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Future high CO_2 in the intertidal may compromise adult barnacle *Semibalanus balanoides* survival and embryonic development rate

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ABSTRACT: The effects of CO_2-induced acidification on survival, shell mineralogy, embryonic development and the timing of larval release were investigated in the intertidal barnacle *Semibalanus balanoides* using an intertidal microcosm system. Compared to that in the control (CO_2 = 344 ppm, pH = 8.07), adult survival was 22% lower in the high-CO_2 treatment (CO_2 = 922 ppm, pH = 7.70) and significant changes in the mineral structure of the adult shell were observed. Embryonic development rate was significantly slower in the high-CO_2 treatment than in the control but still resembled 'natural' rates seen in populations found in similar locations. There was an estimated 19 d delay in development under high-CO_2 conditions, which resulted in a 60% reduction in the number of nauplii reaching hatching stage at the time when over 50% of the control nauplii had hatched. We conclude that ocean acidification could potentially further compromise embryonic development in a species already stressed by temperature, which could in turn impact naupliar development and recruitment. *S. balanoides*, the adults of which live in a highly variable environment, has been shown to be detrimentally impacted by a chronic change in chemical conditions (pH lowered beyond the current range) over a crucial period in their life cycle. Under experimental high-CO_2 conditions, some adults were able to survive and larvae were able to hatch. This may indicate that there is still potential for organisms to find suitable habitats and for populations to develop and survive.

KEY WORDS: Ocean acidification · pH · Barnacle · Embryonic development · Carbon dioxide · Climate change · Intertidal · Larvae

INTRODUCTION

Ocean acidification, which is the decline in ocean pH occurring due to increasing atmospheric CO_2 concentration, is currently thought to impact most significantly those organisms that produce calcium carbonate structures (e.g. Gattuso et al. 1998, Langdon et al. 2000, Kleypas et al. 2006). In addition to a decrease in pH, the dissolution of more CO_2 in seawater also causes a change in chemistry leading to a decrease in the availability of carbonate ions (CO_3^{2-}). This can in turn lead to an increase in the dissolution of calcium carbonate minerals ($CaCO_3$). Organisms that use $CaCO_3$ to form shells may also be susceptible to dissolution.

Coastal shelf seas experience a greater range of pH than open oceans due to terrestrial influences such as river run-off and nutrient enrichment as well as large temperature and salinity fluctuations (Hinga 2002, Blackford & Gilbert 2007). Intertidal systems can experience even greater pH fluctuations, e.g. in rockpools (Morris & Taylor 1983), but knowledge of the environmental conditions in the overlying water during high tide is virtually non-existent (although see Agnew & Taylor 1986 for an example of diurnal fluctuations in pH of 7.5 to 8.5 and Wootton et al. 2008 for recent data for the coastal zone showing a diurnal range of ~0.7 pH units and a seasonal range of ~1 pH unit). Little is known about how sessile organisms in the coastal zone might respond to chronic changes in pH due to current and future increases in CO_2. Sessile organisms are important contributors to the intertidal ecosystem, providing habitat, food and nutrient cycling, yet they are

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unable to move away from unfavourable environmental conditions and may be more at risk from extreme events (Newell 1979). Fluctuating environmental conditions may provide some relief from unfavourable conditions for sessile organisms that show plasticity in their behaviour and/or physiology (Newell 1979). Ocean acidification lowers the overall pH range, which could reduce the duration and efficacy of these short-term periods of respite. Under the IPCC IS92 (Intergovernmental Panel on Climate Change, IS92 Emission Scenarios) CO_2 scenario (CO_2 concentration = 1000 ppm), ocean pH is expected to decrease by 0.3 units by 2100 (Caldeira & Wickett 2003, Orr et al. 2005, Blackford & Gilbert 2007). Low-pH water is already encroaching on coastal shelf seas in upwelling areas such as the west coast of North America, where pH can fall below 7.75 on a seasonal basis. These waters are undersaturated with respect to aragonite and near saturation with respect to calcite, aragonite and calcite being 2 common polymorphs of biogenic $CaCO_3$. Hence, these waters are corrosive to aragonite-forming marine organisms (Feely et al. 2008) and potentially harmful to calcite-forming organisms such as barnacles.

The barnacle *Semibalanus balanoides* is an important space occupier on rocky shores of northern Europe and America. Its calcareous shell plates provide protection from predators, abrasion, and desiccation (Rainbow 1984). *S. balanoides* is a cross-fertilising hermaphrodite, which develops its egg masses within its shell cavity. Fertilisation in the UK normally occurs by mid-November and the embryos develop over the winter period before hatching between February (near the southern limits of its geographic range, southwest England and northern Portugal) and May (at the northern limits, north UK and Norway) (Barnes 1957, Crisp 1962). Crisp (1959) recorded *S. balanoides in vivo* development times at Brixham (south Devon) and Bangor (north Wales), demonstrating no appreciable divergence in timing in early stages but nearly twice as rapid development of embryos after Stage 8 (as defined by Crisp 1954) in Brixham as in Bangor. Laboratory studies have shown that the development rate of these embryos is temperature-dependent, with a maximum development time occurring at ~14°C, which is also impacted by the availability of oxygen within the egg cavity (Crisp 1959, Lucas & Crisp 1987). Adults of this species undergo a period of lowered metabolism and activity in winter (Rainbow 1984), during which they carry out oxygenation within the mantle cavity by flushing it with seawater during periods of immersion (Barnes et al. 1963). *S. balanoides* offers an opportunity to examine the impacts of ocean acidification on an important space occupier, particularly an ability to focus on the development of eggs and larvae, which have been shown in other species to be highly vulnerable to elevated CO_2.

Previous studies investigating the impacts of CO_2-induced acidification on larvae and eggs have shown detrimental impacts on development, growth and survival (Kikkawa et al. 2004, Kurihara & Shirayama 2004, Kurihara et al. 2004, 2007, Dupont et al. 2008, Havenhand et al. 2008). However, there have been no specific investigations on the impacts of high CO_2 on embryonic development in *Semibalanus balanoides* and on survival of the adults through this crucial period in their life cycle, when they have minimal food and predominantly rely on lipid reserves for energy. The aim of the current study was to determine whether *S. balanoides*, a species normally exposed to a fluctuating environment, is likely to be impacted by ocean acidification scenarios realistic for the next 100 yr; and particularly whether the embryos contained within the calcium carbonate shell cavity, which may be subject to increased dissolution under high-CO_2 conditions, would develop normally. The study was conducted using microcosms which simulated immersion and emersion on the shore (Findlay et al. 2008) to investigate the effect of elevated CO_2 on (1) the survival of sexually mature adults during the embryonic production and development period, (2) the calcium and magnesium contents of the adult shells as a measure of changes in their calcium carbonate shells, (3) the timing of the appearance of different developmental stages of the embryos within the adults, and (4) the timing of the subsequent release of free-swimming nauplii.

MATERIALS AND METHODS

Experimental setup. *Semibalanus balanoides* adults on small rock chips were collected from the mid-shore at Looe, England (50° 20′ N, 004° 27′ W) on 23 November 2007. At least 2 rock chips were placed at random into each of 4 microcosms (30 × 15 × 20 cm) in a constant-temperature room so that each microcosm contained >400 ind. (living and dead). Two microcosms were set at control pH (8.07, CO_2 = 346 ppm) and 2 were set as high-CO_2 treatments (pH 7.70, CO_2 = 922 ppm). The CO_2 concentration, and hence pH level, was maintained in each microcosm using a CO_2 mixing system exactly as described by Findlay et al. (2008), which involved bubbling the microcosm with premixed high-CO_2 air. pH (NBS scale, Mettler-Toledo pH meter), dissolved inorganic carbon (DIC) (Ciba-Corning 965D Total CO_2 Analyser, Olympic Analytical Service), CO_2 (Licor LI-6262 CO_2 analyser), temperature and salinity (WTW LF197 combination temperature and salinity probe) were recorded weekly. Total

alkalinity, bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), and the saturation states (Ω) for aragonite and calcite were all calculated from pH and DIC using CO2sys (Pierrot et al. 2006), with dissociation constants from Mehrbach et al. (1973) refit by Dickson & Millero (1987) and KSO_4 using Dickson (1990). The microcosms worked on a tidal system (Findlay et al. 2008) with tide times programmed weekly based on the local Plymouth tide times (salinity = 35, water flow = 10 ml min^{-1}). Air temperature in the controlled-temperature room was set so that water temperature followed the Plymouth sea surface temperature. Light conditions (Polylux XL 58 W) were set to within 15 min of the sunrise/sunset times for London, UK, on a weekly basis (roughly 8 h on:16 h off cycle in December, 9 h on:15 h off cycle in January and 10 hr on:14 h off cycle in February). Natural, filtered (10 µm) seawater was used in the system and was replenished twice weekly to avoid salinity increases through evaporation. The experiment ran for 104 d.

Adult survival and shell mineralogy. Changes in barnacle abundance on each rock chip were recorded using a digital camera (FujiFilm A510 FinePix) which was maintained in consistent alignment using a stand. The photographic images were analysed (Image-Pro Plus v.4.5, Media Cybernetics) to estimate both abundance and survival. Barnacle survival was estimated from the images taken at the beginning and end of the experiment by counting living and dead individuals, accounting for individuals removed for sampling. Prior to photography, individuals were gently touched to check whether they were able to close their operculum, and were counted as dead when the operculum had remained open or the shell was empty.

Adult survival, which was recorded as a proportion, was square root arcsine transformed, tested for normality using the Kolmogorov-Smirnov test, and homogeneity of variances was examined using Levene's test. A 1-way nested ANOVA was used to determine any CO_2 treatment effects, with microcosms being nested within CO_2 treatment (n = 2). All statistical analysis was performed using Minitab 15.1.0.0 (Minitab 2006).

The calcium carbonate composition of the shell was estimated by analysing the calcium (Ca) and magnesium (Mg) ion concentrations as a proxy for any changes in calcification or dissolution. Live individuals produce calcium carbonate (calcify) during shell growth, although there may also be some dissolution of the shells; this dissolution, as discussed in the Introduction, may be enhanced in high-CO_2 conditions. Ca and Mg ions are abundant in seawater and hence are not limiting. Formation of $CaCO_3$ involves combining inorganic carbon with Ca and some Mg is also often incorporated to form Mg-$CaCO_3$. Therefore, any observed changes in Ca and Mg should indicate how the shell structure changes over time through calcification and dissolution. The shells of 10 ind. were haphazardly selected from each microcosm at the end of the experiment. Shells of 10 ind. that were noted as dead at the start of the experiment were also analysed for the concentration of Ca and Mg ions at the end of the experiment. Comparing the concentration in dead animals with that in live animals provides an estimate of a barnacle's ability to calcify relative to any dissolution effects since calcification does not take place in dead individuals. Concentrations of both cations were measured using methods described by Spicer & Eriksson (2003); briefly, this involved dissolving the shells in 10% nitric acid after drying and weighing, and then using an inductively coupled plasma (ICP) optical emissions spectrometer (Varian 725-ES) to measure Ca and Mg simultaneously. The proportion of Ca and Mg in the shell was calculated using the mass of the shell and the volume of acid used in the digestion.

Calcium and magnesium, which were recorded as proportions (cation concentration [mg l^{-1}]:total shell concentration [mg l^{-1}]), were square root arcsine transformed. The Ca:Mg ratio was calculated (mg Ca l^{-1}:mg Mg l^{-1}), all 3 datasets were tested for normality using the Kolmogorov-Smirnov test, and homogeneity of variances was examined using Levene's test. A 2-way nested ANOVA (n = 10) was then used to test for differences between control and high-CO_2 treatments and between live and dead barnacles, with microcosm being nested within CO_2 treatment.

Embryonic development. Embryos were maintained within live adult *Semibalanus balanoides* as naturally fertilised broods. On 8 occasions during the course of the experiment (Days 0 [23 November 2007], 7, 24, 42, 56, 70, 91 and 104), barnacles were removed haphazardly until 20 adults with egg masses were found from each microcosm. After isolating the egg masses in seawater, the embryonic development of 20 eggs from each egg mass at each sampling time was determined under low magnification (40×). Developmental stages were assigned using the classification of Achituv & Barnes (1976):

U: unfertilised, I: early development from being newly laid to having few divisions (equivalent to Stages 1 to 4 in Crisp 1954), II: multicellular (equivalent to Stages 5 to 7 in Crisp 1954), III: limb buds developing (equivalent to Stages 8 to 10 in Crisp 1954), IV: nauplii with eye apparent (equivalent to Stages 11 to 12 in Crisp 1954), and IVh: nauplii hatching (equivalent to Stage 13 in Crisp 1954); following Crisp (1954), the embryos were left for 5 min in seawater on the 2 last sampling points (Days 91 and 104); if any embryos hatched, they were counted as Stage IVh.

Egg development, which was recorded as the proportion of eggs at each stage (I, II, III, IV and IVh) from

20 ind. at each sampling time, was square root arcsine transformed and tested for normality using the Kolmogorov-Smirnov test, and homogeneity of variances was examined using Levene's test. A repeated measures ANOVA (n = 20) was then performed to determine the effect of CO_2 treatment and time (Day 7, 28, 40, 56, 70 and 104), with microcosm being nested within CO_2 treatment.

Development rate was assessed by first calculating the time at which 50% of the sampled eggs reached each stage, which was in turn calculated by fitting a logistic growth function (as the best model fit) to the embryonic stage data and calculating the time to 50% development. Records of the time taken for each stage to achieve 50% development were analysed using PERMANOVA (Primer-E) (Anderson 2001) with a nested (replicate microcosms) regression design (developmental Stages I to IVh) to test for differences between pH treatments. Time to 50% development for each stage was transformed ($d^{0.5}$) to produce a linear fit (best model fit with maximum R^2) whose gradient was taken as the rate of development. Linear regression analysis was then applied to each data set and a 2-tailed *t*-test of the regression was used to assess differences between the slopes of the control and high-CO_2 treatments.

RESULTS

Environmental conditions

The pH was maintained at a mean (±95% CI) of 8.07 (±0.03) and 7.70 (±0.03) in the control and high-CO_2 treatments, respectively. Dissolved inorganic carbon (DIC) was on average 1888 (±0) and 2045 (±83) µmol kg^{-1} in the control and high-CO_2 treatments, respectively. Total alkalinity was not significantly different between treatments, with averages of 2086 (±101) and 2115 (±95) µEq kg^{-1} in the control and high-CO_2 treatments, respectively (see Findlay et al. 2008 for exploration of the variability, control and reproducibility of the carbonate parameter measurements). There was a slight increase in pH towards the end of the experiment (~Day 84) in both control and high-CO_2 treatments (Fig. 1) due to an increase in salinity at this time. CO_2 concentration was maintained at a mean (±95% CI) of 346 (±27) and 922 (±72) ppm in the control and high-CO_2 treatments, respectively. The high-CO_2 treatment was undersaturated with respect to aragonite ($\Omega < 1$) and calcite was near saturation ($\Omega = 1$) throughout. Mean water temperature was 11.9°C in both treatments, but was set to track local sea surface temperature and hence decreased from 13°C in November to 10°C in February (Fig. 1a).

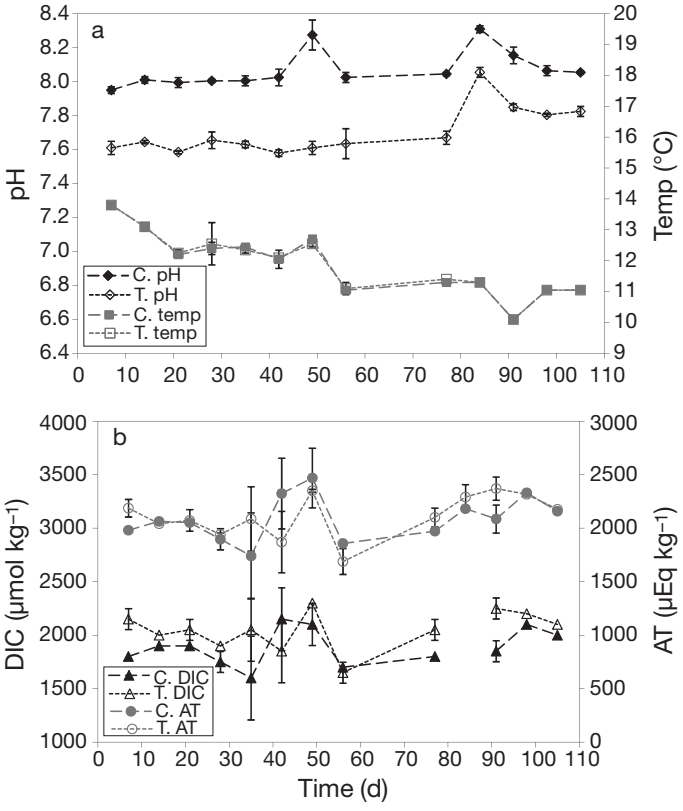

Fig. 1. (a) pH_{NBS} (◆) and temperature (■) in control (C; filled symbols) and high-CO_2 (T; open symbols) microcosms over the experimental period, and (b) dissolved inorganic carbon (DIC; ▲) and total alkalinity (AT; ●) in control (C; filled symbols) and high-CO_2 microcosms (T; open symbols) over the experimental period. Error bars: 95% CIs

Adult survival

Adult survival was significantly lower ($p = 0.017$, df = 1) in the high-CO_2 treatment than in the control (mean (±95% CI) of 47 (±4.62) vs. 69 (±4.28)%) after 104 d (Fig. 2). There were no significant microcosm effects in any of the endpoint measures (survival, mineralogy or development).

Adult shell mineralogy

Calcium. The proportion of calcium increased from the control to the high-CO_2 treatment in the live barnacles but decreased from the control to the high-CO_2 treatment in the dead barnacles (Fig. 3a). This indicates that there might have been some dissolution that was compensated for by calcification in live barnacles. However, there was no significant difference in the proportion of calcium between the treatments or between live and dead barnacles.

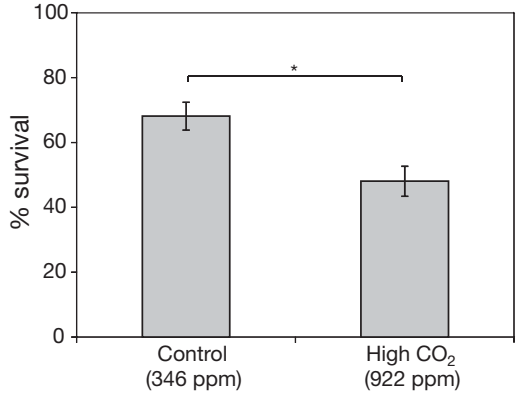

Fig. 2. *Semibalanus balanoides*. Mean percentage of adults surviving in the control (CO_2: 346 ppm) and high-CO_2 (922 ppm) microcosms. Bar with asterisk: significant difference (p = 0.017, df = 1, n = 2). Error bars: 95% CIs

Magnesium. There was a significant difference in the proportion of magnesium between the control and high-CO_2 treatment (p = 0.000, df = 1) and a small significant difference in magnesium between the live and dead barnacles (p = 0.048, df = 1); the significance was small due to large variability in the data. There was no significant interaction between the treatment and whether the barnacle was dead or alive. The proportion of magnesium decreased from the control to the high-CO_2 treatment in both live and dead barnacles (Fig. 3b).

Ca:Mg ratio. There was a significant difference in the Ca:Mg ratio between CO_2 treatments (p = 0.000, df = 1) and between live and dead barnacles (p = 0.000, df = 1), with a significant interaction effect (p = 0.032, df = 1). The Ca:Mg ratio increased from the control to the high-CO_2 treatment in both live and dead barnacles but this increase was greater in living barnacles (Fig. 3c).

Embryonic development

More than 50% of the eggs in egg masses were fertilised at the start of the experiment (Day 0) and all the eggs from fertilised animals had reached Stage I by Day 7. ANOVA (Table 1) indicated effects of both time and CO_2 concentration on the development of embryos through each stage (Fig. 4a–e); however, differences resulting from elevated CO_2 occurred most significantly at Stages III, IV and IVh. On Day 104, ~50% of the embryos had hatched in the control compared to only <20% in the high-CO_2 treatment (Fig. 4e).

The estimated rate of development (Fig. 4f) was significantly greater (see Table 2) in the control (0.24 stages $d^{-0.5}$) than in the high-CO_2 treatment (0.22 stages $d^{-0.5}$) for Stages I to IV. In the high-CO_2 treatment, the time to hatching (Stage IVh) was delayed by 18.95 d. The time to hatching in the control was not significantly slower than that observed by Crisp (1959) at Brixham (0.26 stages $d^{-0.5}$), whereas hatching in the high-CO_2 treatment was significantly slower than at Brixham (regression analysis: t(3.60), t(0.05, 2.447), 2-tailed, df = 6). The development rate in the control was significantly greater than Crisp's (1959) data from Bangor (0.20 stages $d^{-0.5}$), but the rate at the high-CO_2 concentration was not significantly greater (regression analysis between slopes of control vs. Bangor data: t(2.69), t(0.05, 2.447), 2-tailed, df = 6; regression analysis between slopes of low pH vs. Bangor data: t(1.48), t(0.05, 2.447), 2-tailed, df = 6). Crisp (1959) observed that hatching took 54.5 d longer for the Bangor than for the Brixham population.

DISCUSSION

At atmospheric CO_2 concentrations predicted for the year 2100 under the IPCC's IS92a scenario, the probability of adult *Semibalanus balanoides* barnacles sur-

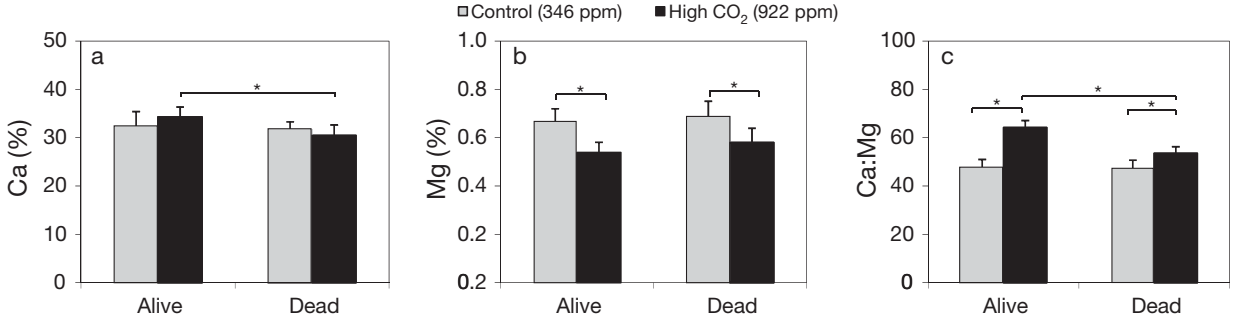

Fig. 3. *Semibalanus balanoides*. Concentrations of (a) calcium and (b) magnesium, and (c) the calcium:magnesium ratio in shells in the control (CO_2: 346 ppm) (light grey bars) and high-CO_2 (922 ppm) (black bars) microcosms, as a percentage of total shell material in barnacles that were alive for the entire experiment and those that were dead at the end of the experiment. Bars with asterisks: significant difference (p < 0.05). Error bars: 95% CIs

Table 1. Nested (microcosm) repeated-measures ANOVA for the proportion of embryos at each stage (I, II, III, IV and IVh) over time. (*) Significant ($p < 0.05$)

Source	df	Seq SS	Adj SS	Adj MS	*F*	p	Significant
Stage I							
pH	1	0.0001	0.0001	0.0001	0.44	0.574	
Microcosm(pH)	2	0.0007	0.0007	0.0003	0	0.996	
Day	5	63.4435	63.4435	12.6887	139.8	0	*
pH × Day	5	0.0007	0.0007	0.0001	0	1	
Stage II							
pH	1	0.0001	0.0001	0.0001	0.44	0.574	
Microcosm(pH)	2	0.0007	0.0007	0.0003	0	0.996	
Day	5	63.4435	63.4435	12.6887	139.8	0	*
pH × Day	5	0.0007	0.0007	0.0001	0	1	
Stage III							
pH	1	1.2349	1.2349	1.2349	1932.45	0.001	*
Microcosm(pH)	2	0.0013	0.0013	0.0006	0.03	0.97	
Day	5	51.6918	51.6918	10.3384	494.65	<0.001	*
pH × Day	5	2.0738	2.0738	0.4148	19.84	<0.001	*
Stage IV							
pH	1	1.49	1.49	1.49	3293.02	<0.001	*
Microcosm(pH)	2	0.001	0.001	0	0.03	0.972	
Day	5	178.969	178.969	35.794	2257.78	<0.001	*
pH × Day	5	1.806	1.806	0.361	22.78	<0.001	*
Stage IVh							
pH	1	1.0462	1.0462	1.0462	222.37	0.004	*
Microcosm(pH)	2	0.0094	0.0094	0.0047	0.59	0.555	
Day	5	19.6909	19.6909	3.9382	493.85	<0.001	*
pH × Day	5	5.2312	5.2312	1.0462	131.2	<0.001	*

Table 2. PERMANOVA for nested (microcosms) regression (stages) at each pH condition, where pH condition 1: control (346 ppm), pH condition 2: high CO_2 (922 ppm), pH condition 3: Crisp (1959) Brixham data, and pH condition 4: Crisp (1959) Bangor data

Source	df	SS	MS	Pseudo-*F*	p (perm)	Unique perms
Stage	3	15801	5267	39751	0.001	998
pH	3	1141.9	380.64	2872.7	0.016	45
Microcosm (pH)	2	0.265	0.1325	1	0.6328	53
Stage × pH	9	922.81	102.53	773.85	0.001	999
Res	6	0.795	0.1325			
Total	23	18608				

viving the winter period was 22% lower than under current (2007/2008) winter sea surface temperature and pH conditions. Adult barnacles appeared to maintain their calcium carbonate mineral structure in the face of increased CO_2 despite the changes in the Ca:Mg ratio, showing that the isolated shell structure should disintegrate as a result of dissolution in corrosive (undersaturated) seawater. The relatively large decline in survival rate could possibly be due to the metabolic cost of maintaining the shell. Embryos developing within the adults developed more slowly due to increased CO_2 levels. Both delayed development and reduced naupliar production, which lead to delayed settlement, have the potential to impact local populations (Pechenik et al. 1993, Jarrett 2003).

Adult shell mineralogy and survival

Under low-CO_2 (control) conditions, a large change in the mineral structure of the shells was not expected in this experiment because adults do not expend much energy on growth during winter and they feed minimally at this time (Barnes et al. 1963). Therefore, in the high-CO_2 treatment where we expect enhanced dissolution, we would predict a loss of calcium carbonate structure. There was, however, no significant difference in the calcium concentration between the control and the high-CO_2 treatments in living individuals, suggesting more energy being invested in shell maintenance in the high-CO_2 treatment than in the control.

There were also significant differences in the mineral structure of the shells between live and dead barnacles: in the high-CO_2 treatment, there was less Ca in dead adult barnacles, resulting in lower Ca:Mg ratios in dead compared to live barnacles. This indicates that dissolution of the dead shells was occurring in the

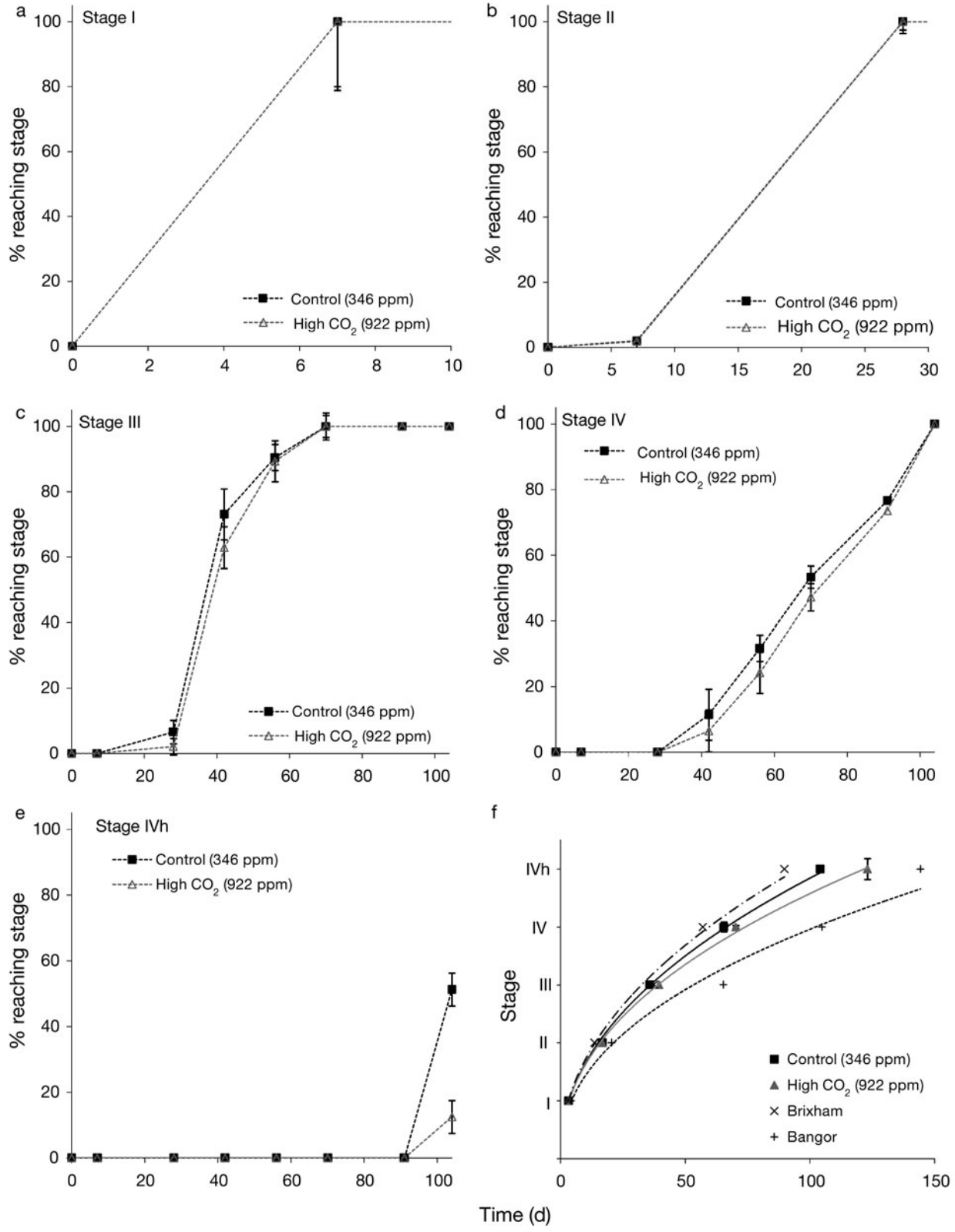

Fig. 4. *Semibalanus balanoides*. Mean percentage of eggs from 20 ind. reaching each stage (stage I – IVh, a to e) at particular time periods (days after start of experiment) in the control (CO_2: 346 ppm) (■) and the high-CO_2 (928 ppm) (△) microcosms. Error bars: 95% CIs. (f) Time for 50% of samples to reach each stage in the control (■) and high-CO_2 (▲) microcosms, and in the Crisp (1959) Brixham (×) and Bangor (+) data, also displaying best fit lines (power equation, $d^{0.5}$)

high-CO_2 treatment, while there was biological precipitation of $CaCO_3$ in the living barnacles.

Barnes et al. (1976) investigated the Ca:Mg ratio in several barnacle species. *Chthamalus depressus* was found to have an increased Ca:Mg ratio in extreme hypobiotic individuals (found in caves and other dark locations) compared to those on the open shore. This increased ratio was accompanied by a reduction in the total organic matter content due to reduced protein. As calcite is the major form of $CaCO_3$ in these organisms, any magnesium present in the shell is held within the lattice matrix but is not tightly bound, hence dissolution will cause ions such as Mg to be lost before the dissolution of $CaCO_3$. Therefore, a larger decrease in Mg compared to Ca was both expected and observed in the high-CO_2 treatments. The absence of photosynthetic organisms in hypobiotic environments could lead to an elevated level of CO_2 in the seawater, which occurs as a net result of high respiration but low photosynthetic rates. This could result in seawater with carbonate chemistry properties similar to those seen in this study and may explain why both high-CO_2 and hypobiotic individuals show similar results.

Wickens (1984) investigated CO_2 impacts on growth and mineralisation in penaeid prawns, and showed an increase in Ca, no change in Mg and hence an increase in the Ca:Mg ratio with increasing CO_2-induced acidification. This agrees with our findings in several respects as we also found an increased Ca:Mg ratio and an increase in Ca, although the latter was not significant at the CO_2 level used here. Increasing CO_2 is accompanied by an increase in HCO_3^-, which is taken up for use both as a buffer to rising haemolymph pH and as a substrate for $CaCO_3$. The decrease in Mg in *Semibalanus balanoides* but not in prawns suggests either a difference in the mechanisms associated with the uptake of Mg into the shell structure (with less Mg being incorporated at lower pH in *S. balanoides*), a difference in relation to external erosion properties or, more likely, a difference in shell mineralogy.

The lower survival of adults in the high-CO_2 treatment could have resulted from either physiological or dissolution effects. Like all other organisms, barnacles expend energy on maintenance, repair, reproduction and respiration (Sibly & Calow 1986). They feed minimally during winter and hence must rely on food reserves (lipid stores) while undergoing a period of lowered metabolism with minimal growth (Barnes et al. 1963). Despite acidosis in extracellular fluids being considered as a 'normal' feature of intertidal barnacles during periods of emersion (e.g. Fyhn et al. 1972), prolonged acidosis resulting from sustained exposure to low-pH seawater, as found for other crustacean species (e.g. Spicer et al. 2007), could lead to disruption of normal physiological processes. Additionally, an increased use of lipid stores for energetic maintenance could result in reduced protein biosynthesis, which in turn could enhance mortality (Barnes et al. 1963). Exposure to corrosive seawater may have led to some dissolution of the shell, as well as created hypercapnic conditions within the organism further stressing the animals and decreasing survival. Further work investigating lipid storage and cirral movement during winter under normal and acidified conditions will aid our understanding of this energy balance, although energetic trade-offs have been shown in other species under hypercapnic conditions (Wood et al. 2008, Findlay et al. 2009).

Embryonic development

Comparing the current study with the investigation of Crisp (1959), it can be seen that the naupliar development rates recorded by Crisp at Brixham, which is geographically the closest site to the barnacle populations used here, were similar to those estimated for the control. Based on this observation, we assume that the 1.5°C increase in temperature over the last 50 yr as a result of global warming (Baxter et al. 2008) has had little impact on development rates. The estimated development rate in the high-CO_2 treatment was significantly slower than in both the control (average winter temperature of 12°C) and in the Brixham population (average winter temperature of 11°C), but was similar to the rate in the Bangor population (average winter temperature of 8°C). There is no information on annual variation in development rates at either Bangor or Brixham. Crisp (1959) suggested that the difference in the rate of development in different locations arises from temperature differences (average winter temperature in Brixham is ~2°C warmer than in Bangor) together with the implication that towards the later stages of development, embryos are more deprived of oxygen or inhibited by excess CO_2 under warmer conditions. Both O_2 and CO_2 are known to impact the rate of egg development (Root 1930, Strathmann & Strathmann 1995, Cohen & Strathmann 1996). Root (1930) demonstrated that the rate of O_2 consumption of *Arbacia* eggs decreased rapidly when CO_2 increased (O_2 consumption decreased by 21% for every ~1315 µatm of CO_2 increase), but only began to decrease below pH 6.25 if the acidity was changed using HCl. This implies that bubbling CO_2 has a much greater effect on egg respiration than using HCl to lower pH, and is in agreement with the results obtained here in which CO_2 had a small yet significant effect at 922 ppm. Mayor et al. (2007) showed that elevated CO_2 (8000 ppm) was associated with nearly an 86% reduction in hatching success of copepods despite

apparently normal growth and reproduction of adults. In our experiments, there was no impact of elevated CO_2 on hatching success; however, there was an estimated 19 d delay in reaching hatching stage.

A change in the rate of embryonic development induced by elevated CO_2 may be important considering that larvae are released into the plankton to coincide with the spring bloom. Adults are triggered to release their larvae by a chemical cue or 'hatching substance' (Clare & Walker 1986) produced when the adult begins feeding. There is considerable interannual and geographic variability in release timing (Barnes 1962, Kendall et al. 1985), therefore effects of delayed development would be greatest in early release years and in areas where spawning tends to be early. In other years, developmentally intact larvae can be held for some time before release takes place. Larval release tends to start at the southern range edge (southwest UK and northern Portugal) as early as February, therefore the already short period between fertilisation and spawning could be problematic. Delaying the time to hatching could prevent synchronisation with the spring bloom and could lead to high mortality resulting from competition with the holoplankton. Even if larvae were able to survive, late settlement would leave juveniles susceptible to additional stresses: on average, the later in the year a larva settles, the greater is its chance of encountering high air and rock temperatures and dying from either heat or desiccation. Hence, the timing of recruitment is crucial, with early and late settlers often having the lowest survival (Kendall et al. 1985, Piñeda et al. 2006).

Here we provide evidence that relatively small changes in CO_2 and pH, to levels that are predicted to occur globally within the next 100 yr (Caldeira & Wickett 2003), can lower the survival of adults and decrease the development rate of embryos. These findings further the work of Kurihara & Shirayama (2004), Dupont et al. (2008), Havenhand et al. (2008) and others who have found impacts on early life stages at realistic levels of future CO_2. However, the CO_2-induced slowing in development rate still falls within the range of rates seen in natural populations. It is known that temperature limits the development rate of *Semibalanus balanoides* embryos at the southern edge of their range (Crisp 1959), but it could be inferred from our results that ocean acidification could further compromise barnacle development at this range edge (cf. synergistic effects of temperature and elevated CO_2 on the thermal limits of the edible crab *Cancer productus* in Metzger et al. 2007). Further work is necessary to establish more exactly how temperature and CO_2 interact over the entire geographic range.

We demonstrate that both the growth and embryonic development of an organism that is normally exposed to seasonal and daily fluctuations in its environment can be significantly affected by lowering pH to levels predicted under future ocean acidification scenarios. Nevertheless, in assessing the significance of these findings, their limitations must be considered. One consideration for this experiment is that we increased CO_2 levels over a period of days, as opposed to increase on yearly timescales seen in nature, which thus removed the possibility that the barnacles might adapt or acclimate over longer time periods. The evidence that some adults were able to survive and their larvae able to hatch in high-CO_2 conditions may indicate that the species is able to find suitable habitats and survive. At the southern edge of its range, the additional temperature stress may make populations more vulnerable to local extinctions.

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