

Global Change Biology (2012), doi: 10.1111/gcb.12109

Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field experiments

JÖRN THOMSEN*¹, ISABEL CASTIES*¹, CHRISTIAN PANSCH†, ARNE KÖRTZINGER† and FRANK MELZNER*

*Marine Ecology, GEOMAR Helmholtz Centre for Ocean Research, Kiel 24105, Germany, †Chemical Oceanography, GEOMAR Helmholtz Centre for Ocean Research, Kiel, 24105 Germany

Abstract

Ocean acidification is expected to decrease calcification rates of bivalves. Nevertheless, in many coastal areas high $p\text{CO}_2$ variability is encountered already today. Kiel Fjord (Western Baltic Sea) is a brackish (12–20 g kg^{-1}) and CO_2 enriched habitat, but the blue mussel *Mytilus edulis* dominates the benthic community. In a coupled field and laboratory study we examined the annual $p\text{CO}_2$ variability in this habitat and the combined effects of elevated $p\text{CO}_2$ and food availability on juvenile *M. edulis* growth and calcification. In the laboratory experiment, mussel growth and calcification were found to chiefly depend on food supply, with only minor impacts of $p\text{CO}_2$ up to 3350 μatm . Kiel Fjord was characterized by strong seasonal $p\text{CO}_2$ variability. During summer, maximal $p\text{CO}_2$ values of 2500 μatm were observed at the surface and >3000 μatm at the bottom. However, the field growth experiment revealed seven times higher growth and calcification rates of *M. edulis* at a high $p\text{CO}_2$ inner fjord field station (mean $p\text{CO}_2$ ca. 1000 μatm) in comparison to a low $p\text{CO}_2$ outer fjord station (ca. 600 μatm). In addition, mussels were able to out-compete the barnacle *Amphibalanus improvisus* at the high $p\text{CO}_2$ site. High mussel productivity at the inner fjord site was enabled by higher particulate organic carbon concentrations. Kiel Fjord is highly impacted by eutrophication, which causes bottom water hypoxia and consequently high seawater $p\text{CO}_2$. At the same time, elevated nutrient concentrations increase the energy availability for filter feeding organisms such as mussels. Thus, *M. edulis* can dominate over a seemingly more acidification resistant species such as *A. improvisus*. We conclude that benthic stages of *M. edulis* tolerate high ambient $p\text{CO}_2$ when food supply is abundant and that important habitat characteristics such as species interactions and energy availability need to be considered to predict species vulnerability to ocean acidification.

Keywords: adaptation, *Amphibalanus improvisus*, calcification, CO_2 enriched ecosystem, community response, energy availability, eutrophication, field study, *Mytilus edulis*, ocean acidification

Received 16 July 2012 and accepted 14 November 2012

Introduction


Increasing atmospheric CO_2 concentrations and subsequent uptake by the oceans lead to increases in seawater CO_2 partial pressures ($p\text{CO}_2$) and decreases in pH and $[\text{CO}_3^{2-}]$ (Cao & Caldeira, 2008). In contrast to the open ocean, where seawater $p\text{CO}_2$ is close to atmospheric values, $p\text{CO}_2$ in coastal habitats is much more variable (Feely *et al.*, 2010; Cai *et al.*, 2011; Melzner *et al.* 2012, Hofmann *et al.*, 2011). Shallow temperate estuaries such as the Western Baltic Sea, with a strong seasonal vertical stratification due to salinity and temperature gradients are characterized by higher and more fluctuating seawater $p\text{CO}_2$ (Thomsen *et al.*, 2010; Melzner *et al.* 2012, Haynert *et al.*, 2012). In these areas, community respiration leads to decreases in $p\text{O}_2$ and increases in $p\text{CO}_2$ below the thermocline. Although this

is a common natural process in the Baltic Sea, it is strongly amplified by high primary production caused by eutrophication from sewage discharge and agriculture (HELCOM, 2009). Upwelling of water masses with high dissolved inorganic carbon content to the surface drastically elevates the $p\text{CO}_2$ in the surface layer of Kiel Fjord during summer and autumn. Due to low alkalinity, calcium carbonate saturation states (Ω) are permanently low and undersaturation frequently occurs (Thomsen *et al.*, 2010; Haynert *et al.*, 2011). Future ocean acidification might lead to very high summer and autumn seawater $p\text{CO}_2$ in such habitats, with regular occurrence of $p\text{CO}_2$ levels >2000 μatm likely by the year 2100 (Melzner *et al.* 2012).

Ocean acidification is expected to lower biomineralization rates of calcifiers and to cause net shell dissolution at high $p\text{CO}_2$ levels in species with insufficient external shell protection (Haynert *et al.*, 2011; Lischka *et al.*, 2011). Nevertheless, several recent studies also revealed unchanged or even increased calcification at high $p\text{CO}_2$ in a range of invertebrates (Ries *et al.*, 2009; **2**

Correspondence: Jörn Thomsen, tel. +49 431 6004466, fax +49 431 6001671, e-mail: jthomsen@geomar.de

¹These authors contributed equally to this work.

	G C B	1 2 1 0 9	B	Dispatch: 14.12.12	Journal: GCB	CE: Sangeetha
	Journal Name	Manuscript No.		Author Received:	No. of pages: 11	PE: Eswari

Thomsen *et al.*, 2010; Pansch *et al.*, 2012b) indicating a considerable potential for acclimation or adaptation to high $p\text{CO}_2$. As most of the present laboratory studies only covered a relatively short fraction of the total lifespan of metazoans, their predictive power is limited. Therefore, CO_2 enriched habitats provide the possibility to investigate long-term acclimation or even adaptation to elevated $p\text{CO}_2$. A gradual decline of the number of calcifying species has been observed along a natural $p\text{CO}_2$ gradient in the Mediterranean (Hall-Spencer *et al.*, 2008), similar observations were made along a tropical $p\text{CO}_2$ gradient (Fabricius *et al.*, 2011). A field study on the US west coast reported a decline in mussel abundance and size with lowered pH during an 8-year period (Wootton *et al.*, 2008). In contrast, benthic metazoan communities in Kiel Fjord are dominated by calcifying invertebrates such as the mussel *Mytilus edulis* and the barnacle *Amphibalanus improvisus* (Thomsen *et al.*, 2010; Pansch *et al.*, 2012a,b). Considering the low Ω and high $p\text{CO}_2$ in this habitat, it is not clear how calcifying species can dominate.

Calcification is a complex, biologically controlled process. In adult mussels, it is not a simple, direct function of seawater $[\text{CO}_3^{2-}]$. Inorganic carbon for biomineralization originates from HCO_3^- that is transported into or generated within cells. Shell CaCO_3 and a diverse set of shell organic matrix molecules are most likely produced intracellularly and are exported to sites of incipient biomineralization (Jacob *et al.*, 2008; Weiner & Addadi, 2011). Furthermore, a number of invertebrates protect their external shells from the surrounding water with chemically robust organic covers such as the periostracum in the case of bivalves (Waite *et al.* 1979). Therefore, shell production is not necessarily exclusively driven by seawater $[\text{CO}_3^{2-}]$, but is an energetically costly process, primarily due to the production of the organic shell components (Palmer, 1992). As a consequence, a relatively large fraction of the energy budget is allocated to shell production. Reduced calcification in adult *M. edulis* may thus simply result from an energetic trade-off. Recent measurements of increased metabolic rates under elevated $p\text{CO}_2$ indicate elevated costs for homeostatic processes. Increased maintenance cost could depress scope for growth and biomineralization (Thomsen & Melzner, 2010). A CO_2 induced shift of the cellular energy budgets towards ion regulatory processes and a reduction in scope for growth have recently demonstrated in marine animals (Deigweier *et al.*, 2009; Stumpp *et al.*, 2011).

Here we present findings of a high dependence of mussel growth and calcification on food supply in laboratory and field experiments. High food supply enables a high CO_2 stress resilience of mussel growth and calcification. This enables *M. edulis* to dominate the high

$p\text{CO}_2$ habitat in Kiel Fjord and out-compete seemingly more CO_2 tolerant organisms. Our study emphasizes the need to carefully consider the main biotic and abiotic habitat characteristics to predict species responses to ocean acidification.

Material and methods

Laboratory study

For the laboratory study, settled post larvae of the genus *Mytilus* were used. The *Mytilus* population in the Baltic Sea is a hybrid population of *M. trossulus* and *M. edulis*, with progressively more *M. trossulus* alleles towards the east (Stuckas *et al.*, 2009). As the available evidence suggests that >80% of alleles in Kiel Fjord animals originate from *M. edulis* (Stuckas *et al.*, 2009; Breusing, Stuckas, Reusch, Melzner unpublished observation). We will for simplicity's sake refer to Kiel mussels as *M. edulis*. To capture freshly settled mussels, 5×5 cm PVC settlement panels, which were roughed on one side using sandpaper (grain 60), were suspended in the fjord (IF station) at 1 m water depth. After 10 days, panels were removed from the fjord and all settled organisms other than 100 settled *M. edulis* larvae were removed with the help of forceps after viewing under a stereo microscope. Subsequently, single plates were transferred into 500 mL aquaria in a 17 °C constant temperature room. For the 7-week laboratory experiment, four $p\text{CO}_2$ treatments, 470, 1020, 2110, and 3350 μatm , and three feeding regimes (low, intermediate and high) were realized. In total, 12 different treatments with seven replicates each were used. The $p\text{CO}_2$ treatment levels used correspond to present day and projected future levels in this area (Thomsen *et al.*, 2010). Aquaria were filled with 0.2 μm filtered and UV-radiated seawater from the fjord and aerated with compressed air with one of four $p\text{CO}_2$ levels (see Thomsen *et al.*, 2010 for details). Water exchange of 400 mL (80%) was performed daily using CO_2 and temperature pre-equilibrated water. Measurements of salinity, temperature, and pH_{NBS} were performed daily in 24 of the 84 aquaria. For carbonate chemistry, 250 mL water samples were taken and pH on the total scale (pH_{T}) and C_{T} were determined using a Metrohm 6.0262.100 electrode and 626 Metrohm pH meter and an AIRICA C_{T} analyser (Marianda, Kiel, Germany). Water samples were analysed within 2 h of sampling. pH_{T} was determined using Tris/HCl and AMP/HCl seawater buffers mixed for a salinity of 15 (Dickson *et al.*, 2007) in a 21 °C water bath. Accuracy of A_{T} and C_{T} measurements was ensured by using certified reference material (Dickson *et al.*, 2003). The carbonate system speciation was calculated using the CO2SYS program (Lewis & Wallace, 1998). For calculations, the KHSO_4 dissociation (Dickson *et al.* 2005) and the carbonate system dissociation constants K_1 and K_2 (Roy *et al.*, 1993) were used.

Three feeding treatments were established by addition of exact cell numbers of *Rhodomonas* sp. algae to the experimental aquaria. At weekly intervals, algae cell concentrations were increased and the number of mussels was reduced to compensate for mussel growth (Supplementary Table S1). Initial algal supply in the different food treatments was 5000, 2500, and

500 cells mL⁻¹, respectively, which was increased up to 40 000, 20 000, and 4000 cells mL⁻¹ during the last week. The ratio of 10 : 5 : 1 between the feedings levels was maintained during the entire experiment. The algae cells were cultured in 0.2 µm filtered seawater with Provasoli's enriched seawater (PES) medium and elevated phosphate (0.036 mmol L⁻¹) and nitrate (0.55 mmol L⁻¹) concentrations as previously described (Thomsen *et al.*, 2010). Once a day, *Rhodomonas* culture densities were measured using a particle counter (Z2 Coulter Particle count and size analyser, Beckman Coulter, Krefeld, Germany) to calculate the culture volume that had to be added to reach the desired cell densities in the aquaria. Weekly, particulate organic carbon (POC) content of the supplied algae and of algae concentrations following 24-h incubation was analysed. For POC determinations, 500 mL water samples were obtained with a water sampler from 1 m depth. Subsequently, samples were filtered on combusted GF/F filters using a vacuum pump and stored at -20 °C. Before analysis, filters were treated with fuming hydrochloric acid, dried (60 °C, 6 h), and wrapped in tin boats. POC determinations were performed using gas chromatography in an elemental analyser Euro Vector 3000 (EuroVector, Milan, Italy). Differences between supplied and remaining POC were used to calculate POC uptake by the mussels. Correlation of measured *Rhodomonas* cell abundance and POC revealed a carbon content of 45 pg C cell⁻¹.

For determination of initial size (shell length, CaCO₃, and total organic content) and weekly growth rates, five to six individuals were sampled at each sampling day from each aquarium and stored in seawater with 4% paraformaldehyde (PFA). Shell length was measured using a stereo microscope equipped with a MicroPublisher 3.3 RTV camera and the image analysis software Image Pro Plus 5.0.1. Final CaCO₃ and total organic content was determined as described above by weighing single specimens using a precision balance (accuracy ± 1 µg, Sartorius, Göttingen, Germany) after drying at 60 °C and ashing at 500 °C for 20 h. In addition, shell free drymass (SFDM) was dissected from carefully opened, frozen specimens and dried at 60 °C. Comparison of SFDM and total organic content (TOC = SFDM + organic shell components, OSC) was performed using a regression of both parameters against shell length.

Energy contents of supplied *Rhodomonas* and seawater POC were estimated using the conversion factor of 46 J mg⁻¹ POC (Salonen *et al.*, 1976). Mussel SFDM and OSC (periostracum + organic shell matrix) was converted to caloric equivalents using the conversion factor 23 J mg⁻¹ (Brey *et al.*, 1988). Similarly, the inorganic shell component (ISC) was converted into energy equivalents using a factor of 2 J mg⁻¹ CaCO₃ (Palmer, 1992).

Field studies

Carbonate chemistry in Kiel Fjord. The carbonate chemistry of Kiel Fjord was monitored on weekly (station 1) and bi-weekly (stations 2–4) cruises using RV Polarfuchs between April 2009 and March 2010. Two samples (500 mL) were taken at each station from both surface (ca. 1 m depth) and bottom

water (1 m above sea floor, i.e. 11–20 m). Seawater samples were poisoned with saturated mercuric chloride solution and stored at room temperature until analysis according to SOP1 (Dickson *et al.*, 2007). Samples were analysed for C_T and A_T by coulometric and potentiometric titration using SOMMA and VINDTA auto analysers respectively (Dickson *et al.*, 2007). In addition, water samples for nutrient determination (PO₄³⁻, H₄SiO₄) were collected in 10 mL Falcon tubes and stored at -20 °C. Analyses were carried out spectrophotometrically using a U-2000 spectrophotometer (Hitachi-Europe, Krefeld, Germany, Hansen and Koroleff 1999). Weekly pH measurements (NBS scale) were conducted at an inner Kiel Fjord (station IF, 54°19.8'N; 10°9.0'E) and bi-weekly at an outer Kiel Fjord station (station OF, 54°25'N, 10°10'O) during the field study using a WTW 340i pH meter and a WTW Sentix 81-electrode. Salinity and temperature were measured at 10 cm depth using a WTW cond 315i salinometer and a TETRACON 325 probe.

***M. edulis* growth experiment.** For the field study, settlement panels were treated in the same way as stated above for the laboratory study. Panels were transferred to the experimental sites and suspended at 1 m depths. Both field sites are characterized by similar salinity, temperature, light exposure, and are sheltered from wave action. The number of replicates was six. However, despite the shelter two panels were lost at station OF during storms. Water chemistry (pH_T, C_T) and POC concentrations were monitored bi-weekly at both stations and analysed as described above. POC concentrations were monitored as an indicator for habitat energy supply. The experiment lasted from July 23th to November 19th 2010 (18 weeks). Panels were removed and frozen. Subsequently, pictures were taken to calculate the percentage of coverage for *M. edulis* and *A. improvisus* on both sides of each panel using imageJ version 1.45. Fouling organisms were determined to the lowest possible taxonomic level. TOC and ISC of the two species comprising >99% of the biomass on the panels (mussels and the barnacles) were determined after drying at 60 °C overnight and ashing at 500 °C for 20 h respectively. In addition, settled post larvae of *M. edulis* were collected at each station every second week starting on August 11th for determination of a shell length growth relationship. Final somatic drymass and shell length and mass were determined from specimens collected on November 19th.

Haemolymph acid-base status. The extracellular acid-base status was determined for *M. edulis* sampled from the field and laboratory experiments. A haemolymph sample (0.4–0.8 mL) was anaerobically drawn from the posterior adductor muscle using gas tight Hamilton syringes and transferred into a 1.5 mL Eppendorf tube. pH_{NBS} was measured using a WTW microelectrode; the measurement became stable after 30 s. C_T was measured using a Corning 965 CO₂ analyser (Olympic Analytical, Malvern, UK), which was calibrated with NaHCO₃ standards. Acid-base parameters pCO₂, [HCO₃⁻] and [CO₃²⁻] were calculated according to the Henderson-Hasselbalch-Equation as described previously (Thomsen *et al.*, 2010). The systematic error of pH determination due to gas exchange was

quantified by monitoring pH changes during 3.5 min after stabilization of the initial value. The pH changed by less than 0.01 units during this period ($n = 11$, Supplementary Fig. S5d). Haemolymph acid-base status was monitored for specimens (shell length about 5 cm) sampled directly at station IF in 2009 (see supplementary Table S7). Animals were transported in ambient water into the lab. To quantify the effect of handling and transportation on acid-bases status, comparisons were made between haemolymph withdrawn immediately from animals after removal from the fjord (<15 s, $n = 5$) and from specimens stored for 5–10 min in ambient water ($n = 6$). No significant differences were observed between both groups for any acid-base parameter (Supplementary Fig. S5a–c, *t*-test, $P > 0.05$). Furthermore, the effect of flow-through and closed experimental setups on extracellular acid-base status was tested. Specimens were kept at $p\text{CO}_2$ levels of 390 and 4000 μatm in either closed (no water exchange) or open, i.e. flow-through (100 mL min^{-1} water exchange), setups for 1 week. Subsequently, extracellular acid-base status and carbonate chemistry of the water was determined as described above. Accumulation of HCO_3^- above the non-bicarbonate buffer line was only observed when animals were kept in laboratory experiments in a closed system without water exchange (Supplementary Figure S5e). In this treatment, shell dissolution due to $\Omega_{\text{aragonite}} < 1$ increased HCO_3^- concentrations of the water and simultaneously increased haemolymph HCO_3^- concentrations (Supplementary Figure S5E, ANOVA, $F_{(3,16)} = 11.4$, $P < 0.01$). In contrast, the flow-through experimental design prevented HCO_3^- accumulation in the water and also in the haemolymph (Supplementary Table S8).

Statistics

Regression analyses were performed using Sigma Plot 10, all other statistics were performed with Statistica 8. Data were analysed for normality using Shapiro-Wilks test. For the laboratory results repeated measures ANOVA and two-way ANOVA were applied. Multiple linear regression models for growth were compared using Akaike Information criterion (AIC). For data analyses of the field study, paired *t*-tests and Mann-Whitney *U*-tests were used.

Results

Laboratory experiment

The carbonate system speciation in the experimental aquaria is given in Supplementary Table S2. Seawater was undersaturated with respect to aragonite in all elevated $p\text{CO}_2$ treatments. Survival rates were not affected by any treatment. At the same time, weekly measurements documented an exponential increment in mussel shell length during the experiment (Fig. 1a–c). Feeding regime and $p\text{CO}_2$ had significant effects on shell length, inorganic shell component (ISC), and total organic component (TOC) growth (Fig. 1d, repeated measures ANOVA: factor food supply: $F_{(14,132)} = 17.86$, $P < 0.01$, factor

$p\text{CO}_2$: $F_{(21,190.1)} = 1.99$, $P < 0.01$, interaction: $F_{(42,313)} = 0.99$, $P > 0.5$). The observed growth was described best using a two factorial linear model (see Fig. 1 caption). However, AIC analysis revealed that single factor models based on food supply, explained most of the variability encountered in the experiment (Supplementary Table S3).

Inorganic shell component (ISC) growth was significantly affected by food level and $p\text{CO}_2$ (Fig. 1d, Two-way ANOVA: factor food supply: $F_{(2,72)} = 40.6$, $P < 0.01$, factor $p\text{CO}_2$: $F_{(3,72)} = 3.27$, $P < 0.05$, interaction: $F_{(6,72)} = 1.07$, $P > 0.5$), whereas total organic component (TOC) growth was significantly influenced by food supply, but not by $p\text{CO}_2$ (Fig. 1d, Two-way ANOVA: factor food supply: $F_{(2,72)} = 51.84$, $P < 0.01$, factor $p\text{CO}_2$: $F_{(3,72)} = 1.19$, $P = 0.32$, interaction: $F_{(6,72)} = 1.02$, $P > 0.5$).

Settled mussels consisted of about 20% organic and 80% inorganic (CaCO_3) material. Approximately 50% of the produced organic mass consisted of structural organic shell material (OSC), i.e. matrix proteins and carbohydrates as well as periostracum. The remaining organic mass was somatic tissue (shell free drymass, SFDM, Supplementary Fig. S1). Thus, shell mass (OSC and ISC) contributed about 90% to the final dry mass of the experimental *M. edulis*. Owing to the lower energy demands for ISC formation, relatively more energy was invested into organic mass growth (Supplementary Fig. S2). We estimate that about 60% of the total energy necessary for body growth was invested into synthesis of organic and inorganic shell components (Supplementary Table S4). Whereas the shell mineralogy of freshly settled *M. edulis* is similar to that of older mussels, the organic shell component is much higher in younger mussels ($>10\%$, Supplementary Table S4) than in fully grown mussels (1–6%, Supplementary Table S5). The proportions of OSC vs. ISC did not significantly change with $p\text{CO}_2$ (Supplementary Table S4). Our study demonstrates the ability of freshly settled *M. edulis* to calcify under highly elevated $p\text{CO}_2$ when nutritional supply is sufficient.

Field studies

Carbonate chemistry in Kiel Fjord. In the field, long-term monitoring confirmed that inner Kiel Fjord (IF) is characterized by elevated mean $p\text{CO}_2$ values and a large seasonal $p\text{CO}_2$ variability. Occasional upwelling of hypoxic bottom water in late summer and autumn leads to increased $p\text{CO}_2$ and lowered pH (Fig. 2, Supplementary Table S6). Surface pH_{NBS} is highly variable, with high values of about 8.0–8.1 during spring and early summer and low values down to about 7.3 in late summer and autumn (Fig. 2b). In addition, pH_{NBS} can vary

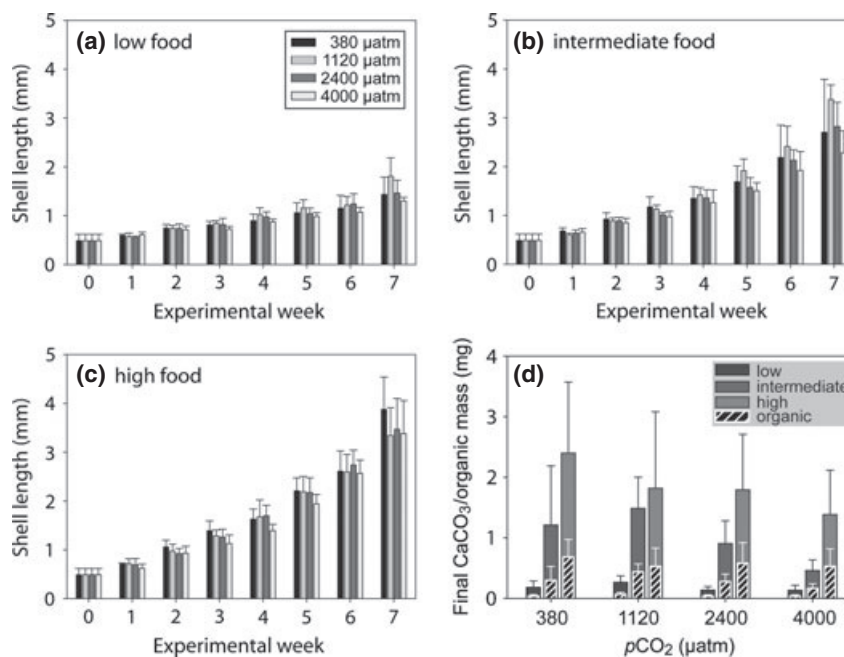


Fig. 1 *Mytilus edulis* shell and somatic growth during the experiment. Weekly measured shell length of mussels from the four $p\text{CO}_2$ and three feeding treatments (a–c). Shell length growth (SLG) could best be described with the following two factorial linear regression: $\text{SLG} (\mu\text{m } 7 \text{ weeks}^{-1}) = 1312.2 (\pm 150.6) - 0.148 (\pm 0.06) p\text{CO}_2 (\mu\text{atm}) + 46.83 (\pm 3.81) \text{ energy supply (J)}$, $R^2 = 0.66$, $F_{(2,81)} = 78.4$, $P < 0.01$, means \pm SE (a–c). Final shell CaCO_3 growth (filled bars) and total organic growth (SFDM + OSC, striped bars, CaCO_3 : Two-way ANOVA: factor energy: $F_{(2,72)} = 40.6$, $P < 0.01$, factor $p\text{CO}_2$: $F_{(3,72)} = 3.27$, $P < 0.05$, interaction: $F_{(6,72)} = 1.07$, $P < 0.5$, total organic: two-way ANOVA: factor energy: $F_{(2,72)} = 51.84$, $P < 0.01$, factor $p\text{CO}_2$: $F_{(3,72)} = 1.19$, $P > 0.05$, interaction: $F_{(6,72)} = 1.02$, $P < 0.5$ (d). $n = 7$ for all treatments. Bars represent means \pm SD.

by more than 0.5 units within a few days. The same was observed for $p\text{CO}_2$. Highest measured surface $p\text{CO}_2$ values were around 2500 μatm in August 2010 (Fig. 2a). Mean annual surface $p\text{CO}_2$ decreased from the inner to outer parts of the fjord (from 706 at station 1 to 447 μatm at Station 4). Highest $p\text{CO}_2$ values were observed between April and November in the inner fjord and in the bottom waters at all stations. Annual mean $p\text{CO}_2$ is around 1000 μatm at >15 m depths in Kiel Fjord and adjacent areas, maximum values of 3400 μatm were recorded in August 2010 (Fig. 2a). Kiel Fjord calcium carbonate saturation state was found to be low during the entire seasonal cycle. Inner fjord surface $\Omega_{\text{aragonite}}$ was lower than 1 in 35 of 55 weeks (64% of all investigated weeks). At water depths >15 m, $\Omega_{\text{aragonite}}$ was <1 almost during the entire investigated period (98% of all investigated weeks).

M. edulis growth experiment. The carbonate system speciation was similar at stations IF and OF during the field *M. edulis* growth study. Mean $p\text{CO}_2$ was variable at both stations, yet significantly higher at station IF (969 μatm , Table 1) in comparison to station OF (599 μatm , Table 1, Fig. 3 + 4f, paired t -test $t = 2.46$, $P < 0.05$, $N = 7$). Therefore, mean pH, Ω_{calcite} and

$\Omega_{\text{aragonite}}$ were also lower at IF (Fig. 4d–e, Table 1). At station IF, POC concentrations were about twice as high as observed at station OF (Fig. 4c, Table 1). During the study period, POC concentrations decreased with time at both stations reflecting the seasonal succession of plankton communities typical for this area. IF shell growth was about 1.1 mm week $^{-1}$ whereas OF mussels grew by only 0.5 mm week $^{-1}$ (Supplementary Fig. S3). Final shell lengths of *M. edulis* grown on the settlement panels were 18.3 and 9.4 mm at stations IF and OF ($U = 166$, $Z = 4.65$, $P < 0.01$) respectively. Shells from both stations were characterized by a similar length–mass relationship (Fig. 4b). Differences were more pronounced for mean shell mass (226 mg vs. 33.0 mg, $U = 166$, $Z = 4.42$, $P < 0.01$) and SFDM (51.1 mg vs. 5.8 mg, $U = 167$, $Z = 4.33$, $P < 0.01$).

Settlement panels were almost completely dominated by *M. edulis* at both stations and only the barnacle *A. improvisus* was able to exclusively cover smaller parts of the panels at station OF (Fig. 3, Supplementary Fig. S4). Few other metazoan taxa were detected on single panels in negligible abundances (Table S4). At station IF, barnacles were fully overgrown by *M. edulis* and a large number was found to be dead underneath the mussels (Table 2). During the experiment, secondary

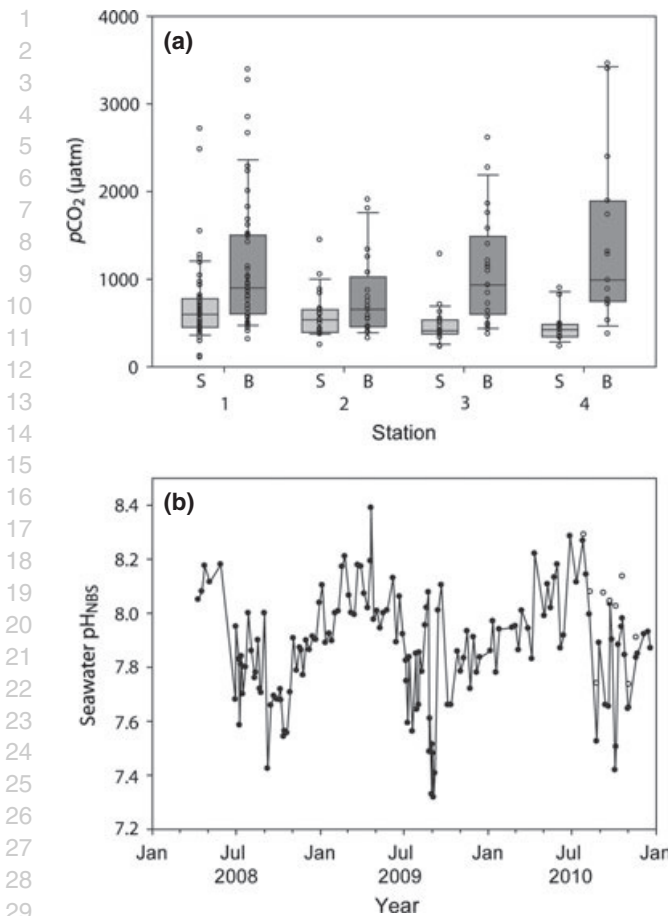


Fig. 2 Box-whisker-plot of Kiel Fjord $p\text{CO}_2$ in 2009/2010 measured at stations 1–4 in surface (light grey, S) and bottom (dark grey, B) water samples. Circles depict individual measurements (a); Time course of surface pH_{NBS} in inner Kiel Fjord (station IF, closed circles) between 2008 and 2010 (data from 2008–2009 adopted from Thomsen *et al.*, 2010) and at station OF during the field experiment (open circles), (b).

settlement of mussel larvae took place and led to final numbers of about 600–700 mussels per settlement panel (area of $2 \times 25 \text{ cm}^2$). Production of calcium carbonate by mussels (101.2 g per panel) at IF exceeded that of OF (13.8 g per panel) panels by a factor of 7. In contrast, barnacle survival (24 % vs. 63 %) and calcification (8.0 g vs. 14.2 g per panel) rates were reduced at station IF compared to OF due to mussel dominance (Table 2, Fig. 4a).

Haemolymph acid-base status. Haemolymph acid-base status was monitored in adult *M. edulis* at locations close to station IF with respect to seawater carbonate chemistry. Our sampling programme revealed that mussel extracellular pH can be described as a linear function of seawater pH (Fig. 5). In August and September, haemolymph pH was significantly reduced to

7.36 and haemolymph $p\text{CO}_2$ increased to $3090 \mu\text{atm}$ as a consequence of low seawater pH and high $p\text{CO}_2$ during upwelling events (Supplementary Table S7). Mean haemolymph $[\text{CO}_3^{2-}]$ was $30.4 \mu\text{mol L}^{-1}$ and ranged between 30 and 66 % of the ambient seawater $[\text{CO}_3^{2-}]$ concentration.

Discussion

Our laboratory experiment results reveal a high tolerance of the earliest benthic stage of *M. edulis* towards elevated $p\text{CO}_2$. For the first time, we could demonstrate that freshly settled *M. edulis* survive high seawater $p\text{CO}_2$ and calcify at high rates when nutritional supply is sufficient. Compared to nutritional supply, $p\text{CO}_2$ has only a minor impact on growth and calcification of settled *M. edulis* in both laboratory and field experiments.

A significant reduction of calcification was only observed at very high $p\text{CO}_2$ of $3350 \mu\text{atm}$ in our laboratory experiment. Our results contrast with studies on freshly settled stages of other bivalve species, which exhibited lower growth and survival, even at moderately elevated $p\text{CO}_2$ below $1500 \mu\text{atm}$ (Talmage & Gobler, 2010).

Our 1-year monitoring of the carbonate system variability confirmed the high annual variability of $p\text{CO}_2$ and pH in the inner parts of Kiel Fjord (Thomsen *et al.*, 2010). Peak $p\text{CO}_2$ observed during summer months in inner Kiel Fjord can reach $>2000 \mu\text{atm}$ – $p\text{CO}_2$ levels that have been shown to cause abnormal development of *M. galloprovincialis* (Kurihara *et al.*, 2008). High and variable seawater $p\text{CO}_2$ is related to occasional upwelling of hypoxic-hypercapnic bottom water driven by southerly winds. Whereas oxygen rapidly equilibrates with the atmosphere, elevated $p\text{CO}_2$ persists in the surface waters for longer time periods. Similarly high degrees of variability in $p\text{CO}_2$ were documented along volcanic CO_2 vents in subtropical and tropical habitats (Hall-Spencer *et al.*, 2008; Fabricius *et al.*, 2011; Rodolfo-Metalpa *et al.*, 2011). However, in these fully marine, naturally acidified systems, seawater remains supersaturated with respect to calcium carbonate when low $p\text{CO}_2$ values are encountered (Hall-Spencer *et al.*, 2008; Fabricius *et al.*, 2011). In contrast, very low CaCO_3 saturation and frequent undersaturation was found in Kiel Fjord during the entire year, as salinity and alkalinity are lower in this brackish system. In Kiel Fjord, high seawater $p\text{CO}_2$ results from aerobic degradation of organic matter in the water masses below the thermocline, an effect which is enhanced by strong eutrophication during the last 50 years (Babenerd, 1991; Melzner *et al.* 2012). Kiel Fjord thus is an example of a highly productive habitat where eutrophication impacts on the carbonate system are already more severe than

Table 1 Abiotic seawater conditions and carbonate system parameters (measured: Sal, T, POC, C_T , pH and calculated: A_T , pCO_2 , Ω_{calcite} and $\Omega_{\text{aragonite}}$) at the field sites (A), final morphometry of individual specimens, plate coverage and $CaCO_3$ production on the settlement panels at the end of the field study (B), values exceeding 100% coverage result from secondary settlement of *Mytilus edulis* spat, see panels in Fig. 3, POC and PON concentration and C : N ratio at the field sites. Values represent means \pm SD

A								
Carbonate system parameters								
Site	Salinity	Temperature °C	C_T $\mu\text{mol kg}^{-1}$	A_T $\mu\text{mol kg}^{-1}$	pH Total scale	pCO_2 μatm	Ω Calcite	Ω Aragonite
Inner Fjord	16.2 ± 2.0	13.2 ± 3.0	1996.5 ± 104.8	2021.1 ± 95.1	7.76 ± 0.23	969 ± 420	1.38 ± 0.68	0.81 ± 0.39
Outer Fjord	16.0 ± 1.4	12.5 ± 2.8	1829.1 ± 99.8	1945.5 ± 56.8	7.93 ± 0.18	599 ± 283	1.79 ± 0.65	1.05 ± 0.38
B								
Single specimens				Settlement plates				
Site	Shell length mm	Shell mass mg	Drymass mg	Mussel coverage %	Barnacle coverage %	Barnacle survival %	Mussel $CaCO_3$ g plate $^{-1}$	Barnacle $CaCO_3$ g plate $^{-1}$
Inner Fjord	18.3 ± 6.8	226.6 ± 169.4	51.1 ± 42.2	166.7 ± 25.8	66.7 ± 44.6	24.2 ± 22.4	101.2 ± 24.3	8.0 ± 2.9
Outer Fjord	9.4 ± 3.4	33.0 ± 22.8	5.8 ± 4.3	87.5 ± 25.0	95.0 ± 10.0	62.5 ± 14.4	13.8 ± 4.9	14.2 ± 4.3
C								
Nutrition								
Site	POC $\mu\text{g l}^{-1}$			PON $\mu\text{g l}^{-1}$			C : N Molar ratio	
Inner Fjord	682 ± 388			92 ± 46			8.8 ± 1.8	
Outer Fjord	342 ± 148			50 ± 23			8.1 ± 0.7	

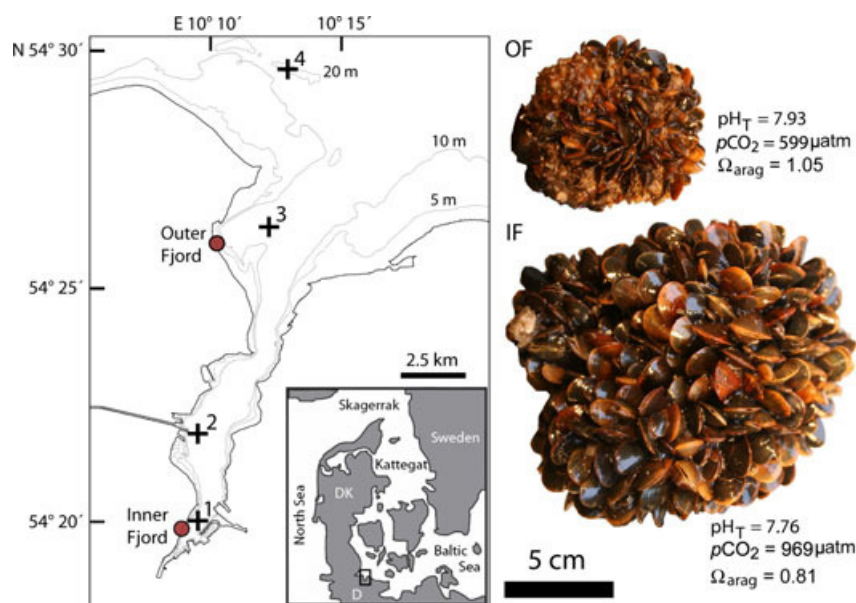


Fig. 3 Map of Kiel Fjord with representative settlement plates from the outer Fjord (OF) and the inner Fjord (IF) and the average carbonate system speciation during the field study, Numbers 1–4 indicate the stations of the carbonate system monitoring program, the brown circles indicate the positions of stations OF and IF, original size of the PVC panels 5 × 5 cm (panels are fully overgrown with mussels and barnacles). Pictures are drawn to scale, scale bar 5 cm.

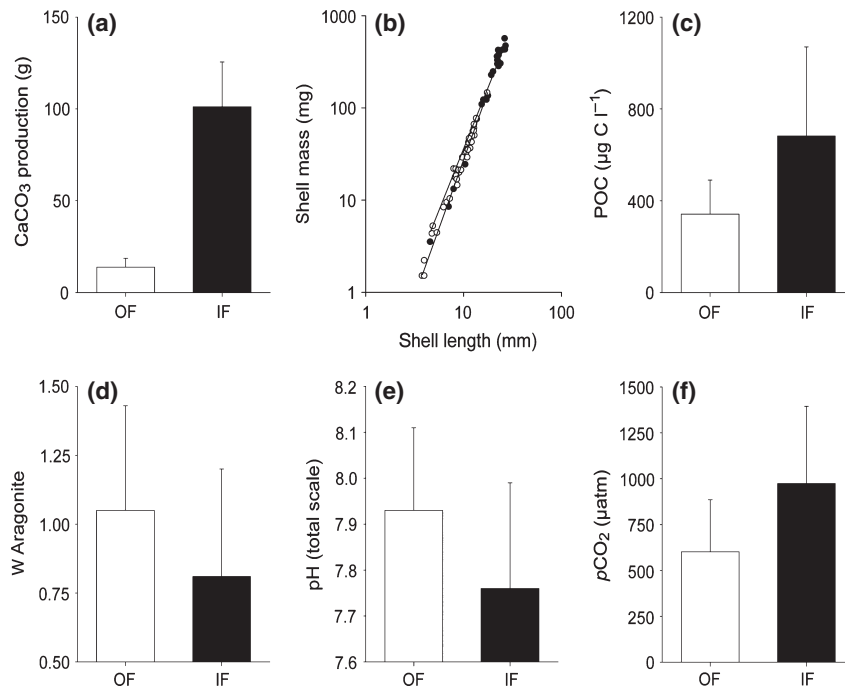


Fig. 4 Results of the field study (outer Fjord: white, inner Fjord: black): Final mean CaCO₃ mass of *Mytilus edulis* per settlement plate (a), Final shell mass and length of individual *M. edulis* grown at the outer and inner Fjord (b), POC concentrations (c), Mean values for abiotic conditions during the experimental period at both sites (d–f), $\Omega_{\text{aragonite}}$ (d), pH_{total} (e), pCO₂ in µatm (f). Bars represent means ± SD.

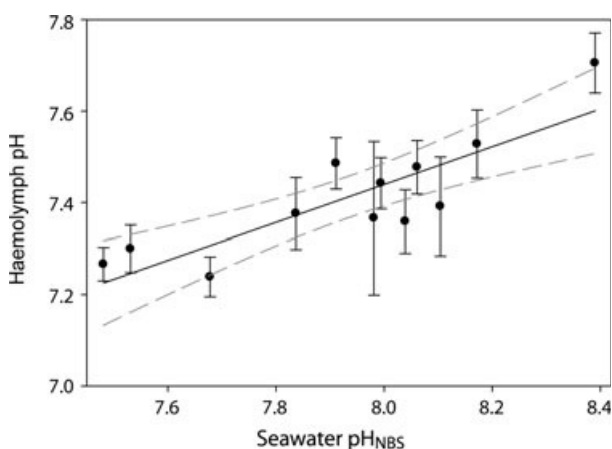


Fig. 5 Relationship of haemolymph pH (pH_{HL}) of *Mytilus edulis* and ambient seawater pH_{NBS} (pH_{SW}), pH_{HL} = 0.413 (± 0.08) × pH_{SW} + 4.14 (± 0.64), $F_{(1,60)} = 26.5$ $P < 0.01$, means ± SE; each data point represents mean ± SD of $n = 5$ mussels from one sampling date.

perturbations that are expected through air-sea CO₂ uptake in the average surface ocean in the next 100 years (Cao & Caldeira, 2008; Borges & Gypens, 2010; Haynert *et al.*, 2012).

The observed fluctuations in seawater pCO₂ over the seasonal cycle directly translate into changes in mussel haemolymph pCO₂. As *M. edulis* does not accumu-

late bicarbonate in its haemolymph to regulate pH (Thomsen *et al.*, 2010; Heinemann *et al.*, 2012), extracellular pH and [CO₃²⁻] respond strongly to changes in seawater pCO₂. However, as an intertidal species *M. edulis* is used to cope with strong fluctuations of extracellular pH – at least on a daily basis (Booth *et al.*, 1984). In contrast, intracellular pH is strongly regulated and maintained close to control levels under acidification stress (Zange *et al.*, 1990; Michaelidis *et al.*, 2005). To maintain intracellular pH under changed extracellular acid-base status cellular ion transport needs to be adjusted, which most probably requires additional energy (Deigweier *et al.*, 2009). This hypothesis is supported by the observation of CO₂-induced elevated metabolic rates in several marine invertebrates with limited pHe regulatory capacity – including *M. edulis* (molluscs: Beniash *et al.*, 2010; Thomsen & Melzner, 2010; echinoderms: Stumpp *et al.*, 2011). Higher maintenance costs for intracellular homeostasis under CO₂ stress might be one main cause for the decrease of scope for growth and consequently, reduced calcification rates (Stumpp *et al.*, 2011). Furthermore, the formation of amorphous calcium carbonate precursor (ACC) material for shell formation occurs in intracellular vesicles (Jacob *et al.*, 2008; Weiner & Addadi, 2011). Excess protons, which are generated intracellularly during calcification, need to be excreted against an increased

extracellular proton concentration under high environmental $p\text{CO}_2$. This might further compromise energy budgets of bivalves under elevated $p\text{CO}_2$.

Due to the relative large shell mass and the high organic content of the shell of small mussels (>10% of shell mass), calcification is an extremely costly process for the earliest life stages. In adult mussels, matrix proteins only contribute about 5% to total shell mass (Jørgensen, 1976). Our findings demonstrate that in early juveniles >60% of the energy used for body growth is required for shell formation, including about 40% for its organic content, that is the shell matrix proteins and carbohydrates and the periostracum. Therefore, downregulation of calcification can contribute significantly to energy conservation during unfavourable, low food periods or during exposure to very high ambient $p\text{CO}_2$.

However, our laboratory experiment indicates that the negative effects of seawater acidification are marginal compared to those of food supply, with no significant reductions in growth and calcification up to a seawater $p\text{CO}_2$ of 2100 μatm . In agreement with the laboratory results, we observed that moderately elevated $p\text{CO}_2$ (ca. 1000 μatm) at field station IF does not lower mussel fitness. Rather, high energy availability enables higher growth and calcification rates and more complete dominance of benthic communities when compared to the lower $p\text{CO}_2$ (ca. 600 μatm) station OF.

These findings are in contrast to other field studies along natural $p\text{CO}_2$ gradients which observed a strong decrease of the productivity of calcifying metazoans (Hall-Spencer *et al.*, 2008; Fabricius *et al.*, 2011). The high POC concentrations in Kiel Fjord in general and at station IF in particular, enable overcompensation of the adverse carbonate system conditions. Higher organic particle concentrations lead to much higher energy uptake at similar filtration rates, which facilitate higher growth rates and net growth efficiencies. This might partly explain the sevenfold increase in shell mass growth at only twofold elevated POC concentration at station IF (Riisgård & Randløv, 1981). In other coastal areas with comparable POC availability (e.g. the North Sea), mussels may also compensate negative effects of high CO_2 through high energy availability. In contrast, in oligotrophic ocean regions such as the Mediterranean Sea, such compensatory effects might not be possible (Rodolfo-Metalpa *et al.*, 2011). Due to the eutrophication process in the Baltic, the benthic community in Kiel Fjord has been subjected to a rapid change towards high ambient $p\text{CO}_2$ within less than 50 years. This has not impacted the dominant position of *M. edulis* in this habitat (Boje, 1965). Thus, *M. edulis* might be inherently tolerant to high and variable $p\text{CO}_2$, or possess an expressed capacity for rapid adaptation to high $p\text{CO}_2$

within about 50 generations. Simulated selection of closely related *M. trossulus* larvae under an elevated $p\text{CO}_2$ regime indicated the possibility for significant adaptation potential within a similar time period (Sunday *et al.*, 2011). Population specific differences in vulnerability to ocean acidification were already observed in oysters and emphasize the need to compare the responses of Kiel Fjord mussels with other populations from less CO_2 enriched areas (Parker *et al.*, 2012).

The results from our field experiment also emphasize the need to consider multi-species responses towards ocean acidification. Barnacles, thought to be more tolerant to acidification than mussels (Pansch *et al.*, 2012a,b), are out-competed at station IF. While CaCO_3 production of both species was similar at station OF, mussel CaCO_3 production exceeded that of barnacles about 12-fold at station IF. This is most probably due to restriction of barnacle food supply by fast growing mussels and very high recruitment intensity. High filtration and 100% clearance rates of particles >4 μm in *M. edulis* (Riisgård & Randløv, 1981), the spatial restriction of the barnacle feeding apparatus in dense mussel assemblages and depletion of planktonic organisms by mussels are probable causes for starvation – induced high mortality (>75%) of barnacles at station IF. Therefore, in Kiel Fjord recruitment success of barnacles is more dependent on competition with mussels than on the direct effects of high $p\text{CO}_2$ (Pansch *et al.*, 2012a,b). Consequently, results from single species laboratory experiments on CO_2 tolerance cannot be extrapolated to the field without considering habitat specific characteristics such as recruitment intensity, competition for space and food availability. In summary, this study demonstrates a high inherent resilience of calcifying benthic communities in an estuarine, eutrophic habitat to elevated seawater $p\text{CO}_2$. Food supply, and not $p\text{CO}_2$, appears to be the primary factor driving biomass and biogenic CaCO_3 production, as well as community structure. Our findings emphasize the need to include additional factors such as habitat energy availability to predict single- and multi-species responses to ocean acidification. Future studies need to address the impact of additional stressors such as temperature increase, as well as the higher sensitivity of the early, planktonic larval stages (Gazeau *et al.*, 2010).

Acknowledgements

The authors thank Dennis Rossoll, Holger Ossenbrügger, Katja Trübenbach, Luise Töpfer, and Helgi Mempel for supporting carbonate chemistry sampling and Susann Grobe, Tobias Steinhoff, Sebastian Fessler, and Vincent Saderne for help with the measurements. Peter Fritsche and Annegret Stuhr provided nutrient data, Aljosa Zavisic measured POC and Philipp Schubert contributed the map of Kiel Fjord. Furthermore, we

acknowledge Ulrike Panknin for maintaining *Rhodomonas* cultures and Renate Schütt for species determination of the settlement panels. The authors thank three anonymous reviewers for their constructive comments on the earlier versions of this manuscript. This study was funded by the DFG Excellence Cluster 'Future Ocean' and the German 'Biological impacts of ocean acidification (BIOACID)' project 3.1.3, funded by the Federal Ministry of Education and Research (BMBF, FKZ 03F0608A).

Statement of authorship: J. T. and F. M. designed the study, J. T., I. C. and C. P. collected the data, J. T., I. C., A. K., and F. M. analysed the data, J. T. and F. M. wrote the manuscript and all authors contributed substantially to revisions.

References

- Babener B (1991) Increasing oxygen deficiency in Kiel Bay (Western Baltic). *Marine Research*, **33**, 121–140.
- Beniash E, Ivanina A, Lieb N, Kurochkin I, Sokolova I, IM (2010) Elevated level of carbon dioxide affects metabolism and shell formation in oysters *Crassostrea virginica*. *Marine Ecology-Progress Series*, **419**, 95–108.
- Boje R (1965) Die Bedeutung von Nahrungsfaktoren für das Wachstum von *Mytilus edulis* L. in der Kieler Förde und im Nord-Ostsee Kanal. *Kieler Meeresforschung*, **21**, 81–100.
- Booth CE, McDonald DG, Walsh PJ (1984) Acid-base balance in the sea mussel, *Mytilus edulis*. I. Effects of hypoxia and air-exposure on hemolymph acid-base status. *Marine Biology Letters*, **5**, 347–358.
- Borges AV, Gypens N (2010) Carbonate chemistry in the coastal zone responds more strongly to eutrophication than to ocean acidification. *Limnology & Oceanography*, **55**, 346–353.
- Brey T, Rumohr H, Ankar S (1988) Energy content of macrobenthic invertebrates: general conversion factors from weight to energy. *Journal of Experimental Marine Biology and Ecology*, **117**, 271–278.
- Cai WJ, Hu X, Huang WJ *et al.* (2011) Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience*, **4**, 1–5.
- Cao L, Caldeira K (2008) Atmospheric CO₂ stabilization and ocean acidification. *Geophysical Research Letters*, **35**, L19609.
- Deigweier K, Hirse T, Bock C *et al.* (2009) Hypercapnia induced shifts in gill energy budgets of Antarctic notothenioids. *Journal of Comparative Physiology B*, **180**, 347–359.
- Dickson AG (1990) Standard potential of the reaction - AgCl(s) + 1/2 H₂ = Ag(s) + HCl (Aq) and the standard acidity constant of the ion HSO₄⁻ in synthetic sea-water from 273.15 to 318.15-K. *Journal of Chemical Thermodynamics*, **22**, 113–127.
- Dickson AG, Afghani JD, Anderson GC (2003) Reference materials for oceanic CO₂ analysis: a method for the certification of total alkalinity. *Marine Chemistry*, **80**, 185–197.
- Dickson AG, Sabine CL, Christian JR (2007) *Guide to Best Practices for Ocean CO₂ Measurements (Vol. 3, p. 191)*. PICES Special Publications, ????????
- Fabricius KE, Langdon C, Uthicke S *et al.* (2011) Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nature Climate Change*, **1**, 165–169.
- Feely RA, Alin SR, Newton J *et al.* (2010) The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. *Estuarine, Coastal and Shelf Science*, **88**, 442–449.
- Gazeau F, Gattuso JP, Dawber C *et al.* (2010) Effect of ocean acidification on the early life stages of the blue mussel *Mytilus edulis*. *Biogeosciences*, **7**, 2051–2060.
- Hall-Spencer JM, Rodolfo-Metalpa R, Martin S *et al.* (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature*, **454**, 96–99.
- Haynert K, Schönfeld J, Riebesell U, Polovodova I (2011) Biometry and dissolution features of the benthic foraminifer *Ammonia aomoriensis* at high pCO₂. *Marine Ecology-Progress Series*, **432**, 53–67.
- Haynert K, Schönfeld J, Polovodova-Asteman I, Thomsen J (2012) The benthic foraminiferal community in a naturally CO₂-rich coastal habitat of the southwestern Baltic Sea. *Biogeosciences*, **9**, 4421–4440.
- Heinemann A, Fietzke J, Melzner F, Böhm F, Thomsen J, Garbe-Schönberg D, Eisenhauer A (2012) Conditions in *Mytilus edulis* extracellular body fluids and shell composition in a pH-treatment experiment: acid-base status, trace elements and δ¹¹B. *Geochemistry Geophysics Geosystems*, **13**, Q01005.
- HELCOM (2009) Eutrophication in the Baltic Sea – an integrated thematic assessment of the effects of nutrient enrichment and eutrophication in the Baltic Sea region. *Baltic Sea Environmental Proceedings*, **115B**, ???–???
- Hofmann GE, Smith JE, Johnson KS *et al.* (2011) High-frequency dynamics of ocean pH: a multi-ecosystem comparison. *PLoS ONE*, **6**, e28983.
- Jacob DE, Soldati AL, Wirth R *et al.* (2008) Nanostructure, composition and mechanisms of bivalve shell growth. *Geochimica et Cosmochimica Acta*, **72**, 5401–5415.
- Jørgensen C (1976) Growth efficiencies and factors controlling size in some mytilid bivalves, especially *Mytilus edulis* L.: review and interpretation. *Ophelia*, **15**, 175–192.
- Kurihara H, Asai T, Kato S, Ishimatsu A (2008) Effects of elevated pCO₂ on early development in the mussel *Mytilus galloprovincialis*. *Aquatic Biology*, **4**, 225–233.
- Lewis E, Wallace DWR (1998) *Program Developed for CO₂ System Calculations*. Oak Ridge National Laboratory ORNL/CDIAC-105, Oak Ridge.
- Lischka S, Büdenbender J, Boxhammer T, Riebesell U (2011) Impact of ocean acidification and elevated temperatures on early juveniles of the polar shelled pteropod *Limacina helicina*: mortality, shell degradation, and shell growth. *Biogeosciences*, **8**, 919–932.
- Michaelidis B, Ouzounis C, Palaras A, Pörtner HO (2005) Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*. *Marine Ecology-Progress Series*, **293**, 109–118.
- Palmer AR (1992) Calcification in marine mollusks – how costly is it. *Proceedings of the National Academy of Science USA*, **89**, 1379–1382.
- Pansch C, Nasrolahi A, Appelhans YS, Wahl M (2012a) Impacts of ocean warming and acidification on the larval development of the barnacle *Amphibalanus improvisus*. *Journal of Experimental Marine Biology and Ecology*, **420–421**, 48–55.
- Pansch C, Nasrolahi A, Appelhans YS, Wahl M (2012b) Tolerance of juvenile barnacles (*Amphibalanus improvisus*) to warming and elevated pCO₂. *Marine Biology*, ???, ???–???, in press. doi: 10.1007/s00227-012-2069-4
- Parker LM, Ross PL, O'Connor WA, Borysko L, Raftos DA, Pörtner HO (2012) Adult exposure influences offspring response to ocean acidification. *Global Change Biology*, **18**, 82–92.
- Ries JB, Cohen AL, McCorkle DM (2009) Marine calcifiers exhibit mixed responses to CO₂-induced ocean acidification. *Geology*, **37**, 1131–1134.
- Riisgard HU, Randløv A (1981) Energy budgets, growth and filtration rates in *Mytilus edulis* at different algal concentrations. *Marine Biology*, **61**, 227–234.
- Rodolfo-Metalpa R, Houlbrèque F, Tambutté É *et al.* (2011) Coral and mollusc resistance to ocean acidification adversely affected by warming. *Nature Climate Change*, **1**, 308–312.
- Roy R, Roy L, Vogel K *et al.* (1993) The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0 to 45°C. *Marine Chemistry*, **44**, 249–267.
- Salonen K, Sarvala J, Hakala I, Viljanen M (1976) The relation of energy and organic carbon in aquatic invertebrates. *Limnology & Oceanography*, **21**, 724–730.
- Smetacek V (1985) The annual cycle of Kiel Bight plankton: a long-term analysis. *Estuaries*, **8**, 145–157.
- Stuckas H, Stoof K, Quesada H, Tiedemann R (2009) Evolutionary implications of discordant clines across the Baltic *Mytilus edulis* hybrid zone (*Mytilus edulis* and *Mytilus trossulus*). *Heredity*, **103**, 1–11.
- Stumpff M, Wren J, Melzner F, Thorndyke MC, Dupont S (2011) CO₂ induced seawater acidification impacts sea urchin larval development I: Elevated metabolic rates decrease scope for growth and induce developmental delay. *Comparative Biochemical Physiology A*, **160**, 331–340.
- Sunday JM, Crim RN, Harley CDG, Hart MW (2011) Quantifying rates of evolutionary adaptation in response to ocean acidification. *PLoS ONE*, **6**, e22881.
- Talmage SC, Gobler CJ (2010) Effects of past, present, and future ocean carbon dioxide concentrations on the growth and survival of larval shellfish. *Proceedings of the National Academy of Science USA*, **107**, 17246–17251.
- Thomsen J, Melzner F (2010) Moderate seawater acidification does not elicit long-term metabolic depression in the blue mussel *Mytilus edulis*. *Marine Biology*, **157**, 2667–2676.
- Thomsen J, Gutowska MA, Saphörster J *et al.* (2010) Calcifying invertebrates succeed in a naturally CO₂ enriched coastal habitat but are threatened by high levels of future acidification. *Biogeosciences*, **7**, 3879–3891.
- Weiner S, Addadi L (2011) Crystallization pathways in biomineralization. *Annual Review of Materials Research*, **41**, 21–40.
- Weiss RF (1974) Carbon dioxide in water and seawater: the solubility of a non-ideal gas. *Marine Chemistry*, **2**, 203–215.
- Wootton JT, Pfister CA, Forester JD (2008) Dynamic patterns and ecological impacts of declining ocean pH in a high-resolution multi-year dataset. *Proceedings of the National Academy of Science USA*, **105**, 18848–18853.
- Zange J, Grieshaber MK, Jans AW (1990) The regulation of intracellular pH estimated by ³¹P-NMR spectroscopy in the anterior byssus retractor muscle of *Mytilus edulis* L. *Journal of Experimental Biology*, **150**, 95–109.