Sour times: seawater acidification effects on growth, feeding behaviour and acid–base status of *Asterias rubens* and *Carcinus maenas*

Yasmin S. Appelhans*, Jörn Thomsen, Christian Pansch, Frank Melzner, Martin Wahl

Marine Ecology, Helmholtz Centre for Ocean Research (GEOMAR), Düsternbrooker Weg 20, Kiel 24105, Germany

ABSTRACT: The impact of seawater acidification on calcifying organisms varies at the species level. If the impact differs between predator and prey in strength and/or sign, trophic interactions may be altered. In the present study, we investigated the impact of 3 different seawater pCO_2 levels (650, 1250 and 3500 µatm) on the acid–base status or the growth of 2 predatory species, the common sea star *Asterias rubens* and the shore crab *Carcinus maenas*, and tested whether the quantity or size of prey consumed is affected. We exposed both the predators and their prey, the blue mussel *Mytilus edulis*, over a time span of 10 wk and subsequently performed feeding experiments. Intermediate acidification levels had no significant effect on growth or consumption in either predator species. The highest acidification level reduced feeding and growth rates in sea stars by 56 %, while in crabs a 41 % decrease in consumption rates of mussels could be demonstrated over the 10 wk experimental period but not in the subsequent shorter feeding assays. Because only a few crabs moulted in the experiment, acidification effects on crab growth could not be investigated. Active extracellular pH compensation by means of bicarbonate accumulation was observed in *C. maenas*, whereas the coelomic fluid pH in *A. rubens* remained uncompensated. Acidification did not provoke a measurable shift in prey size preferred by either predator. Mussels exposed to elevated pCO_2 were preferred by previously untreated *A. rubens* but not by *C. maenas*. The observed effects on species interactions were weak even at the high acidification levels expected in the future in marginal marine habitats such as the Baltic Sea. Our results indicate that when stress effects are similar (and weak) on interacting species, biotic interactions may remain unaffected.

KEY WORDS: Acidification · pH · CO_2 · Interactions · Predation · Sea star · Crab · Mussel

INTRODUCTION

The brackish western Baltic Sea is a relatively species-poor environment. Two of the main benthic predators in the western Baltic are the common sea star *Asterias rubens* and the shore crab *Carcinus maenas*. A large proportion of their prey consists of the highly abundant blue mussel *Mytilus edulis*. Regionally, the bivalve can cover 95 to 100 % of all suitable substrate, especially if predators are absent

(e.g. Kautsky & van der Maarel 1990, Reusch & Chapman 1997). Adult crabs and sea stars, however, consume mussels only to the threshold size of ~48 mm shell length, beyond which size refuge is obtained (Sommer et al. 1999, Enderlein et al. 2003, Laudien & Wahl 2004). The protective effect could be due to large size, strong adductor muscles and/or more robust shells. Any factor prolonging the time that a mussel spends in sub-threshold size properties diminishes its chances for survival. Reduced growth

can lead to shifts in trophic interactions with increasing overall predation impact on the mussel population (e.g. Enderlein & Wahl 2004). In addition, when changing environmental conditions affect the feeding rates and/or prey size range of sea stars and crabs, shifts in trophic interactions with community-wide consequences may be expected.

The predicted ocean acidification (IPCC 2007) is one of the environmental shifts with this potential. Seawater acidification at a global and long-term scale is caused by the anthropogenic increase of atmospheric CO_2, which, at smaller spatial and temporal scales, may be overridden by biological and biogeochemical processes (Thomsen et al. 2010). Until the end of the century, oceanic pH decreases of 0.3 to 0.4 units (equivalent to atmospheric CO_2 levels of 650 µatm and above) can be expected (Feely et al. 2004, Caldeira & Wickett 2005). Due to the unique structure of the Baltic Sea with its generally low salinity but strong stratification and pronounced oxygen minimum zones occurring in the summer below the pycnocline, pCO_2 levels here can be naturally high, especially in benthic regions. The surface water pCO_2 in this region occasionally exceeds values of 2300 µatm and is thus higher than expected for the open ocean within the next 300 yr (Thomsen et al. 2010). Recent model calculations indicate that with increasing atmospheric CO_2 levels, Baltic surface pCO_2 will rise overproportionally. Thus, with a doubling of atmospheric concentrations from currently 385 to future 761 µatm, surface water pCO_2 is expected to seasonally reach peak values of >4000 µatm in the study area (Thomsen et al. 2010). Moreover, the pH in this region is expected to fluctuate more than under open ocean conditions because CO_2 effects are not buffered as strongly, mainly due to the lower concentration of HCO_3^- and thus lower total alkalinity (A_T) (Beldowski et al. 2010). These properties (low salinity and thus low A_T) are found in many estuarine and coastal systems. Research to date has focused mainly on pelagic open ocean conditions and may be of little relevance to benthic organisms (cf. Beldowski et al. 2010).

It has traditionally been assumed that seawater acidification will most severely affect calcifying organisms (e.g. Fabry 2008). In fact, several recent studies have shown that acidified conditions may affect (in one direction or other) early development (Kurihara et al. 2004, 2007, Kurihara & Shirayama 2004, Dupont et al. 2008, Ellis et al. 2009), growth and calcification (Michaelidis et al. 2005, Gazeau et al. 2007, Kurihara et al. 2008, Wood et al. 2008, Gooding et al. 2009, Thomsen et al. 2010) and diverse physiological traits (Michaelidis et al. 2005, Metzger et al. 2007, Miles et al. 2007, Thomsen et al. 2010, Thomsen & Melzner 2010) of calcifying echinoderms, crustaceans and bivalves.

The strength of acidification impacts on calcifiers varies at the species level (Hall-Spencer et al. 2008, Melzner et al. 2009, Gutowska et al. 2010, Hendriks et al. 2010, Kroeker et al. 2010, Whiteley 2011). This variation is due in part to variable combinations of calcite, aragonite and organic matrix in the respective skeletons. While the exoskeleton of crustaceans consists solely of relatively stable calcite, the shell of mussels, such as *Mytilus edulis*, is composed partly of calcite and partly of the more soluble aragonite. The skeleton of echinoderms consists of Mg-calcite, a form of carbonate even more soluble than aragonite (Andersson et al. 2008). Moreover, as suggested by Melzner et al. (2009), highly active organisms, such as crabs, due to their high ion-regulatory and extracellular fluid buffering capacity, may be less sensitive to seawater acidification than less active groups (e.g. sea stars and mussels). Also, species from groups that compensate their extracellular fluid pH by active accumulation of HCO_3^-, such as cephalopods (Gutowska et al. 2008) or teleost fish (Checkley et al. 2009), hypercalcify when being chronically exposed to acidified seawater. This response is thought to be mostly due to the excess amount of accumulated HCO_3^-, which could lead to an increase in calcification via a change in ionic driving forces across calcifying epithelia (Gutowska et al. 2010). Finally, the amount of organic material covering the outer skeleton layer may influence the sensitivity towards acidification in the different groups (e.g. Tunnicliffe et al. 2009).

The feeding modes of the 2 predator species differ. *Carcinus maenas* crushes the shells to consume the flesh, whereas *Asterias rubens* slowly pulls the shells apart and digests the soft body in its everted stomach. With increasing shell size, mussel consumption is reduced, by crabs due to handleability and a larger stability of the molluscan shell, by starfish by an increased strength of the muscle adductor muscle, i.e. the amount of myofibrils in smooth muscles and energy available to prolong the catch phase (e.g. Twarog 1967), during which mussels keep their shells closed with minimal energy investment. The different modes of feeding of the 2 predator species may be differently affected by acidification. A study of the brittlestar *Amphiura filiformis* by Wood et al. (2008) proposed that not only carbonate structures but also the quantity of muscle tissue might be influenced by acidification. If the amount of smooth mus-

cle fibres in the adductor muscles of bivalves is reduced, or if for any reason the catch mechanism is disturbed, sea star feeding should be facilitated— unless their muscles, too, are weakened. If the calcification of mussel shells is reduced and shells become less stable, crabs may be able to consume a larger quantity and larger sizes of mussels per unit time, again unless they themselves are negatively affected by acidification.

The aim of the present study was to test whether seawater acidification affects the susceptibility of *Mytilus edulis* to predation by *Asterias rubens* and *Carcinus maenas*, the main controlling factors of this mussel species in the western Baltic. We attempted to differentiate between the influence of acidification on predators and on prey. We hypothesised that (1) if interacting species are differently affected by acidification, their trophic interaction should shift, and (2) acidification on the consumer side should affect sea stars (noncompensating) more than crabs (compensating). To date, very few studies have focused on the predator–prey interaction between organisms under acidified conditions (Bibby et al. 2007, Gooding et al. 2009), although such indirect effects of stress may be substantially stronger than their direct impact (Wahl 2008).

MATERIALS AND METHODS

Sampling

All of the organisms investigated were sampled in the Kiel Fjord (western Baltic Sea) via dredging or manual collection in April 2009. The size spectra of selected test animals at the start of the experiment were as follows: *Asterias rubens* 15.5 ± 3.5 g wet wt and 4.5 ± 0.5 cm arm length; *Carcinus maenas* 4.5 ± 0.5 cm carapace width; *Mytilus edulis* (3 classes) 2.00 ± 0.15 cm, 3.00 ± 0.15 cm and 4.00 ± 0.15 cm shell length (SL).

Experimental design

The experimental units (EUs) consisted of 2 l plastic aquaria connected to a flow-through system of seawater from Kiel Fjord. The water was pre-treated in 3 interconnected header-tanks via bubbling with pressurized air into which different concentrations of CO_2 (ambient conditions, 1120 µatm and 4000 µatm) were mixed. Water from the header-tanks then streamed into the EUs at a flow rate of 5 to 6 l h^{-1} and was additionally bubbled with the above-mentioned CO_2 and air mixtures. In this way, we established 3 different treatment levels (Table 1). Due to the naturally acidified conditions of water in the Kiel Fjord (see above), the lowest treatment levels (with a mean of 650 µatm, see Table 1) were substantially higher than the currently ~380 µatm CO_2 prevailing in the atmosphere.

To each single EU, we added either one crab, one sea star or 20 mussels of all 3 size classes (60 mussels in total per EU). The 3 treatment levels were replicated 10 times for each prey or consumer species, resulting in a setup of 90 EUs. In each EU containing mussels, 3 random mussels of each size class were individually marked to allow for growth monitoring. *Asterias rubens* were fed ad libitum with mussels of 3.50 ± 0.35 cm SL, and *Carcinus maenas* were fed ad libitum with mussels of 2.50 ± 0.35 cm SL. The mussels were fed with 10 ml of suspended plankton food (DT's Live Marine Phytoplankton Premium Blend) per EU 3 times a week. During feeding, the flow-through of the mussel EUs was stopped for 2 h. The experimental period lasted for 10 wk.

The carbonate system was determined via the coulometric analysis of total inorganic carbon (C_T; SOMMA System autoanalyser) and A_T (potentiometric titration, VINDTA autoanalyser) at the beginning, intermediate phase and end of the experiment in each 3 representative EUs per respective treatment level and species (9 per measurement day). Calculations of the carbonate system speciation were performed using the

Table 1. Water carbonate system for the different treatment levels. Water pCO$_2$, $\Omega_{calcite}$ and $\Omega_{aragonite}$ were calculated using measured C_T and A_T values as well as temperature and salinity values of the respective experimental units on the day of measuring with the CO2SYS macro for low salinities (Pierrot et al. 2006). Values are means ± SE. A_T: total alkalinity, C_T: total inorganic carbon, Ω: saturation state, SW: seawater

| pCO$_2$ of pressurized air (µatm)	C_T (µmol kgSW^{-1})	A_T (µmol kgSW^{-1})	pH (NBS scale)	Temp. (°C)	Salinity	pCO$_2$ (µatm)	$\Omega_{calcite}$	$\Omega_{aragonite}$
		Measurements					Calculations	
Ambient (>387)	1999.2 ± 18.0	2046.4 ± 18.6	8.06 ± 0.005	12.9 ± 0.07	14.8 ± 0.13	654.2 ± 54.0	1.64 ± 0.17	0.96 ± 0.10
1120	2076.2 ± 20.3	2051.60 ± 20.7	7.84 ± 0.006	12.9 ± 0.07	14.8 ± 0.13	1245.7 ± 85.8	0.91 ± 0.10	0.53 ± 0.06
4000	2208.8 ± 24.0	2060.54 ± 19.5	7.36 ± 0.008	12.9 ± 0.07	14.8 ± 0.13	3466.9 ± 155.6	0.34 ± 0.03	0.20 ± 0.02
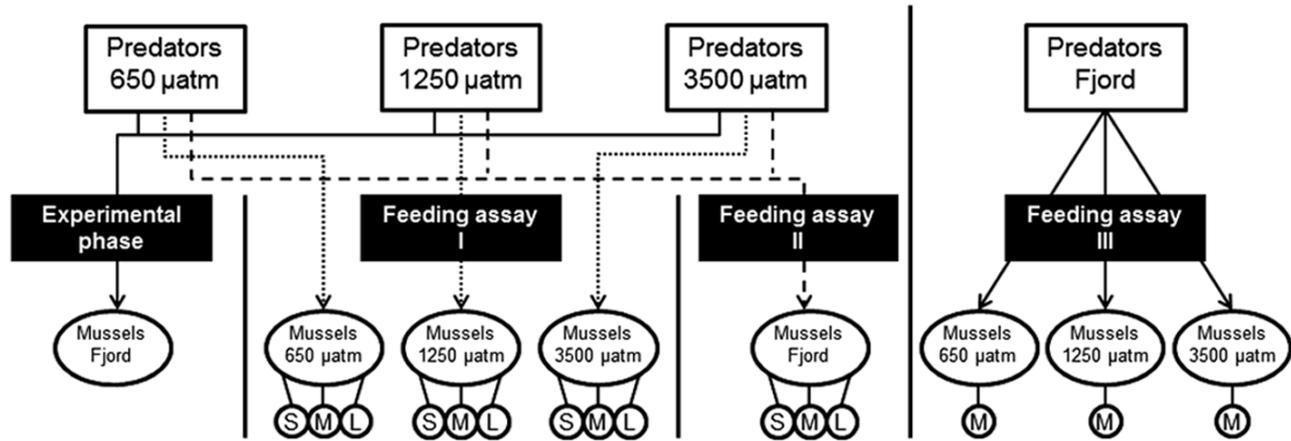
Fig. 1. Schematic overview of the experimental setup. After the experimental phase, *Asterias rubens* and *Carcinus maenas* were fed with *Mytilus edulis* classified into 3 sizes—S: 2.00 ± 0.15 cm shell length (SL); M: 3.00 ± 0.15 cm SL; L: 4.00 ± 0.15 cm SL. During the experimental period, *A. rubens* and *C. maenas* were fed with mussels of 3.50 ± 0.35 cm SL, and 2.50 ± 0.35 cm SL, respectively

CO2SYS macro for low salinities (modified by Kört-zinger after Pierrot et al. 2006). The dissociation constants K_1 and K_2 were chosen according to Roy et al. (1993). Additionally, the pH and salinity in the EUs was measured weekly with a hand-held pH meter (WTW 340i pH-analyser, WTW SenTix 81 measuring chain, using the NBS scale) and salinometer (WTW cond 315i, WTW TETRACON 325 measuring chain). The concentration of NH_4^+ was determined in each of the 3 EUs per pCO_2 level at the end of the experimental period using a JBL NH_4^+ aquarium kit.

Growth

Biomass increase (wet wt) of the sea stars was quantified weekly, weighing each individual 3 times after gently blotting it dry with a paper towel. Mussel growth (increase in shell length) was documented as the shell length increase between the beginning and end of the experimental period. Crab growth could not be monitored because only 1 crab moulted during the experimental period.

Feeding assays

Consumption of mussels by sea stars and crabs during the experimental period was monitored weekly. After the 10 wk of treatment at different pCO_2 levels, we performed 3 different feeding assays (FA I, II and III; see Fig. 1 for an overview). In FA I, predators were starved for 1 d before being offered 3 mussel individuals from each of the 3 different size classes (i.e. 9 mussels) simultaneously, with consumers and prey stemming from identical experimental conditions. The number and size class of mussels consumed was monitored daily over 6 d.

The predators were then again starved for 1 d, after which FA II was performed, which was identical to FA I, except that the prey were not pre-treated but collected from Kiel Fjord the day before. These mussels served as external references to assess which proportion of the effects observed in Assay I were due to the predators rather than the prey. Consumption in FA II was monitored daily over 6 d, as before.

In FA III, consumers collected from Kiel Fjord (10 sea stars and 10 crabs starved for 3 d) were offered 3 prey individuals of the medium size class (3.00 ± 0.15 cm) from each of the 3 treatment levels (i.e. 9 mussels). In FA III, the predators served as external references to estimate which proportion of the effects observed in FA I were attributable to the treated mussels. Monitoring of FA III took place over 6 d as well, but only the data obtained during Day 1 were analysed, when one of the predators had consumed all 3 mussels of one treatment level.

All of the FAs took place under the respective CO_2 conditions to which the predators had been previously exposed.

Acid–base status

Following the FAs, the extracellular acid–base status of the consumer species was assessed to deter-

mine the regulative capacities of the species. *Asterias rubens* was starved for 7 d, then the tip of an arm was cut, and the coelomic fluid was collected in a cap. Crabs were starved for 3 d, then haemolymph was drawn bubble-free with a syringe from the infra-branchial sinus without contact to air. The pH was measured using a microelectrode (WTW Mic-D and WTW pH 340i), and C_T was measured using a CO_2 analyser (Corning 965). The pCO_2 and HCO_3^- concentrations of the body fluids were calculated from the measured pH and C_T with the rearranged forms of the Henderson-Hasselbalch equation:

$$pCO_2 = C_T \times (10^{pH-pK_1} \times \alpha_{CO_2} + \alpha_{CO_2})^{-1} \quad (1)$$

$$[HCO_3^-] = 10^{pH-pK_1} \times \alpha_{CO_2} \times pCO_2 \quad (2)$$

For *Carcinus maenas*, the α_{CO_2} (CO_2 solubility) and pK_1 (the first apparent dissociation constant) were calculated from Truchot (1986) for ambient salinity and temperature. The α_{CO_2} summed to 0.000375 Pa, and the pK_1 summed to 6.065. In *Asterias rubens*, α_{CO_2} was calculated from Weiss (1974). The pK_1 was calculated from a linear correlation of pK and pH (pK = −0.1331pH + 7.2481, r² = 0.3047) determined *in vitro* for coelomic fluid at a given experimental salinity and temperature. For this purpose, 600 µl samples of body fluid pooled from 10 animals were equilibrated with known pCO_2 levels of 560, 1400, 4000 and 6000 µatm for 1 h. Afterwards, the pH and C_T of the samples were determined as described above. pK_1 was calculated using the equation:

$$pK_1 = pH - \log\left(\frac{C_T}{pCO_2 \times \alpha_{CO_2}} - 1\right) \quad (3)$$

The α_{CO_2} used for calculation was 0.0003888 Pa, and the pK_1 levels were 6.18 ± 0.01 for the 650 µatm level, 6.19 ± 0.01 for 1250 µatm and 6.21 ± 0.01 for 3500 µatm.

The non-bicarbonate buffer line was determined *in vitro* by correlation of the measured pH and calculated HCO_3^- concentration in equilibrated body fluid samples.

Mussel properties

The mussels not used in the FAs were frozen at −20°C after the experimental period. Ten mussels of each size class and treatment level were later defrosted to determine the dry soft-tissue weight of the mussels at the respective treatment level and to correlate that weight to consumption rates. The others were used to determine adductor muscle dry wt, shell weight and maximum breaking resistance of shells. Pilot assays had shown that a freezing/thawing treatment did not cause detectable differences in shell stability.

To determine the adductor muscle dry wt, shell weight and maximum breaking resistance of shells, 3 mussels of each size class per EU were defrosted and opened with a scalpel. The adductor muscles were dissected, dried for 24 h at 80°C until a constant weight was reached and weighed individually. The shells were dried with a paper towel, left to surface-dry at room temperature for 2 h and then weighed individually. The breaking resistance was determined with a TAXT2i texture analyser (25-1 measuring cell, Stable Micro Systems), using a cylinder of 2 mm diameter and a speed of 1.00 mm s⁻¹, measuring the maximum force necessary for breaking the shell. For these measurements, the shells were positioned opening downwards, with the rim of the shell on a neoprene sheet to ensure homogeneous distribution of forces and the cylinder position above the highest point of the shell. Both the left and the right shell half were broken, and the higher value of the 2 was used for analysis.

Statistical analyses

The total mussel consumption of the predators during the experimental time, the total mussel consumption in FAs I and II, the growth of all 3 species and the adductor muscle weight, shell weight and maximal breaking resistance of the mussels were analysed using a 1-way ANOVA, followed by Tukey′s HSD post-hoc analysis. The size range of the mussels consumed in FAs I and II was compared using a repeated-measures ANOVA (following Yun et al. 2007). Data were tested for normality using the Shapiro-Wilks *W*-test before further statistical analysis. If the data were non-normally distributed, the Box-Cox procedure was used to identify the simplest transformation to achieve normality. Percentage data were arcsine transformed. Data were tested for homogeneity of variances using Levene′s test. If normality and homoscedasticity could not be achieved, we used parametric tests but lowered the α level to 0.01 (following Wakefield & Murray 1998). The data of FA III were analysed using resampling and a Monte Carlo simulation with 100 permutations (compare e.g. Rohde et al. 2004). Data of coelomic fluid and haemolymph acid−base status were plotted in pH-bicarbonate (Davenport) diagrams.

RESULTS

Experimental conditions

The mean ($\pm$SE) pCO_2 in the EUs at the 3 treatment levels was 654.2 $\pm$ 54.0, 1245.7 $\pm$ 85.8 and 3466.9 $\pm$ 155.6 at 650, 1250 and 3500 μatm (equivalent to 66, 127 and 356 Pa), respectively, during the course of the experimental period (Table 1). Seawater $[NH_4^+]$ was below 0.2 mg l^{-1} in all cases.

None of the sea stars died during the experiment. One crab per treatment level died during the 10 wk experimental period. One crab at the highest treatment level moulted 9 wk into the experiment. Four (650 μatm), 3 (1250 μatm) and 5 (3500 μatm) of the 6 female crabs per treatment level spawned in Week 4 or 5 of the experimental period.

Growth

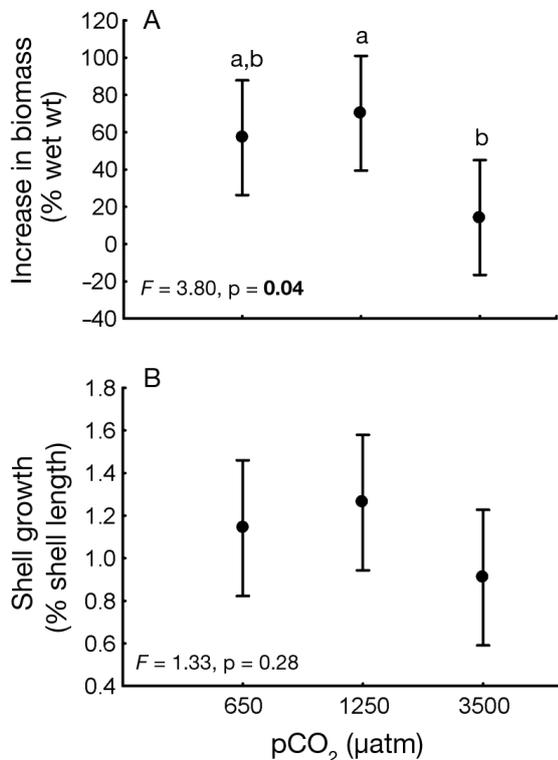Growth of *Asterias rubens* over the 10 wk experimental phase was significantly lower at the

Fig. 2. (A) *Asterias rubens* and (B) *Mytilus edulis*. Mean growth during the 10 wk experimental phase at different treatment levels. Vertical bars denote $\pm$95% CI. Results from 1-way ANOVAs followed by post-hoc testing (Tukey's HSD). Groups with different letters in (A) are significantly different at p $\leq$ 0.05 (**bold**)

high (3500 μatm) than at the intermediate pCO_2 (1250 μatm) concentrations. Growth did not differ between the 650 and 1250 and between the 650 and 3500 μatm treatments (Fig. 2A, Table 2). Growth of *Mytilus edulis* (Fig. 2B, Table 2) was generally very low and not significantly affected by acidification. Crab growth was not assessed.

Consumption

Feeding behaviour of *Asterias rubens* during the experimental phase differed significantly between the pCO_2 treatment levels, with highest consumption at the intermediate level (1250 μatm) and lowest consumption at the highest (3500 μatm) treatment level (Fig. 3A, Table 2). The same pattern was found in the total consumption by sea stars in FA I, in which pre-treated mussels were fed to the pre-treated sea stars (Fig. 3B, Table 2), as in FA II, in which untreated mussels were offered to the pre-treated *A. rubens* (Fig. 3C, Table 2). The prey-size preference exhibited by sea stars was not significantly affected by acidification in both FA I (Fig. 4A, Table 2) and FA II (Fig. 4B, Table 2). In FA III, in which pre-treated mussels were offered to untreated sea stars, significantly more mussels treated at 3500 μatm were consumed within 24 h than mussels pre-treated with lower pCO_2 levels (Fig. 3D).

In contrast to the sea stars, significant acidification effects on *Carcinus maenas* feeding were detected in the experimental phase only (Fig. 5A, Table 2). Here, consumption was significantly lower at 3500 μatm than at the other levels. Acidification had no measurable effect on the trophic interaction between identically pre-treated mussels and crabs (FA I, Fig. 5B, Table 2), on the feeding behaviour of crabs (FA II, Fig. 5C, Table 2) or on the susceptibility of mussels to crab predation (FA III, Fig. 5D). Similar to the sea stars, the differently treated crabs did not differ in prey size preference in FA I (Fig. 6A, Table 2) or II (Fig. 6B, Table 2).

Acid–base status

The coelomic fluid pH (pHe) of *Asterias rubens* decreased with increasing seawater pCO_2 along the non-bicarbonate buffer line (Fig. 7A, Table 2). We did not observe extracellular HCO_3 accumulation deviating from solely passive increments, as presented in the pH-bicarbonate (Davenport) diagram. In contrast, the haemolymph pH of *Carcinus maenas* was maintained at control pH levels of 7.82, indepen-

Table 2. ANOVA with different response variables and the dependent factor pCO$_2$ measured during the acclimatisation phase and the subsequent feeding assays (FA) and 3 different pCO$_2$ treatments. For non-parametric data, the α level was adjusted to 0.01. Statistically significant results are **bold**. pHe: coelomic fluid pH

|	α	df	MS	F	p	Partial η^2
Asterias rubens						
Increase in biomass	0.05	2	8563.71	3.80	**0.04**	0.22
Consumption during experiment	0.05	2	375.23	6.37	**0.01**	0.32
FA I, total consumption	0.05	2	67340.00	4.13	**0.03**	0.23
FA II, total consumption	0.01	2	208276.00	4.95	**0.01**	0.27
FA I, consumption per mussel size class	0.01	4	1.47	1.98	0.11	0.13
FA II, consumption per mussel size class	0.01	4	0.34	0.68	0.61	0.05
pHe	0.01	2	0.20	40.67	**<0.01**	0.75
HCO$_3^-$e	0.01	2	0.07	3.08	0.06	0.19
Carcinus maenas						
Increase in biomass	0.01	2	786.81	0.85	0.44	0.07
Consumption during experiment	0.05	2	1911.26	3.93	**0.03**	0.25
FA I, total consumption	0.01	2	9505.00	0.31	0.73	0.03
FA II, total consumption	0.01	2	57996.00	0.42	0.66	0.03
FA I, consumption per mussel size class	0.01	4	1.04	2.38	0.06	0.17
FA II, consumption per mussel size class	0.01	4	0.73	1.33	0.27	0.10
pHe	0.01	2	<0.01	0.12	0.88	0.01
HCO$_3^-$e	0.01	2	34.00	5.67	**0.01**	0.35
Mytilus edulis						
Increase in shell length	0.01	2	0.32	1.33	0.28	0.09
Adductor muscle weight	0.01	2	<0.01	0.14	0.87	0.01
Shell weight	0.01	2	4.10	1.12	0.34	0.08
Breaking resistance	0.05	2	<0.01	6.70	**<0.01**	0.33
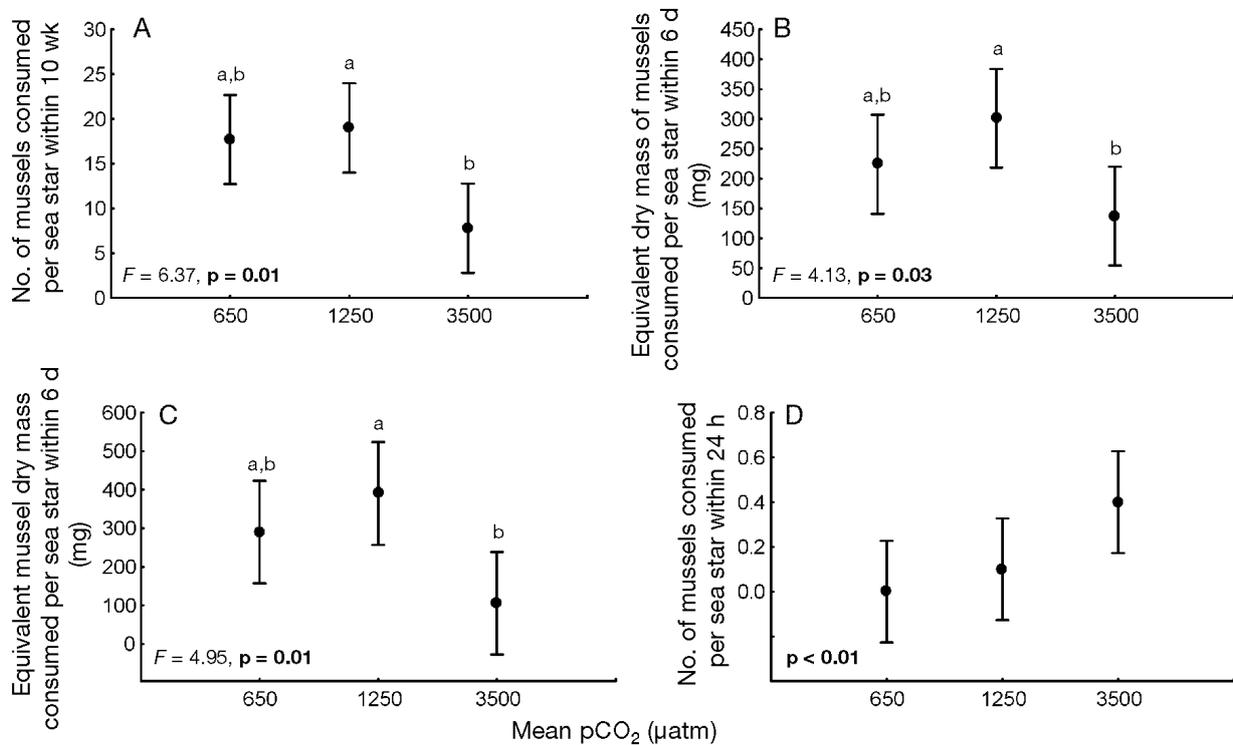
Fig. 3. *Asterias rubens*. Mean mussel consumption (A) during the 10 wk experimental period, (B) in feeding assay (FA) I (treated sea stars, treated mussels), (C) in FA II (treated sea stars, untreated mussels) and (D) in FA III (untreated sea stars, treated mussels) over treatment levels. Vertical bars denote ±95% CI. (A–C) Results from 1-way ANOVAs followed by post-hoc testing (Tukey's HSD). Groups with different letters are significantly different in (A,B) at p ≤ 0.05 or (C) p ≤ 0.01. (D) Results from re-sampling and Monte Carlo simulation. Statistically significant p values are **bold**
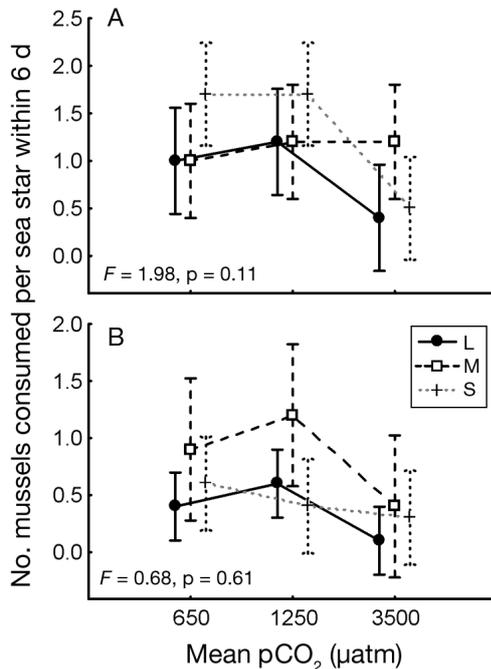
Fig. 4. *Asterias rubens*. Mean consumption of differently sized mussels in (A) feeding assay (FA) I (treated sea stars, treated mussels) and (B) FA II (treated sea stars, untreated mussels) over treatment levels. See Fig. 1 for mussel size classes. Vertical bars denote ±95% CI. Results from repeated-measures ANOVAs

dently of the pCO_2 treatment level, which resulted from active elevation of $[HCO_3^-]$ from 7.9 to 10.4 and 11.7 mM, in the 1250 and 3500 µatm CO_2 treatments, respectively (Fig. 7B, Table 2).

Mussel properties

The dry mass of the adductor muscles (Fig. 8A, Table 2) and the shell mass (Fig. 8B, Table 2) of *Mytilus edulis* did not significantly differ among the treatment levels, although a trend toward a lower shell mass with increasing seawater pCO_2 was observed. The mean maximum breaking resistance of mussel shells was significantly lowered by ~20% at the highest level of 3500 µatm (Fig. 8C, Table 2).

DISCUSSION

Acidification effects on species interactions were mostly weak even at the high acidification levels (expected for marginal marine habitats like the Baltic Sea) where there were direct effects on the prey (e.g. increasingly fragile shell), concurrent effects on the predators (e.g. lower consumption or slower growth)
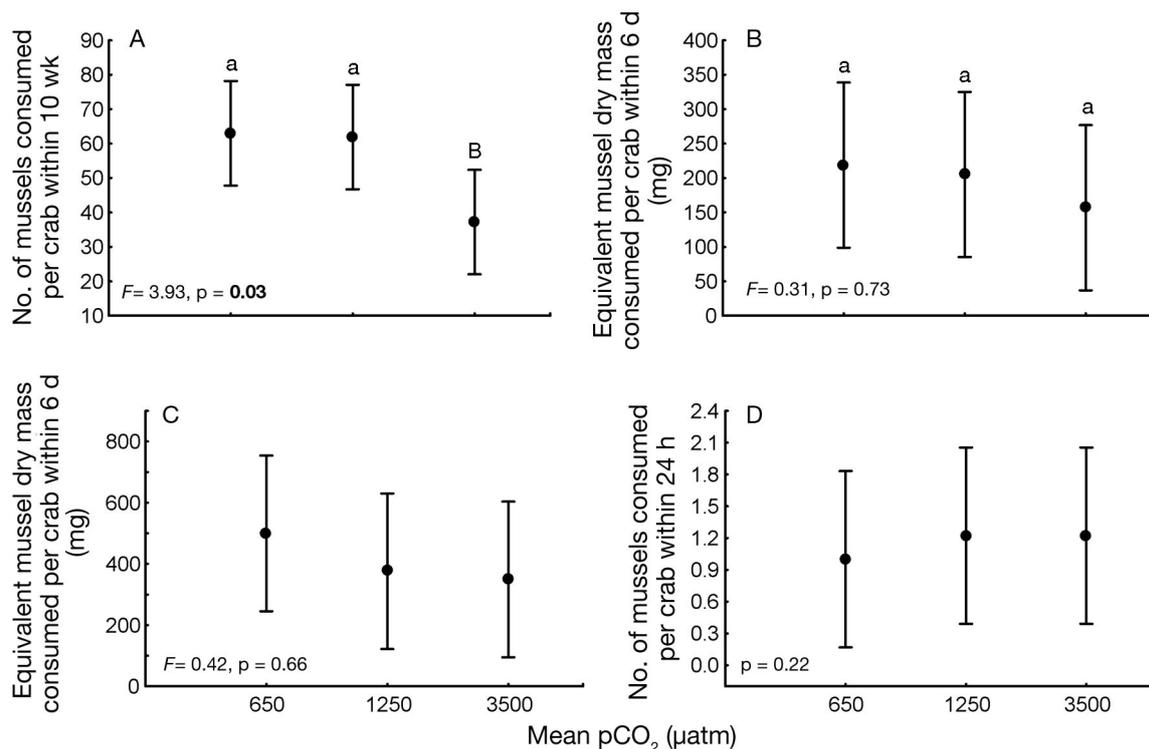

Fig. 5. *Carcinus maenas*. Mean mussel consumption (A) during the 10 wk experimental period, (B) in feeding assay (FA) I (treated crabs, treated mussels), (C) in FA II (treated crabs, untreated mussels) and (D) in FA III (untreated crabs, treated mussels) over treatment levels. Vertical bars denote ±95% CI. Results in (A–C) from 1-way ANOVAs followed by post-hoc testing (Tukey's HSD). Groups with different letters in (A) are significantly different at p ≤ 0.05 (**bold**). (D) Results from re-sampling and Monte Carlo simulation
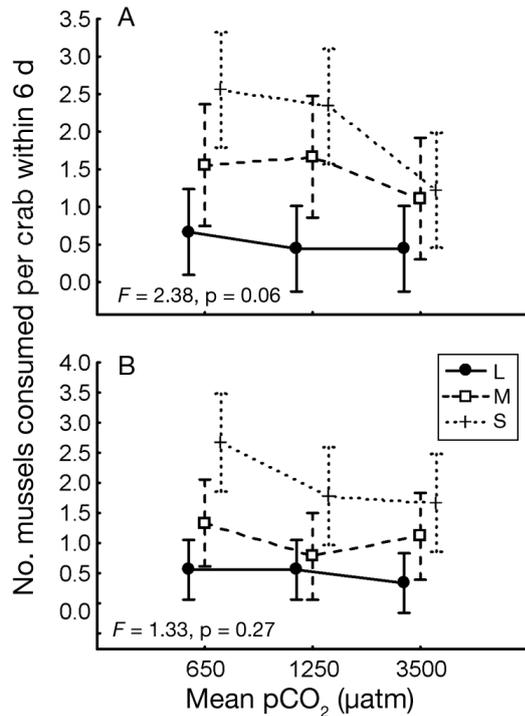
Fig. 6. *Carcinus maenas*. Mean consumption of differently sized mussels in (A) feeding assay I (treated crabs, treated mussels) and (B) feeding assay II (treated crabs, untreated mussels) over treatment levels. See Fig. 1 for mussel size classes. Vertical bars denote ±95% CI. Results from repeated-measures ANOVAs

tended to neutralize any shifts in trophic interactions.

Even though the seawater was undersaturated in aragonite in all treatment levels and in calcite in the 2 higher pCO_2 levels, both predator organisms were able to survive under acidified conditions. These observations are in line with recent acidification studies on crabs and echinoderms (Dupont & Thorndyke 2008, Wood et al. 2008, Gooding et al. 2009, Dupont et al. 2010a, 2010b, Whiteley 2011). Furthermore, no significant responses of feeding rate and/or growth were observed under moderate (1250 µatm) seawater acidification scenarios.

Asterias rubens

Feeding and growth of *Asterias rubens* were only negatively affected at the highest CO_2 level (3500 µatm), whereas intermediate acidification (1250 µatm) even seemed to slightly enhance growth and consumption. A natural pre-adaptation to acidified conditions could explain this pattern. Because the benthic habitat of sea stars, especially in eutrophic coastal zones, is often an area of high decomposition,

the CO_2 levels in these regions are often substantially higher than in the pelagic environment (Feely et al. 2008, Melzner et al. 2009, Thomsen et al. 2010). The mean pH at 10 m depth in Kiel Fjord was 7.7 (±0.2 SD, corresponding to a pCO_2 of ~1400 µatm) between May and October 2009 (Wahl et al. 2010) and was probably periodically lower at the sediment–water interface (see also Thomsen et al. 2010). Consistent with our findings, Gooding et al. (2009) report that the sea star *Pisaster ochraceus* grew significantly better and tended to consume more mussels under intermediate acidification levels (780 µatm), which, however, were closer to our natural 'low' than to our intermediate level. Also, larvae and juveniles of the sea star *Crossaster papposus* showed faster growth and development under a pH of 7.7 than under a pH of 8.1 (Dupont et al. 2010a). Our findings indicate that moderate acidification (up to at least 1250 µatm) does not stress *A. rubens* enough to significantly reduce feeding rates, although abiotic stress in general can lead to reduced feeding rates in asteroids. For example, strong osmotic stress in the sea star *Luidia clathrata* (abrupt salinity reduction from 28 to 17) has been shown to lead to depressed feeding rates over 30 d (Forcucci & Lawrence 1986).

Sea star tolerance to acidification was reduced at very high levels of acidification, as reflected in reduced growth and consumption rates. The underlying cause is not clear. However, recent studies have demonstrated that acidification can lead to energy budget re-allocations that result in a reduction of the scope for growth in larval and adult echinoderms (Stumpp et al. 2011). Excess energy might be required for the maintenance of intracellular pH when the extracellular pH is decreased (e.g. Michaelidis et al. 2005, Thomsen & Melzner 2010), thus limiting the amount of energy that can be invested into digestion, assimilation and growth. In addition, the low pHe could impact the energy available for calcification. However, energetic trade-offs are mostly relevant under energy-limited conditions, which we attempted to avoid in our experimental design. It is also possible that decreased feeding rates were connected to a reduced functionality of digestive enzymes, if stomach pH was affected by seawater acidification or if more energy had to be invested into stomach pH regulation (which we did not assess). The stomach pH of adult asteroids has been shown to be between 7.3 and 7.5, and an optimum of enzymatic activity was found at a similar pH (Irving 1926). The change in extracellular and environmental pH in our study might have caused a decrease in enzyme activity.
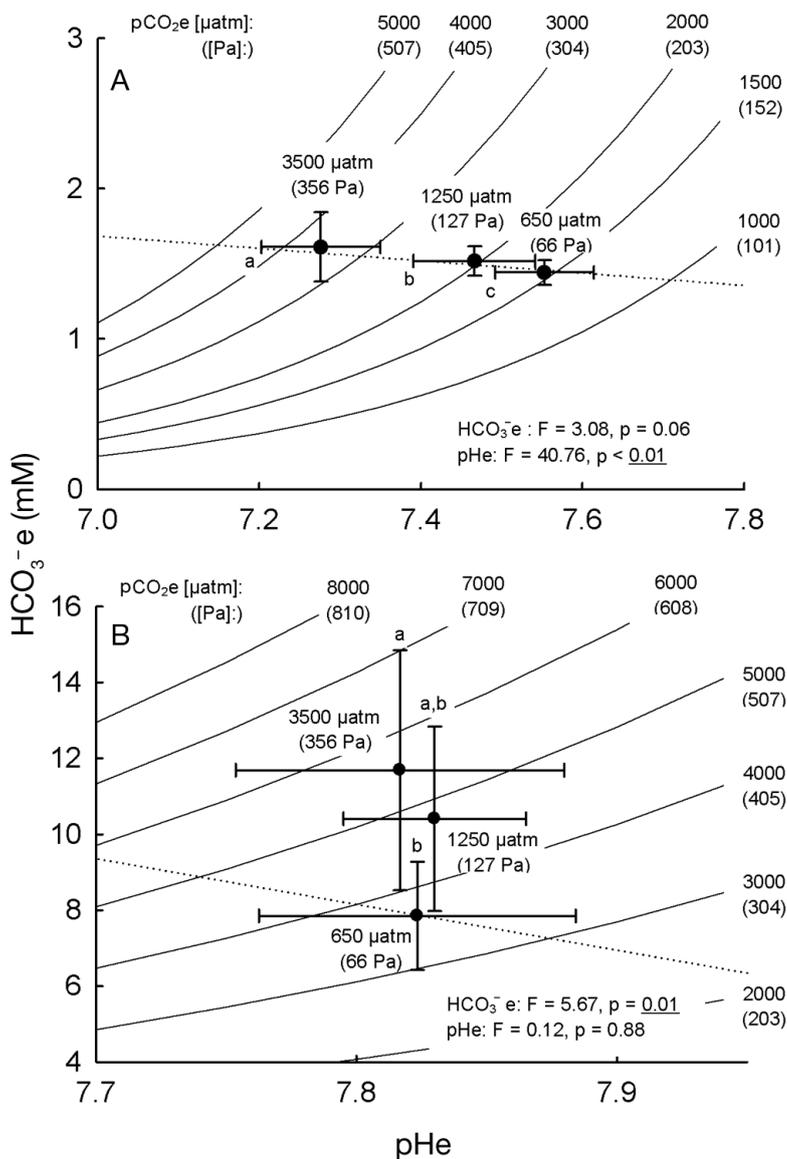
Fig. 7. pH-bicarbonate (Davenport) diagrams of (A) coelomic fluid of *Asterias rubens* and (B) haemolymph of *Carcinus maenas* at the different treatment levels. Vertical and horizontal bars are standard deviations. Isopleths display pCO_2. Dotted line indicates non-bicarbonate buffer line. Statistical results from 1-way ANOVAs. Groups with identical letters do not differ in (a) extracellular pH (pHe) and (b) extracellular HCO_3^- (HCO_3^-e) at $p \leq 0.01$

Carcinus maenas

Similar to *Asterias rubens*, the shore crab *Carcinus maenas* was not influenced in feeding at moderate (1250 µatm) seawater acidification. At the highest pCO_2, a significant reduction in feeding was observed during the comparatively long period of the experiment but not in the subsequent feeding assays. Because only one of the crabs moulted during the entire study period, we cannot be certain whether or

not the growth of crabs is affected by acidification. However, a relative insensitivity of decapod crustaceans towards short-term acidification stress has been demonstrated (e.g. Spicer et al. 2007) and has been explained by their high regulatory capacity. In contrast, Kurihara et al. (2008) found significantly lower moulting frequencies and growth of the shrimp *Palaemon pacificus* when exposed to CO_2 levels of 1900 µatm over a longer time period of 30 wk. Our results clearly show that the crabs, in contrast to sea stars, can compensate pHe when facing substantial pH shifts in their environment. A compensation of respiratory acidosis caused by hypercapnia has been demonstrated before, and its mechanisms were explained by accumulation of HCO_3^- in the haemolymph (Truchot 1979). While short-term pHe regulation in crabs is probably necessary for efficient haemocyanin-mediated blood oxygen transport (e.g. Lallier & Truchot 1989), it cannot be excluded that, in the long term, the excess amount of energy invested in pHe regulation negatively impacts energy allocation to other processes, such as reproduction, growth or even feeding processes (e.g. digestion or prey handling). Again, this would mainly apply for energy-limited conditions.

Only one of the crabs moulted during the experimental period in our study. This long intermoult period is not unusual for the crab size used in our experiments and the prevailing water temperature (Adelung 1971, Klein Breteler 1975, Hartnoll 2001). Monitoring growth rates and moulting intervals over a longer time period (6 to 12 mo) may therefore be necessary to test whether acidification impacts energy allocation into somatic growth in *Carcinus maenas* and whether calcification effects increase with increasing time exposure to high pCO_2 seawater and the concomitant increases in haemolymph $[HCO_3^-]$.

In the previously mentioned study by Kurihara et al. (2008), no differences in feeding rates were found between shrimp treated at ambient conditions, 1000 and 1900 µatm. However, previous studies have shown the handling time of mussels by *Carcinus maenas* to be extended and thus the consumption rates reduced when crabs were kept under other stressful conditions, such as hypoxia (Brante & Hughes 2001). Melzner et al. (2009) proposed that organisms with high and highly fluctu-
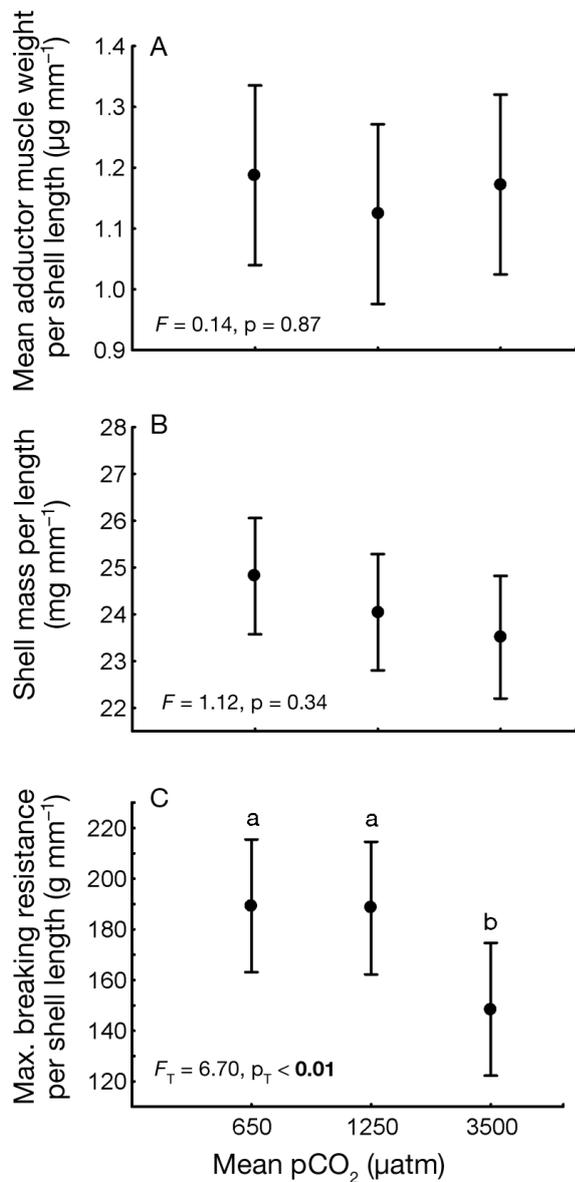
Fig. 8. *Mytilus edulis*. (A) Mean adductor muscle dry weight per shell length, (B) mean shell weight per shell length and (C) mean maximal breaking resistance of the mussel shell after the experimental period. Vertical bars denote ± 95% CI. Results from 1-way ANOVAs followed by post-hoc testing (Tukey's HSD). Groups with different letters are significantly different in (C) at $p \leq 0.05$ (**bold**). Subscript T: value derived from transformed data

mediate treatment suffered from respiratory acidosis, but mussel flesh consumption tended to increase. Also, a few other studies on organisms of different phyla show that metabolic rates may remain constant or even increase under conditions of acidification in organisms that do not compensate pHe (Beniash et al. 2010, Comeau et al. 2010, Lannig et al. 2010, Thomsen & Melzner 2010). The crabs in our experiments, although completely compensating pHe, demonstrated decreased food consumption during the experimental period at 3500 µatm, indicating that impacts of elevated seawater pCO_2 are not exclusively related to extracellular acid–base status.

Influence of the prey organism

The fact that the consumption of mussels pretreated under the highest acidification levels were targeted by untreated sea stars suggests that the adductor muscle strength of the mussels might have been weakened. Wood et al. (2008) proposed that the change in the Ca^{2+} to arm mass ratio in regenerated arms of brittle stars indicates higher levels of calcification but less muscle tissue biomass when the organisms are grown under acidified conditions (pH of 7.7, 7.3 and 6.8). In the present study, no differences in posterior adductor muscle mass were detected among the treatment levels. Possibly, rather than degrading muscle, acidification affected the catch phase of the smooth muscle by an as yet unknown cause. Surprisingly, despite a significant decrease in the breaking resistance of the shell, mussels did not become more susceptible to crab predation under high acidification levels. Because the consumption efficacy of the crabs was not impacted by acidification, as verified by offering reference prey in FA II, the decrease in shell stability was either too small to enhance vulnerability or was compensated for by other as yet unknown factors. It has previously been suggested that in addition to shell stability (Kossak 2006), the handleability of a mussel is a crucial factor limiting crab consumption (Enderlein et al. 2003).

Although it is possible that our results concerning mussel performance are slightly biased by generally low growth rates, which might have been caused by food quality and quantity (Melzner et al. 2011), our results suggest that the influence on the size and amount of mussels consumed is more dependent on the recent history of the predators than their prey.

ating metabolic rates (thus, high fluctuations in extracellular fluid pCO_2) should, on average, cope better with increases in environmental pCO_2. This concept, however, is based on the assumption that uncompensated pHe leads to metabolic depression (Pörtner et al. 2004), which does not seem to hold true in all cases. In our study, sea stars in the inter-

Conclusions

All 3 species show a shift in certain traits when exposed to high levels of acidification. Mussel shells become more brittle, sea stars (which cannot compensate pHe) grow slower, and both sea stars and crabs (the latter capable of pHe compensation) feed less under strong acidification. Interestingly, the enhanced vulnerability of mussels seems to be neutralised by the decreased consumption of the predators under high acidification. These results illustrate that different stress effects on interacting species may not only enhance but also buffer community level effects. The overall weak effects of substantial acidification on the species studied here confirm the expectation of enhanced tolerance of hypercapnic hypoxia in the low-salinity and periodically hypoxic Baltic (Thomsen et al. (2010).

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