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Community interactions dampen acidification effects in a coastal plankton system

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ABSTRACT: Changing seawater chemistry towards reduced pH as a result of increasing atmospheric carbon dioxide (CO_2) is affecting oceanic organisms, particularly calcifying species. Responses of non-calcifying consumers are highly variable and mainly mediated through indirect ocean acidification effects induced by changing the biochemical content of their prey, as shown within single species and simple 2-trophic level systems. However, it can be expected that indirect CO_2 impacts observed at the single species level are compensated at the ecosystem level by species richness and complex trophic interactions. A dampening of CO_2-effects can be further expected for coastal communities adapted to strong natural fluctuations in pCO_2, typical for productive coastal habitats. Here we show that a plankton community of the Kiel Fjord was tolerant to CO_2 partial pressure (pCO_2) levels projected for the end of this century (<1400 µatm), and only subtle differences were observed at the extremely high value of 4000 µatm. We found similar phyto- and microzooplankton biomass and copepod abundance and egg production across all CO_2 treatment levels. Stoichiometric phytoplankton food quality was minimally different at the highest pCO_2 treatment, but was far from being potentially limiting for copepods. These results are in contrast to studies that include only a single species, which observe strong indirect CO_2 effects for herbivores and suggest limitations of biological responses at the level of organism to community. Although this coastal plankton community was highly tolerant to high fluctuations in pCO_2, increase in hypoxia and CO_2 uptake by the ocean can aggravate acidification and may lead to pH changes outside the range presently experienced by coastal organisms.

KEY WORDS: Ocean acidification · Copepods · Phytoplankton · Mesocosm · Plankton community · Microzooplankton · Reproduction

INTRODUCTION

The reduction of ocean pH and shift in seawater carbon chemistry caused by increasing atmospheric carbon dioxide (CO_2), termed ocean acidification (OA), is affecting a wide range of marine organisms (Fabry et al. 2008, Cooley et al. 2009). In particular, calcifying organisms respond sensitively to elevated CO_2 levels (Engel et al. 2008, de Nooijer et al. 2009, Beaufort et al. 2011), whereas biological effects of OA on non-calcifying organisms are mixed and often highly species-specific (Doney et al. 2009, Whiteley 2011). Elevated levels of CO_2 are expected to increase growth rates of photosynthetic organisms, a result that has been reported for some phytoplankton species (Kim et al. 2006, Fu et al. 2007) — although not consistently for a wide range of planktonic species. The responsiveness of phytoplankton to OA may depend on species-specific differences in the ability to use CO_2 and bicarbonate (HCO_3) as a carbon source (Van de Waal et al. 2011). For zooplankton, direct physiological effects of CO_2 on growth and reproduction are typically experienced only at extremely high levels of CO_2 partial pressure (pCO_2) that exceed the projected increase for this century (Kurihara & Ishimatsu 2008, Nielsen et al. 2010, Whiteley 2011). The strongest response to OA within non-calcifying primary consumers has been shown in

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simple 2 trophic level food chains through changes in nutritional prey quality that cause an imbalance between phytoplankton elemental and biochemical composition and consumer nutrient demand for somatic growth (Urabe et al. 2003, Rossoll et al. 2012). Increasing CO_2 supply can stimulate carbon fixation by photosynthetic organisms and limit mineral nutrients of nitrogen (N) and phosphorus (P) and consequently reduce the nutrient content relative to carbon (Urabe et al. 2003, Bellerby et al. 2008, Engel et al. 2008). Similarly, as shown in a simple 2-species-food chain with a diatom and a copepod species, both species were insensitive to pCO_2 changes under current conditions, but at elevated pCO_2 (750 µatm) polyunsaturated fatty acids were reduced in the diatoms and led to a decrease in copepod egg production (Rossoll et al. 2012). While these studies suggest strong indirect OA effects on zooplankton crustacean, it can be expected that differential sensitivity to pCO_2 at the community and ecosystem level may compensate for indirect impacts observed at the single species level.

Experimental OA studies are often limited to simplified systems (but see Riebesell et al. 2008) including monocultures and simple 2 trophic interactions whereby a consumer species encounters a single prey species. These experimental setups exclude much of the structural complexity, ecophysiological variability, and genetic diversity encountered in natural communities (Paasche 2001, Lohbeck et al. 2012). Most experiments also fail to account for evolutionary adaptation that may mitigate adverse effects (Lohbeck et al. 2012). *In situ*, herbivores have the opportunity to choose between different phytoplankton food types that have different responsiveness to pCO_2, which can mitigate effects imposed by monocultures (Urabe & Waki 2009). In addition, most copepods considered traditionally 'herbivorous' are in fact omnivorous, which means that heterotrophic protists (often ciliates or heterotrophic dinoflagellates) can form a substantial part of their diet. Consumption of heterotrophic protists might compensate for biochemical deficiencies of algae, even if the heterotrophic protists form a relatively small portion of the available food spectrum (Klein Breteler et al. 2004, Ptacnik et al. 2004). Thus it can be expected that more complex communities might dampen CO_2 effects, while the tolerance to pCO_2 and pH might be lower for monocultures and simple 2-species interactions may amplify single-species effects.

Species richness and complex trophic interactions might provide a dampening of some effects caused by CO_2, particularly in communities that experience strong natural fluctuations in pCO_2. Coastal environments encounter often large amplitudes in pCO_2 due to large fluxes of organic and inorganic carbon from river runoff, leading to wider pH variation in coastal systems compared to the open ocean (Hinga 2002). In addition, variation in pCO_2 is more severe in estuarine brackish systems due to lower alkalinity and hence reduced buffer capacity (Melzner et al. 2013). Strong diel and seasonal shifts in the balance of photosynthesis and respiration can also lead to short term and seasonal pCO_2 fluctuations that far exceed the atmospheric signal predicted for the next 100 yr (IPCC 2007). Adaptations to a wide pCO_2 range is particularly relevant for coastal plankton in nutrient rich areas, where respiration in deep layers and subsequent upwelling of CO_2 enriched water result in acidification of surface waters (Feely et al. 2008). High CO_2 fluctuations are characteristic for the western Baltic Sea, where seasonal monthly mean values of pCO_2 range from ~500 µatm (March) to 2500 µatm (September) and short term variability might even span the range from 300 to 4500 µatm (Melzner et al. 2013). In comparison, atmospheric pCO_2 is expected to rise from current 390 µatm to values of 700 to 1000 µatm, and pH_{NIST} is expected to decrease from the present ~8.07 to 7.73 by the end of this century (IPCC 2007, Cao & Caldeira 2008, Gosling et al. 2011).

Here we test the tolerance of an experimentally enclosed natural plankton community of the Baltic Sea to elevated pCO_2 levels and show the development of 3 trophic levels grown at present pCO_2 (380 µatm), future levels by 2100 according to IPCC predictions (840, 1120, and 1400 µatm) and at a high pCO_2 level (4000 µatm) that served as a proof of principle for CO_2 responses. The former 3 levels are within the mean trend of seasonal variation within the western Baltic Sea, while the latter value has only been reached during a few measurements in late summer and early fall (Melzner et al. 2013). We hypothesized that (1) higher aggregate phytoplankton responses (e.g. total biomass) to acidification will be less pronounced than single species responses, (2) the copepod *Acartia tonsa* will respond less sensitively to OA compared to a 2-species experiment that excluded community complexity (Rossoll et al. 2012), and (3) most of the observed effects will be driven by the differences between the 1400 and 4000 µatm pCO_2 treatment, while differences between the 380 and 1400 µatm treatments will be minor. To test these hypotheses, bloom dynamics and elemental composition of primary producers were measured as well as development and reproduction of the planktonic copepod *A. tonsa*.

MATERIALS AND METHODS

Mesocosm setup and CO_2 manipulation

Baltic seawater from Kiel Fjord containing the natural summer (August 2009) phyto- and microzooplankton community was pumped into a 1500 l stock tank and subsequently distributed to 12 mesocosms of 300 l volume each, depth of 1 m and diameter of 1.5 m. The mesocosms were set up in temperature-controlled culture rooms and kept at constant temperature of 18°C and salinity of 18.1. Mesocosms were initially manipulated with CO_2-enriched air with 5 different pCO_2 levels: 3 × 380, 1 × 840, 2 × 1120, 2 × 1400 and 2 × 4000 µatm. In the initial phase, mesocosm seawater was bubbled with corresponding CO_2-enriched air for 48 h using wooden aqua stone-endings (Knudsen Aquaristik) to ensure maximum distribution. After the initial phase, direct CO_2 supplied into the water was stopped and the headspace of each mesocosm was aerated continuously over the whole experimental time with the targeted CO_2 level. To avoid outgassing, the top of each mesocosm was closed with plexiglass and fixed with elastic clamps. The light supply was set to simulate an average August day with sunrise at 05:17 h and sunset at 18:43 h using 6 fluorescent tubes (5 × JBL Solar Tropic, each 4000 K; 1 × JBL Solar Natur, 9000 K); duration of sunrise/sunset was set to 2 h 39 min. Total light energy per day was 20.575 kW m^{-2}, which corresponds to ~4 m water depth on sunny days. Total incubation time for the experiment was 28 d.

The natural plankton community was allowed to acclimatize to changing CO_2 levels for 1 wk. On Day 8 a plankton bloom was initiated by additions of sodium dihydrogen phosphate (NaH_2PO_4), sodium silicate (Na_2SiO_3) and sodium nitrate ($NaNO_3$) to reach dissolved concentrations of 35 µmol l^{-1} inorganic nitrogen, 40 µmol l^{-1} silicate and 2.2 µmol l^{-1} phosphate. On Day 11 of the experiment, *Acartia tonsa* nauplii (Stage 2) from a stock culture were added to the mesocosms to reach a starting density of ~40 ind. l^{-1}. For this purpose, *A. tonsa* eggs from indoor cultures were incubated in 6 incubation buckets (10 l) with CO_2 pre-treated (as per the above CO_2 levels) and filtrated (0.2 µm) seawater until hatching at 18°C and development to Stage 2.

Sampling and sample analysis

Salinity, temperature, and pH (NIST scale) of the mesocosm water were monitored daily using a WTW 340i pH-analyzer connected to a SENTIX-81 electrode. Water samples were taken from each mesocosm 3 times per week using a silicone tube. Before sampling, each mesocosm was smoothly stirred with a Secchi disc to resuspend settled phytoplankton and protozoan cells. Samples of dissolved inorganic carbon (DIC) for measuring initial pCO_2 were taken at the beginning of the experiment, filtered with 0.2 µm pre-filters via syringe and stored in 2 ml brown flasks at 4°C until analysis (described in Rossoll et al. 2012).

Total alkalinity (TA) samples of 50 ml volume were taken weekly and immediately poisoned with mercury chloride. TA was analyzed potentiometrically in duplicate with an open-cell titration technique according to Dickson et al. (2003) with an average precision between duplicate measurements of ≤4 µmol kg^{-1}. Seawater samples of 10 to 12 g filtered on GF/F were exactly weighed (1416B MP8-1, Sartorius). Titration was conducted using an automatic titrator (Titrando 808, Metrohm); hydrochloric acid (HCl) with a concentration of 0.005 N served as titrand. Dissolved inorganic nutrients (nitrite/nitrate, ammonium, silicate and phosphate) were measured according to standard protocols (see Fig. 1 for measured nutrient concentrations over the duration of the experiment).

A volume of 150 to 200 ml of sample water was filtered onto pre-combusted Whatman GF/F filters for particulate organic carbon (POC), nitrogen (PON), phosphoros (POP) and chlorophyll *a* (chl *a*) measurements, respectively. POC and PON were analyzed using an elemental analyser; POP was analyzed by the ammonium molybdate method (Grasshoff et al. 1983) and chl *a* using a spectrophotometer (Hitachi U–2900) with the absorption equation of Strickland & Parsons (1968) after acetone extraction. Phytoplankton and protozoan samples of 250 ml were fixed with Lugol's iodine for microscopic analysis according to Utermöhl's (1958) method. Mesozooplankton was sampled 2 times per week (Mondays and Fridays) using a plankton net (64 µm mesh size, 6 cm diameter), fixed with formalin, and identified to life stages.

Copepod egg production experiment

After 28 d of mesocosm incubation, adult *Acartia tonsa* females were isolated from each mesocosm and transferred into 500 ml chambers filled with CO_2-enriched seawater of the related mesocosms and under the same conditions as the mesocosm (e.g. closed chambers with no headspace). To sepa-

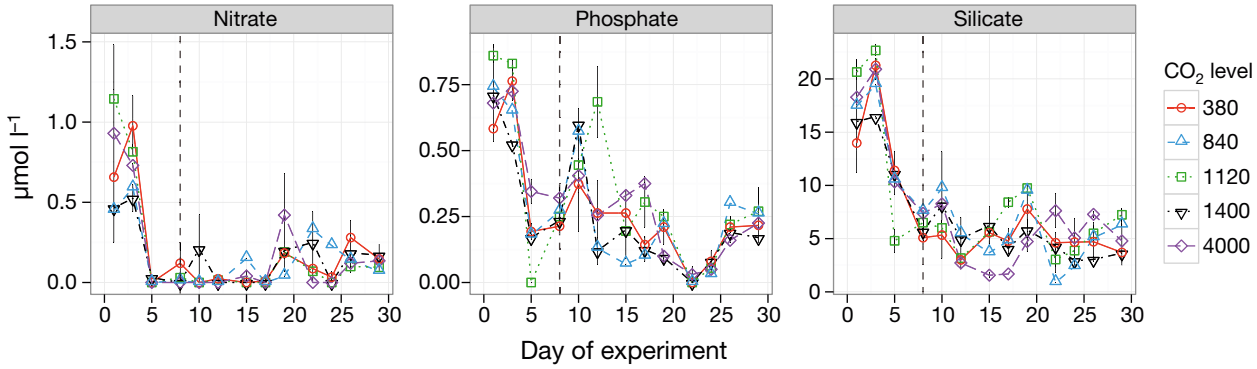

Fig. 1. Mean (±SE) nutrient (nitrate, phosphate, silicate) concentration within CO_2 treatment levels over the duration of the experiment. Dashed lines indicate the day of nutrient addition (Day 8)

rate the copepods from produced eggs, a mesh of 250 µm separated the chambers. Egg chambers each with 5 females per treatment were set up as follows: 10 × 380, 9 × 840, 9 × 1120, 10 × 1400 and 10 × 4000 µatm pCO_2 with 3 to 5 replicate for each mesocosm. Living female copepods were separated from the egg chambers after 24 h incubation and all eggs and hatched nauplii were transferred into 20 ml airtight hatching chambers for another incubation of 48 h, followed by formalin preservation to avoid further development or disintegration in the aftermath. Nauplii, hatched and empty eggs of the hatching chambers were categorized and nauplii were further analyzed for developmental malfunctions.

Differences in plankton variables between treatments were tested using analysis of variance (ANOVA). A Tukey HSD post hoc test was used to assess differences among treatments.

RESULTS

Carbonate system

Starting pCO_2 concentration of the Baltic seawater used for mesocosm filling was 1600 µatm with a mean (±SD) pH_{NIST} of 7.61 ± 0.01 and TA of 2023 ± 10 µmol kg^{-1} across all mesocosms (Fig. 2). After 2 d of CO_2 adjustment, all mesocosms reached the CO_2 target levels of 380, 840, 1120, 1400 and 4000 µatm as indicated by the difference in pH. Measured pH values varied over the duration of the experiment and were strongly associated with phytoplankton bloom development (Figs. 2A & 3A). Given that pCO_2 was manipulated only at the beginning of the experiment and the pH was allowed to vary with plankton metabolism, the treatments were not discrete pCO_2 manipulations. Nevertheless, pH differences were maintained within CO_2 treatments, particularly

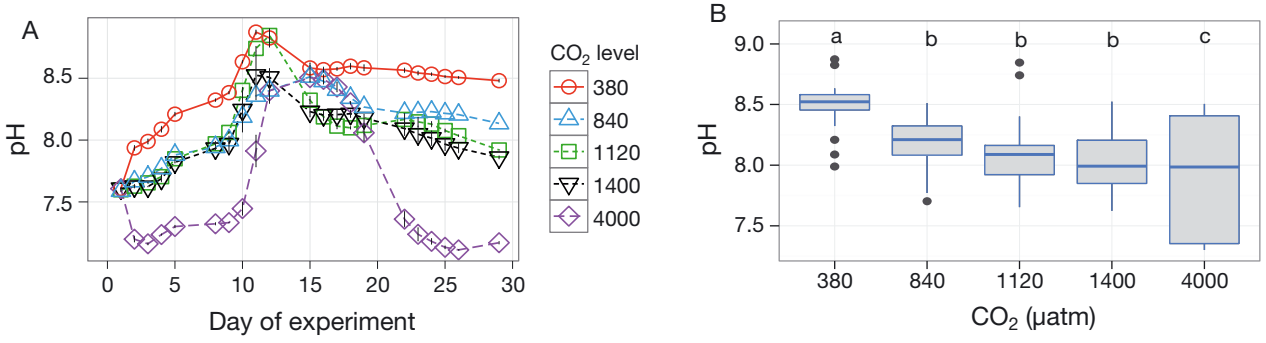

Fig. 2. Mean (±SE) pH_{NIST} values over the course of the experiment across CO_2 treatment levels. (A) Temporal development of pH. (B) Daily measured pH over the duration of the experiment. Bars: 50th percentile; boxes: 25th and 75th percentiles; whiskers: 10th and 90th percentiles; black points: outliers. Letters above bars (a,b,c) represent significant differences based on a Tukey HSD test

between the low (380 µatm), medium (840, 1120, 1400 µatm) and high (4000 µatm) pCO_2 levels, suggesting reduced outgassing (Fig. 2B). TA was on average 2058 ± 24 µmol kg^{-1} in all treatments during the whole experiment (data not shown).

Phytoplankton and seston elemental composition

Following the addition of nutrients on Day 8, chl *a* concentration increased from ~5 µg l^{-1} to ~40 µg l^{-1} on Day 10 at the lowest pCO_2 (380 and 840 µatm) and to ~30 µg l^{-1} at intermediate pCO_2 (1120 and 1140 µatm) treatment levels, whereas phytoplankton peak timing was delayed for 2 d at pCO_2 4000 µatm and reached a peak concentration of ~30 µg l^{-1} (Fig. 3A). C:N ratios were comparable between the 380 and 1400 µatm treatments over the duration of the experiment. During the bloom to postbloom period, C:N ratios increased significantly ($p = 0.001$) from an average of ~7 to 14 at the highest CO_2 level (4000 µtam) (Fig. 3B). Conversely, C:P ratios were lowest at CO_2 values of 380 and 840 µatm, and increased from ~115 to 125 at higher CO_2 levels during the bloom to postbloom period (Fig. 3C). However, this difference was not significant.

Phytoplankton biomass followed the same pattern as chl *a* concentration and increased after nutrient addition. The phytoplankton bloom was short-lived and the temporal development matched closely between mesocosms within the pCO_2 treatment levels of 380 to 1400 µatm (Fig. 4A). At 4000 µatm phytoplankton biomass showed a delayed response to nutrient addition with a lag time of about 5 d and a comparable peak magnitude to lower pCO_2 treatments. Phytoplankton community composition and peak magnitude was similar between treatments and was dominated by diatoms, mainly *Skeletonema* sp. and *Leptocylindrus* sp., and to a lesser extent by dinoflagellates (Figs. 5 & 6). Microzooplankton biomass was dominated by ciliates (scuticociliates, strobilidiid ciliates, *Euplotes* sp.) and increased after the

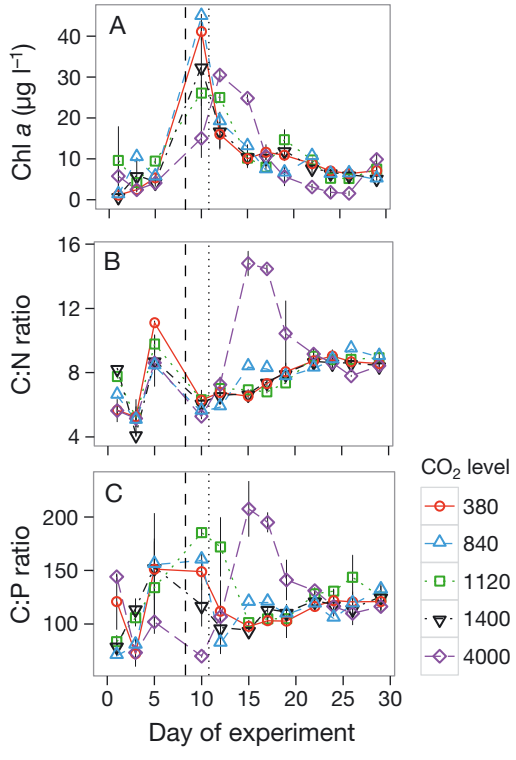

Fig. 3. Temporal development of mean (±SE) chl *a* and elemental composition over the duration of the experiment at different CO_2 treatment levels (µatm). (A) Chl *a* concentration, (B) particulate organic carbon to nitrogen molar ratio (C:N) and (C) carbon to phosphate molar ratio (C:P). Dashed lines indicate the day of nutrient addition (Day 8), dotted lines the addition of copepod nauplii (Day 11)

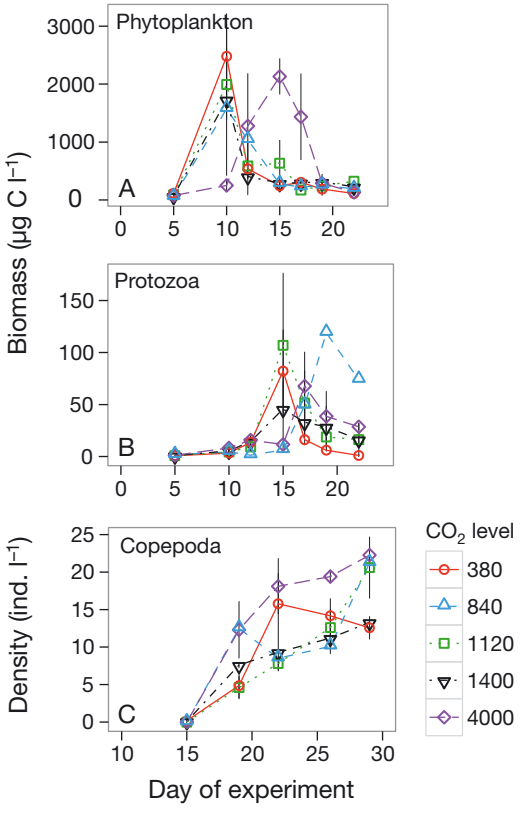

Fig. 4. Mean (±SE) (A) phytoplankton biomass, (B) protozoa biomass and (C) copepod *Acartia tonsa* density over the course of the experiment among CO_2 treatment levels (µatm). Copepod densities were counted after nauplii addition on Day 11

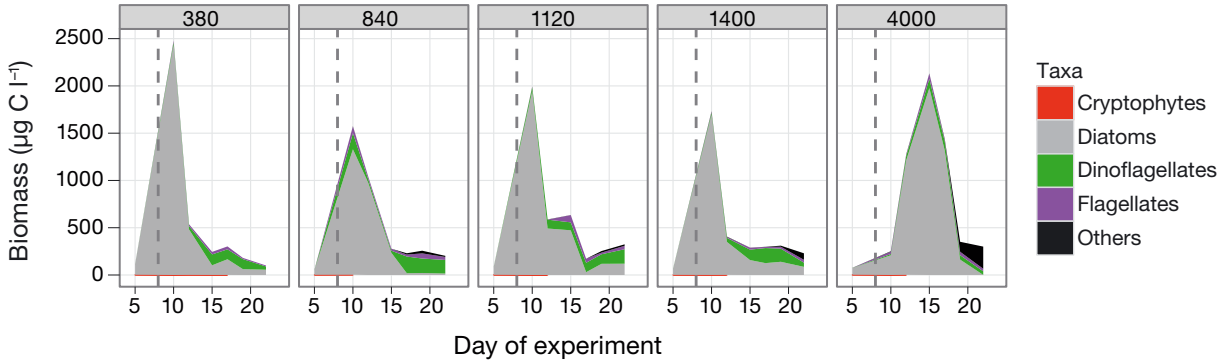

Fig. 5. Phytoplankton development of major taxonomic groups within CO_2 treatment levels (µatm). Dashed lines indicate the day of nutrient addition (Day 8)

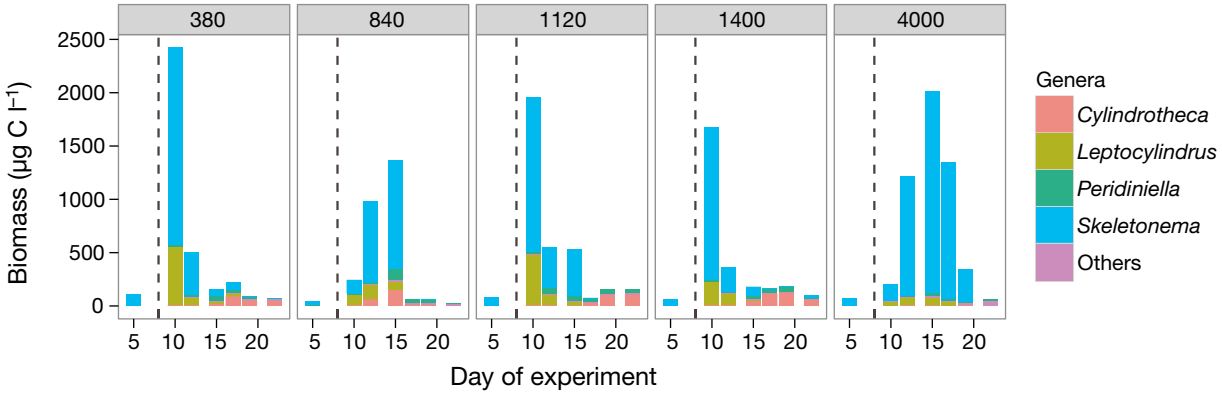

Fig. 6. Mean biomass of phytoplankton genera across different pCO_2 treatment levels (µatm). Dashed lines indicate the day of nutrient addition (Day 8)

phytoplankton bloom. The treatments of 380 to 1400 µtam reached similar microzooplankton biomass concentrations on experimental Day 15 (Fig. 4B). Similar to phytoplankton, protozoa peak biomass was delayed for ~5 d at 4000 µatm. Average protozoan biomass during the whole experiment was comparable across the CO_2 gradient ($p > 0.1$).

Abundance of *Acartia tonsa* increased after nauplii addition in all CO_2 treatment levels (Fig. 4C). Average abundances over the copepod growth period were consistent between pCO_2 treatments of 380 to 1400 µatm (~11 ind. l^{-1}) and density was higher with ~18 ind. l^{-1} at 4000 µatm. The majority of individuals reached the adult Stage at Day 26 and development was similar between CO_2 levels (Fig. 7A). Egg production of *A. tonsa* was lowest at 380 and 840 µatm (~25 ± 10 eggs female^{-1} d^{-1}) and showed a tendency of increasing production at higher pCO_2 with highest egg production of 52 ± 19 eggs female^{-1} d^{-1} at 4000 µatm (Fig. 7B), but this was not statistically significant. These relatively high egg production rates (for comparison see Holste & Peck 2006) suggest that *A. tonsa* was not limited by food availability after the bloom at the end of the experiment. Egg hatching success was on average 60 ± 19 % and did not vary across pCO_2 treatments (data not shown).

DISCUSSION

Predicting marine community vulnerability to OA is challenging, as experimental setups often limit biological or ecological complexity and diversity when compared to natural systems (Thomsen et al. 2010). However, understanding OA effects at ecosystem level is important as complex communities could either dampen or aggravate CO_2 effects experienced at the single species level. Moreover, in productive coastal habitats, such as the Eastern Pacific (Feely et al. 2008) and the Baltic Sea (Thomsen et al. 2010) that experience high natural pCO_2 fluctuations, evolutionary adaptation may favor genotypes that are less pH sensitive compared to oceanic species (Melzner et al. 2013). Using a natural coastal plankton commu-

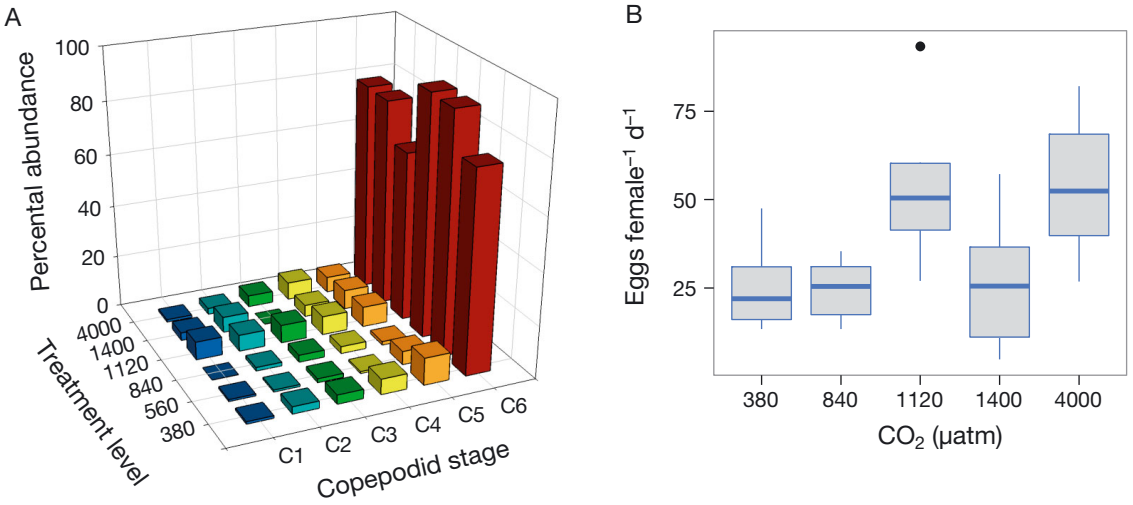

Fig. 7. Development and egg production of *Acartia tonsa*. (A) Age distribution on Day 26 of the experiment across CO_2 treatment levels (µatm) and (B) copepod egg production at the end of the experiment (Day 28), for 8 to 10 egg chambers per CO_2 level with 5 female copepods per chamber. Boxplot features as in Fig. 2B

nity from Kiel Fjord we observed only subtle changes (in plankton dynamics, reproduction and elemental composition) from initial pCO_2 manipulations to high CO_2 treatment levels. The resilience of the plankton community to OA can likely be explained by the naturally large seasonal and daily variance of pH and CO_2 experienced by the community in this productive low-salinity region, suggesting that this community is adapted to strong pCO_2 fluctuations.

Supporting our above conjecture, the pCO_2 value measured in Kiel Fjord at the start of the experiment was 1600 µatm. The Western Baltic Sea (Kiel Bay) experiences large seasonal fluctuations in pCO_2 ranging on average from about 500 µatm during winter to 2500 µatm during summer, and seasonal pH changes of ~8 to 7.5. High pCO_2 fluctuations are a result of lower alkalinity and consequently lower buffering capacity of brackish water compared to seawater. In addition, nutrient enrichment strongly drive pCO_2 to more extreme values by promoting plankton blooms that remove inorganic C from the water, and subsequent remineralization lowers the pH through the generation of CO_2. Consequently, high natural pCO_2 values — as observed at the beginning of this experiment — are not unusual for this coastal community. However, this also indicates that the lower CO_2 treatments were in fact deacidification treatments compared to the natural environment.

Our assumption was that higher aggregate phytoplankton responses (e.g. total biomass) to acidification will be less pronounced than single species responses (hypothesis 1). In fact, neither the single species nor the total biomass parameter showed a significant response to CO_2 enrichment. There was a tendency of reduced phytoplankton biomass at the highest pCO_2 treatment, which is likely due to an immediate imposed stress to the community or increased grazing pressure. The delayed phytoplankton peak at 4000 µatm suggests that the immediate injection of CO_2 induced a temporary stress for phytoplankton growth; after a few days, however, growth returned to normal levels. Our findings are in accordance with other studies that found no significant changes in growth, taxonomic shifts, photosynthetic activity or total PON and POC of coastal phytoplankton communities within realistic future predicted OA scenarios (Berge et al. 2010, Nielsen et al. 2010). However, truly oceanic species and calcifying organisms might be sensitive to anthropogenic CO_2 changes (Müller et al. 2010) as diel and seasonal CO_2 variability are generally lower in oligotrophic oceans compared to high productive coastal sites. Calcifying organisms depend on the saturation state of calcium carbonate ($CaCO_3$), which decreases with increasing CO_2 levels and resulting lower pH of seawater (Beaufort et al. 2011). In general, the current state of knowledge reveals either little change or enhanced primary productivity with elevated CO_2 and sparse information about significant changes in dominant non-calcifying phytoplankton species. Comparable to phytoplankton, neither total microzooplankton biomass nor species composition was significantly influenced by the CO_2 treatments. Direct CO_2 effects

on microzooplankton physiology are not to be expected (Nielsen et al. 2010), instead indirect OA effects through the variation in prey dominance would be more likely since microzooplankton such as ciliates show high biomass-specific grazing rates on algae (Nejstgaard et al. 2001).

The hypothesis (2) that *Acartia tonsa* will respond less sensitively to OA compared to a 2-species experiments that excluded community complexity (Rossoll et al. 2012) was supported. In our study, marine copepods of *A. tonsa* seemed not to be affected by the different OA scenarios, neither directly due to decreased pH nor indirectly through possible food quality changes of the algae food source as indicated by abundance, development and egg production. The tendency towards higher egg production at high pCO_2 level is most likely a response to the delayed phytoplankton bloom, since *A. tonsa* has no lipid reserves and thus more food was available when the adult stage was reached. Lack of direct acidification effects in *Acartia* species across pCO_2 up to 5000 µatm were shown in other experiments (Kurihara et al. 2004, Kurihara & Ishimatsu 2008). These studies observed declining egg production rates of female adult copepods at pCO_2 of 5000 µatm or higher, which is far from predicted future scenarios but may be experienced in coastal habitat with upwelling of corrosive water. An adverse indirect effect might be due to CO_2-driven changes in food quality, which can be transmitted to higher trophic levels and result in stunted development and reproduction. In this context a decline of total fatty acid concentrations and the concentration of specific unsaturated fatty acids in CO_2 treated food algae were observed by Rossoll et al. (2012). The decline of unsaturated fatty acids at high pCO_2 resulted in a significant decline in egg production rates of female *A. tonsa*. Since our study did not involve fatty acid measurements, we cannot exclude a change in the fatty acid profiles of algae or copepods. However, given that egg production did not decline at high CO_2 level and that egg production is closely related with unsaturated fatty acid concentration (Hazzard & Kleppel 2003), we expect no significant changes in prey and copepod fatty acid composition. The contrasting response to the study by Rossoll et al. (2012) is likely due to the simple 2-species experiment where a monoculture of *Thalassiosira pseudonana* was used as food source. The biochemical response of phytoplankton species to CO_2 may differ greatly between species and/or taxonomic groups, and the species-specific response will further strongly dependent on other environmental variables (Joint et al. 2011).

In a more complex community the adverse CO_2 effect of single specific algae might be mitigated by a major selection of several algae species, which could serve as potential food source for copepods. In a freshwater acidification study, *Daphnia* individuals maintained high growth rates when fed with high CO_2 cultured mixed algae consisting mainly of diatoms (Urabe & Waki 2009). This compensation effect was given even under lowered P and N contents, which was also consistent with our particulate organic measurements. In spite of the increase of C:N ration to ~9.3 at the highest CO_2 level, stoichiometric food inadequacy can be ruled out in our experiment because this value is far from being potentially limiting for copepods. The seston C:N ratios in our experiment overlap with typical copepod C:N ratios (Walve & Larsson 1999) and only C:N ratios clearly in excess of zooplankton biomass ratios can be limiting, because part of food C will be needed for respiration and not for production (Sterner & Elser 2002). Seston C:P ratio of 115 to 130 are far beyond the P-demand of any zooplankton species and below the threshold ratio for relatively P-poor copepods. Copepods have an additional option to cope with dietary deficiencies of certain food algae: copepods can actively select between equal sized food particles based on chemical quality (DeMott 1988). In addition, most copepods can compensate nutritional inadequacy of algae by a partial or complete shift to a protozoan diet (Klein Breteler et al. 2004, Ptacnik et al. 2004).

Our hypothesis (3) was partially confirmed: most of the observed effects were driven by the difference between the 1400 and 4000 µatm treatment, while differences between the 380 and 1400 µatm treatments were minor and not significant. The only significant difference was the increase of seston C:N ratios at 4000 µatm during the bloom to postbloom period. However, this increase was too small to have any effect on other ecosystem components. This suggests that this coastal community is adapted to high levels of CO_2, and phytoplankton has the capacity to recover immediately after exposure to CO_2-induced stress. Crustaceans are in general physiologically adjusted to strong pCO_2 fluctuations. Further, copepods are strong iono- and osmoregulating species and have compensatory mechanisms to respond to acid-base disruptions, which give them the ability to cope with OA (Whiteley 2011).

A caveat of our study was that the Baltic Sea community used in this experiment experienced high natural pCO_2 levels that are outside the range of predicted pCO_2 increase for the open ocean by the end

of this century. Consequently, except for the extreme pCO_2 treatment level, most of our treatments were in fact de-acidification treatments for the microzooplankton community — but not for *A. tonsa*, which originated from a stock culture. Recent studies indicate that such large pCO_2 fluctuations are not unusual for productive estuarine systems, which have lower buffering capacity to seasonal variation in pCO_2 (Melzner et al. 2013). This suggests that to assess the sensitivity of coastal communities, future experiments need to include pCO_2 treatments that go beyond the worst scenarios projected for the open ocean (Caldeira & Wickett 2003). Moreover, increase in hypoxia and CO_2 uptake by the ocean can aggravate acidification and lead to exponential increase in pCO_2, which might be outside the experienced range for coastal organisms (Feely et al. 2008, Thomsen et al. 2010). Coastal communities such as the Western Baltic Sea already experience higher pCO_2 and pH fluctuations than open ocean plankton will encounter by the end of the century. Hence, coastal communities are not very appropriate model systems to test the sensitivity of open ocean communities to OA, as open ocean organisms are adapted to much more stable pCO_2 conditions.

Although the coastal plankton community used in our experiment was highly resilient to initial OA manipulation, extreme pCO_2 levels frequently observed in productive coastal systems may impose adverse effects for longer-lived and calcifying organisms that do not have the ability to adjust to rapidly acidifying water. This study indicates that the variety of biological responses, both competitive and synergistic, at the organism and population level might prevent extrapolation to the community and ecosystem level: biotic interactions might lead to a dampening (as shown here) or amplification of single species effects. In this context, manipulative acidification experiments on the community level are required for an improved comprehension of marine ecosystem responses to OA.

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