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RESEARCH ARTICLE

Effects of ocean acidification on early life-history stages of the intertidal porcelain crab *Petrolisthes cinctipes*

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SUMMARY

Intertidal zone organisms naturally experience daily fluctuations in pH, presently reaching values beyond what is predicted for open ocean surface waters from ocean acidification (OA) by the year 2100, and thus present an opportunity to study the pH sensitivity of organisms that are presumably adapted to an acidified environment. The intertidal zone porcelain crab, *Petrolisthes cinctipes*, was used to study physiological responses to low pH in embryonic, larval and newly recruited juvenile life-history stages. In these crabs, embryonic development occurs in the pH-variable intertidal zone (pH 6.9–9.5), larvae mature in the more stable pelagic environment (pH 7.9–8.2), and juvenile crabs settle back into the pH-variable intertidal zone. We examined survival, cardiac performance, energetics and morphology in embryonic, larval and juvenile crabs exposed to two pH conditions (pH 7.9 and 7.6). Embryos and larvae were split by brood between the pH treatments for 9 days to examine brood-specific responses to low pH. Hatching success did not differ between pH conditions, but ranged from 30% to 95% among broods. Larval survival was not affected by acidification, but juvenile survival was reduced by ~30% after longer (40 days) exposure to low pH. Embryonic and larval heart rates were 37% and 20% lower at low pH, and there was a brood-specific response in embryos. Embryos did not increase in volume under acidified conditions, compared with a 15% increase in ambient conditions. We conclude that sustained exposure to low pH could be detrimental to *P. cinctipes* embryos and larvae despite the fact that embryos are regularly exposed to naturally fluctuating hypercapnic water in the intertidal zone. Importantly, our results indicate that early life-history stage responses to OA may be brood specific through as yet undetermined mechanisms.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/216/8/1405/DC1

Key words: crustacean larvae, embryo, juvenile, pH, hypercapnia, cardiac performance, heart rate, sub-lethal effect.

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INTRODUCTION

Atmospheric CO_2 is predicted to be three to four times higher than pre-industrial levels by the end of the 21st century as a result of increased anthropogenic CO_2 emissions (Feely et al., 2008; Orr et al., 2005; Meehl et al., 2007). Increased atmospheric CO_2 leads to increased CO_2 dissolution in ocean surface waters, and is altering seawater carbonate chemistry and pH at rates not seen in the past 300 million years (Sabine et al., 2004), a phenomenon termed ocean acidification (OA) (Caldeira and Wickett, 2003; Meehl et al., 2007). How marine biota respond to OA will depend on their current tolerance to elevated CO_2 conditions, and the ecological ramifications of OA are considered to include changes in local biodiversity and community composition (Kleypas et al., 2006; Guinotte and Fabry, 2008; Pörtner and Farrell, 2008; Widdicombe and Spicer, 2008). OA may affect integrated physiological performance parameters such as metabolism, growth and reproduction (Whiteley, 2011; Melzner et al., 2009; Barry et al., 2011) as a consequence of fundamental requirements for intracellular pH homeostasis (Pörtner et al., 2011).

Organisms naturally experiencing high CO_2, such as intertidal zone species, facilitate the assessment of physiological performance under pH conditions that are more variable than those of offshore habitats (Pörtner et al., 2011; Hofmann et al., 2011; Findlay et al., 2009). Previous studies have shown tidepool pH values ranging from 6.5 to 9.5 (Morris and Taylor, 1983; Truchot, 1986; Wootton et al., 2008), thus reaching pH levels lower than global predictions for surface water during the next century (Morris and Taylor, 1983; Truchot, 1986; Wootton et al., 2008). In future climate change scenarios, tidepool and near-shore pH extremes may be further reduced by increased upwelling intensity of hypercapnic waters (Hofmann et al., 2011; Feely et al., 2008).

Reduced survival, growth, reproduction and metabolic rate have been observed under OA conditions in intertidal species of crustaceans and snails (Kurihara et al., 2008; Melatunan et al., 2011; Findlay et al., 2009). In contrast, physiological performance in other species, including teleost fish and some brachyuran crabs, is unaffected (Small et al., 2010; Melzner et al., 2009) or even enhanced by OA (Moulin et al., 2010; Dupont et al., 2010). For intertidal zone organisms, the largest responses to OA may be observed in early life-history stages (i.e. embryonic, larval or juvenile) (Kurihara, 2008; Ross et al., 2011) as these life stages are particularly energetically demanding (Barry et al., 2011). Though responses to OA in early life-history stages may vary, negative effects have been observed in mollusks, echinoderms and crustaceans, including reduced survival, growth and recruitment, as well as changes in developmental timing (Kurihara et al., 2008; Dupont et al., 2008; Crim et al., 2011; Findlay et al., 2009; Walther et al., 2010). The persistence or failure of a population will be determined by the

successful completion of all life stages (Byrne, 2011), emphasizing the importance of assessing sensitivity to OA in adult as well as early developmental life-history stages.

Porcelain crabs, genus *Petrolisthes* (Decapoda; Anomura), are common and abundant inhabitants of the California rocky shore (Haig, 1960). *Petrolisthes cinctipes* (J. W. Randall 1840) early life-history stages occur in distinct pH environments: embryos and newly settled juveniles live in the pH-variable intertidal zone, whereas larval stages spend around 45 days offshore in pelagic regions where the pH is more stable (Gonor, 1970; Gonor and Gonor, 1973; Shanks and Eckert, 2005). We hypothesized that embryonic and juvenile stages posses mechanisms to tolerate environmental acidification, and thus would have muted responses to low pH as compared with zoea I larval stages, which do not experience pH variability in their natural habitat. The specific aim of this study was to investigate whether responses to OA differ among developmental stages by evaluating survival, morphology and cardiac performance of embryonic, larval and juvenile crabs of *P. cinctipes* under continuous exposure to low pH water.

MATERIALS AND METHODS

Water chemistry

Seawater collected in Half Moon Bay, CA, USA, was delivered to the Romberg Tiburon Center by a commercial vendor (SeaPure Inc., El Granada, CA, USA). Water was kept in temperature-controlled insulated reservoirs (~500 l) with continuous circulation and filtration (0.35 μm, no. FH803, Aquatic Eco-Systems Inc., Apopka, FL, USA). Water equilibrated with the atmosphere by continuously bubbling with air was used as the 'ambient pH' condition (pH 7.93±0.06). Water bubbled with CO_2 was used as the 'low pH' condition (pH 7.58±0.06) (modified from Widdicombe and Needham, 2007). A pH controller (Duo ORpH Controller no. CP2311, Captive Purity Inc., Marine Depot, Garden Grove, CA, USA; Accumet pH Submersible Combination Electrode, Fisher Scientific no. 13-620-AP56, Pittsburgh, PA, USA) connected to a solenoid valve on the CO_2 gas regulator was used to deliver CO_2 when the pH exceeded 7.6.

Total alkalinity (TA) was assessed weekly during the length of the experiments and calculated from linear Gran plots (Gran, 1952; Dickson, 1981) of potentiometric measurements (Metrohm dosimat 765 pH-Meter, Herisau, Switzerland) using certified Dickson references (http://andrew.ucsd.edu). Seawater pH was monitored daily with electrodes (see above) and measured spectrophotometrically twice per week using *m*-Cresol Purple sodium salt dye (PharmaSpec UV-1700, Shimadzu, Columbia, MD, USA; Sigma-Aldrich no. 211761, St Louis, MO, USA; pH reference: Tris buffer certified Dickson reference) using the modified protocol of DOE (DOE, 1994). Salinity and temperature were measured daily using a conductivity meter (YSI Model 30-25FT, Yellow Springs, OH, USA). The 'seacarb' package in R (v. 2.14.0) was used to calculate the carbonate chemistry of the water using TA and pH inputs, and applying the 'carb' and 'phinsi' functions (Lavigne and Gattuso, 2011) (Table 1).

Table 1. Abiotic conditions measured or calculated during the experiments

	Variable	*N*	Ambient pH	Low pH
Measured	pH	20	7.93±0.06	7.58±0.06
	Salinity	84	32±0.9	32±1.0
	Alkalinity (μmol kg^{-1})	9	2380±98	2364±105
Calculated*	P_{CO_2} (μatm)	9	574±105	1361±199
	DIC (μmol kg^{-1})	9	2235±95	2338±104
	HCO_3^- (μmol kg^{-1})	9	2124±122	2255±124
	CO_3^{2-} (μmol kg^{-1})	9	118±14	57±9

Values are means ± s.d.
DIC, dissolved inorganic carbon.
*Calculations were made using the 'carb' function in 'seacarb' R package using 'flag 8'. Total pH scale, Kf (from Pérez and Fraga, 1987), k1, k2 (from Lueker et al., 2000) and Ks (from Dickson, 1990).

Animal maintenance

Late-stage gravid females (*N*=22) and newly settled juveniles (*N*=169) of *P. cinctipes* were collected during low tide at Pacifica, CA, USA (37°35′48″N, 122°30′34″W) between March and June 2011 (supplementary material Table S1) and brought to the Romberg Tiburon Center, where they were maintained in ambient conditions (13±0.2°C, 33±1 salinity and pH 7.93±0.06). Gravid females were held individually in 385 ml acrylic cylinders (~7 cm diameter and 24 cm height) with 500 μm Nytex mesh bottoms through which water flowed continuously. Embryo and larval experiments were performed in broods from *N*=22 females. Embryos were removed from females 2–4 weeks before hatching, based on embryo coloration and the appearance of eyespots, and split between treatments. Larval experiments were performed on newly hatched individuals. Broods were kept separate in all larval and embryonic experiments. Juvenile experiments were conducted on field-collected newly settled specimens that were roughly 2 mm carapace width. For all three life-history stages, individuals were placed in either acrylic cylinders or 50 ml conical tubes with mesh bottoms (as specified below per experiment) and randomly assigned to different sealed plastic boxes containing water at low and ambient pH. The number of individuals per cylinder was standardized as much as possible, but the number of cylinders per brood varied depending on the number of embryos or larvae in the brood (supplementary material Table S1). Daily 100% water changes were performed by transferring the cylinders with individuals to a new plastic box containing pre-equilibrated water from the reservoirs. Larvae and juveniles were fed daily *ad libitum* with a mixture of newly hatched *Artemia franciscana* nauplii (SF Bay strain, Brine Shrimp Direct, Ogden, UT, USA), lab-cultured live rotifers and Shellfish Diet 1800 (a mixture of four marine microalgae: 30% *Isochrysis*, 20% *Pavlova*, 20% *Tetraselmis* and 30% *Thalassiosira weissflogii*, Reed Mariculture Inc., Campbell, CA, USA).

Survival and hatching success

Hatching success, a proxy for embryonic survival, was determined separately in *N*=6 broods (*N*=90–200 embryos per brood per treatment) by placing 50 embryos per cylinder, with *N*=3–4 cylinders per treatment (i.e. three cylinders per treatment where used if the brood had at least 300 embryos) at ~8 days pre-hatching for each brood in each treatment, and counting the daily number of hatchlings (supplementary material Table S1). To determine larval survival, we used larvae from *N*=10 broods (*N*=24–123 larvae per brood per treatment) during a 9 day exposure to low pH (supplementary material Table S1). Newly hatched larvae were placed in 50 ml conical tubes (as described above) containing *N*=3–10 larvae per treatment and survival assessment and removal of dead individuals was performed daily. The total number of larvae screened was 473 in ambient pH and 468 in low pH. Juveniles were housed individually in 50 ml conical tubes and survival was determined by daily assessment during a ~40 day exposure (*N*=84 and 85 in ambient pH and low pH, respectively).

Cardiac performance

Heart rate (f_H) and stroke volume (V_S) were determined from video recordings of embryos and larvae. Calcification of juvenile carapaces prevented imaging of cardiac activity. Larvae were immobilized by attaching the rostral spine to a greased (petroleum jelly, Vaseline) glass capillary tube that was mounted to a glass microscope slide and placed in a Petri dish (o.d.=10 cm, Fisherbrand no. 08-757-913, Fisher Scientific, Pittsburgh, PA, USA) containing seawater at the treatment pH. Embryonic measurements did not require immobilization as each individual was simply placed in a dorsal view position using a scaled slide. The Petri dish was maintained at 13°C by placing it in a water-jacketed, aluminum block connected to a recirculating water bath (NESLAB RTE 7, Thermo Scientific, Pittsburgh, PA, USA). The block sat on the stage of a stereoscope (Scope Nikon SMZ1500, Nikon Instruments Inc., Melville, NY, USA). After a 10 min recovery period, each individual was imaged at 70× magnification and recorded at a rate of 25 frames s^{-1} for 2 min using a digital camera (Nikon D3100, Nikon Inc.).

Videos were parsed into 10 s segments and slowed to 25% of original speed (Apple iMovie '11 software v. 9.0.4) to allow heartbeats to be accurately counted. For each individual, the heart rate was assessed in N=5 segments, and those rates were averaged to calculate an individual f_H. Specimens with a vertically aligned dorsal position were used to determine V_S by averaging N=10 cardiac cycles (heart beats) per individual using frame-by-frame analysis. The number of broods and individuals used per treatment is presented in supplementary material Table S1. V_S was calculated as the difference between the end-diastolic volume (EDV) and end-systolic volume (ESV) (Eqn 1), assessed using ImageJ (v. 1.45).

$$V_S = \text{EDV} - \text{ESV} . \quad (1)$$

EDV and ESV were modeled as prolate spheroids (Eqn 2) (Harper and Reiber, 2004; Storch et al., 2009):

$$\text{Volume} = 4/3\pi\, ab^2 , \quad (2)$$

where a is the radius of major diameter and b is the radius of minor diameter. Individual cardiac output ($\dot{Q}$) was determined as the product of V_S and f_H (Eqn 3) (Harper and Reiber, 2004):

$$\dot{Q} = V_S \times f_H , \quad (3)$$

where $\dot{Q}$ is in nl min^{-1}, V_S is in nl $beat^{-1}$ and f_H is in beats min^{-1}.

Yolk consumption

Embryo yolk consumption rate was determined in N=54 embryos from N=2 broods (supplementary material Table S1) split between the two treatments and imaged after 5, 9 and 20 days in the experimental conditions (representing 20, 16 and 5 days pre-hatching). To estimate yolk volume at each time point, lateral and dorsal view photographs were analyzed using the color threshold function in ImageJ (Eqn 4) (supplementary material Fig. S1):

$$\text{Yolk volume} = 4/3\pi\, cd^2 , \quad (4)$$

where c is the dorsal yolk area and d is the maximum radius of a lateral fitted ellipse.

Larval activity

Larval activity, defined as the rate of maxilliped movement, was determined in a subset of larvae randomly chosen from five broods (supplementary material Table S1). The same videography procedure used for the cardiac assessment was used to record the maxilliped movement. Videos with full lateral views of the maxillipeds were used. For each individual, maxilliped beats were averaged across N=5 video segments of 10 s duration.

Morphometrics

Morphometric analyses were conducted from photographs of live specimens taken with the same stereoscope and camera used in the video recordings. Dimensions of distinctive structures [embryonic measurements: volume and ellipticity (major diameter/minor diameter); larval measurements: dorsal carapace length, anterior carapace width, rostrum spine width, telson length] were determined with ImageJ (supplementary material Figs S2, S3). Embryos were measured following exposure to ambient or acidified water for 5 or 9 days and larvae were measured after 9 days of exposure to their respective pH conditions.

Statistical analysis

Data were tested for normality and homoscedasticity using Shapiro–Wilk and variance tests, except for survival data, where a different approach was used (described below). If parametric requirements were met, one-way ANOVA tests were performed. If parametric requirements were not met, data transformation or non-parametric Kruskal–Wallis tests were applied. For heart rate analyses, larval and embryonic f_H data were square-root transformed and then analyzed using a nested one-way ANOVA, using brood as the nested variable. Embryonic morphometrics were analyzed by applying the Kruskal–Wallis test at each time point separately.

Survival was analyzed following previous methods (Bewick et al., 2004) and survival curves were created and compared between the treatments using the Kaplan–Meier log-rank test within the R 'survival' package (Therneau, 2011). Survival curves were constructed by pooling larval survival data from all 10 broods. Juvenile survival data were pooled across N=4 trials before analysis. Rate of yolk consumption was determined using linear regression. All statistical analyses and construction of plots were performed using R v. 2.14.0 (R Development Core Team, 2011) and the package 'ggplot2' (Wickham, 2009).

RESULTS

Hatching success and survival

Embryos hatched 6–10 days after placement in treatment conditions. Hatching success ranged from <30% to >95% among broods, independent of pH treatment (supplementary material Fig. S4A). No difference in mean hatching success was observed between pH conditions when embryos from all broods were pooled together (ANOVA $F_{1,10}$=0.006, P=0.94). A brood-specific response to acidification was observed; some broods had 40% lower hatching success relative to the mean survival at ambient pH, while others had 20% higher success at ambient pH (supplementary material Fig. S4B).

No significant differences in larval or juvenile survival were detected after 9 days in the treatments (Kaplan–Meier log-rank test, χ^2_1=2.7, P=0.102, Fig. 1). However during the 9 days of observation, daily larval survival was routinely lower in the low pH treatment (Fig. 1A). At day 9 there was less than 50% larval survival in both conditions. Juveniles kept at low pH showed significantly lower survival than those in the ambient pH condition over the entire 40 day period (Kaplan–Meier log-rank test, χ^2_1=7.1, P=0.008) (Fig. 1B). Almost no juvenile mortality was observed in either treatment during the first week.

Cardiac performance

Embryonic f_H was 37.4% lower in the acidified condition across all broods (ANOVA: $F_{1,45}$=5.84, P=0.02) (Fig. 2A). However, there was

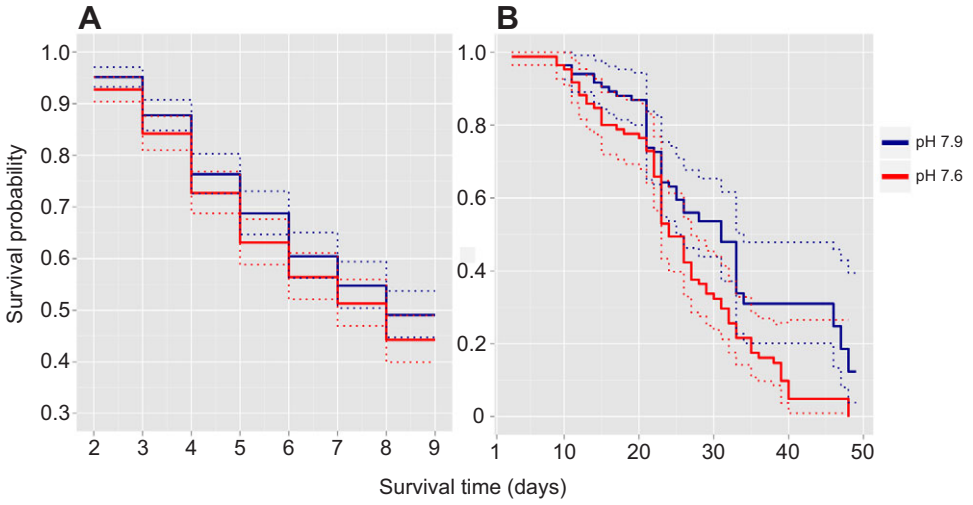

Fig. 1. Kaplan–Meier survival probability of *Petrolisthes cinctipes*. (A) Larvae from 10 broods (ambient pH N_{t0}=473, low pH N_{t0}=468, where N_{t0} is the number at time t=0). (B) Juveniles from four trials (ambient pH N_{t0}=85, low pH N_{t0}=84). Dotted line represents 95% confidence interval.

significant variation among broods in the degree of response to low pH, with embryonic f_H showing a 20–65% reduction across broods (Fig. 2B). In the embryonic stage, an interaction between the response and the brood was found (ANOVA nested by N=5 broods, $F_{8,45}$=2.70, P=0.02; ambient N=26, low N=29). Overall, larval f_H was 20.9% lower in the acidified condition (ANOVA: $F_{1,64}$=4.64, P=0.04) (Fig. 2A), and in several broods was reduced by 20–65% (Fig. 2B), though the overall effect of brood was not statistically significant.

Mean cardiac performance in embryonic and larval stages was reduced at low pH, though differences were not statistically significant (Fig. 3; supplementary material Table S2). Embryonic cardiac output ($\dot{Q}$) and stroke volume (V_S) were reduced by 52% and 14%, respectively, in low pH (ANOVA: $\dot{Q}$ $F_{1,10}$=2.88, P=0.12; V_S $F_{1,8}$=1.67, P=0.23) compared with ambient pH (supplementary material Fig. S5). Larval cardiac performance was also affected, with $\dot{Q}$ and V_S reduced by 20% and 7%, respectively (ANOVA: $\dot{Q}$ $F_{1,14}$=0.33, P=0.58; V_S $F_{1,14}$=0.17, P=0.69; supplementary material Fig. S5).

Yolk consumption

Embryonic yolk was depleted at the same rate (0.004 mm^3 day^{-1}) in both pH treatments (time: t-value=–16.22, P<0.001; pH: t-value=0.73, P=0.47). Yolk volume decreased in both conditions from 0.072±0.004 mm^3 (~20 days pre-hatching, 5 days in the treatments) to 0.012±0.001 mm^3 (~5 days pre-hatching, 20 days in the treatments; supplementary material Fig. S6).

Larval activity

Maxilliped activity ranged from 271 to 545 beats min^{-1} in the ambient pH treatment and was between 169 and 464 beats min^{-1} in low pH. Mean maxilliped frequency was 15% lower under acidified conditions, but the difference was not statistically significant (ANOVA: $F_{1,12}$=1.17, P=0.30; supplementary material Fig. S7).

Morphometrics

Embryo volume increased in the ambient pH condition from 0.27±0.02 to 0.31±0.04 mm^3 between 6 days pre-hatching (5 days in the treatments) and 2 days pre-hatching (9 days in the treatments, Fig. 4). In contrast, no changes in embryo volume were observed under low pH (0.28±0.03 mm^3 at 5 and 9 days, Fig. 4). Embryo ellipticity was not affected by pH, indicating that embryos maintained the same shape in both pH conditions. Non-parametric statistical analysis, treating the two time points independently, did not show a significant effect of pH on embryo volume (supplementary material Table S3). Larval morphology was not affected by low pH after 9 days in acidified water (supplementary material Table S4).

DISCUSSION

In this study, we assessed physiological performance of porcelain crab early life-history stages (embryo, larval, juvenile) following exposure to acidified water in order to understand possible variation in responses to OA related to natural exposure to different pH environments (i.e. intertidal zone and offshore pelagic). Physiological responses to low pH are discussed in two broad categories: survival and metabolic performance. Overall, we conclude that future ocean pH decline may result in sub-lethal physiological rate reductions in *P. cinctipes* early life-history stages, and that long-term exposure to acidified water may be detrimental for organisms that are presently tolerant to natural pH variability. Additionally, there is a suggestion of brood-specific responses to low pH, highlighting the necessity of including the role of parental effects in early life-history stages in response to environmental change in future investigations.

Survival and hatching success

Increased sensitivity to OA during early developmental stages has been observed in shrimp, crabs, snails and other marine invertebrates (Kurihara et al., 2008; Walther et al., 2009; Ellis et al., 2009; Melzner et al., 2009). In our study, survival of embryonic, larval and juvenile porcelain crabs, *P. cinctipes*, was not affected by low pH after 9 days of continuous exposure. However, embryonic morphometrics data suggest suppressed development under elevated CO_2 as embryos did not increase their volume in low pH. Embryonic volume increase before hatching is the norm in most, if not all, crustaceans [e.g. *Hyas araneus* (Petersen and Anger, 1997); *Eupagurus bernhardus*, *Ligia oceanica*, *Artemia salina* (Pandian and Schumann, 1967); *Crangon crangon* (Pandian, 1970); and *Homarus gammarus* (Pandian, 1970)]. Pre-hatching volume increases are due to changes in water intake and/or production of metabolic water from lipid catabolism (Petersen and Anger, 1997). Our study suggests a disruption of this natural pre-hatching process caused by changes in the pH of the surrounding water, though the disruption did not alter hatching success.

Reduced survival of juveniles under low pH was only evident during a longer-term exposure (more than 40 days), demonstrating that exposure to a continuous low pH can be detrimental for

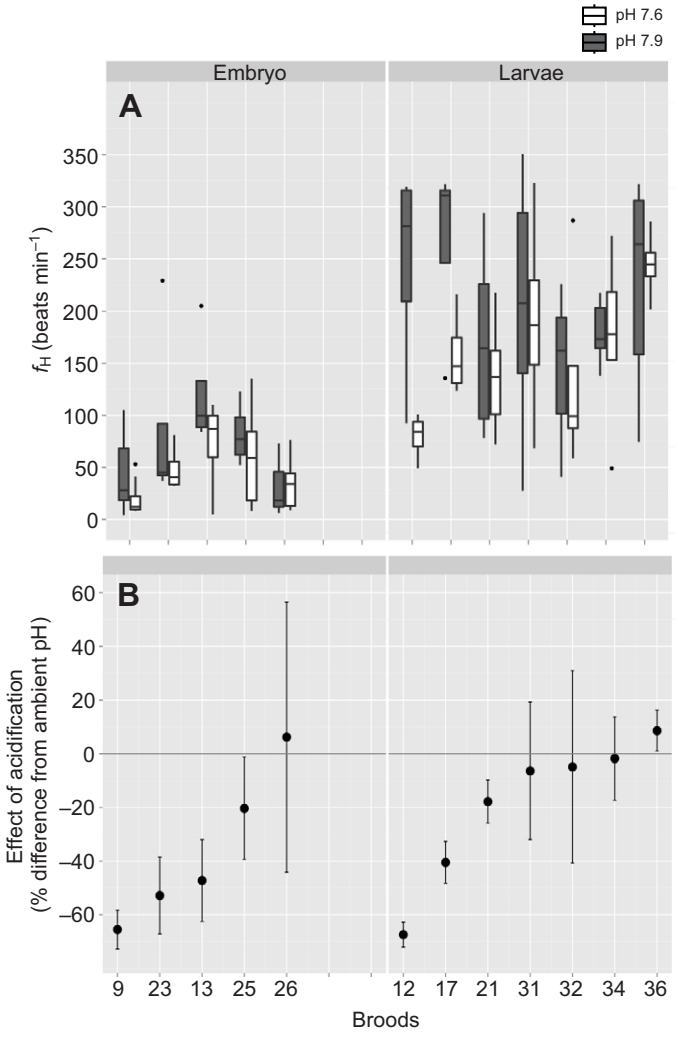

Fig. 2. (A) Heart rate (f_H) of *P. cinctipes* embryos (*N*=26–29 per treatment) and larvae (*N*=38–40 per treatment) pooled from five and seven broods, respectively, and measured after 8–10 days in the pH treatments. (B) Effect of low pH on heart rate of embryos and larvae (*N*=4–8 per brood) of independent broods after 8–10 days in the treatments, shown as the percentage change from ambient pH values. Means ± s.e.m.

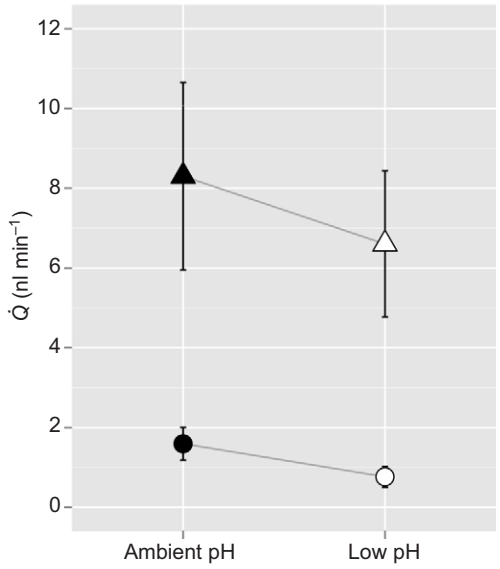

Fig. 3. Cardiac output ($\dot{Q}$) of *P. cinctipes* embryos (circles) and larvae (triangles) under two pH conditions: ambient (pH 7.9) and low (pH 7.6). Means ± s.e.m., *N*=5–10 individuals per stage per treatment (from *N*=4–6 broods).

organisms that are tolerant of extreme pH variability in their natural habitat. Future OA and climate change scenarios predict prolonged, extreme upwelling events amplified by unusual weather conditions (Meehl et al., 2007). This threat is specifically relevant along the California coast, where acidified deep waters upwell close to the coast, directly affecting coastal ecosystems (Feely et al., 2008; Morgan et al., 2009; Yu et al., 2011). Our data suggest that long-term upwelling events and/or the superimposition of high levels of anthropogenic CO_2 could represent a threat to species that presently appear to be quite tolerant to natural short-term changes in their environment (Pörtner et al., 2011).

Metabolic performance

Environmental hypercapnia associated with ocean acidification affects physiological processes such as acid–base regulation, cardiovascular function and metabolic activity, and results in reductions in growth, reproduction and survival (Ishimatsu and Kita, 1999; Albright, 2011). Low external pH causes extracellular acidosis, reducing hemocyanin O_2 affinity and potentially damaging cardiac cells, leading to CO_2 toxicity, muscle contraction failure and metabolic depression (Pörtner et al., 2011; Ishimatsu et al., 2004; Ishimatsu et al., 2008; Widdicombe and Spicer, 2008; Gesser and Poupa, 1983). Embryos and larvae of *P. cinctipes* exhibited metabolic depression, as measured by a significantly reduced heart rate under acidification, which is likely due to extracellular acidosis and associated cardiac muscle failure. Many intertidal zone organisms use metabolic depression as a mechanism to survive short-term sub-optimal conditions; however, this mechanism could have negative effects during long-term exposure to stressful scenarios.

Extracellular pH homeostasis involves regulation of the concentration of bicarbonate in extracellular fluids by bicarbonate ion transport across the cell membrane or from the surrounding environment (Pörtner et al., 2011). Though active transport mechanisms require cellular energy, in this study no additional energetic demands were observed in embryonic crabs held at low pH, as inferred by equal embryonic yolk consumption rates across treatments. Thus, energy used for pH homeostasis-related transport may be allocated from other processes (e.g. heart rate) (Barry et al., 2011). The reallocation of energy during stressful periods is considered a first response and will allow organisms to maintain short-term homeostasis (Melzner et al., 2009; Pörtner et al., 2011), though if sustained may compromise growth or reproduction (Albright, 2011).

To survive and maintain homeostasis under stressful conditions, an organism must have the capacity to control cardiovascular function (Reiber, 1997). Recent studies have shown heart rate reduction under acidification in many marine organisms [e.g. *Littorina obtusata* (Ellis et al., 2009); *Hyas araneus* (Walther et al., 2009)], but only a few studies have analyzed the capacity of individuals to modify parameters of cardiac function independently (stroke volume, heart rate and cardiac output) in order to compensate for environmental change (Harper and Reiber, 2004; Orlando and Pinder, 1995; Spicer, 2001). A reduction in cardiac output under sustained stressful conditions was observed in embryos and larvae of *P. cinctipes*, driven primarily by a reduction in heart rate with no adjustment of stroke volume for compensation. This may result

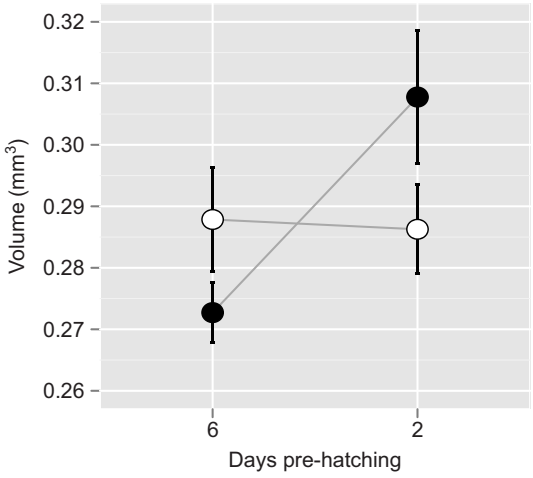

Fig. 4. *Petrolisthes cinctipes* embryo volume under two pH conditions (filled circles, ambient pH; open circles, low pH). Measurements were taken 6 and 2 days pre-hatching, representing 5 and 9 days in the treatments, respectively. Means ± s.e.m., *N*=13–15 from three females per group per time point.

in lower oxygen availability and CO_2 removal at a cellular level, potentially contributing to an overall metabolic depression. Our results are consistent with additional findings (Carter et al., 2013) that the oxygen consumption rate of *P. cinctipes* embryos was reduced under acidification.

Consistent with reduced heart rate and a hypothesized compromise in muscle function under acidification, *P. cinctipes* larvae showed slower maxilliped activity. Reduced maxilliped beating may negatively impact important processes such as ventilation and swimming, potentially increasing vulnerability to predation and decreasing feeding performance (Larimer, 1964; Batterton and Cameron, 1978).

In many groups of organisms, the experiences of the breeding parents can influence the viability and quality of the offspring (Sibert et al., 2004; McCormick and Gagliano, 2008) and their tolerance of environmental stress (Parker et al., 2012; McCormik and Gagliano, 2008). Parental genetic input, energy allocated to eggs and maternal behavior (e.g. aeration, cleaning and protection) (Levi et al., 1999) during embryo brooding may determine the sensitivity of independent broods and their ability to tolerate extreme conditions. Factors like density-dependent stress in the breeding females of *Pomacentrus amboinensis* had a negative effect on larval size (McCormick, 2006). Adult shrimp *Palaemon pacificus* and copepods reduce egg production under acidified water (Kurihara et al., 2008; Zhang et al., 2011).

Consistent with previous studies, we observed that the response (heart rate) to low pH varied among females (broods) (e.g. Chan et al., 2011; Carter et al., 2013) and this variation was strongest during the embryonic stage, suggesting maternal and/or paternal effects on some physiological responses to elevated CO_2. In our study design, treatment and brood were not truly independent, but the substantial variation between broods across multiple experiments provides considerable support for the possibility of brood-specific responses to OA. Different responses to acidification among broods could be related to microenvironmental variation within the intertidal zone. If females are exposed to diverse conditions (e.g. food availability, abiotic parameters) during gametogenesis or early embryogenesis, this could result in variation in genetic or energetic allocation and so alter the offspring's resilience to environment changes. Even though many marine organisms have long generation times, reducing the adaptation capacity to face rapid changes in their environment, some recent studies have shown that the variability in the responses could represent potential for adaptation in some groups (Sunday et al., 2011; Parker et al., 2012). Variation in response to acidified water among broods observed in this study suggests a potential for adaptation; however, further analysis needs to be done in order to understand the heritability of tolerance capacities.

This study shows that continuous acidification may be detrimental to intertidal zone organisms that naturally experience fluctuating hypercapnic conditions. Greater understanding of sub-lethal responses, like the reduction in cardiac performance shown by *P. cinctipes*, and the interaction between parentage and the stress response are of great importance as we attempt to predict changes in intertidal zone community structure under future OA scenarios.

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AUTHOR CONTRIBUTIONS

L.C.-O. led all aspects of conception, design and execution of the study, interpretation of the findings, and drafting and revising the article. H.A.C., N.A.M. and J.H.S. participated in aspects of conception, design and execution of the study, interpretation of the findings, and drafting and revising the article.

COMPETING INTERESTS

No competing interests declared.

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