < 20 µm	Chl <i>a</i>	1	−4.603	< 0.00001	GAMM
<i>Strobilidium</i> sp., < 20 µm	<i>f</i> CO ₂ × Temp	1	−3.600	0.0005	GAMM
<i>Strobilidium</i> sp., < 20 µm	<i>f</i> CO ₂ × Chl <i>a</i>	1	3.926	0.0002	GAMM
Shannon index <i>H</i>	Temp	1	3.652	0.0004	GAMM
Shannon index <i>H</i>	<i>f</i> CO ₂	1	2.824	0.006	GAMM
Shannon index <i>H</i>	<i>f</i> CO ₂ × Temp	1	−3.454	0.0008	GAMM
Mesozooplankton					
MZP total abundance	Temp	31	−1.155	0.257	GLM
MZP total abundance	<i>f</i> CO ₂	31	−0.025	0.980	GLM
MZP total abundance	Chl <i>a</i>	31	0.550	0.586	GLM
MZP total abundance	<i>f</i> CO ₂ × Temp	31	0.947	0.351	GLM
MZP total abundance	<i>f</i> CO ₂ × Chl <i>a</i>	31	−1.081	0.288	GLM
<i>Bosmina</i> sp.	Chlor <i>a</i>	1	0.76	0.453	GAMM
<i>Bosmina</i> sp. ratio empty to full brood chambers	Temp	1	−3.572	0.001	GAMM
<i>Bosmina</i> sp. ratio empty to full brood chambers	<i>f</i> CO ₂	1	−2.684	0.011	GAMM
<i>Bosmina</i> sp. ratio empty to full brood chambers	Chl <i>a</i>	1	−3.980	0.0004	GAMM
<i>Bosmina</i> sp. ratio empty to full brood chambers	<i>f</i> CO ₂ × Chl <i>a</i>	1	2.738	0.01	GAMM
Shannon index <i>H</i>	Chl <i>a</i>	1	−0.555	0.582	GAMM

plii and cyprids). Only very rarely was a mysid found and specimens of this order were also included in the Crustacea group. The main occurrence of Crustacea was from t_{-1} until t_{10} , contributing between 10 and 2 % to the total zooplankton community during this time. The others group always contributed less than 0.5 % to the total abundance.

In all mesocosms, the Shannon diversity index was highest at the beginning of the experiment (t_3 : 1.78–1.89) and decreased continuously with time reaching the lowest values on the last sampling day (t_{45} : 0.23–0.5). This indicates that towards the second half of the experiment and at the end the dominance of single species or groups increased.

3.2.3 Copepoda

Eurytemora sp. was the dominant copepod species in the zooplankton community over the entire period. *Acartia* sp. occurred regularly but in much lower abundances. *Temora* sp. occurred only in very low numbers mainly during the first part of the experiment (Fig. 7). The abundance of *Eurytemora* sp. was relatively low at the beginning (82–2496 ind. m^{−3}). Peak abundances were reached around days t_{17} and t_{24} (19 192–32 297 ind. m^{−3}) and then declined. Dur-

ing the course of the experiment, *Acartia* sp. varied in numbers between 117 and 4624 ind. m^{−3} and did not show clear abundance peaks in most of the mesocosms. *Temora* sp. was present the whole time (though not always in all mesocosms) but always in low abundances ranging between 330 and 3 ind. m^{−3} among all mesocosms. Copepod nauplii occurred during the entire experiment duration with peak abundance between t_{10} and t_{24} (9003–33 555 ind. m^{−3}).

The three copepod species were determined by copepodite stages (CI–CV) and adult females and males (Fig. 7b). *Eurytemora* sp. copepodites CI–CV were present in high proportions during almost the whole period of time up to > 90 %. Adult females and males had their minimum during the abundance peak of this species (t_{17} – t_{31}) but occurred during the entire study period, indicating more or less continuous reproduction in all mesocosms. At the beginning and towards the end of the study, most of *Acartia* sp. were in the copepodite stage CI–CV. Adult females and males occurred during the whole period of time and had maximum proportions halfway through the experiment (t_{17} , t_{24}). During this time, reproduction took place indicated by the following increase in copepodite stages during the second half of the study. The stage distribution of *Temora* sp. was similar to *Acartia* sp., with

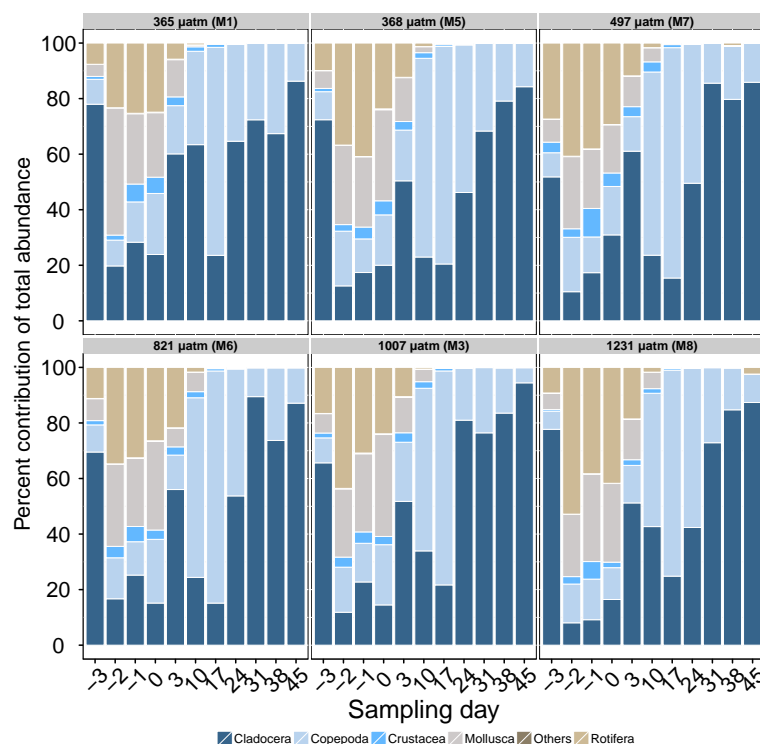


Figure 6. Percent contribution of mesozooplankton main taxonomic groups.

a peak of copepodite stages CI–CV on the first and the last sampling days. Most of the time, however, adult females and males dominated.

3.2.4 Cladocera

Four species of cladocera were found in the mesocosms: *Bosmina* sp., *Podon* sp., *Evadne* sp., and *Daphnia* sp. *Daphnia* sp. occurred only rarely in very low abundances ($<0.5\%$ contribution to total cladocera, abundance range: $2.6\text{--}12.8\text{ ind. m}^{-3}$). *Evadne* sp. had maximum abundances between t_3 and t_{10} ($184\text{--}3893\text{ ind. m}^{-3}$) and contributed up to 38% to this group during the first days of the experiment but noticeably decreased in importance later. *Podon* sp. dominated among the cladocerans at the beginning of the experiment accounting for more than 80% of the total abundance until day t_{10} (max. numbers: $43\,688\text{--}15\,272\text{ ind. m}^{-3}$). By day t_{17} *Bosmina* sp. reached more than a 90% share until termination of the experiment. Peak abundance of *Bosmina* sp. occurred between t_{24} and t_{38} and was substantially higher in the medium-range CO_2 mesocosms M7 ($497\text{ }\mu\text{atm}$), M6 ($821\text{ }\mu\text{atm}$), and M3 ($1007\text{ }\mu\text{atm}$) ($138\,394$, $114\,169$, $127\,080\text{ ind. m}^{-3}$, respectively) compared to the two controls, M1 and M5, and the highest CO_2 mesocosm (M8, $1231\text{ }\mu\text{atm}$) ($72\,020$, $58\,107$, $63\,182\text{ ind. m}^{-3}$, respectively) (Fig. 8, only *Bosmina* sp. is shown).

The counting of the two dominant cladoceran species *Podon* sp. and *Bosmina* sp. was divided into organisms

with empty brood chambers and organisms bearing embryos and/or resting eggs in their brood chambers to inspect for a possible direct or indirect effect of CO_2 on asexual or sexual reproduction. A ratio was subsequently calculated; see above. Mostly, the percent contribution of organisms with filled brood chambers varied between 40 and 10% in all mesocosms during the study period. Only during the very first days, did *Bosmina* sp. with filled chambers have contributions of up to 67% (not shown). The ratio of *Bosmina* brood chambers varied during peak occurrence ($t_{24}\text{--}t_{31}$) between 3.47 (M8) and 17.18 (M7) (Fig. 8). During times of high *Podon* sp. abundances, the share of this organism with full brood chambers varied roughly between about 25 and 50% . *Podon* actively reproduced during the first days of the experiment, indicated by a low ratio of organisms with empty-to-full brood chambers ($0.79\text{--}2.77$), whereas the lowest reproductive activity occurred on t_{17} and t_{24} ($5.09\text{--}33.10$) (not shown).

3.2.5 Statistical analyses mesozooplankton

For total abundance of mesozooplankton we determined no significant relationship with $f\text{CO}_2$ or any of the other explanatory variables (temperature, chlorophyll a) (Table 1).

The Cladocera *Bosmina* sp. showed distinct abundance peaks in M7, M6, and M3 with approx. $110\text{--}130\text{ ind. }10^3\text{ m}^{-3}$ higher numbers between t_{24} and t_{31} compared to the two control mesocosms and M8. The GLM

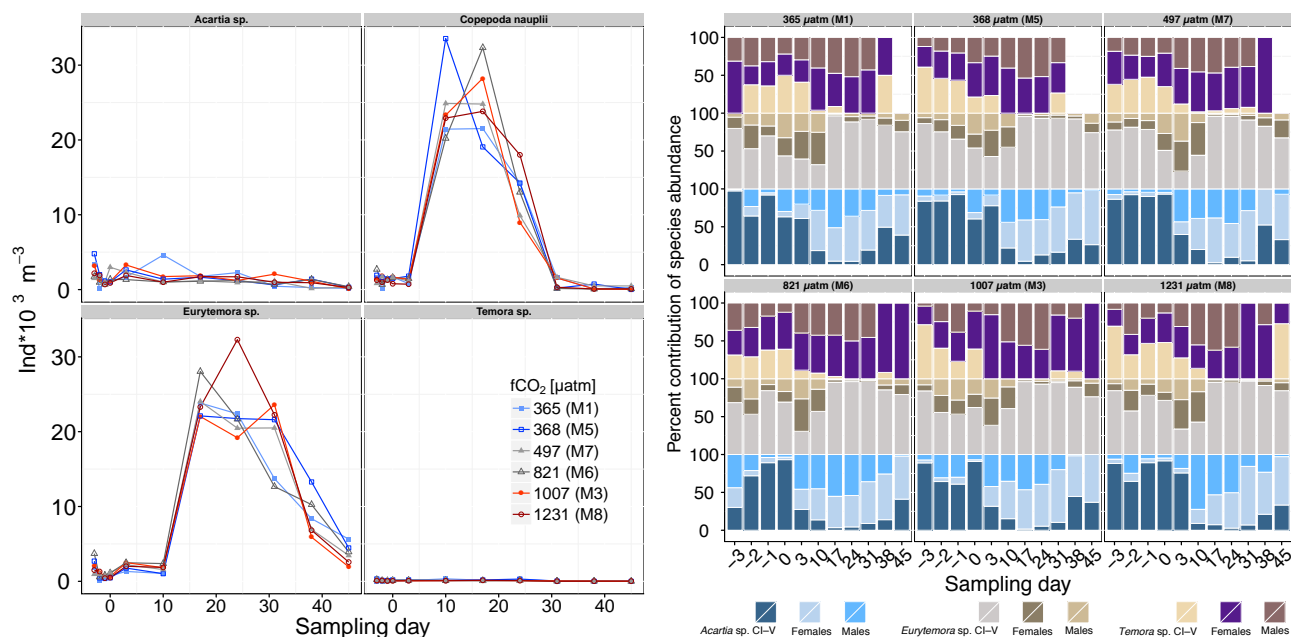


Figure 7. Left: abundance of the dominant copepod species *Acartia* sp., *Eurytemora* sp., *Temora* sp., and copepod nauplii. Right: percent contribution of different stages of dominant copepods.

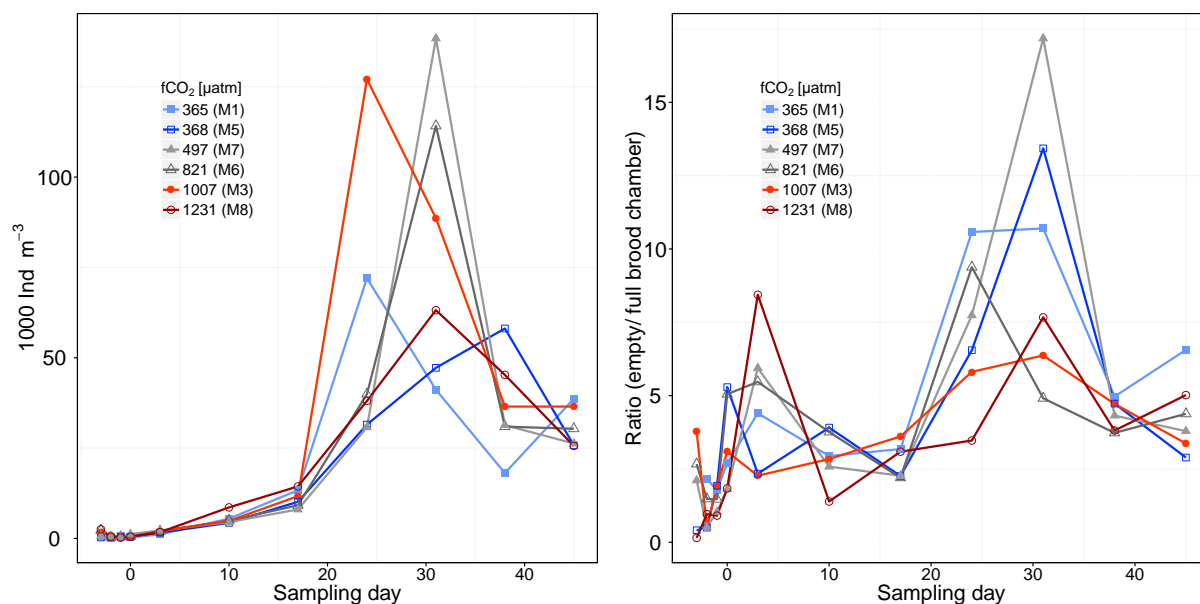


Figure 8. Left: total abundance of the most dominant cladoceran species *Bosmina* sp. Right: ratio of *Bosmina* with empty brood chambers to organisms with full brood chambers. Note: figure shows all data, but statistics were done on data from t_3 to t_{45} only to assure equally spaced data.

model revealed neither a significant relation of the total abundance of *Bosmina* sp. with $f\text{CO}_2$ nor temperature. Chlorophyll *a* concentration was determined to significantly affect the *Bosmina* occurrence, but model validation showed heterogeneity of the residuals mostly due to experiment day.

Running the GAMM with a smoother on the experiment day did not confirm this result.

GAMM analysis of the ratio between *Bosmina* with empty brood chambers to organisms with full brood chambers yielded a significance of all three main terms as well as a significant interaction term between $f\text{CO}_2$ and chlorophyll *a*

($p = 0.01$). Some minor residual structures remained after GAMM on the *Bosmina* ratio that should be kept in mind with respect to resulting p values (Zuur et al., 2009).

According to a GAMM applied to the Shannon diversity index H , neither of the factors significantly affected MZP species diversity.

3.2.6 Predator–prey relationships

Pearson correlation coefficients larger than ± 0.7 are listed in Table 2 and shown in the Supplement (Figs. S1–S2). *Myrionecta rubra* and *Bosmina* sp. turned out to be of particular importance in this study. Therefore, in the following sections, we focus on the correlations of these two species with particular phytoplankton and bacteria groups, respectively. *M. rubra* positively correlated with Cryptophytes and heterotrophic Dinoflagellates, whereas the species negatively correlated with Cyanobacteria and low-DNA bacteria. Pearson correlation for the different size classes of *M. rubra* were very similar when determined for all $f\text{CO}_2$ levels (0.8, 1.0, 0.9) or low (0.8, 0.9, 0.8) and high (0.8, 1.0, 0.9) levels separately. *Bosmina* sp. showed a strong positive correlation with Cyanobacteria (0.7). Figure 9 depicts the succession of the two species in relation to the mentioned potential prey organisms during the course of the experiment.

4 Discussion

4.1 Ciliates

4.1.1 Ciliate succession

The ciliate abundance and species succession in our experiment corresponded well with the description by Kivi (1986) of annual succession of protozooplankton in Tvärminne–Storfjärden. In May, shortly after the chlorophyll maximum, Kivi (1986) observed the highest protozoan biomass, whereas a minimum was found in June–July 2 weeks after the spring bloom (mostly ciliates and heterotrophic dinoflagellates). Dominant ciliates during the summer months were *Lohmaniella* spp. or small *Strombidium* spp. (35 μm). *Myrionecta rubra* was always present, with maximum abundance in late spring. *Lohmaniella* spp. also occurred in the present study but was classified with *Strobilidium* spp. ($\leq 20 \mu\text{m}$) due to difficulties with clear identification. However, most of the Strobilids $\leq 20 \mu\text{m}$ probably belonged to *Lohmaniella* spp. In our study, the ciliate community was dominated by the primarily photoautotrophic ciliate *M. rubra* (= *Mesodinium rubrum*, Lohmann (1908); Jankowski (1976) (Mesodiniidae, Litostomatea)) most of the time (Lindholm, 1985). Only towards the end of our experiment, when small *Strombidium* spp. such as *Strombidium* cf. *epidemum* occurred with similar abundances as *M. rubra*, did heterotrophic ciliates become more important in the ciliate community. *M. rubra* is also a common species in the Baltic Sea, with maximum re-

ported densities of 26 600 cells L^{-1} in the Arkona Basin usually above the thermocline and associated with the euphotic layer (Setälä and Kivi, 2003). Maximum total ciliate densities in the entrance of the Gulf of Finland varied between 10 000 and 50 000 cells L^{-1} in 1988 and 1990, respectively, and hence are in the same range as in our study. They also consisted of the same typical species or groups (Setälä and Kivi, 2003).

4.1.2 Changes in ciliate species diversity

Previous studies on sensitivities of MiZP communities towards ocean acidification are inconsistent. For example, Rose et al. (2009) reported on significant changes in MiZP abundance and community composition in the open North Atlantic Ocean between their single-factor (only temperature) and two-factor (temperature and CO_2) experiments and concluded that a combination of direct and indirect (bottom-up) effects were responsible for the changes observed. Mesocosm studies off the coast of Norway and in the Arctic revealed no effect of different CO_2 concentrations on the MiZP community either with respect to abundance or community composition (Suffrian et al., 2008; Nielsen et al., 2010; Aberle et al., 2013). In the latter study, positive effects on the autotrophic biomass with higher and lower CO_2 concentrations were found for dinoflagellates and prasinophytes and haptophytes, but these effects did not translate to the MiZP level (Schulz et al., 2013).

We found no significant relation between ciliate total abundance and $f\text{CO}_2$ concentration, but total abundance was significantly affected by temperature. Moreover, there seemed to be a trend with respect to species diversity H towards a higher dominance of single species with increasing temperature and $f\text{CO}_2$. Most likely, small species or genera are responsible for this change in diversity. During the first days of the experiment (t_5 , t_5 – t_9 , and t_7 – t_{13}) small species such as *Balanion comatum*, *Strombidium* cf. *epidemum*, and *Strobilidium* sp. ($< 20 \mu\text{m}$), respectively, showed some distinct differences in abundance between the three higher- and lower- $f\text{CO}_2$ mesocosms. While *B. comatum* occurred at higher abundance in the control mesocosms and the lowest CO_2 enrichment level (M7, 497 μatm), *S. cf. epidemum* and *Strobilidium* sp. had higher abundances in the three high- CO_2 mesocosms. Later in the experiment, between t_{19} and t_{31} , the small size class *Myrionecta rubra*, for example, occurred in much higher numbers in the mesocosms with the three highest $f\text{CO}_2$ concentrations. For the species mentioned, significant relations were determined for all factors included in our analyses, except for *Balanion comatum*, which showed no significant response to chlorophyll a and *Strombidium* cf. *epidemum*, which only showed a significant relation with chlorophyll a . Rose et al. (2009) also reported on increased dominance of smaller taxa (mostly *Lohmaniella* sp. among ciliates) during the course of their experiment, but it was dependent on a combination of dif-

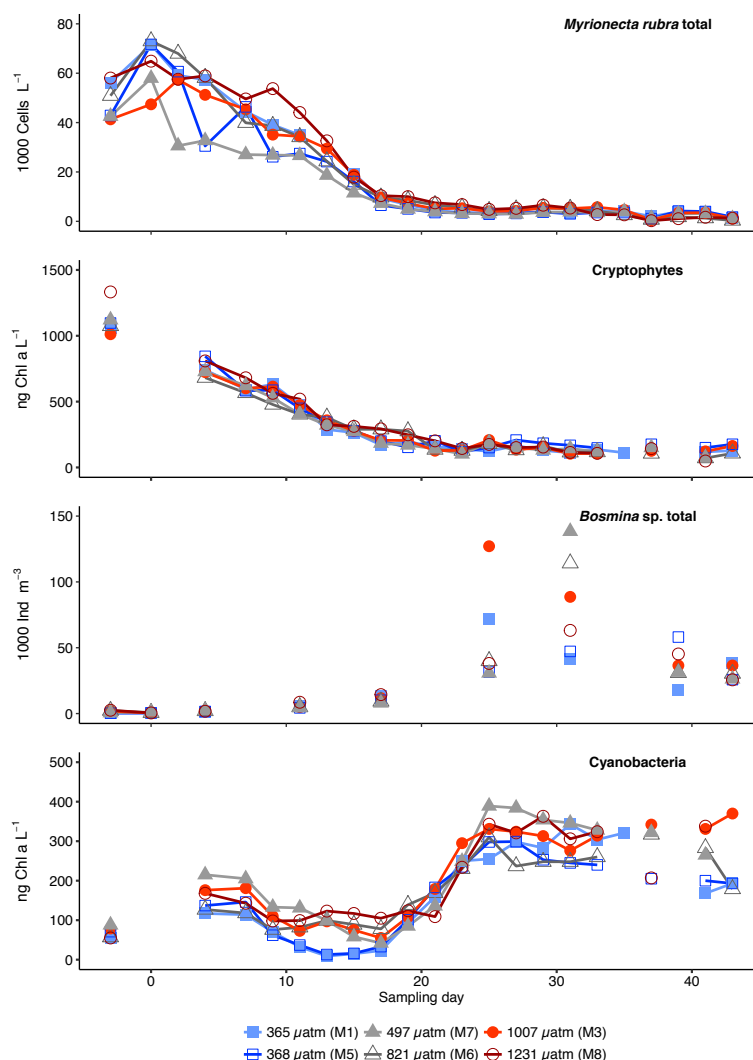


Figure 9. Succession of total cell numbers of *Myrionecta rubra*, total biomass of Cryptophytes, total abundance of *Bosmina* sp., and total biomass of Cyanobacteria during the course of the experiment. According to temperature variations and the first CO₂ manipulation, different experimental phases were defined: phase 0 = t_{-5} to t_0 , phase I = t_1 to t_{16} , phase II = t_{17} to t_{30} , phase III = t_{31} to t_{43} . Note that there is one missing value in M1 on t_{13} .

ferent factors, i.e., temperature, CO₂, and changes in the top-down control. Finally, they conclude on a more general effect of temperature on MiZP abundance and community composition. A relationship between temperature and Shannon diversity H of ciliate communities and heterotrophic ciliates was also shown by Setälä and Kivi (2003) and Aberle et al. (2007). In contrast to our present study, Aberle et al. found H to increase with higher temperature, and it was larger ciliates (mostly *Strobilidium* species) that caused the community shift. Like Rose et al. (2009), the temperature effect determined in the present study is most likely of a more general nature related to the natural succession of ciliates during the summer season.

Although some of the species mentioned above significantly correlated with chlorophyll a concentrations (*Strobi-*

lidium sp., *Strobilidium* sp.), chlorophyll a had no significant effect on species diversity H . This is most likely due to the occurrence of species with different (heterotrophic–autotrophic) food preferences during the course of the experiment. Species diversity was lowest during phases I and II, which was due to the dominance of the mixotroph *Myrionecta rubra*. Later in the experiment when chlorophyll a concentrations had decreased, *M. rubra* still occurred with lower cell numbers, but other ciliates like the mixotrophic *Strobilidium* sp. also increased in abundance and as a consequence H increased. Members of the genus *Strobilidium* feed on a variety of organisms, including bacteria and nano- and dinoflagellates (Fenchel and Jonsson, 1988; Ichinotsuka et al., 2006; Stoecker et al., 2009). Furthermore, this experiment was conducted during the post-bloom phase. Possi-

Table 2. Pearson correlation for various predator–prey relationships. Listed are only correlations ≥ 0.7 . The pairwise correlation plots for all group combinations and the Pearson correlation coefficients can be seen from the supplemental material (Figs. S1–S2). het Dino.: heterotrophic dinoflagellates, excl.: excluded. For *Myrionecta rubra*, Pearson correlation was determined combined for all $f\text{CO}_2$ levels and also separate for low (365, 368, 497 μatm) and high (821, 1007, 1231 μatm) $f\text{CO}_2$ levels.

Predator–Prey	Pearson correlation	$f\text{CO}_2$ levels	Data
Ciliates–Bacteria, Phytoplankton groups			
<i>Myrionecta rubra</i> < 10 μm –Cyanobacteria	–0.7	high	this study, Paul et al. (2015)
<i>Myrionecta rubra</i> < 10 μm –low-DNA bacteria	–0.7–0.7–0.7	all–low–high	this study, Crawford et al. (2016)
<i>Myrionecta rubra</i> < 10 μm –Picoeukaryotes III	–0.7–0.7	low–high	this study, Crawford et al. (2016)
<i>Myrionecta rubra</i> < 10 μm –Synechococcus	–0.7	high	this study, Crawford et al. (2016)
<i>Myrionecta rubra</i> < 10 μm –Cryptophytes	0.8–0.8–0.8	all–low–high	this study, Paul et al. (2015)
<i>Myrionecta rubra</i> 10–20 μm –Cryptophytes	1.0–0.9–1.0	all–low–high	this study, Paul et al. (2015)
<i>Myrionecta rubra</i> > 20 μm –Cryptophytes	0.9–0.8–0.9	all–low–high	this study, Paul et al. (2015)
<i>Myrionecta rubra</i> < 10 μm –het. Dino.	0.8	all	this study, A. Stühr (unpublished)
<i>Myrionecta rubra</i> 10–20 μm –het. Dino.	0.7	all	this study, A. Stühr (unpublished)
<i>Myrionecta rubra</i> < 10 μm –het. Dino. (<i>Ebria</i> sp. excl.)	0.8	all	this study, A. Stühr (unpublished)
<i>Myrionecta rubra</i> 10–20 μm –het. Dino. (<i>Ebria</i> sp. excl.)	0.7	all	this study, A. Stühr (unpublished)
<i>Myrionecta rubra</i> > 20 μm –het. Dino. (<i>Ebria</i> sp. excl.)	0.7	all	this study, A. Stühr (unpublished)
<i>Balanion comatum</i> –Cryptophytes	0.8	all	this study, Paul et al. (2015)
<i>Mesodinium</i> sp.–Euglenophytes	0.7	all	this study, Paul et al. (2015)
<i>Rimostrombidium</i> sp.–Cryptophytes	0.8	all	this study, Paul et al. (2015)
Tintinnids sp.–Cryptophytes	0.7	all	this study, Paul et al. (2015)
<i>Spathidium</i> sp.–Euglenophytes	0.7	all	this study, Paul et al. (2015)
Mesozooplankton–Bacteria, Phytoplankton groups, Ciliates			
<i>Podon</i> sp.–Cryptophytes	0.9	all	this study, Paul et al. (2015)
<i>Bosmina</i> sp.–Cyanobacteria	0.7	all	this study, Paul et al. (2015)
<i>Podon</i> sp.–het. Dino.	0.7	all	this study, A. Stühr (unpublished)
<i>Podon</i> sp.–het. Dino. (<i>Ebria</i> sp. excl.)	0.7	all	this study, A. Stühr (unpublished)
<i>Eurytemora</i> sp.–Picoeukaryotes II	0.7	all	this study, Crawford et al. (2016)
<i>Eurytemora</i> sp.–Cryptophytes	–0.7	all	this study, Paul et al. (2015)
Copepod nauplii–Euglenophytes	0.7	all	this study, Paul et al. (2015)
Copepod nauplii–Nanoeukaryotes II	0.8	all	this study, Crawford et al. (2016)
<i>Podon</i> sp.– <i>Balanion comatum</i>	0.8	all	this study

bly, if our experiment also covered the phytoplankton peak-bloom phase and H was determined over the whole duration from the peak- to the post-bloom phase, the relationship between H and chlorophyll a would be more pronounced.

4.1.3 *Myrionecta rubra*

Increased abundances of the mixotrophic ciliate *Myrionecta rubra* ($\leq 10 \mu\text{m}$) in the high- CO_2 mesocosms coincided well with increased chlorophyll a concentrations at high CO_2 levels during phases II and III and attributed up to 90 % to picophytoplankton ($\leq 2 \mu\text{m}$). The relative contribution of the 2–20 μm size fraction to total chlorophyll a was estimated as about 20 % (Paul et al., 2015). Blooms of *M. rubra* can contribute significantly to chlorophyll a values and primary production in estuaries, fjords, and upwelling areas. *M. rubra* robs plastids from Cryptophytes (Lindholm, 1985; Gustafson Jr. et al., 2000, and references therein). Cryptophytes were among the main contributors to total chlorophyll a in particular during phase I (Paul et al., 2015).

Moreover, small picoeukaryotes (PICO III) of approximately 2.9 μm cell diameter, most likely representing cryptophytes, had the highest abundances during phases II and III and showed a distinct negative correlation with $f\text{CO}_2$ (Crawford et al., 2016). Cryptophyte biomass decreased from t_3 to t_{17} (Paul et al., 2015), as did the total abundance of *M. rubra*, but the small-size-class cells remained and developed a distinct difference in abundance between the higher- and lower- CO_2 mesocosms during phase II. Growth and photosynthetic performance of *M. rubra* is ultimately dependent on the availability of cryptophytes, but the ciliate can sustain for long periods without feeding by functioning as a phototroph. It also has the ability to control cryptophyte plastid division and synthesize chlorophyll (Johnson and Stoecker, 2005; Johnson et al., 2006). Photosynthetic performance of *M. rubra* may have been stimulated by elevated CO_2 concentrations, and thus this ciliate may be “co-responsible” for the CO_2 -driven total chlorophyll a differences observed during phases II and III. Consequently, higher cell numbers of small-sized *M. rubra* at elevated CO_2 may be a combination

of indirect and direct CO₂ effects through (1) availability of cryptophytes, in particular during phase I, and (2) through a CO₂-mediated higher photosynthetic rate of *M. rubra* supporting its own growth. Losses of PICO III during phase II were largely due to microzooplankton grazing (Crawford et al., 2016). In further support of our assumption are the strong positive Pearson correlations between *M. rubra*, cryptophytes, and Dinophyta suggesting a high grazing pressure of *M. rubra*. During phase II Dinophyta showed a significant decrease in relative biomass at increasing CO₂ concentrations consistently with the CO₂-stimulated increase of small *M. rubra* (Bermúdez et al., 2016). Overall, a CO₂ effect on *M. rubra* was only visible during the post-bloom phase when cell numbers were rather low compared to initial numbers. However, it is possible that differences were already established before but we were not able to see them because we only looked at abundances not processes.

4.2 Mesozooplankton

4.2.1 Mesozooplankton succession

The MZP community enclosed in the mesocosms reflected fairly well the natural succession of MZP in Tvärminne–Storfjärden where rotifers, cladocerans, and calanoid copepods comprise the major zooplankton taxa (Kivi, 1986; Viitasalo, 1992; Koski et al., 1999). Usually rotifers numerically dominate in spring–early summer (*Synchaeta* sp.) and reach a second peak in midsummer–autumn (*Keratella* sp.). The calanoid copepods *Acartia bifilosa* and *Eurytemora affinis* show two abundance peaks, in mid-June and mid-September, respectively, and *Temora longicornis* occurs only at low numbers year round. Cladocerans peak in summer (August–September) with *Bosmina longispina maritima* clearly dominating among *Podon* spp. and *Evadne nordmanni*. The highest MZP biomass is built up in summer (August–September) (Kivi, 1986; Viitasalo, 1992; Koski et al., 1999).

The species composition in the mesocosms resembled natural conditions well and were dominated by the most common and successful genus or species known for the Gulf of Finland and the Tvärminne region, such as *Acartia bifilosa*, *Eurytemora affinis*, and *Bosmina longispina maritima*. Due to the rather late start of our mesocosm experiment after the spring phytoplankton bloom, the usual peak of *Synchaeta* sp. – also one of the most successful species (i.e., *Synchaeta baltica*, Viitasalo, 1992) – in spring–early summer was barely visible during the first days. Later rotifers still occurred until termination but were not of great importance anymore.

Total population densities known for mesozooplankton in the Tvärminne area more or less coincide with abundances found in the mesocosms and range from median values between ~ 22 000 to ~ 40 000 ind. m⁻³, with occasional peak abundance for *Acartia bifilosa* and *Bosmina* sp. of up

to 45 000 and 82 000 ind. m⁻³, respectively. Average peak abundance of *Acartia bifilosa* and *Bosmina* sp. during a period from 1967 to 1984 was ~ 10 000 and ~ 20 000 ind. m⁻³, respectively (Viitasalo et al., 1995; Viitasalo, 1992). Between *t*₂₄ and *t*₃₁, however, some exceptionally high numbers (> 150 000 ind. m⁻³) occurred in the mesocosms mainly attributed to extremely high occurrence of *Bosmina* sp. Even higher densities exceeding 1 000 000 ind. m⁻³ during blooms of blue-green algae are known for *B. fatalis* in a eutrophic lake in Japan (Hanazato and Yasuno, 1987). The MZP community in the surrounding water did not entirely correspond with the mesocosms over the course of the experiment. While the dominance of particular species corresponded quite well until *t*₃, it diverged progressively after *t*₁₀ when the occurrence of colonies of blue-green algae (*Aphanizomenon*) and Rotifera were higher in the surrounding water than in the mesocosms, and the abundance of copepods and cladocerans was comparatively lower (S. Lischka, personal observation during this study, 2012). This is most likely a result of isolation of the mesocosm bags from surrounding water mass exchange and incoming plankton communities and selective advantage of single species in the mesocosms.

4.2.2 Copepods

This study is one of the first to follow MZP community development subjected to ocean acidification scenarios projected for this century in a close-to-natural holistic plankton community (IPCC, 2013; Riebesell et al., 2008, 2013b). Previous studies using the same mesocosm setup investigated effects on an Arctic MZP community and found no significant difference in either total abundance, single-taxa abundance, or species diversity (Niehoff et al., 2013; Riebesell et al., 2013a).

Copepods comprised one of the two dominant taxonomic groups in the present study and the mesocosm approach allowed investigation of CO₂ effects on the succession of all different life stages from eggs to reproducing adults. While copepods are thought to be rather robust against ocean acidification, with negative effects not usually occurring until *p*CO₂ levels far beyond projections for the end of this century (Kurihara et al., 2004; Mayor et al., 2007; Weydmann et al., 2012; McConville et al., 2013; Almén et al., 2016), more recent studies give evidence that copepod sensitivity may be highly stage dependent. Thus, their sensitivity may be mostly underestimated due to the fact that most studies until now only considered adult-stage copepods (Cripps et al., 2014). Over the CO₂ range projected for this century, we found no distinct abundance differences for either of the species. The permanent occurrence of adult males and females together with copepodite stages and nauplii suggests more or less continuous reproduction. Concurrent lab experiments investigating the effect of CO₂ on reproductive success of *Eurytemora affinis* are in agreement with the observations from the mesocosms (Almén et al., 2016, this issue). Incubated *Acartia bi-*

filosa showed that $f\text{CO}_2$ did not affect egg production, but slight negative effects on egg hatching and development were found and adult females were smaller in the two highest- CO_2 mesocosms (Vehmaa et al., 2016, this issue). Our results are also in line with Niehoff et al. (2013), who do not describe any apparent CO_2 effect on an Arctic MZP community including copepods. Copepods in the study region naturally experience $f\text{CO}_2$, pH, and temperature fluctuations of more than 0.5 pH units and 5 °C temperature during daily vertical migrations, which is more than the predicted climate change for the year 2100. These copepods are probably well adapted to short-term physicochemical changes (Lewis et al., 2013; Almén et al., 2014).

4.2.3 Cladocera – OA effect on *Bosmina* spp. through increased food availability?

Most conspicuous differences found in mesozooplankton abundance are due to the cladoceran *Bosmina* sp. between t_{24} and t_{31} . In three of the four CO_2 -enriched mesocosms (497, 821, 1007 μatm) peak numbers were twice, or even more than twice, as high compared to the control and the highest- CO_2 mesocosms, though a significant relation with $f\text{CO}_2$ could not be proved. Nevertheless, this striking difference may possibly point to an indirect CO_2 effect through higher food availability under high CO_2 .

Cladocerans are highly reproductive at times of favorable environmental conditions. The life span of *Bosmina* spp. varies between 20 and 25 days, age of first reproduction is between 4 and 7 days (food dependent), and populations can increase twofold within 5–10 days (Purasjoki, 1958; Kankaala and Wulff, 1981; Hanazato and Yasuno, 1987; Biswas et al., 2014). Population dynamics of *Bosmina longirostris* are highly food sensitive, with food quantity and quality having a significant effect on growth, net reproductive rate, and rate of population increase to shorten lifetime by up to 10 days (Kankaala and Wulff, 1981; Hanazato and Yasuno, 1987; Urabe, 1991). Cladocerans are opportunistic feeders that graze on nano- and microplankton, bacteria (including Cyanobacteria), and detritus (Purasjoki, 1958; Hanazato and Yasuno, 1985; Work and Havens, 2003; Kluijver et al., 2012). *Bosmina* tolerate low pH in acidic lakes well (Uimonen-Simola and Tolonen, 1987).

The abovementioned population increase of *Bosmina* in the mesocosms coincides with significant CO_2 -mediated differences during phase II in Cyanobacteria during the respective days (Paul et al., 2015). The population increase may represent favorable food conditions for this species, enhancing asexual reproduction in particular in the elevated- CO_2 mesocosms. The highly positive correlation between Cyanobacteria and *Bosmina* sp. supports this assumption. Only M8, the mesocosm with the highest CO_2 concentration, diverged from this trend. Peak abundance in all mesocosms occurred only on 1 sampling day. That is, it did not stay high for a longer period but was low on the preced-

ing sampling day and had already dropped on the following sampling day. The drop in population size that occurred earlier than to be expected from *Bosmina* life span of around 20 days was possibly due to high mortality and/or change to sexual reproduction producing resting eggs. Therefore, a possible explanation of why *Bosmina* in M8 did not follow the trend observed in the other CO_2 -elevated mesocosms may be that due to the rather low possible sampling frequency (every 7 days), the actual abundance peak was missed (Riebesell et al., 2013a). Reason for mortality could be in response to the overall drop in available food during phases II and III and/or a stress response due to extreme densities or reproductive rates of *Bosmina* itself. It is known that *Bosmina* sp. can die earlier when it has higher reproductive rates and switch to sexual reproduction to produce resting eggs at population densities that are too high (so called “crowding phenomenon”) (Purasjoki, 1958; Acharya et al., 2005). In Kankaala (1983), *Bosmina* started sexual reproduction at around 4500 ind. m^{-3} , which is about 1–2 orders of magnitude less than observed peak numbers in the mesocosms.

The significant results we found for the ratio of *Bosmina* with empty brood chambers to full brood chambers strongly suggest that organisms in the high- CO_2 mesocosms had higher reproductive activities during the time of actual peak abundance. In particular, *Bosmina* in M8 and M3 (two highest CO_2 levels) had continuously low brood chamber ratios (i.e., large proportion of actively reproducing organisms in the population) from t_{10} onwards (with the ratio in M8 mostly even lower than in M3). This supports our assumption that we may have missed sampling the abundance peak of *Bosmina* in M8, possibly obstructing the proof of a significant indirect $f\text{CO}_2$ effect on *Bosmina* abundance through increased food availability.

4.2.4 Predator–prey relationships

We have some evidence for $f\text{CO}_2$ -stimulated predator–prey relationships between *Myrionecta rubra*–Cryptophytes and *Bosmina* sp.–Cyanobacteria, though the mixotrophic ciliate *M. rubra* may also have benefitted directly from elevated $f\text{CO}_2$ concentrations (see above). With respect to *Balanion comatum*, *Strombidium* cf. *epidemum*, and *Strobilidium* sp., the $f\text{CO}_2$ -related abundance differences during particular phases of the experiment cannot be explained through enhanced predator–prey relationships.

Although our results show no direct significant CO_2 effect on *Bosmina* abundance, we cannot rule out that growth and reproduction was stimulated by increased Cyanobacteria availability at elevated CO_2 , mostly during phases II and III, or from increased heterotrophic bacterial production (Hornick et al., 2016). This would point to an indirect CO_2 effect that was masked as a consequence of sampling frequency that was too low not allowing to adequately capture the population dynamics of this short-lived and highly adjustable genus. For the study region, microbial loop has been shown to be of

particular importance during late summer and autumn when most of the secondary production, including fish, is fueled by carbon channeled from the microbial loop to crustacean zooplankton (Uitto et al., 1997; Koski et al., 1999). In the present study, heterotrophic bacterial production and biovolume was strongly linked to phytoplankton dynamics and suggested several indirect responses to $f\text{CO}_2$ (Hornick et al., 2016). Enhanced bacterial grazing, and thus stimulated microbial loop, was assumed to be related to higher $f\text{CO}_2$ (Crawford et al., 2016). This was mostly reflected in relatively high rates of cell-specific bacterial protein production of particle-associated heterotrophic bacteria throughout the entire experiment, though they only contributed a minor fraction to the overall heterotrophic bacterial biovolume (Hornick et al., 2016). Filter-feeding cladocerans directly feed on bacteria and flagellates and effectively transfer carbon from the microbial loop to higher trophic levels. In the eastern and western Gulf of Finland, as well as in the southern Baltic Sea, *Bosmina longispina* can be the dominant prey for herring (*Clupea harengus*), sprat (*Sprattus sprattus*), and three-spined stickleback (*Gasterosteus aculeatus*) (Casini et al., 2004; Peltonen et al., 2004). Larger herring feed more on Mysids during autumn, which in turn can effectively prey on cladocerans, including *Bosmina* sp. (Rudstam et al., 1992). Increased bacterial production (Hornick et al., 2016) may have provided optimal feeding conditions to favor reproduction of *Bosmina* sp. at elevated $f\text{CO}_2$, but unfortunately our data cannot provide sufficient proof.

A more recent publication by Wikner and Andersson (2012) states that increased microbial heterotrophy decreases trophic transfer efficiency of biomass to higher trophic levels. This work investigated the influence of increased river discharge through increased precipitation on phytoplankton biomass production and found a shift in the carbon flow towards microbial heterotrophy. This shift was mainly due to an increase in freshwater and riverine organic carbon supply despite a concomitant increase in nutrients. As already mentioned above, during phase III increasing importance of production of the microbial food web related to higher $f\text{CO}_2$ was found in the present study (Paul et al., 2015; Crawford et al., 2016; Hornick et al., 2016) concomitant with the abundance peak of the cladoceran *Bosmina* sp. In plankton communities comprised of species able to effectively graze on bacteria such as *Bosmina* sp., trophic transfer to higher trophic levels may not necessarily decrease but could still be enhanced.

The results described for *M. rubra* and *Bosmina* should also be robust if biomass estimates were considered. The significant response of *M. rubra* to CO_2 was determined for cells of the same size class (all mostly $10\mu\text{m}$), i.e., biomass-based results would scale proportionally with cell numbers. In the case of *Bosmina*, the significant CO_2 relation was found for the ratio of embryo-bearing to non-embryo-bearing organisms, which is an abundance- and/or biomass-independent measure. Regarding a possible indirect CO_2 ef-

fect that we suggested for *Bosmina* sp., abundance increased more than twofold in the mesocosms mentioned and consisted of different-sized individuals. A respective increase in biomass would probably be smaller compared to the abundance increase but certainly still existent.

5 Conclusions

This study describes $f\text{CO}_2$ -related effects on the zooplankton community level in a close-to-natural plankton community for the first time. Some ciliate species, as well as the species diversity of ciliates, responded to elevated $f\text{CO}_2$ levels. On the mesozooplankton level, significant $f\text{CO}_2$ effects were only found for the ratio of empty brood chambers to full brood chambers of the cladocera *Bosmina* sp., but an indirect effect on *Bosmina* abundance via food seems likely. Although for the ciliates, in particular the mixotroph *Myrionecta rubra*, the magnitude of change in abundance was rather minor since effects were observed only in the post-bloom phase, and for the cladoceran *Bosmina* sp. a $f\text{CO}_2$ effect could only be carefully assumed, our study has shown that ocean acidification effects can potentially translate from the primary production level up to higher trophic levels. This is certainly not a general consequence, but it is probably highly dependent on the species composition of a pelagic community, i.e., the presence of species that have the ability to quickly respond to changes in food availability and composition with increased reproduction or cell division, such as the highly flexible cladocerans or the mixotroph ciliate *Myrionecta rubra*.

6 Data availability

The ciliate and mesozooplankton data are available at doi:10.1594/PANGAEA.871119 (Lischka et al., 2017). Other data used in this work are available from Paul et al. (2016, doi:10.1594/PANGAEA.863032) and Crawford et al. (2016).

The Supplement related to this article is available online at doi:10.5194/bg-14-447-2017-supplement.

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